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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-572

Microbiology Review(s)

Division of Anti-Infective Drug Products
Clinical Microbiological Review # 1

NDA: 21-572 **Dates Completed: September 5, 2003**

Applicant (NDA):
Cubist Pharmaceuticals, Inc.
65 Hayden Avenue
Lexington, MA 02421
781-860-8660

Therapeutic Type: Daptomycin for injection

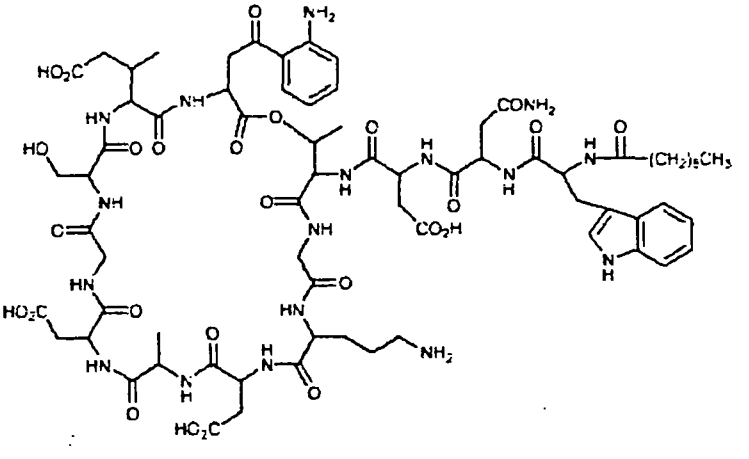
Submissions Reviewed: NDA 21,572

Providing for: Treatment of complicated skin structure infections (cSSSI)

Product Name(s):
Proprietary: Cubicin ®
Non-proprietary: Daptomycin

Chemical name: *N*-decanoyl-L-tryptophyl-L-asparaginyl-L-aspartyl-L-threonylglycyl-L-ornithyl-L-aspartyl-D-alanyl-L-aspartylglycyl-D-seryl-*threo*-3-methyl-L-glutamyl-3-anthraniloyl-L-alanine ε_1-lactone.

Structural formula:



Molecular formula: $C_{72}H_{101}N_{17}O_{26}$; the molecular weight is 1620.67.

Dosage form: Four mg/kg administered over a 30-minute period by intravenous infusion in 0.9% sodium chloride injection, USP once every 24 hours for 7-14 days.

Route(s) of administration: Injection

Pharmacological Category: Anti-Infective

Dispensed: Rx__X____ OTC_____

Initial Submission Dates

Received by CDER: September 12, 2003
Received by Reviewer: September 12, 2003
Review Completed: September 12, 2003

Related Documents: NDA 21,572; IND 57,693

Remarks:

This is an amendment to the original review of the clinical microbiology portion of an NDA submission from Cubist Pharmaceutical, Inc. for Cubicin. This drug is intended to treat **complicated skin and skin structure infections** caused by *S. aureus* (methicillin-susceptible and –resistant strains), *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae* subsp. *equisimilis*, [redacted] and *Enterococcus faecalis* (vancomycin-susceptible strains only). However, based on discussion within the review team and as negotiated with the Applicant, the [redacted] has been excluded as a pathogen for the indication.

This review addresses the modification of the breakpoints for the Streptococci species listed in the product package insert. The original susceptible breakpoint negotiated with Cubist Pharmaceuticals, Inc. of [redacted] for Streptococci have been renegotiated to ≤0.5 µg/mL, since the [redacted] is now deleted from the indications section of the package insert. It is concluded by the review team that this organism is not a pathogen for complicated skin and skin structure infections. Thus we need to change the breakpoint to reflect the susceptibility of the pathogens to be approved in the indications section of the package insert for daptomycin.

The basis of our argument rests upon the following points:

- Analysis of the in vitro spectrum of activity as presented in the original review does not support the breakpoint of — unless the — is included in the analysis.
- However, it has been determined by the review team that the — should be excluded from the analysis because they are not considered pathogens for the indication of complicated skin and skin structure infections sought by the Applicant.
- Analysis of the in vitro spectrum of activity dataset excluding — supports the breakpoint of ≤0.5 µg/mL.
- In addition to this dataset, the surveillance information clearly supports a breakpoint of ≤0.5 µg/mL. Evaluation of this data shows that the vast majority of pathogens had MICs less than 0.5 µg/mL. Thus, pathogens with MICs greater than 0.5 µg/mL are rare.
- Although pharmacokinetic/pharmacodynamic studies were performed, the majority of the studies were performed with *Streptococcus pneumoniae*, an organism not sought as a pathogen for the proposed indication. Some studies were performed with *S. pyogenes*; these data are used to provide part of the information necessary to make decisions on breakpoints. These data are not the final arbitrators of breakpoint determinations but augment existing evidence.
- Evaluation of the clinical data was also performed to determine the final breakpoint for Streptococci species. If we look at Microbiological Review #1 and specifically at Table 47 (page 59) which describes clinical and microbiological success rates by MIC, we clearly see that there are no clinical or microbiological experiences to support a breakpoint of — In fact we have little evidence to demonstrate the efficacy of daptomycin for pathogens with susceptible MICs of 0.5 µg/mL. Most of these data demonstrate clinical and microbiological efficacy for pathogens with MICs of ≤0.25 µg/mL. Since a majority of the clinical and microbiological experiences are with MICs at this dilution, and the error of the assay can be ± one tube dilution, the breakpoint supported by the data is ≤0.5 µg/mL. This is consistent with the practice of setting breakpoints that are one dilution higher than the clinical and microbiological experiences.
- These arguments were conveyed to the Applicant in a teleconference dated September 11, 2003 at which time final agreement was reached that the breakpoint of ≤0.5 µg/mL would be established for Streptococci species. They conceded the discussion and sent their final product package insert with the susceptible breakpoint of ≤0.5 µg/mL.

Conclusions/Recommendations:

The Microbiology portion of this submission is approvable but with the indicated changes to the Microbiology Section of the Package Insert. Specifically, the susceptible breakpoint of ≤0.5 µg/mL for the Streptococci species listed in the indications section of the package insert and as described in the Microbiology section should be adopted.

Peter Coderre Ph.D.
Microbiology Reviewer

Albert T. Sheldon, Jr. Ph.D.
Microbiology Team Leader

Cc: Original NDA No. 021-572
Microbiologist, HFD-520
File name: 21572-Strept BPs.doc N21572_RD#2.doc

Smicro/ATSheldon
RD#1 Initialed 6/10/03, RD#2 Initialed 8/27/03 ATS; Final Initialed 8//03ATS

DepDir/LGavrilovich

Cc: Original NDA # 21-572
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HFD-520/DepDir/LGavrilovich
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HFD-520/Pharm/
HFD-520/Chem/
HFD-520/CSO/
HFD-520
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HFD-635

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/s/

Albert Sheldon
9/12/03 11:24:28 AM
MICROBIOLOGIST

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9/12/03 11:34:59 AM
MEDICAL OFFICER

Division of Anti-Infective Drug Products
Clinical Microbiological Review # 1

NDA: 21-572 **Dates Completed: September 5, 2003**

Applicant (NDA):
Cubist Pharmaceuticals, Inc.
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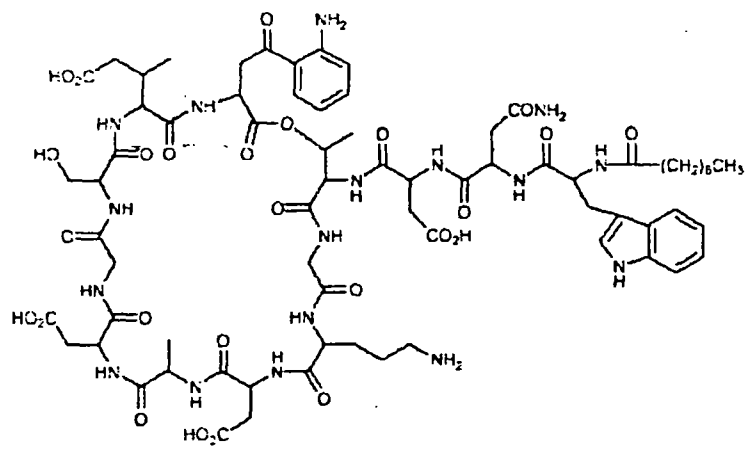

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Pharmacological Category: Anti-Infective

Dispensed: Rx__X____ OTC_____

Initial Submission Dates

Received by CDER: December 19, 2002
Received by Reviewer: December 30, 2002
Review Completed: September 5, 2003

Related Documents: IND 57,693

Remarks:

This is a review of the clinical microbiology portion of an NDA submission from Cubist Pharmaceutical, Inc. for Cubicin. This drug is intended to treat **complicated skin and skin structure infections** caused by *S. aureus* (methicillin-susceptible and –resistant strains), *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae* subsp. *equisimilis*, — and *Enterococcus faecalis* (vancomycin-susceptible strains only).

Conclusions/Recommendations:

The Microbiology portion of this submission is approvable but with the indicated changes to the Microbiology Section of the Package Insert.

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INTRODUCTION

Daptomycin is a lipopeptide antibiotic derived from *Streptomyces roseospor* that represents a new class of agents. The spectrum of activity is similar to those of vancomycin and teicoplanin, and has bactericidal activity against most Gram-positive pathogens including the clinically significant species of staphylococci, streptococci, and enterococci. The characteristics of daptomycin that distinguish it from vancomycin and teicoplanin are its concentration-dependent bactericidal activity against enterococci and staphylococci, its novel mechanism of action and its requirement for ionized calcium. The Applicant has provided the microbiology data that they believe will help to support their request for the following indication:

> **Complicated skin and skin structure infection** caused by *S. aureus* (methicillin-susceptible and –resistant strains), *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae* subsp. *equisimilis*, ——— ———) and *Enterococcus faecalis* (vancomycin-susceptible strains only).

The Applicant also proposes the interpretative criteria presented in Table 1 for the following pathogens that cause complicated skin and skin structure infections.

Table 1: Provisional interpretive criteria for susceptibility to daptomycin

[

]

PRECLINICAL EFFICACY (*IN VITRO*)

MECHANISM OF ACTION

Daptomycin inserts directly into the cytoplasmic membrane of Gram-positive cells [1]. This action is calcium-dependent and results in dissipation of the membrane potential [2, 3]. Depolarization of the membrane is followed rapidly by the arrest of bacterial DNA, RNA, and protein synthesis, and cell death. The conditions, rates and dose responses

associated with daptomycin-induced depolarization are consistently associated with its bactericidal activity. A possible mechanism for the depolarization has been demonstrated through the release of potassium ions from bacterial cells exposed to daptomycin. An early report suggested that daptomycin acts through the inhibition of lipoteichoic acid synthesis [4, 5]; however, data that are more recent indicate that event is secondary to membrane depolarization [6].

Binding and fractionation studies using ^{14}C-daptomycin in *S. aureus* and human tissue culture cells suggest that daptomycin inserts tightly into the membrane of Gram-positive pathogens, but is only loosely associated with the membranes of mammalian cells [9, 10].

Upon binding, daptomycin gradually dissipates the membrane potential of *S. aureus*, requiring 30 to 60 minutes for full depolarization compared to less than 5 minutes for the pore-forming antibiotic nisin. Viability decreased in parallel to the changes in potential.

The Applicant has provided data that further support the mechanism of action. These data can be found in of the Microbiology Section 6.1 of the briefing package.

Effects of miscellaneous factors on activity

The antibacterial activity of daptomycin requires the presence of free (ionized) calcium. This requirement cannot be met by other inorganic or organic cations [8, 27]. A solution of 50 mg/L calcium has a free (ionized) calcium concentration of 1.1 mM, which is close to the normal range for human serum (1.15 - 1.31 mM)[7]. Current NCCLS standards specify that Mueller-Hinton Broth (MHB) used for susceptibility testing should contain 25 mg/L calcium [17]; the proposed standard for daptomycin testing is 50 mg/L calcium [13]. These studies indicate that daptomycin MICs are more accurate and reproducible when the media contain 50 mg/L calcium and are artifactually elevated 2- to 8-fold when the media contain 25 mg/L calcium [60, 63, 64].

Table 2 displays the distribution, median, and geometric mean of the daptomycin MICs at the two calcium concentrations for each of three major genera of Gram-positive pathogens (staphylococci, streptococci, and enterococci). Using the proposed standard media for susceptibility testing (MHB supplemented with 50 mg/L calcium), >99% of staphylococci were inhibited by 1 µg/ml of daptomycin; >99% of streptococci by 0.5 µg/ml; and 92% of enterococci by 2 µg/ml.

As noted above, the current NCCLS standard media for susceptibility testing are Mueller-Hinton broth or agar supplemented with 25 mg/L calcium [17]. On the basis of the data presented from the Applicant and similar results from other laboratories, the proposed NCCLS recommendation is that daptomycin susceptibility testing of rapidly growing, aerobic Gram- positive organisms be performed using Mueller-Hinton broth media with 50 mg/L calcium. This level of supplementation provides a physiologic concentration of free (ionized) calcium and ensures that *in vitro* susceptibility measurements are accurate and reproducible. Media typically used for susceptibility testing of anaerobes, e.g., Brucella blood agar, are also deficient in Ca^{2+} ions and must be supplemented to physiological levels of Ca^{2+} ions for use with daptomycin.

Table 2: Distribution of daptomycin MICs for three different genera of bacteria tested in MHB with two different levels of calcium supplementation[a]

Daptomycin MIC (µg/ml)	*Staphylococcus* spp. (N= 1,094)		*Streptococcus* spp. (N= 1,096)		*Enterococcus* spp. (N=550)	
	No. of strains at each MIC when tested with different calcium supplement[b]					
	25 mg/L	50 mg/L	25 mg/L	50 mg/L	25 mg/L	50 mg/L
>16					1	
16					14	
8	2		1	1	109	4
4	2		3	1	232	40
2	39	3	12	2	130	172
1	741	16	155	6	51	201
0.5	251	339	597	38	5	106
0.25	53	656	82	351	6	18
0.12	5	73	216	430	1	6
0.06		5	28	114		2
0.03		1	1	140	1	1
0.016	1	1	1	13		
0.008						
Median MIC	1	0.25	0.5	0.12	4	1
Geometric mean MIC	0.81	0.30	0.38	0.13	3.34	1.12

a. Cation-adjusted Mueller-Hinton Broth with calcium supplemented to 25 or 50 mg/L as indicated.

The Applicant has supplied additional data that explain the effects of calcium that can be found in the Microbiology Section 6.4 of the briefing package.

Daptomycin powder is stable for at least 2 years at refrigerated temperatures (4°C±3°C). Daptomycin in water or phosphate buffer solutions stored frozen (-20°C) was stable for at least three months with little degradation (as determined by —

The Applicant has supplied evidence for the stability of daptomycin in various microbiological broth media used for susceptibility testing and assessed at concentrations of 2 and 8 µg/ml. Daptomycin showed good stability in the presence of 5% lysed horse blood over the course of the experiments.

Daptomycin is appreciably bound to serum proteins (approx. 90%). As expected, susceptibility testing of daptomycin in the presence of serum proteins was associated with an increase in the MIC.

Further data on the effects of serum proteins was provided by the Applicant in Microbiology section 6.4 of the briefing package.

The *in vitro* antibacterial activity of daptomycin is reduced by increases in the inoculum density. The effect of the inoculum density on the activity of daptomycin against isolates of *S. aureus*, *S. epidermidis* and *E. faecalis* was studied by several independent investigators [21, 29]. The daptomycin MIC of these isolates increase 2- to 8-fold as the inoculum density increased from 10^3 to 10^6 CFU/ml (Table 3).

Table 3: Effect of inoculum density of the *in vitro* activity of daptomycin

Isolate (Antibiotic Susceptibility)	Log_{10} Inoculum Density (CFU/ml) 3	4	5	6
			MIC µg/ml	
S. pneumoniae SSL #25 (Pen-S)[a]	0.25	0.5	1	1
S. pneumoniae SSL#27 (Pen-R)	0.5	1	1	1
Staphylococcus aureus #784 (Meth-R)	1	2	2	8
Staphylococcus aureus SSL#758 (Meth-R)	1	1	2	8
Staphylococcus spp.[b] SSL#638 (Van-I)	1	1	2	4
S. aureus ATCC 29212 (Meth-S)	0.5	1	2	8
Enterococcus faecium SSL#501 (Van-R)	0.125	0.5	2	4
E. faecalis ATCC 29213 (Van-S)	1	2	4	8

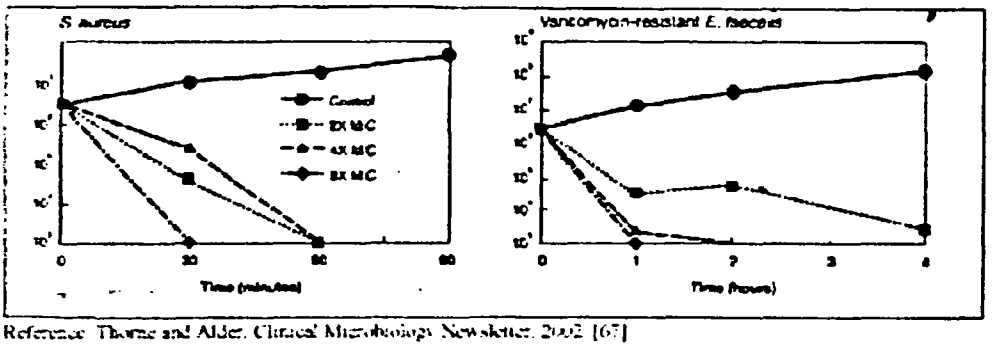
a. Tested in MH agar supplemented with 5% sheep blood, incubated in CO_2
b. Coagulase-negative staphylococci

Bactericidal activity

Time-kill curves illustrating the bactericidal activity of daptomycin against *S. aureus* and *E. faecalis* are shown in Figure 3. For *S. aureus* (including MRSA), daptomycin (at 2x to 4x MIC) achieves a 3-log_{10} reduction in viable organisms in less than 30 min. Daptomycin is bactericidal within 1 hour at 4xMIC against VRE. Vancomycin is generally bacteriostatic against enterococci using the same methodology.

Figure 3: Bactericidal effects of daptomycin

Figure 6-11: Bactericidal effects of daptomycin



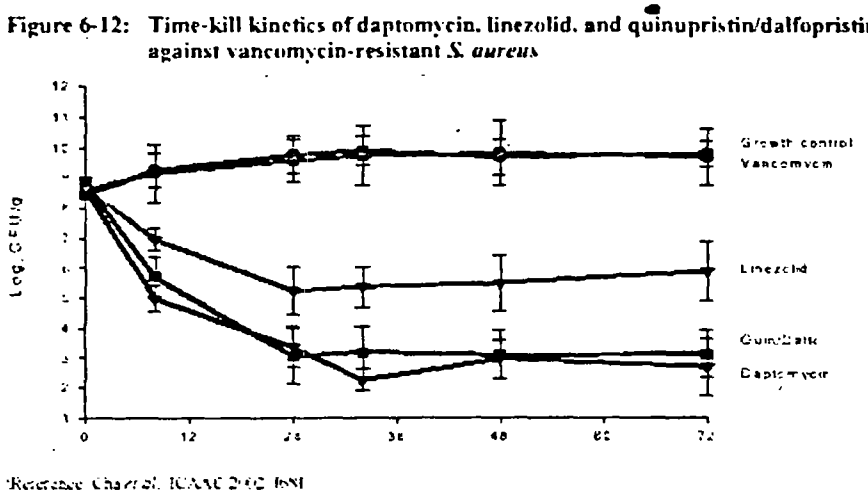
Reference: Thorne and Alder. Clinical Microbiology Newsletter. 2002 [67]

Fuchs *et al.* [82] determined the bactericidal activity of daptomycin as compared to those of vancomycin, quinupristin/dalfopristin and linezolid against 108 isolates of staphylococci (3 GISA, 25 MRSA, 40 MRSE, 25 MSSA, 4 MSSE and 11 *S. haemolyticus*). Eighty-three percent of the isolates showed MBC/MIC ratio of 1 (MICs =MBCs); 15% showed MBC/MIC ratio= 2 (within 1 dilution), and 2 isolates (one MRSA, and one MRSE) showed MBC/MIC ratio = 4 (2 dilutions apart). The compilation of MIC and MBC data from this study is shown in Table 6-16.

Recently Cha *et al* [31] investigated the bactericidal activity of daptomycin, linezolid, and quinupristin/dalfopristin against the first reported isolate of VRSA [78] in an *in vitro*

pharmacodynamic model with simulated endocardial vegetations (Figure 2). Daptomycin and quinupristin/dalfopristin achieved 99.9% kill against the VRSA isolate by 8 hours (first timepoint analyzed) and maintained bactericidal activity for the duration of the experiments (i.e. 72 hours). Linezolid did not achieve 99.9% kill until 24 hours, vancomycin had no activity against the isolate.

Figure 2: Bactericidal effects of daptomycin, linezolid, and quinupristin/dafopristin against vancomycin-resistant *S. aureus*.



Using MBC/MIC ratios, Snydman *et al.* determined that daptomycin was bactericidal for 82% (14/17) of *E. faecium* (VRE) strains [76]. Similar findings were obtained by Fuchs *et al.* against 44 enterococcal isolates (16 *E. faecalis*, 9 vancomycin-resistant, 7 vancomycin-susceptible; and 28 *E. faecium*, 19 vancomycin-resistant, 9 vancomycin-susceptible)[32]. Time-kill studies determining the bactericidal activity of daptomycin against enterococci showed that daptomycin is bactericidal against enterococci, with a rate of kill for VRE that appears to be slower than that observed for MRSA. Akins and Rybak, using the pharmacodynamic *in vitro* model demonstrated bactericidal activity of daptomycin against an *E. faecium* (VRE) isolate [81].

Transmission Electron Microscopy (TEM) was used to better assess the early structural effects of daptomycin on *S. aureus* strain 42 MRSA exposed to four concentrations of daptomycin [33]. Cells exposed to 2 µg/ml demonstrated no killing and begin to recover and grow at approximately 2hr. Cells exposed to 4 µg/ml showed killing during the first hour.

Table 4: MICs and MBCs of daptomycin and 3 comparator agents against 108 Staphylococci isolates

Species (resistance phenotype)	No. of Strains	Antimicrobial Agent	MIC Range (µg/ml)	MBC Range (µg/ml)	MBC/MIC[a] (dilutions)
S. aureus (methicillin-susceptible)	25	Daptomycin			1 - 2
		Vancomycin			1 - 2
		Linezolid			>16 - >64
		Quinupristin/dalfopristin			1 - >16
S. aureus (methicillin-resistant)	25	Daptomycin			1 - 4
		Vancomycin			1 - 2
		Linezolid			16 - >32
		Quinupristin/dalfopristin			>4 - >16
S. aureus (glycopeptide intermediate-susceptible; GISA)	3	Daptomycin			1 - 2
		Vancomycin			1 - >4
		Linezolid			8.0 - >32
		Quinupristin/dalfopristin			1 - >8
S. epidermidis (methicillin-susceptible)	4	Daptomycin			1 - 2
		Vancomycin			1 - 1
		Linezolid			32 - >128
		Quinupristin/dalfopristin			1 - >32
S. epidermidis (methicillin-resistant)	40	Daptomycin			1 - 4
		Vancomycin			1 - 2
		Linezolid			1 - >128
		Quinupristin/dalfopristin			1 - >32
S. haemolyticus	11	Daptomycin			1 - 2
		Vancomycin			1 - 2
		Linezolid			1 - >64
		Quinupristin/dalfopristin			1 - >16

a. MBC/MIC < 4 is interpreted as bactericidal activity; MBC/MIC > 4 is interpreted as lack of bactericidal activity

SPECTRUM OF ACTIVITY OF DAPTOMYCIN

Daptomycin is a potent antibiotic with a spectrum of activity that includes many clinically significant species of aerobic and anaerobic Gram-positive pathogens. The *in vitro* activity of daptomycin has been assessed against more than 21,000 clinical isolates from throughout the US and Europe. These studies include a series of carefully controlled, large-scale profiling surveys using standard NCCLS recommended methodology [17], except that microdilution was performed using MH broth supplemented with 50 mg/L calcium chloride, as recommended by NCCLS for daptomycin susceptibility testing (Table 3, pp. 118-119, Document M100-S12 [13]). As detailed below (see Section 6.4.1), accurate *in vitro* assessment of daptomycin activity requires physiologic levels of free (ionized) calcium.

The Applicant has provided a table (Table 5) which summarizes the spectrum of activity of daptomycin.

Table 5: Summary of the spectrum of activity of daptomycin

Species of Microorganism	N	MIC Range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Aerobes (Gram-positive)				
Bacillus anthracis	13		2.0	2.0
Bacillus spp.	8		-	-
Corynebacterium jeikeium	68		0.25	0.25 - 0.5
Other Corynebacterium spp.	42		0.06	0.25
Enterococcus avium	10		-	-
Enterococcus casseliflavus	1		-	-
Enterococcus faecalis (Susceptibility not specified)	951		0.25 - 1.0	0.25 - 4.0
Enterococcus faecalis (Vancomycin-susceptible)	4310		0.5 - 1.0	0.5 - 2.0
Enterococcus faecalis (Vancomycin-resistant)	131		0.5 - 2.0	0.5 - 4
Enterococcus faecalis (Vancomycin-intermediate)	5		-	-
Enterococcus faecium (Susceptibility not specified)	875		1.0 - 4.0	1.0 - 8.0
Enterococcus faecium (Vancomycin-susceptible)	590		1.0 - 2.0	2.0 - 4.0
Enterococcus faecium (Vancomycin-resistant)	525		1.0 - 4.0	2.0 - 4.0
Enterococcus faecium (Vancomycin-intermediate)	9		-	-
Enterococcus gallinarum	11		-	-
Enterococcus spp. (Vancomycin-susceptible)	216		<0.5 - 2.0	4.0 - 4.0
Enterococcus spp. (Vancomycin-intermediate)	6		-	-
Enterococcus spp. (Vancomycin-resistant)	18		4.0 - 4.0	4.0 - 4.0
Enterococcus spp. (Susceptibility not specified)	109		1.0 - 1.0	4.0 - 4.0
Lactobacillus spp. (grown aerobically)	15		-	-
Lactococcus coprophilus	2		-	-
Leuconostoc spp.	4		-	-
Listeria monocytogenes	32		4.0	4.0
Pediococcus pentosaceus	2		-	-
Staphylococcus aureus (methicillin-susceptible; MSSA)	2440		0.125 - 0.5	0.125 - 1.0
Staphylococcus aureus (methicillin-resistant; MRSA)	1378		0.125 - 1.0	0.13 - 1.0
Staphylococcus aureus (vancomycin-intermediate; VISA)	8		-	-
Staphylococcus aureus (vancomycin-resistant; VRSA)	2		-	-
Staphylococcus epidermidis (methicillin-susceptible;	101		0.13 - 0.25	0.25 0.5
Staphylococcus epidermidis (methicillin-resistant; MRSE)	105		0.13 - 0.25	0.25 - 0.25
Staphylococcus spp. (vancomycin-intermediate)	4		-	-
Staphylococcus haemolyticus	102		0.125 - 0.25	0.25 - 0.5
Staphylococcus saprophyticus	36		0.5 - 0.5	0.5 - 0.5
Staphylococcus spp., coagulase negative (methicillin-susceptible)	1101		0.12 - 0.25	0.25 - 0.5
Staphylococcus spp., coagulase negative (methicillin-resistant)	1779		0.25 - 0.5	0.5 - 1.0
Streptococcus agalactiae (Group B)	983		0.125 - 0.5	0.25 - 0.5
Streptococcus anginosus	6		-	-
Streptococcus β–hemolytic (not grouped or speciated)	100		-	-
Streptococcus bovis	12		-	-
Streptococcus gordonii	5		-	-
Streptococcus intermedius	8		-	-
Streptococcus milleri	49		0.25 - 0.5	0.5 - 1.0
Streptococcus mitis	16		0.5 - 0.5	1.0 - 1.0
Streptococcus oralis	30		0.5 - 0.5	1.0 - 1.0
Streptococcus pneumoniae (penicillin susceptibility not specified)	428		0.125 - 0.25	0.25 - 0.5
Streptococcus pneumoniae (penicillin-susceptible)	1894		0.03 - 0.12	0.125 - 0.25
Species of Microorganism	**N**	**MIC Range**	**MIC_{50}**	**MIC_{90}**

		(µg/ml)	(µg/ml)	(µg/ml)
Streptococcus pneumoniae (penicillin-intermediate)	638		0.12 - 0.25	0.25 - 1.0
Streptococcus pneumoniae (penicillin-resistant)	454		0.12 - 0.25	0.125 - 1.0
Streptococcus pyogenes (Group A)	950		0.015 - 0.06	0.06 - 0.12
Streptococcus salivarius	10		-	-
Streptococcus sanguis	18		0.5 - 0.5	1.0 - 1.0
Streptococcus parasanguis	2		-	-
Streptococcus vestibularis	1		-	-
Streptococcus spp., Group C, F, and G	59		0.03 - 0.06	0.06 - 0.06
Viridans *Streptococcus* Group (not speciated)	593		0.25 - 0.5	1.0 - 1.0
Anaerobes (Gram-positive)				
Clostridium difficile	18		0.5	1.0
Clostridium innocuum	19		2.0	4.0
Clostridium perfringens	11		0.5	0.5
Clostridium ramosum	15		16.0	16.0
Other *Clostridium* species	25		0.5	2.0
Lactobacillus species	37		1.0	16.0
PeptoStreptococcus asaccharolyticus	10		0.02	0.06
PeptoStreptococcus magnus	7		-	-
PeptoStreptococcus micros	7		-	-
Propionibacterium spp.	15		0.5	2.0
Total [N]	**21,703**			

Daptomycin possesses potent *in vitro* activity against the most common aerobic Gram-positive pathogens [14, 15, 63, 71, 72, 75, 76], including staphylococci and enterococci resistant to methicillin, vancomycin, linezolid, and/or quinupristin-dalfopristin (e.g., MSSA, MRSA, MRSS, GISA, VSE, VRE). The two recently reported isolates of vancomycin-resistant *S. aureus* (VRSA) [18, 78] are susceptible to daptomycin with a MIC of 0.5 and 1 µg/ml [23, 79]. Table 6 presents the *in vitro* activity of daptomycin against 4,429 *S. aureus* isolates, including 1,378 MRSA, and 3,371 coagulase-negative staphylococci. Daptomycin was active against all isolates of *S. aureus*, with a MIC range of =0.015-2 µg/ml. Based on the SECURE studies; the MIC_{90} for all *S. aureus* is 0.5µg/ml (see Table 6). Resistance to methicillin did not affect the potency of daptomycin. Daptomycin was also very active against coagulase-negative *Staphylococcus* spp. including *S. epidermidis*, with MIC_{90} of 0.5 µg/ml [Table 6].

Over the past several years, staphylococcal isolates with reduced susceptibility to glycopeptides have emerged [19]. In general, these isolates have also been resistant to methicillin and other ß-lactam antibiotics. The Applicant has provided data that shows daptomycin has potent antimicrobial activity against the glycopeptide intermediate-susceptible staphylococci (GISE). This data can be found in Microbiology Section 6.2 of the briefing package.

Table 6: Activity of Daptomycin against Staphylococci

Species of Microorganism	N	MIC Range µg/ml	MIC_{50} µg/ml	MIC_{90} µg/ml
Staphylococcus aureus (methicillin-susceptible) (MSSA)	2440		0.125 -0.5	0.125- 1.0
Staphylococcus aureus (methicillin-resistant) (MRSA)	1378		0.125 - 1.0	0.13 - 1.0
Staphylococcus aureus (methicillin not specified)	601		0.25	0.25 - 0.5
Staphylococcus aureus (vancomycin-intermediate) (VISA)	8		-	-
Staphylococcus aureus (vancomycin-resistant) (VRSA)	2		-	-
Staphylococcus epidermidis (methicillin-susceptible) (MSSE)	101		0.13 - 0.25	0.25 - 0.5
Staphylococcus epidermidis (methicillin-resistant) (MRSE)	105		0.13 - 0.25	0.25 - .25
Staphylococcus epidermidis (methicillin not specified)	40		0.25 - 0.5	0.5 - 0.5
Staphylococcus spp., (vancomycin- intermediate)	4		-	-
Staphylococcus haemolyticus	102		0.125 - 0.25	0.25 - 0.5
Staphylococcus saprophyticus	36		0.5 - 0.5	0.5 - 0.5
Staphylococcus spp., coagulase negative (methicillin-susceptible)	1101		0.12 - 0.25	0.25 - 0.5
Staphylococcus spp., coagulase negative (methicillin-resistant)	1779		0.25 - 0.5	0.5 - 1.0
Staphylococcus spp., coagulase negative (methicillin not specified)	103		0.25	0.5
Total	**7800**			

- Recently, daptomycin was tested against a set of 57 *S. aureus* and 31 coagulase-negative staphylococci with reduced vancomycin susceptibility obtained between 1996 and 2001 [20]. The results are displayed in Table 7. The susceptibility of vancomycin-resistant clinical isolates of *S. aureus* to daptomycin is displayed in Table 8.

Table 7: Distribution of Daptomycin MIC against Staphylococci Collected from Project ICARE Hospitals

#Species (vancomycin susceptibility)	Vancomycin MIC	N	No. of Isolates with Daptomycin MIC (µg/ml) =0.12	0.25	0.5	1	2	4	8
S. aureus		57							
Intermediate (VISA)	8 µg/ml	3			1	2			
Decreased (DSV)	4 µg/ml	16				6	5	2	3
Susceptible (VS)	2 µg/ml	38	1	9	19	6	2	1	
Coagulase-Negative Staphylococci		31							
Intermediate (VISA)	8 µg/ml	1			1				
Decreased (DSV)	4 µg/ml	16	1		3	5	5	2	
Susceptible (VS)	2 µg/ml	14	1		10	2	1		

Shaded area represents MIC_{50}

Table 8: Daptomycin Susceptibility against Vancomycin-Resistant *Staphylococcus aureus* Isolate (VRSA)

Strain (Geographical Source)	Daptomycin MIC µg/ml	Vancomycin MIC µg/ml
S. aureus (Michigan)	1.0	>128
S. aureus (Pennsylvania)	0.5	>64

enterococci, including 5,397 *E. faecalis* 1,999 *E. faecium*, 11 *E. gallinarum*, 10 *E. avium*, 1 *E. casseliflavus* and 349 other *Enterococcus* spp. Based on the SECURE studies, the MIC_{90} for vancomycin- susceptible and -resistant *E. faecalis* is 2 µg/ml (Table 6). In general, daptomycin was slightly more active against *E. faecalis* than against *E. faecium*. The Applicant asserts that these data support the selection of provisional susceptible breakpoint of 8 µg/ml for *E. faecalis* as detailed in Table 1.

Table 10: Activity of Daptomycin against Enterococci

Species of Microorganism	N	MIC Range µg/ml	MIC_{50} µg/ml	MIC_{90} µg/ml
Enterococcus avium	10		-	-
Enterococcus casseliflavus	1		-	-
Enterococcus faecalis (vancomycin-susceptible)	4310		0.5 - 1.0	0.5 - 2.0
Enterococcus faecalis (vancomycin-intermediate)	5		-	-
Enterococcus faecalis (vancomycin-resistant)	131		0.5 - 2.0	0.5 - 4
Enterococcus faecalis (susceptibility not specified)	951		0.25 1.0	0.25 - 4.0
Enterococcus faecium (vancomycin-susceptible)	590		1.0 - 2.0	2.0 - 4.0
Enterococcus faecium (vancomycin-intermediate)	9		-	-
Enterococcus faecium (vancomycin-resistant)	525		1.0 - 4.0	2.0 - 4.0
Enterococcus faecium (susceptibility not specified)	875		1.0 - 4.0	1.0 - 8.0
Enterococcus gallinarum	11		-	-
Enterococcus spp. (vancomycin-susceptible)	216		<0.5 - 2.0	4.0 - 4.0
Enterococcus spp. (vancomycin-intermediate)	6		-	-
Enterococcus spp. (vancomycin-resistant)	18		4.0 - 4.0	4.0 - 4.0
Enterococcus spp. (susceptibility not specified)	109		1.0 - 1.0	4.0 - 4.0
Total [N]	**7,767**			

The Applicant has provided a table (Table 11) demonstrating the activity of daptomycin against Gram-positive aerobic bacteria. These data are from two of the largest daptomycin profiling studies, SECURE US and SECURE EU. The MIC_{50} and MIC_{90} values generated for this set of > 12,000 isolates provide a basis of comparison for the other smaller *in vitro* studies presented. Table 11 compares the MIC distribution for these isolates by geographic region and species. The activity of daptomycin against prevalent Gram-positive pathogens was similar in both the US and Europe. Within and between both regions, daptomycin MICs were similar for antibiotic-resistant and -susceptible isolates within a species, including oxacillin-resistant staphylococci and vancomycin-resistant enterococci.

Table 11: Daptomycin Activity against Selected Pathogens by Geographic Region from the SECURE Surveillance Studies

Organism Resistance phenotype	Geographic region	N=	MIC Range (µg/mL)	MIC_{50}	MIC_{90}
S. aureus					
Oxacillin-Susceptible	US	713		0.25	0.25
	EU	888		0.25	0.5
Oxacillin-Resistant	US	305		0.25	0.5
	EU	334		0.25	0.5
Coagulase-negative Staphylococci					
Oxacillin-Susceptible	US	356		0.25	0.5
	EU	486		0.25	0.5
Oxacillin-Resistant	US	770		0.25	0.5
	EU	554		0.25	0.5
E. faecalis					
Vancomycin-Susceptible	US	2,049		1.0	2.0
	EU	1,798		1.0	2.0
Vancomycin-Resistant	US	40		1.0	2.0
	EU	40		1.0	2.0
E. faecium					
Vancomycin-Susceptible	US	147		2.0	4.0
	EU	333		2.0	4.0
Vancomycin-Resistant	US	219		2.0	4.0
	EU	114		2.0	4.0
S. pneumoniae					
Penicillin-Susceptible	US	728		0.12	0.12
	EU	619		0.12	0.25
Penicillin-Intermediate	US	248		0.12	0.25
	EU	165		0.12	0.25
Penicillin-Resistant	US	187		0.12	0.12
	EU	81		0.12	0.25
S. agalactiae	US	273		0.12	0.25
	EU	367		0.25	0.25
S. pyogenes	US	484		0.03	0.06
viridans streptococci group	US	369		0.25	1.0

Daptomycin displayed activity against 206 isolates of other Gram-positive aerobic bacteria such as *Bacillus anthracis*, *Bacillus* spp., *Corynebacterium* spp., *Lactococcus* spp., *Leuconostoc* spp., *Listeria monocytogenes*, *Micrococcus* spp. and *Pediococcus* spp. Daptomycin showed good activity against *B. anthracis* including the Ames strain. Daptomycin exhibited very good activity against isolates of *Corynebacterium jeikeium*, which is the most commonly isolated *Corynebacterium* species and often resistant to most β- lactam antibiotics (see Table 5).

Gram positive anaerobes

Daptomycin exhibited *in vitro* activity against Gram-positive anaerobic species, such as *Peptostreptococcus* spp., *Clostridium perfringens, Clostridium difficile*, other *Clostridium* spp., and anaerobic Gram-positive non-spore forming bacilli such as *Bifidobacterium* spp., *Eubacterium lentum*, other *Eubacterium* spp., *Lactobacillus* spp., *Propionibacterium acnes* and other *Propionibacterium* spp [68] (Table 5). Daptomycin showed excellent activity against 37 isolated Gram-positive anaerobic cocci that included 10 *PeptoStreptococcus*

asaccharolyticus, 7 *P. magnus*, 7 *P. micros*, 9 *P. anaerobius*, 3 *P. prevotti* and 1 *Gemella morbillorum*. Daptomycin showed good to moderate activity against other *Clostridium* spp. including isolates of *C. innocuum* that were resistant to vancomycin [68].

Daptomycin displayed activity against 135 anaerobic non-spore forming Gram-positive bacilli. This group of anaerobic bacteria included 133 isolates, 22 *Actinomyces* spp. isolates, 13 *Bifidobacterium* spp., 48 *Eubacterium* spp., 37 *Lactobacillus* spp., and 15 *Propionibacterium* spp [68]. All 15 strains of *Propionibacterium* spp. were susceptible to daptomycin at < 2 µg/ml. This organism is known to cause serious skin infections and appears very susceptible to daptomycin *in vitro* [68].

Gram negative bacteria

As with aerobic Gram-negative bacteria, daptomycin demonstrates poor *in vitro* activity against anaerobic Gram-negative organisms [80]. For that reason, these organisms will not be discussed further.

Comparison of daptomycin and other antimicrobial agents

The Applicant has supplied a comparison of the *in vitro* activity of daptomycin to that of vancomycin, teicoplanin, linezolid, quinupristin/dalfopristin (Q/D) and against staphylococcal, *S. pneumoniae*, *Streptococcus* spp., and enterococcal isolates and these data are displayed in Table 12, Table 13, Table 14, and Table 15, respectively. For most Gram-positive aerobic bacteria, the activity of daptomycin (weight to weight basis) is equivalent or superior (by 2 to 4 fold) to that of other agents. Among Gram-positive anaerobic bacteria, daptomycin exceeded or equaled the activity of vancomycin against *Clostridium difficile*, *Clostridium perfringens*, *Eubacterium* spp. *PeptoStreptococcus assacharolyticus* and *Peptococcus micros-magnus* Group. Daptomycin was more active than linezolid, Q/D or the β-lactam antibiotics against *C. difficile* and *C. perfringens*.

Daptomycin and Q/D were the most active antibiotics against *S. aureus* and *S. epidermidis*, independent of the methicillin-susceptibility of the isolates. The MIC_{90} values of daptomycin against *S. epidermidis* (MSSE and MRSE) were 0.25 and 0.5 µg/ml. Quinupristin/dalfopristin showed similarly low MIC_{90} values. Both antibiotics were more active than vancomycin and linezolid, MIC_{90} of 1 - 2 µg/ml and 1 - 4 µg/ml, respectively. Daptomycin, vancomycin and teicoplanin showed excellent activity against all *S. pneumoniae* isolates, independent of their susceptibility to penicillin, with a MIC_{90} < 1 µg/ml. Vancomycin, teicoplanin and quinupristin/dalfopristin also showed good activity against these isolates. Linezolid was somewhat less active than the other agents, particularly against *S. agalactiae* and *S. pyogenes*.

Table 12: *In vitro* activity of daptomycin and comparative agents against staphylococci

Species	N	Daptomycin	Vancomycin	Teicoplanin	Linezolid	Q/D	Penicillin
S.*aureus*	27	1	1	-	-	-	-
(MSSA)	50	0.125	1	-	4	1	-
	57	1	1	1	-	-	-
	51	0.25	1	-	-	-	16
	50	0.5	2	1	2	0.25	-
	375	0.5	≤1.0	-	-	-	>2.0
	713	0.25	1	0.5	4	0.25	0
	888	0.5	1	1	2	0.25	-
	229	0.125	1	0.5	4	0.25	-
S. aureus	54	1	2	-	-	-	-
(MRSA)	50	0.125	1	-	4	1	-
	51	1	2	1	-	-	-
	29	0.25	1	-	-	-	64
	50	0.5	1	0.5	2	0.5	-
	172	0.5	≤1.0	-	-	-	>2.0
	305	0.5	1	1	4	0.5	-
	334	0.5	1	2	2	0.5	-
	78	0.5	1	4	4	1	-
	225	1	1	-	2	0.5	-
S. aureus	102	0.5	2	4	-	-	64
(Methicillin not specified)	499	0.25	1.0	0.5	2.0	1.0	-
S. aureus (GISA)	8	≤0.25 - 2	2 - 8	1 - 32	1 - 2	0.5 - 1	-
S.*aureus* (VRSA)	1	1	1024	32	2	≤1	-
S. epidermidis	24	0.5	1	-	4	0.5	-
(MSSE)	37	0.25	2	-	-	-	8
	39	0.25	2	8	2	0.25	-
S. epidermidis	24	0.25	1	-	4	0.25	-
(MRSE)	30	0.25	2	-	-	-	16
	50	0.25	2	16	2	0.25	-
S. epidermidis	20	0.5	2	2	1	0.25	-
(Methicillin susceptibility not specified)	20	0.5	2	-	-	-	>2.0
S. haemolyticus	20	0.5	2	16	-	-	-
	25	0.25	2	-	-	-	128
	52	0.25	2	32	2	0.5	-
	5	0.125 - 0.25	≤1.0 - 2.0	-	-	-	≤0.03 - >2.0
S. saprophyticus	30	0.5	2	2	2	0.5	-
	6	0.25 - 0.5	≤1.0 - 2.0	-	-	-	≤0.03 - >2.0
Staphylococcus spp.	38	0.5	2	4	-	-	-
coagulase negative	174	0.5	2	-	-	-	2
(MSSS)	356	0.5	1	4	2	0.25	-
	486	0.5	2	4	2	0.25	-
	47	0.25	1	2	2	0.5	-
Staphylococcus, spp.	29	1	4	-	-	-	-
coagulase negative	61	1	2	8	-	-	-
(MRSS)	334	0.5	2	-	-	-	>2
	770	0.5	2	8	2	0.5	-
	554	0.5	2	8	2	0.25	-
	31	0.5	2	8	1	1	-
Staphylococcus, spp.	103	0.5	2	16	-	-	>128
(Methicillin not specified)							
Staphylococcus spp	4	0.5 - 1	8	16 - 32	2	≤0.25 - 0.5	-

(GISS)

a. Values shown are MIC_{90} ; for n<10, values represent MIC range
b. Include 3 *S. epidermidis*, 1 *S. haemolyticus*

Table 13: *In vitro* activity of daptomycin and comparative agents against *S. pneumoniae*[a]

Species	N	Daptomycin	Vancomycin	Teicoplanin	Linezolid	Q/D	Penicillin
S. pneumoniae	16	0.125	0.25	-	-	-	-
(Penicillin susceptible)	158	0.125	0.5	0.06	-	-	-
MIC <0.06 µg/ml	373	0.25	≤1.0	-	-	-	-
	728	0.125	0.25	-	1	0.5	-
	619	0.25	0.5	-	1	0.5	-
S. pneumoniae	21	1	0.5	-	-	-	-
(Penicilin intermediate)	111	0.25	0.5	0.125	-	-	-
MIC = 0.12 - 1.0 µg/ml	93	0.25	≤1.0	-	-	-	-
	248	0.25	0.25	-	1	0.5	-
	165	0.25	0.25		1	0.5	-
S. pneumoniae	24	1	0.5	-	-	-	-
(Penicillin resistant)	52	0.25	0.5	0.125	-	-	-
MIC > 2 µg/ml	110	0.25	≤1.0	-	-	-	-
	187	0.125	0.25	-	1	0.5	-
	81	0.25	0.5	-	1	0.5	-
S. pneumoniae	50	0.25	0.5	-	-	-	0.5
(Penicillin susceptibility not specified)	99	0.25	0.5	0.06	1	0.5	-
	100	0.5	0.5	0.06	-	-	4
	179	0.25	0.25	-	1	0.5	1

a. Values shown are MIC_{90} ; for n<10, values represent MIC range

Table 14: *In vitro* activity of daptomycin and comparative agents against *Streptococcus* spp. other than *Streptococcus pneumoniae*[a]

Species	N	Daptomycin	Vancomycin	Teicoplanin	Linezolid	Q/D	Penicillin
S. agalactiae	31	0.25	0.5	0.25	-	-	-
(β-hemolytic Group B)	206	0.25	≤1.0	-	-	-	0.06
	50	0.25	0.5	-	-	-	0.06
	273	0.25	0.25	-	1	0.25	0.06
	367	0.25	0.5	-	1	0.25	0.06
	56	0.5	0.5	-	2	0.5	0.06
S. pyogenes	51	0.06	0.5	0.12	-	-	-
(β -hemolytic Group A)	238	0.06	≤1.0	-	-	-	≤0.03
	50	0.06	0.5	-	-	-	0.016
	10	0.12	0.5	-	-	-	-
	484	0.06	0.25	-	1	<0.12	≤0.03
	117	0.06	0.25	-	1	<0.12	≤0.03
Streptococcus spp. β-hemolytic (Group not specified)	100	0.25	0.5	0.12	-	-	0.06
Group C	9	0.03 - 0.25	0.25 - 0.5	0.015 - 0.25	-	-	-
	5	0.03 - 0.5	≤1.0 - 2.0	-	-	-	≤0.03
Group G	10	0.06	0.25	0.25	-	-	-
	21	0.06	≤1.0	-	-	-	≤0.03
Group F	6	0.03 - 0.5	≤1.0	-	-	-	≤0.03 - 0.06
Group C, G	8	0.03 - 0.06	0.25 - 0.5	-	-	-	0.008 - 0.016
Viridans Group	37	1	1	0.25	-	-	-
(Not speciated)	15	1	≤1.0	-	-	-	2
	66	1	1	0.5	-	-	0.5
	369	1	0.5	-	1	1	2
	106	1	0.5	-	1	1	1
Viridans Streptococci Group (Speciated)							
S. anginosus	6	0.12 - 0.5	0.5 -1	-	-	-	-
S. bovis	6	0.03 - 0.06	≤1.0	≤0.03 - 0.06	6	0.016 - 0.5	0.25 - 0.5
S. gordonii	5	0.5 - 1	0.5 - 1	-	-	-	-
S. intermedius	8	0.12 - 2	0.5 - 1	-	0.5 - 2	0.25 - 1	-
S. milleri	30	1	1	0.06	1	0.5	-
	13	0.5	≤1.0	0.06			
	6	0.12 - 1	1 - 1	-	1 - 2	0.25 - 1	-
S. mitis	10	0.25-1	0.5-1	-	0.5-2	0.25-1	-
	6	0.25 - 1	0.5 - 1	-	-	-	-
S. mutans	4	0.06 - 1	0.25 1	-	-	-	-
S. oralis	11	0.25-2	0.5-1	-	1-2	0.5-2	-
	9	0.12 - 1	0.5 1	-	-	-	-
S. parasanguis	2	0.5 - 0.5	0.5- 0.5	-	-	-	-
S. salivarius	8	0.12 - 0.5	0.5- 1	-	1 - 2	0.25 - 1	-
	2	0.03 - 0.12	0.25- 0.5	-	-	-	-
S. sanguis	8	0.25 - 1	0.5 1	-	1 - 1	0.25 - 1	-
	10	1	1	-	-	-	-
S. vestibularis	1	0.5	1	-	-	-	-

a. Values shown are MIC_{90} ; for n<10, values represent MIC range
b. Q/D = quinipristin/dalfopristin

Table 15: *In vitro* activity of daptomycin and comparative agents against enterococci[a]

Species	N	Daptomycin	Vancomycin	Teicoplanin	Linezolid	Q/D[b]	Penicillin
E. faecalis	20	1	2	-	-	-	-
(Vancomycin susceptible)	36	1	2	0.25	-	-	-
	377	2	2	-	-	-	>2.0
	30	0.5	2	-	-	-	4
	2049	2	2	0.12	2	8	1 (Amp)
	1798	2	2	0.25	2	8	1 (Amp)
E. faecalis	3	0.5 - 2	8- 16	≤0.03 - 0.12	1 - 2	2 - 8	1- 1(Amp)
(Vancomycin intermediate)	2	0.5 - 1	8 - 16	0.12 - 0.25	1 - 2	8 - 8	0.5 - 1(Amp)
E. faecalis	14	1	>64	64	-	-	-
(Vancomycin resistant)	10	4	>16	-	-	-	>2.0
	20	0.5	>128	-	-	-	8
	40	2	>256	>64	2	16	2 (Amp)
	40	2	>256	>64	2	16	2 (Amp)
	25	1	64	-	4	16	-
	24	2	>128	64	2	4	-
E. faecalis	334	4	2	0.25	2	16	-
(Vancomycin susceptibility not	506	2	4	0.5	2	8	-
specified)	62	0.25	>128	≤0.5	-	>8	2 (Amp)
E. faecium	28	2	2	1	-	-	-
(Vancomycin susceptible)	50	4	2	-	-	-	>2.0
	32	2	1	-	-	-	128
	147	4	1	0.5	2	2	64 (Amp)
	333	4	2	0.5	2	2	64 (Amp)
E. faecium	2	2 - 2	16 - 16	0.25 - 4	2 - 2	0.5 - 0.5	64 - 128
(Amp) (Vancomycin intermediate)	7	1 - 4	16 -16	0.12 - 1	1 - 2	0.25 - 2	32 - 128
(Amp)							
E. faecium	23	2	>64	-	-	-	-
(Vancomycin resistant)	18	2	>128	-	-	-	>128
	34	2	>64	>64	-	-	-
	90	4	>16	-	-	-	>2.0
	219	4	>256	>64	2	1	128 (Amp)
	114	4	>256	>64	2	2	128 (Amp)
E. faecium	25	4	64	-	4	4	-
(Vancomycin susceptibility not	25	2	>128	32	2	0.25	-
specified)	220	8	256	32	4	2	-
	502	2	>128	64	2	2	-
	103	1	>128	>32	-	4	>128
(Amp)							
Enterococcus spp.	79	4	4	0.25	2	4	8 (Amp)
(Vancomycin S)	137	4	4	0.5	2	4	64 (Amp)
Enterococcus spp.							

Species	N	Daptomycin	Vancomycin	Teicoplanin	Linezolid	Q/D[b]	Penicillin
(Vancomycin I) (Amp)	6	2 - 4	8 -16	0.25 - 0.5	1 - 2	0.5 - 2	0.5 - 32
Enterococcus spp. (Amp)	1	2	256	16	2	4	≤0.25
(Vancomycin R)	17	4	>256	>64	2	4	128 (Amp)
Enterococcus spp. (Vancomycin susceptibility not specified)	6	0.03 - 4	≤1.0 - 16	-	-	-	0.12 - 2
	103	4	>128	64	-	-	>128
E. avium	1	0.5	0.5	-	-	-	-
	9	0.25 - 4	≤1.0 - >16	-	-	-	1 - >2
E. casseliflavus	1	0.25	8	-	-	-	-
E. gallinarum	3	0.03 - 2	8 - 8	-	-	-	-
	8	1 - 8	≤1.0 - >16	-	-	-	1 - >2

a. Values shown are MIC90; for n<10, values represent MIC range
b. Q/D = quinipristin/dalfopristin

MECHANISMS OF RESISTANCE

There are no known transferable elements that confer resistance to daptomycin. Development of resistance to daptomycin is rare among susceptible Gram-positive organisms. No spontaneously resistant mutants were obtained for any clinical and laboratory isolates after single passage however, stable resistant organisms have been isolated after multiple (n=20) passages in liquid media with progressively increasing concentrations (initiated from sub-MIC levels) and following chemical mutagenesis. The daptomycin MICs for these isolates were 8 to 32-fold higher than the parental isolates. Several different phenotypic classes of mutants were found. Some mutant isolates grew at normal rates and were virulent in a mouse model of infection. Other mutants had significant growth defects *in vitro* and had lost virulence *in vivo* [24].

Studies in both *S. aureus* and *E. faecalis* demonstrated the involvement of different genetic determinants in mediating reduced susceptibility to daptomycin [25]. In *S. aureus*, two genes potentially affecting cytoplasmic membrane composition, cardiolipin synthetase *(cls)* and oxacillin resistance-related *fmtC/mprF* protein have been implicated and confirmed by sub-cloning experiments. The upregulated *fmtC/mprF* protein is associated with a two-fold increase in daptomycin MIC; down-regulation is associated with an increase in oxacillin resistance [26]. In *E. faecalis* a DNA-binding response regulator has been associated with a reduction in susceptibility to daptomycin. Daptomycin resistance is not mediated by one common genetic determinant and data from genetic studies implicate involvement of different genetic loci in these two genera [24, 25].

Consistent with the above studies, the emergence of resistance has been rare across the entire set of phase 2/3 clinical trials conducted by Lilly and Cubist. In one case, the organism was a *S. aureus* from a subject with endocarditis; in the other, an *E. faecalis* from a subject with an infected chronic decubitis ulcer.

Daptomycin demonstrates potent *in vitro* activity against a broad range of Gram-positive resistant pathogens including multi-drug resistant strains and strains with characterized resistance mechanisms. Cross-resistance between daptomycin and other antibiotic classes

has not been reported and is unlikely to occur since daptomycin represents a new class of antibiotics, the lipopeptides, and possesses a mechanism of action that is distinct from other antibiotics, including ß-lactams, aminoglycosides, glycopeptides, macrolides, streptogramins and oxazolidinones. In all these studies, the MICs were interpreted as susceptible, intermediate, or resistant according to NCCLS 2000 published breakpoints (M100-S10), where available [22].

Verhoef and associates studied *S. aureus* isolates from a special collection of strains with identified genetic determinants mediating resistance to methicillin, fluoroquinolones and tetracycline [69] (see Table 16). There was no apparent cross resistance.

Table 16: *In vitro* activity of daptomycin against *Staphylococcus aureus* strains with characterized resistance genes

No Isolates	Antibiotic Resistance	Genotype	MIC Range µg/ml	MIC_{50} µg/ml	MIC_{90} µg/ml
38	Methicillin	*mec*A		0.25	0.5
3	Linezolid	G2576T mutation in (23S rDNA)		-	-
14	Quinolone	Ser80Phe/Glu88Lys*		0.125	0.25
13	Quinolone	Ser80Phe/Ser84Leu		0.125	0.25
9	Quinolone	Ser80Tyr/Glu88Lys		0.125	-
13	Quinolone	Ser80Tyr/Ser84Leu		0.125	0.5
18	Tetracycline	*tet* K		0.125	0.5
34	Tetracycline	*tet*M		0.125	0.25
7	Tetracycline	*tet*K + *tet* M		0.25	-
2	Vancomycin/Methicillin	*van*A/ *mec*A		-	-

Total N=150
*Specific amino acid change in grlA/gyrA (topoisomerase IV and DNA gyrase)

The SECURE multicenter profiling studies conducted in the US and Europe characterized phenotypic resistance of 2240 *Staphylococcus aureus* isolates using antibiograms based on testing with the following five antibiotics: oxacillin, erythromycin, gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole. The MIC_{90} of daptomycin against all US strains, oxacillin-susceptible as well as -resistant, was 0.5 µg/ml. Resistance to oxacillin was seen in 53.3% (554/1,040) of the European isolates. Resistance rates to other antimicrobials against the European coagulase negative isolates were: 30.6% to ciprofloxacin, 14.6% to gentamicin and 34.1% to erythromycin. Daptomycin MIC_{90}s were consistent at 0.5g/ml against both US and European coagulase-negative *Staphylococcus* isolates regardless of methicillin (oxacillin) susceptibility phenotypes.

Verhoef and associates [69] also examined the activity of daptomycin against a set of streptococci with characterized genetic mechanisms of resistance. The test isolates included 61 *S. pneumoniae* and 62 other streptococcal species (see Table 17).

Table 17: *In vitro* activity of daptomycin against *Streptococcus pneumoniae* and *Streptococcus* spp. strains with characterized resistance genes

Organism	No. Isolates	Antibiotic Resistance	Resistance Genotype	MIC Range µg/ml
S pneumoniae	20	Quinolone	Changes associated with gyrA, gyrB, parC, and/or parES	0.12 - 1
	12	Tetracycline	tetM	
	9	MLS[a]		
	10	(Clindamycin and		
	11	erythromycin)	ermB	
	7	MLS (Erythromycin)	mefE	
	7	MLS, tetracycline	ermB[b] tetM	
	6	MLS, tetracycline	mefE[b] tetM	
Subtotal	**61**			
S. agalactiae	1	MLS	ermA	
	1	MLS	ermA, ermC, ermTR	
	3	MLS	ermB	
	2	MLS	ermA, ermTR	
S. bovis	11	MLS	ermB	
	1	MLS	ermB, ermTR	
	1	MLS	ermB, ermC	
S. equisimilis	1	MLS	ermC, ermTR	
	1	MLS	ermB	
S. intermedius	1	MLS	ermB, ermTR	
S. pyogenes	2	MLS	ermB	
	2	MLS	ermTR	
	1	MLS	mefE	
S. mitis	3	Quinolone	(No change gyrA, parC, gyrB, parES)	
	5	Quinolone	Change gyrB (No change: gyrA, parC, parES)	
	2	Quinolone	Change gyrA, parC, gyrB; (no change parES)	
	2	Quinolone	Change gyrA, parC; (no change gyrB, parES)	
	5	MLS[a]	ermB	
	1	MLS	ermA,mefE	
	5	MLS	mefE	
S. oralis	4	MLS	mefE	
S. salivarius	2	MLS	ermB	
	1	MLS	mefE	
S. sanguis	1	Quinolone	(No change gyrA, parC, gyrB, parES)	
	1	Quinolone	Change gyrA, parC (no change gyrB or parES)	
	2	Quinolone	Change gyrB (No change gyrA, parC, parES)	
Total	**123**			

a. MLS = resistance to macrolides, lincosamides and/or streptogramins
b. Changes in the same region might involve different amino acids

The distribution of daptomycin MICs for 2,028 *S. pneumoniae* isolates (1,163 from the United States and 865 from Europe) classified for MDR phenotype are found in the SECURE reports [74]. The overall rate of penicillin resistance among the US isolates was 16.1% (187/1,163). Erythromycin resistance among these isolates was 31.8% and resistance to trimethoprim-sulfamethoxazole (SXT) was 30.8%. (Data for penicillin resistance among *S. pneumoniae* for the SECURE studies is shown in Table 11). The MIC_{90} for daptomycin for all US isolates (non-MDR and MDR) was 0.12 µg/ml; none of the isolates had a MIC >0.5 µg/ml. Penicillin resistance among the European *S. pneumoniae* was 9.4%. For the European isolates, resistance rates for other antimicrobials were: 24.8% to erythromycin, 18% for clindamycin, 19.0% for SXT and 18.2% for cefuroxime (data not shown). The MIC_{90} values of daptomycin for European isolates (MDR as well as non-MDR) were one dilution higher than for the US isolates (0.25 vs. 0.12 µg/ml, respectively). Resistance to other antimicrobials among US and European isolates of *S. pneumoniae* showed no effect on the potency of daptomycin [73, 74].

The daptomycin MIC distribution patterns for 640 *S. agalactiae* are shown in the SECURE reports [73, 74]. The activity of daptomycin was unaffected by resistance to erythromycin, clindamycin or levofloxacin [73, 74]. In addition, daptomycin showed excellent activity against *S. pyogenes* isolates regardless of resistance to erythromycin [73]. The activity of daptomycin against the viridans streptococci group was unaffected by resistance of the isolates to penicillin, erythromycin, clindamycin or levofloxacin.

The Applicant has supplied data from two separate studies that determined the *in vitro* activity of daptomycin against 159 enterococci with genetically characterized resistance genes (see Table 18). The activity of daptomycin against the enterococcal isolates with genetically characterized resistance was quite similar to the activity exhibited against the isolates from multiregional regional profiling studies (Table 10).

Table 18: *In vitro* activity of daptomycin against *Enterococcus* spp. with characterized resistance genes

Organism	# Isolates	Antibiotic Resistance	Genotype /Inactive enzyme	MIC Range µg/ml	MIC_{50} µg/ml	MIC_{90} µg/ml
E. faecalis	25	Vancomycin	van A		1	1
	1	Vancomycin	van B		-	-
	7	Vancomycin	van A		0.5	-
	4	High level gentamicin	APH(3)		0.5	-
	11	High level gentamicin	AAC(6)/APH(2)		1	2
	17	High level gentamicin	AAC(6)/APH(2)APH(3)		2	4
	3	Linezolid	G2528U		-	-
E. faecium	19	Vancomycin	van A		2	4
	27	Vancomycin	van A		4	4
	2	High level gentamicin	APH(3)		-	-
	5	High level gentamicin	AAC(6)/APH(2)		8	-
	9	High level gentamicin	AAC(6)/APH(2)APH(3)		4	-
	24	Linezolid	23S rDNA		-	-
E. casseliflavous	1	Vancomycin	van A		-	-
	1	Vancomycin	van C		-	-
E. durans	1	Vancomycin	van A		-	-
E. gallinarum	2	Vancomycin	van C		-	-

The *in vitro* activity of daptomycin against phenotypically characterized *E. faecalis* and *E. faecium* isolates as to multiple-drug-resistance (MDR or non-MDR) has been reported in the SECURE reports [73, 74]. Daptomycin showed consistent activity against *E. faecalis* and *E. faecium*, regardless of the MDR status. The activity of daptomycin among US and European isolates of *Enterococcus* spp. was unaffected by their resistance to vancomycin, ampicillin or ciprofloxacin.

POST-ANTIBIOTIC EFFECT (PAE)

Daptomycin produces a concentration-dependent post-antibiotic effect of several hours against Gram-positive bacteria. Bush *et al.* showed a PAE up to 6 hours against *E. faecalis* and *S. aureus* following exposure to daptomycin concentrations ranging from 0.25-16 µg/ml [34]. Exposure to 15 µg/ml of daptomycin for 2 hours produced a PAE of 2.4-5.3 hours for four clinical *S. aureus* isolates and 3.5-3.9 hours for two clinical isolates of *E. faecalis*, as measured by viable cell counts. In similar experiments using bioluminescence measurements of bacterial ATP rather than viable cell counts, Hanberger *et al.* [7] showed a PAE of 6.3-6.7 hours for strains of both *S. aureus* and *E. faecalis*. These investigators also noted that effective regrowth time (following the PAE) was concentration dependent [7, 34].

In studies using the murine thigh model, PAE was defined as the interval beginning when serum concentration of daptomycin fell below MIC values and ending when bacterial growth occurred. In the murine thigh model, daptomycin exhibited a concentration-dependant PAE of 5 and 10 h for *S. aureus* and *S. pneumoniae*, respectively, over a range of daptomycin dosages of 0.21 to 400 mg/kg [35].

SYNERGY STUDIES

An early *in vitro* interaction study of daptomycin with 25 other antimicrobials against 70 clinical isolates demonstrated that most effects were additive or indifferent [36] (see Table 19). Synergistic interactions occurred most frequently with gentamicin (37% of isolates tested) and amikacin (23%). Among the bacterial strains tested, the enterococci showed the greatest propensity for synergistic effects between daptomycin and other drugs. No antagonism was observed in this study.

Table 19: Daptomycin interactions with antimicrobial agents *in vitro*

Daptomycin in combination with:	% Interactions[a]			
	Synergistic	Additive	Indifferent	Antagonistic
pipercillin	5.7	45.7	48.6	0
mezlocillin	2.8	47.1	50.0	0
cefotiam	0	80.0	20.0	0
cefotaxime	17.1	42.9	40.0	0
cefamandole	1.4	61.4	37.1	0
gentamicin	37.1	52.9	10.0	0
amikacin	22.9	44.3	32.9	0
ampicillin	1.4	44.3	54.3	0
apalcillin	0	58.6	41.4	0
augmentin	0	52.9	47.1	0
aztreonam[b]	0	18.6	52.9	0
ceftriaxone	10.0	34.3	55.7	0

	Synergistic	Additive	Indifferent	Antagonistic
ciprofloxacin	1.4	38.6	60.0	0
enoxacin	0	28.6	71.4	0
ofloxacin[b]	0	24.3	61.4	0
tobramycin	1.4	31.4	67.1	0
cefoxitin	0	58.6	41.4	0
cefuroxim	7.1	42.9	50.0	0
imipenem	8.6	61.4	30.0	0
ticarcillin + clavulanate[b]	0	45.7	48.6	0
ceftizoxime[b]	5.7	40.0	41.4	0
cefmenoxime	11.4	40.0	48.6	0
penicillin G	1.4	58.6	40.0	0
ceftazidime[b]	0	38.6	58.6	0
cefazolin	0	50.0	50.0	0

a. Total 70 isolates: 10 each of *S. aureus*, *S. epidermidis*, *S. pyogenes*, *E. faecalis*, *E. faecium*, *S. pneumoniae*, and viridans streptococcal group

b. Not all isolates tested with this combination.

PRECLINICAL EFFICACY *(IN VIVO)*

A series of animal studies were performed to characterize the *in vivo* efficacy and pharmacokinetic profile of daptomycin. These data were used in pharmacodynamic investigations to correlate efficacy with pharmacokinetic parameters of daptomycin. Monte Carlo analysis is conducted to predict daptomycin clinical performance against *S. aureus, Streptococcus* spp., and *E. faecalis*. The Monte Carlo analysis used the standard of pharmacodynamic performance applied to the variables of anti-microbial potency and human pharmacokinetics of daptomycin.

Preclinical pharmacokinetics studies were conducted in mice, rats, rabbits, guinea pigs, dogs, and monkeys. Tissue distribution and accumulation of daptomycin was characterized in rats while metabolism and excretion were characterized in multiple animal species. Following bolus i.v. administration, daptomycin exhibited primarily first order kinetics independent of animal species. C_{max} and AUC were dose proportional and predictable. The apparent plasma elimination half-life ($t_{1/2}$) was 1 - 2 hours in rodents and 2 - 4 hours in dogs and monkeys. Binding to plasma proteins was 87-94%, which is comparable to binding in humans. Total clearance (CLTB) was low. These results suggest that daptomycin resides primarily in the extracellular vascular space. In animal studies, daptomycin was eliminated primarily by renal excretion. Daptomycin did not inhibit or induce human hepatocyte cytochrome P450 isoenzymes, indicating a low potential for metabolic drug interactions.

In clinical pharmacokinetic studies, daptomycin demonstrated linear pharmacokinetics over the clinical i.v. dosages of 4 to 6 mg/kg. At the clinical dosage of 4 mg/kg q24h, a steady state C_{max} was 57.8 µg/ml and AUC_{0-24} was 493.5 µg x hr/ml. The half-life in plasma was 8.1 hours.

The efficacy of daptomycin was demonstrated against the important Gram-positive pathogens including MSSA, MRSA, *Streptococcus* spp., and vancomycin-resistant and susceptible *E. faecalis*, and *E. faecium*. The efficacy of daptomycin was demonstrated in

mice, rats, hamsters, rabbits, and guinea pigs. The infections studied included bacteremia, endocarditis, soft tissue (thigh), lung, and kidney. Efficacy was measured by increase in survival or by multi-log10 reductions in bacterial burden in the target organ. Daptomycin produced efficacy against these pathogens at dosages, which produced AUC0-24 exposures attainable at the clinical dosage of 4 mg/kg q24h in humans.

Pharmacodynamics were characterized in mouse models of soft tissue (thigh) infection using *S. aureus*, *S. pneumoniae*, and *E. faecalis*. Potential bacterial breakpoints were investigated in a pharmacodynamic model of murine pyelonephritis using *E. faecalis* and *E. faecium* with a range of MIC values (see pp 33-34 and Table 29). These pharmacodynamic studies indicated that C_{max} and AUC were the pharmacokinetic parameters that predicted efficacy, and that once daily dosing was an effective dosing schedule. Efficacy was obtained at clinically relevant AUC0-24hr exposures. Monte Carlo modeling indicated a high probability of efficacy with the exposure generated by the 4 mg/kg q24h clinical dose against *S. aureus, Streptococcus* spp., and *E. faecalis*.

COMPARATIVE PHARMACOKINETICS IN DIFFERENT SPECIES

Pharmacokinetic studies of daptomycin were conducted primarily in F344 rats with additional data from ICR mice, beagle dogs, and rhesus monkeys. The majority of pharmacokinetic studies were conducted in males and no notable gender differences in pharmacokinetics were apparent in any species tested (rats, dogs, or monkeys). A summary of the ADME trials is provided in Table 20.

Daptomycin exhibits linear kinetics in animals following i.v. injection across a dose range of 1 to 150 mg/kg. Daptomycin is ~90% bound to plasma proteins, but the binding is reversible and has low avidity. It exhibits both a low plasma clearance and low volume of distribution. Unbound daptomycin distributes rapidly to more highly vascularized tissues, but does not appear to partition into cells or penetrate either the blood-brain barrier or the placental barrier. In the rat, daptomycin appears to distribute preferentially to the kidneys. No metabolites were detected in plasma, and no change in pharmacokinetics or appreciable accumulation was observed upon repeated administration. Daptomycin is cleared primarily by the kidneys. Based on daptomycin's pharmacokinetic profile, it has limited potential for adverse drug interactions.

Upon bolus i.v. injection, daptomycin exhibits first order kinetics, independent of animal species. The daptomycin plasma profile following i.v. injection is consistent with a two-compartment model with a rapid distribution phase ($t_{1/2}$ = approximately 7 min) and slower elimination phase. The terminal plasma half-life ($t_{1/2}$) ranged from 1 - 2 hours in rodents to 2 - 4 hours in dogs and monkeys, as shown in Table 21. C_{max} and AUC were dose proportional.

Table 20: Studies conducted to assess ADME of daptomycin in animals[a]

Type of study	Test system	Route of Administration
Pharmacokinetic Profile		
Single-dose absorption	Mouse, rat, dog, monkey	i.v. bolus injection
Single-dose absorption	rat	s.c.
Single-dose absorption	Rat	oral gavage
Distribution		
Single-dose tissue distribution (quantitative tissue distribution, qualitative whole body autoradiography, renal localization)	Rat	i.v. bolus injection
Repeat-dose tissue distribution	Rat	i.v. bolus injection
Repeat-dose tissue distribution	Pregnant rat	i.v. bolus injection
Single-dose tissue distribution (quantitative tissue distribution, gastrointestinal tract localization)	Rat	Oral gavage
Metabolism		
single-dose *in vivo*		
plasma and urine	Mouse, rat, dog, monkey	i.v. bolus injection
respired air	Rat	i.v. bolus injection
renal localization	Rat	i.v. bolus injection
In vitro Cytochrome P450 induction & inhibition	Human hepatocytes	*In vitro*
Excretion		
Single-dose excretion		
(Urine and feces)	Rat	i.v. bolus injection
(Respired air)	Rat	i.v. bolus injection
Repeat-dose excretion (balance study)	Rat	i.v. bolus injection
Single-dose excretion	Rat	Oral gavage
Pharmacokinetic Drug Interactions		
Interaction with tobramycin	Dog	i.v. bolus injection
Other		
Pharmacokinetics and excretion in model of renal impairment	Rat	s.c.

Table 21: Daptomycin pharmacokinetics in different species (single-dose, i.v.)[a]

Species	$T_{1/2}$ (h)	CL_{TB} (kg/h/kg)	V_d (kg/kg)
Mouse	1.2-1.8	39	80-162
Rat	1.0-2.4	33-102	57-145
Monkey[b]	2.9-4.6	17-20	86-108
Dog	1.9-4.2	19-41	59-166
Human	9	7-9	90-100

a. Reference: See section 6.3 of the Pharmacology and Toxicology Section

b. At doses of 1-25 mg/kg; nonlinearity (decreased clearance) was observed at 200 mg/kg and values at this dose level are not included.

Note: With the exception of the human data, plasma concentrations of daptomycin were measured by a microbiological assay using *Micrococcus luteus*

Data for the pharmacokinetics for mouse, rat and rabbit were provided by the Applicant in Tables 22, 23, and 24, respectively. Neither dogs nor monkeys were used in any efficacy studies.

Table 22: Dose-relationship of daptomycin pharmacokinetics in mice (single-dose, i.v.)

Dose (mg/kg)	C_{max} (µg/ml)	$t_{1/2}$ (hour)	AUC (µg-h/ml)	CL_{TB} (kg/h/kg)	V_d (kg/kg)
15[a]	187	1.8	380	-	-
100[b]	1107	1.2	1051	96.0	162

Table 23: Dose-relationship of daptomycin pharmacokinetics in rats (single-dose, i.v.)

Dose (mg/kg)	C_{max} (µg/ml)	$t_{1/2}$ (hour)	$AUC_{0-\infty}$ (µg-h/ml)	CL_{TB} (kg/h/kg)	V_d (kg/kg)
2[a]	12	1.2	25	81	135
10[a]	66	1.1	119	84	130
10[b]	110	0.8	105	96	106
20[b]	271	2.5	448	42	129
25[b]	526	1.6	698	36	76
50[b]	563	1.3	566	88	145
75[b]	854	2.2	1770	42	102
150[b]	1943	2.9	5383	30	113

Table 24: Serum concentrations in rabbits

I.V. Dose / Duration	Serum Concentration (µg/ml)	Hours post-dose	Infection status
10 mg/kg twice daily for 3 or 6 days	31 ± 1.34	1 h	Infected (endocarditis)
	2.38 ± 0.4	18 h	
10 mg/kg q24h for 2 to 4 days	49 ± 12	1 h	Infected (endocarditis)
	3.1 ± 2.0	24 h	
8 mg/kg q8h for 4 days	Mean: 76.9	1 h	Infected (endocarditis)
	Mean: 20.4	8 h	

Non-intravenous parenteral administration of daptomycin by subcutaneous (s.c.), intraperitoneal (i.p.), or intramuscular (i.m.) injection was utilized in numerous animal model efficacy studies, as well as certain toxicity studies. At therapeutic doses, the plasma concentrations following administration by these routes appeared indicative of relatively high bioavailability. The data are presented below by species due to the similarity of pharmacokinetics across routes.

In mice, the systemic bioavailability after s.c. administration of 1 to 25 mg/kg was 94 to 100%; at 100 mg/kg, bioavailability was 76.4% [52]. The terminal half-lives were 0.9 - 1.4 h and 1.8 h following s.c. and i.p. administration, respectively [35, 46, 52]. In a murine thigh model of *S. aureus* infection, daptomycin kinetics was relatively linear across s.c. doses of 10 and 40 mg/kg with no change in elimination half-life [35]. In a similar model, peak concentration and AUC were linear across an i.p. dose range of 1 to 20 mg/kg [39].

After s.c. administration of daptomycin to rats, serum concentrations peaked at 2 hours post- dose [38]. Peak concentrations were proportional to dose after s.c. injection of doses ranging from 1 to 25 mg/kg. Bioavailability at s.c. doses of 25, 75, and 150 mg/kg were 88, 95, and 43%, respectively, and half-life increased from 2.8 h at 25 mg/kg to 9 h at 150 mg/kg, suggesting saturation of absorption at high dose levels.

Guinea pigs receiving a 20 mg/kg i.p. dose of daptomycin had plasma levels of 38 µg/ml at 20 minutes post-dose [39]. After s.c. injection at dose levels of 25, 75, and 150 mg/kg, daptomycin was reported to be well absorbed; concentrations of daptomycin in the blood and lymph peaked at 4 and 8 hours post-dose, respectively. After i.m. administration of daptomycin to healthy rabbits, serum concentrations peaked 2 to 3 hours post-dose [40]. The terminal half-life of daptomycin was reported to be 7.75 hours after a single s.c. injection of 4 mg/kg and 5.8 hours after a single s.c. injection of 10 mg/kg [11, 12]. As shown in Table 7-6, peak serum concentrations at timepoints after i.m. and s.c. injection ranged from 43.6 to 120 µg/ml, which is in the range of Cmax values obtained in humans by the 4mg/kg (57.8g/ml), 6mg/kg (98.6 µg/ml), and 8mg/kg (133 µg/ml) dosages (Table 26).

Table 25: Serum concentrations in rabbits following non-intravenous parenteral administration

Route	Dose	Hours post-dose	Serum Concentration (µg/ml)	Infection status
i.m.	20 mg/kg	2 h[a]	>120	Control
		12 h	~50	
s.c.	10 mg/kg	a	43.6	Control
s.c.	10 mg/kg	2 ha	43.6 ± 1.5	Control
N.S.b	10 mg/kg q12h for 8 days	1 h	66.4 ± 18.0	Infected
		8 h	16.8 ± 6.6	(endocarditis)
N.S.b	12 mg/kg q8h for 8 days	1 h	92.8 ± 33.1	Infected
		12 h	43.3 ± 23.8	(endocarditis)

a. Peak
c. Route not specified

Table 26: Daptomycin pharmacokinetic parameters at steady-state[a]

Dose	C_{max} (µg/ml)	AUC_{0-24} (µg.h/ml)	$T_{1/2}$ (h)
4 mg/kg	57.8 (3.0)	494 (75)	8.1 (1.0)
6 mg/kg	98.6 (12)	747 (91)	8.9 (1.3)
8 mg/kg	133 (13.5)	1130 (117)	9.0 (1.2)

a. Mean (standard deviation)

Daptomycin was not well absorbed when administered either orally or locally. Oral absorption of daptomycin is low, probably due to poor gastrointestinal permeability. After a single oral dose of radiolabeled daptomycin to rats at a dose of 10 or 20 mg/kg, the Cmax (50 to 200 ng-eq/ml) and AUC (1 µg-h/ml) were low. Oral bioavailability is estimated to be 1% or less. Local administration of daptomycin to sequestered compartments led to high local concentrations without demonstrable systemic exposure.

HUMAN PHARMACOKINETICS

A range of daptomycin biopharmaceutic studies was conducted by Lilly and Cubist. The studies included a distribution and metabolism study using radiolabeled daptomycin, *in vivo* protein binding studies, single and multiple dose pharmacokinetic studies using different doses of daptomycin, a study in subjects with varying degrees of renal

impairment, and a drug interaction studies. The dosage used in the pivotal phase 3 cSSSI trials was 4 mg/kg q24h, the proposed dosage.

The protein binding studies indicated that the binding of daptomycin was not concentration dependent over the range of 2.5 to 80 µg/ml *in vitro* and 0.15 to 30 µg/ml *in vivo*. In addition, pH (7.0-7.4), temperature (25 or 37°C), and freezing and thawing over a period of two months did not affect protein binding. Daptomycin protein binding is ~90%. There was no relationship between the quantity of albumin or protein in the serum and the percentage of daptomycin bound. Daptomycin binding to serum proteins was independent of dose, concentration and multiple daily dosing.

Daptomycin pharmacokinetics was studied in both single dose and multiple dose studies. Single-dose pharmacokinetics of daptomycin was established principally from the Cubist-sponsored study, DAP-00-02. The study demonstrated that daptomycin pharmacokinetics is dose proportional between the 4 mg/kg and 6 mg/kg dose. Mean values for Cmax were 54.6, 86.4, and 116.3 µg/ml for the 4, 6, and 8 mg/kg doses, respectively. Mean values for $AUC_{0\text{-}24h}$ were 494, 747, and 1130 µg x hr/ml, respectively.

Multiple-dose pharmacokinetics of daptomycin were established principally from the Cubist- sponsored study, DAP-00-02, which examined daptomycin dose regimens of 4, 6, and 8 mg/kg q24h x 7d and 8 mg/kg q24h x 14d. The values at steady state are shown in Table 27. At steady state, the mean values for Cmax were 57.8, 98.6, and 133 µg/ml for the 4, 6, and 8 mg/kg doses, respectively. The mean values for $AUC_{0\text{-}24h}$ were 494, 747, and 1130 µg x hr/ml, respectively.

Table 27: Daptomycin pharmacokinetic parameters at steady-state

Dose	C_{max} (µg/ml)	$AUC_{0\text{-}24}$ (µg.h/ml)	$T_{1/2}$ (h)
4 mg/kg	57.8 (3.0)	494 (75)	8.1 (1.0)
6 mg/kg	98.6 (12)	747 (91)	8.9 (1.3)
8 mg/kg	133 (13.5)	1130 (117)	9.0 (1.2)

Tissue Distribution

The ADME studies of daptomycin suggest that upon i.v. injection, daptomycin binds extensively to plasma proteins. Unbound daptomycin distributes rapidly from the plasma with a $t_{1/2}$ of approximately 7 minutes. In the rat, daptomycin penetrates into the more highly vascularized tissues but does not appear to penetrate across the blood-brain barrier or the placental barrier. The half-life in tissues is greater than that in plasma. In the rat, daptomycin appears to distribute preferentially to the kidneys, reflecting the vascularization of the tissue as well as renal concentration of the drug during excretion. This renal tissue localization effect appears to be species-specific. Daptomycin exhibits plasma protein binding across species. *In vitro* analyses showed that approximately 90% of daptomycin is bound in mouse, rabbit and human serum [37, 42, 46].

Protein binding in mouse serum and human serum was independent of daptomycin

concentration between 2 to 80 µg/ml [30, 46]. Binding was primarily to albumin and readily reversible, as indicated by the dissociation constant (K_d) of 90.3 µM (146 µg/ml)[30].

Daptomycin does not distribute into red blood cells. These results are consistent with the inability of daptomycin to penetrate into bacteria, [28] its weak binding to mammalian cell membranes [10] and clinical data obtained with radiolabeled material. Daptomycin bound weakly to human cells and was spontaneously shed in large proportions. These findings suggest that daptomycin stays predominantly in the extracellular space.

In rats, daptomycin was widely distributed throughout the body after a single i.v. dose. ^{14}C-radiolabeled daptomycin demonstrated rapid distribution within 0.25h after dose administration. In most tissues, the half-life ranged from 1.57 to 4.42 hours, as compared with 1.54 hours in plasma. With the exception of the kidney, tissue concentrations after a single injection correlated with vascularization and decreased in a time-dependent manner.

Daptomycin demonstrated distribution into well-vascularized tissues. After single and multiple dosages of ^{14}C-radiolabeled daptomycin to rats, the kidneys, liver, lung, and blood all demonstrated high concentrations. Daptomycin appeared to accumulate in the kidneys of rats.

Daptomycin exhibits low penetration into poorly vascularized tissues. After a single dose of ^{14}C-radiolabeled daptomycin to rats, the bone, eye, and white fat were among the tissues with the lowest concentrations of radioactivity. Daptomycin does not appear to penetrate the blood-brain barrier.

A clinical trial (DAP-00-04) evaluated the penetration of daptomycin into the inflammatory fluid in cantharides-induces skin blisters. Pharmacokinetic analyses were based on data from six subjects who received a single i.v. infusion of daptomycin at 4 mg/kg. Daptomycin penetrated the inflammatory exudate moderately rapidly, with mean 1- and 2-hour concentrations of 9.4 µg/ml and 14.5 µg/ml, respectively. T_{max} in the inflammatory fluid occurred approximately 3 hours later than in plasma (3.7 hours vs. 0.5 hours) with a Cmax of 27.6 µg/ml. The elimination half-life of daptomycin from the inflammatory exudates was highly variable, ranging from 6.3 hours to 30.9 hours, with a mean of 17.3 hours. The mean AUC_{0-24} in the inflammatory exudates was 318.2 µg x hr/ml. The penetration of daptomycin into inflammatory exudates, calculated as AUC_{0-24} inflammatory fluid/AUC_{0-24} plasma, was 68.4%.

Metabolism

Daptomycin undergoes limited metabolism and is eliminated primarily as non-metabolized (intact) active drug. In addition, daptomycin does not appear to affect hepatic cytochrome (CYT) P450 drug metabolizing enzymes.

Daptomycin has no apparent inhibitory or stimulatory effects on hepatic drug metabolizing enzymes. Daptomycin caused no biologically significant inhibition or induction of the activities of cytochrome P450 isoforms 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 in

isolated human hepatocytes. These *in vitro* P450 results are consistent with *ex vivo* findings in rats.

Excretion

Daptomycin was excreted primarily in the urine in all species examined. Approximately 70 to 80% of the administered radioactivity was recovered in the urine by 48 hours post-dose in the mouse, rat, dog, and monkey (see Table 28). These results are also in agreement with those obtained during clinical trials. The presence of high concentrations of active daptomycin in the urine suggests the potential for treatment of urinary tract infections.

Table 28: Excretion of daptomycin in animals receiving 10 mg/kg ^{14}C-[tryptophan]-daptomycin (single-dose, i.v.)

	% Dose excreted in 48 hours	
Species (N)	**Urine**	**Feces**
ICR Mouse (4)	70.0	9.0
Fischer 344 Rat (2/3)[a]	68.4	10.4
Beagle Dog (2)	77.2	5.3
Monkey (2)	75.4	3.2
Human	~78	~5

a. Urinary data is available for 2 rats; fecal data is available for 3 rats

Discussion of pharmacokinetics, distribution, and excretion

Daptomycin exhibits a linear and highly predictable pharmacokinetic profile following intravenous administration. For all species evaluated, plasma clearance, volume of distribution, and terminal half-life are dose-independent across a wide dose range (1 to 150 mg/kg). The pharmacokinetic profile and key pharmacokinetic parameters do not exhibit any strain or gender differences and are not altered upon repeated daily administration for up to 6 months. Approximately 90% of daptomycin is bound to plasma proteins, contributing to its low plasma clearance and volume of distribution and extending the overall systemic half-life of total.

The Vd is consistent with that of plasma and interstitial fluid. Daptomycin does not appear to penetrate inside red blood cells or across the blood-brain barrier. Based on studies in rats, the half-life of daptomycin in tissues is greater than that in plasma; however, as plasma levels decline, tissue levels fall to below detectable levels and do not accumulate upon repeated once daily administration.

Daptomycin exhibits limited metabolism by spontaneous or enzymatic degradation, or by hepatic metabolic pathways. No metabolites were detected in the plasma, consistent with findings in man. Daptomcyin does not inhibit or induce any of the key cytochrome P450 isozymes and, therefore, has low potential to affect the hepatic metabolism of concomitantly administered drugs. Daptomycin is excreted primarily by the kidney.

PHARMACODYNAMICS

Pharmacodynamics in animals

Pharmacodynamic investigations were conducted using mouse models of thigh infections in neutropenic mice and renal infections in normal mice. The pharmacodynamic parameters

that best correlated with efficacy were AUC/MIC or C_{max}/MIC, whereas time above MIC did not correlate with efficacy. [42, 44]. This profile is consistent with the concentration-dependent bactericidal activity of daptomycin *in vitro*.

The AUC/MIC ratios needed to achieve a static response against a thigh infection in immunosuppressed mice differed for different pathogenic species. Against *S. aureus*, *S. pneumoniae*, and *E. faecium*, the mean total AUC/MIC ratios of 438, 160, and 1.7, respectively, were needed to achieve a static response. The corresponding C_{max} /MIC ratios of 70.6, and 24 produced a static response against *S. aureus* and *S. pneumoniae* (see Table 29) [42].

Table 29: AUC/MIC and C_{max} /MIC ratios to achieve a static effect against *S. aureus*, *S. pneumoniae*, and *E. faecium* in neutropenic mouse thigh infections

Bacteria	Daptomycin MIC	AUC/MIC	Cmax/MIC
S. aureus 25923	0.5	388	59
S. aureus 33591	0.5	537	93.6
S. aureus 29213	0.5	420	66.2
S. aureus 6538p	0.5	409	63.6
S. aureus			
Mean ± SD		438 ± 67	70.6 ± 15.6
S. pneumoniae 10813	0.12	168	25.1
S. pneumoniae 145	0.12	74.7	11.8
S. pneumoniae 1293	0.12	203	30.5
S. pneumoniae 1199	0.12	117	17.4
S. pneumoniae 1396	0.12	237	36.5
S. pneumoniae 673	0.25	199	29.8
S. pneumoniae 1325	0.25	182	27.3
S. pneumoniae 49619	0.25	126	18.9
S. pneumoniae 1020	0.25	129	19.4
S. pneumoniae			
Mean ± SD		160 ± 51	24 ± 7.6
E. faecium VA 20	2	0.94	0.14
E. faecium VA 21	2	1.67	0.25

The Applicant has provided a table with data for another series of pharmacodynamic studies performed using thigh infections in neutropenic mice. In these studies, the static free AUC/MIC ratios were calculated against *S. aureus* and *E. faecalis* (see Table 30).

Table 30: Free AUC/MIC values to achieve a static response in thigh infection in neutropenic mice

Isolate	MIC	Free AUC/MIC[a]	Total AUC/MIC
MRSA 54	0.25	11.9	119
MRSA 56	0.5	18.4	184
E. fcecalis 64 (VRE)	2	4.8	48
E. faecalis 70	1	13.2	132

a. Assuming a protein binding rate of 90%

In another dose fractionation study with neutropenic mice, efficacy correlated most closely with AUC/MIC against an *S. aureus* thigh infection (see Table 31). Efficacy was not

improved by dose fractionation as compared with once-daily administration. A static effect was reached at an AUC/MIC ratio of 43 against *S. aureus*. These pharmacodynamic studies suggest that clinical administration of daptomycin once daily could be used to optimize efficacy. The dosage regime of once daily also optimizes the safety of daptomycin.

Table 31: Pharmacodynamic efficacy of daptomycin against *Staphylococcus aureus* thigh infection in mice

Total dose of daptomycin (mg/kg/24h)	Treatment Regimen[a]	C_{max}/MIC[b]	Time (h) above MIC per 24 h	AUC/MIC	*S. aureus* density (log_{10}/g)[c]
2.5	2.5 mg/kg q24h SC	8.28	5.0	20.06	6.54
	1.25 mg/kg q12h SC	4.14	9.1	21.28	6.83
	0.625 mg/kg q6h SC	2.07	17.2	23.72	6.61
5.6	5.6 mg/kg q24h SC	18.54	6.08	43.41	5.12
	2.8 mg/kg q12h SC	9.27	10.17	44.63	4.96
	1.4 mg/kg q6h SC	4.63	18.34	47.08	5.02
15.0	15.0 mg/kg q24h SC	49.65	9.44	114.24	3.73
	7.5 mg/kg q12h SC	24.83	13.52	115.46	3.82
	3.75 mg/kg q6h SC	12.41	21.34	117.90	3.68

a. The first dose was administered 2 hours post-infection; the duration of treatment was 24h
b. The MIC for *S. aureus* ATCC 29213 was 1.0 µg/ml in 100% mouse serum
c. Density at sacrifice number of hours after last dose.

Due to difficulty in obtaining an active enterococcal infection of thigh tissue, daptomycin efficacy against *E. faecalis* and *E. faecium* was studied in a murine model of renal infection. The Applicant has supplied additional data as Table 7-12 and Figure 7-2 of the Microbiology Section of the briefing document. These trials demonstrated efficacy as measured by a 2 log_{10} reduction in bacterial count in renal tissue. Clinical enterococci with MIC values of 0.5 to 8 µg/ml were evaluated. Efficacy was produced by daptomycin at AUC exposures obtainable at the clinical dose of 4 mg/kg. The pharmacodynamic parameter needed to obtain a mean 2 log_{10} reduction in bacterial CFU was a total AUC/MIC ratio of 42.7.

Comparison of the pharmacodynamic results for enterococci from the thigh and renal models is complicated by two important variables: enterococci have low pathogenicity for intact muscle and tissue in rodents and the kidney is the route of excretion for daptomycin. Markedly higher daptomycin dosages were required to treat the renal enterococcal infections (AUC/MIC = 42.7) compared to the exposures needed for thigh infections (AUC/MIC = 1.7). The drug exposures required in the renal model to treat *E. faecalis* with MIC values of 0.5 to 8 µg/ml merits consideration in break point evaluations.

These pharmacodynamic studies support the once daily dosing of daptomycin to achieve a high Cmax and a large AUC_{0-24} value to maximize efficacy. The clinical dosage of 4 mg/kg q24h which produces a mean C_{max} of 57.8 µg/ml, and a mean AUC_{0-24} of 494 µg-hr/ml, achieves the pharmacodynamic values predictive of efficacy against *S. aureus*, *Streptococcus* spp., and *E. faecalis*. The four *in vivo* pharmacodynamic studies can be used to project potential break points for daptomycin against *S. aureus*, *Streptococcus* spp. and

E. faecalis. These are calculated by using the AUC/MIC values for efficacy generated against these three bacterial species in the four studies and comparing them to the mean pharmacokinetic exposures of 494 µg x hr/ml produced clinically at 4 mg/kg (see Table 32).

Table 32: Projection of potential break points using animal pharmacodynamic data and human pharmacokinetic exposures

Pathogen	Study	AUC/MIC for static response	Adjusted total AUC/MIC	Maximum MIC breakpoint[a]
S. aureus	Louie *et al* [46]	43[b]	172	2.9
S. aureus	Safdar [35]	438	438	1.1
S. aureus	Dandekar [45]	18[c]	180	2.7
Enterococcus spp	Safdar [35]	1.7	1.7	NA[d]
Enterococcus spp	Dandekar [45]	13.2[c]	132[c]	3.7
		4.8[c]	48[c]	10.3
Enterococcus spp	Alder [47]	43.4	43.4	11.3
Streptococcus spp.	Safdar [35]	160	160	3.1

a. Based on a human $AUC_{0\text{-}24}$ of 494 µg x hr/ml produced by the 4 mg/kg q24h dose.
b. MIC =1.0 performed in serum, break point calculated using a MIC = 0.25 in broth.
c. AUC of free drug. based on 90% serum binding as used in the study.
d. Not applicable, isolates were insufficiently pathogenic

Monte Carlo Analysis

Monte Carlo analysis was performed to calculate probabilities of daptomycin achieving AUC/MIC criteria (see Table 33). A total of 10,000 simulations were done for each pathogen group. The two variables were applied against the total AUC/MIC pharmacodynamic standards established in mouse models of thigh and renal infection (see Table 32). AUC/MIC ratios of 180, 160, and 132 were used for *S. aureus*, *Streptococcus* spp., and *E. faecalis*, respectively. The AUC/MIC criteria are listed in Table 33. These analyses were conducted by M. Rybak (Wayne State University, MI) using @Risk 4.0. These Monte Carlo analyses indicate a high probability of daptomycin achieving the AUC/MIC criteria for efficacy against the three pathogen groups with a range of susceptibilities determined through surveillance. Probabilities of achieving required AUC/MIC ratios were calculated against *S. aureus, Streptococcus* spp., and *E. faecalis*.

Table 33: Parameters used in Monte Carlo analysis of daptomycin efficacy

Pathogen	MIC µg/ml Mean ± SD	Human PK µg-hr/ml Mean ± SD	Pharmacodynamic criteria AUC/MIC	Probability of meeting criteria
S. aureus	0.27 ± 0.11	493.5 ± 75.4	180	99.9%
Streptococcus spp.[d]	0.21 ± 0.22	493.5 ± 75.4	160	99.9%
E. faecalis[e]	1.0 ± 0.88	493.5 ± 75.4	132	96.9%

a. See Sahm *et al* [73]; Sahm *et al*; [74]
b. See Human Pharmacokinetics and Bioavailability Section, Table 5-5 of the Briefing Package.
c. Pharmacodynamic parameter from Dandekar *et al*, 2002 [45]
d. Pharmacodynamic parameter from Safdar *et al*, 1999 [44]
e. Pharmacodynamic parameter from Dandekar *et al*, 2002 [45]

The *in vitro* and animal pharmacodynamic studies demonstrated the potential for efficacy at daptomycin concentrations achieved clinically. Both C_{max} /MIC and AUC/MIC were pharmacodynamic predictors for efficacy, which correlates with the concentration dependent cidality of daptomycin. The difference in the speed of the daptomycin bactericidal response against staphylococci and streptococci (30 minutes) compared to enterococci (2 hours) may contribute to both C_{max} and AUC emerging as parameters to drive efficacy. Very rapid bactericidal action will tend to correlate better with C_{max}, while longer bactericidal exposure times may correlate better with AUC. With the long half-life of daptomycin, a high C_{max} will lead to a large AUC, and a large AUC is a good predictor of a high C_{max}.

Monte Carlo analysis was used to calculate the probable clinical efficacy of daptomycin against *S. aureus, E. faecalis*, and *Streptococcus* spp. group including *S. pyogenes, S. agalactiae,* and viridans streptococci group. For *S. aureus*, there was a calculated > 99.99% probability of achieving the AUC/MIC criteria of 180, based on human PK AUC distribution and *S. aureus* MIC. For *Streptococcus* spp., there was a 99.93% probability of achieving the AUC/MIC criteria of 160. For *E. faecalis*, there was a 96.88% probability of achieving the AUC/MIC criteria of 132.

No formal human pharmacodynamic trials were conducted with daptomycin.

***IN VIVO* ANIMAL MODELS OF EFFICACY**

The efficacy of daptomycin was assessed in animal models of infection. The daptomycin spectrum of activity includes most pathogenic Gram-positive bacteria including *Staphylococcus aureus*, *Streptococcus* spp., *Enterococcus faecalis*, and *Enterococcus faecium*. Daptomycin is also active against MRSA, PRSP, and VRE. The rapid *in vitro* cidal activity of daptomycin, along with its long half-life, wide tissue distribution, and extended post-antibiotic effect (PAE), suggest clinical potential for the treatment of infections in different anatomical sites.

Daptomycin efficacy was demonstrated in animal models of infection including the proposed clinical indication of skin and skin structure infection. The models of infection tested included septicemia, skin and soft tissue, pulmonary, endocarditis, and renal infections in mice, rats, rabbits, guinea pigs and hamsters. Studies on pulmonary, endocarditis and renal infections will not be discussed further (see Microbiology sections 7.9.3, 7.9.4, and 7.9.5). Efficacy was demonstrated against *S. aureus,* including MRSA, *S. pneumoniae*, including penicillin-resistant isolates, and *E. faecalis* and *E. faecium,* including vancomycin-resistant isolates. The efficacy of daptomycin in these investigations generally paralleled *in vitro* potency and was typically achieved at dosages lower than those required for vancomycin efficacy (see Table 34).

Animal pharmacodynamic studies demonstrated that the parameters that correlate best with efficacy of daptomycin are AUC/MIC and C_{max}/MIC consistent with the concentration-dependent bactericidal activity of daptomycin. Efficacy was not related to time above MIC. These pharmacodynamic data support the use of a once-daily clinical regimen of daptomycin and suggest that efficacy will be achieved at a dose of 4 mg/kg q24h.

Table 34: Animal models demonstrating efficacy of daptomycin

Animal System	Infection	Bacteria Tested
Mouse	Bacteremia	MSSA, MRSA, MRSE, *S. pneumoniae*, *Streptococcus pyogenes*, *E. faecalis*
	Soft tissue	MSSA, MRSA, *S. pneumoniae*, *E. faecium*
	Hematogenous pneumonia	*S. pneumoniae*, *S. aureus*
	Kidney	*E. faecalis*, *E. faecium*
Guinea pig	Soft tissue	MSSA, MRSA, MRSE
Hamster	Lung	MRSA
Rat	Kidney	*E. faecalis*
	Endocarditis	MRSA, MRSE, MSSA, *E. faecalis*, *E. faecium*
Rabbit	Endocarditis	MRSA, MSSA, MRSE, *Enterococcus* spp.

Skin and skin structure infections

Daptomycin efficacy was demonstrated in animal models of drug-susceptible and drug-resistant *S. aureus*, *S. pneumoniae*, and *E. faecium* infections in soft tissue and bacterial abscess infections. Efficacy was obtained at drug exposures in mice similar to those obtained clinically in humans. In addition, prophylactic activity of daptomycin was observed in a model of surgical wound infections in guinea pigs.

Daptomycin efficacy was demonstrated in immunosuppressed mouse models of bacterial thigh infection. The thigh model investigations encompassed the major pathogens for which daptomycin is targeted clinically, including MSSA, MRSA, streptococci, and enterococci. Efficacy was evaluated based on reduction of bacterial count in thigh tissue. Static doses for various organisms ranged from 0.2 to 29 mg/kg/day (see Table 29).

Daptomycin also demonstrated efficacy against challenging bacterial abscess infections. Initial studies determined that the bactericidal activity of daptomycin is enhanced under anaerobic conditions [44, 49, 50]. In a rat model of MRSA or MSSA bacterial abscess, daptomycin therapy (10 mg/kg q12h) produced 5 to 7 $\log_{10}$ reductions against *S. aureus* strains, consistent with its rapid bactericidal activity, whereas vancomycin treatment was significantly less effective in reducing bacterial counts [51].

Daptomycin also demonstrated prophylactic efficacy. In a guinea pig model of surgical wound infections, lesions were induced by intradermal inoculation of MSSA, MRSA, or MRSE [39]. Pretreatment with a single intraperitoneal (i.p.) dose of daptomycin at 20 mg/kg was effective in reducing the lesion size by 30 to 55% for all staphylococci isolates tested. The efficacy of daptomycin was comparable to that of vancomycin, and superior to that of nafcillin [39].

Bacteremia

Daptomycin was effective in treating a variety of drug-resistant and -susceptible Gram-positive bacterial strains in lethal challenge models. The efficacy of daptomycin was consistent with the *in vitro* potency demonstrated against *S. aureus*, *S. pneumoniae* and *Enterococcus* spp. Daptomycin demonstrated greater efficacy and potency than several clinically relevant comparator agents in models of Gram-positive bacteremia.

Daptomycin dosages of 0.6 to 19.3 mg/kg/day protected normal and irradiated immunosuppressed mice challenged i.p. with lethal bacterial concentrations (see Table 39).

Table 39: *In vitro* and *in vivo* antibacterial activity of daptomycin in lethal mouse septicemia trials

Organism	*In vitro* MIC (µg/ml)	*In vivo* ED_{50} (mg/kg)[a]
E. faecalis #80 (VRE)	2.5	1.2
S. aureus 3055 (Pen Strain MSSA)	0.5	1.36
S. aureus ST56 (MRSA)	2	1
S. aureus ST57 (MRSA)	1	9.6
S. aureus ST59 (MRSA)	1	4.2
S. aureus ST60 (MRSA)	0.5	2.7
S. aureus ST201 (MRSA)	0.5	0.6
S. aureus ST210 (MRSA)	1	2.1
S. aureus ST329 (MRSA)	0.5	8.1
S. epidermidis ST277 (MRSE)	1	14
S. epidermidis ST278 (MRSE)	1	19
S. pneumoniae Park 1	0.12	0.3
S. pyogenes C203	0.06	0.1

a. Dosage to produce 50% survival, each of two SC doses separated by 4 h.

Eradication of infections with *Streptococcus pyogenes*, *S. pneumoniae*, MSSA, and vancomycin-resistant *Enterococcus faecalis* was achieved at effective dosages (50% effective dosage, ED_{50}) values of less than 2 mg/kg. The slightly higher doses required to achieve efficacy against MRSA and MRSE are likely related to the immunosuppression of the host required to establish systemic infection with these strains. The estimated AUCs at the effective dosages in these murine models are less than the clinical AUC value of 494 µg x hr/ml.

Daptomycin also protected mice against disseminated VRE infection significantly better than either vancomycin or ciprofloxacin [52]. In contrast, neither vancomycin nor ciprofloxacin protected the animals from VRE.

In a murine model of bacteremia with drug-susceptible *S. aureus*, daptomycin treatment significantly increased survival [53]. The therapeutic effectiveness of daptomycin was equal or superior to that of vancomycin as evaluated by both survival and sterilization of septicemia.

Daptomycin is rapidly bactericidal *in vitro* and *in vivo* against *S. aureus*. Mice were infected intraperitoneally with *S. aureus* Xen-1, an MRSA isolate with a luciferase plasmid construct, which causes the bacteria to appear luminescent while alive. Viable concentrations of bacteria appear more strongly luminescent, allowing the monitoring of antibiotic bactericidal activity in real time. Daptomycin or vancomycin was administered one hour after infection and groups of five mice were imaged at different time points. Daptomycin appeared to be more rapidly bactericidal than vancomycin in this trial. Dosages of 50 mg/kg of daptomycin or vancomycin both caused reductions in luminescent signal of *S. aureus* relative to the signal from mice treated with saline. However,

daptomycin caused approximately 90% reduction in signal over two hours, compared to a reduction of 60-70% for vancomycin.

Drug Combination Studies

Use of daptomycin in combination therapy has shown potential in several animal models of infection. In a neutropenic rat model of MRSA bacteremia, survival of rats treated with the combination of daptomycin (5 mg/kg q12h) and amikacin (30 mg/kg q6h) was significantly greater than for rats treated with either drug alone [56]. In a model of delayed therapy against MRSA or MSSA bacterial abscess, the combination of daptomycin plus rifampin or daptomycin plus rifampin and tobramycin was more effective than any of the drugs alone. All drugs were administered at 20 mg/kg q12h i.p. [50].

In rat models of enterococcal pyelonephritis, the success of combination therapy of daptomycin and gentamicin was dose-dependent. However, use of an increased dosage of gentamicin (~ 4 to 6 mg/kg IM twice daily) resulted in a combination therapy that was more effective than either drug alone in reducing bacterial counts in kidney tissue [43].

- In animal models of endocarditis, synergism of daptomycin and gentamicin was observed for some isolates when daptomycin was administered in combination with gentamicin [40, 41]. No positive or negative interaction was observed when daptomycin was administered with rifampin for treatment of endocarditis [54]. However, the combination was better than either drug alone [57]. In some studies, daptomycin as a single therapy was more effective than the combination therapy of ampicillin plus either sulbactam or gentamicin [40, 55].

Conclusions – animal models of efficacy

In animal models, daptomycin has been shown to be effective against Gram-positive infections in the bloodstream, skin and soft tissue, kidney, and lung. These models included clinically relevant drug-resistant bacterial infections, such as MRSA-induced bacteremia, and VRE- induced bacteremia and pyelonephritis. The efficacy demonstrated against these infections was reflective of daptomycin's microbiological characteristics of rapid cidal activity, low MICs against both drug-susceptible and drug-resistant strains, extended PAE, and long serum half-life. Efficacy in animals was achieved at estimated exposure levels equal to or less than that at the clinical dose of 4 mg/kg q24h. In addition, the efficacy of daptomycin was usually greater than or equal to that of current therapies. These results suggest that daptomycin could have significant utility for the treatment of Gram-positive infections at multiple sites.

Combination therapy of daptomycin plus an aminoglycoside has shown potential for enhanced efficacy in comparison with monotherapy.

Pharmacodynamic studies conducted in infected neutropenic mice demonstrated that the efficacy after once-daily administration of daptomycin is comparable to that observed after dose fractionation. AUC/MIC and C_{max}/MIC correlated better with efficacy than t > MIC, consistent with the concentration dependent bactericidal activity of daptomycin. The results of these studies support the clinical schedule of once-daily administration of daptomycin.

The animal pharmacology data are consistent with the clinical trial results demonstrating the efficacy of daptomycin for treatment of complicated skin and skin structure infections by Gram- positive organisms.

CLINICAL EFFICACY

CLINICAL LABORATORY SUSCEPTIBILITY TEST METHODS

Disk Diffusion Testing

Daptomycin requires physiologic levels of free (ionized) calcium for accurate *in vitro* testing and this requirement is described for both susceptibility test broths and agar media. The NCCLS has accepted the tentative MIC and disk zone QC ranges as developed by Dr. Arthur Barry, Clinical Microbiology Institute (CMI), in a study for Cubist [60]. The earlier Lilly sponsored study by Jones and Barry [58] provides support for disk content as well as providing a historical reference of the consistent potency of daptomycin for Gram-positive pathogens.

The preliminary results, tested by NCCLS standardized methods, favored the use of the 30 µg disk [59]. These authors proposed zone diameter breakpoints for daptomycin of =16 mm for susceptible (MICs, =2 µg/ml) and =12 mm for resistant strains (MICs, =8 µg/ml) using 30 µg disks (the same potency as for vancomycin and teicoplanin).

The tentative QC zone size ranges were determined following analysis of data collected at ten testing facilities using two lots of DAP (daptomycin) disks, one lot of vancomycin disks, and three different lots of Mueller-Hinton medium. Six hundred zone diameters were examined for each control strain. Vancomycin zone diameters had to fall within the acceptable NCCLS range for daptomycin testing to be valid. Zone size limits proposed for the disk diffusion tests of daptomycin were calculated based on the median statistic of zone diameters [61]. The results shown in Table 8-1 were published following their approval at the June 1999 NCCLS meeting [62]. NCCLS M 23 states that the ideal disk is one that provides zone diameters greater than 15mm and less than 45mm for most susceptible strains. The 30 µg disks appear to produce zone diameters within the ideal diameter range [62].

Table 8-1: Zone size limits proposed for disk diffusion tests of daptomycin

Organism	Zone Diameter (mm)	No. of Zones Tested	No. of Zones in Range	% in Range
S. aureus ATCC 25923	18 - 23 mm	600	593	98.8%
S. pneumoniae ATCC 49619	19 - 26 mm	600	590	98.3%

Broth Dilution Testing

Barry *et al* [60] performed the standardization of MIC testing using broth microdilution following the method outlined by the M7 NCCLS Guidelines. The only exception to the standard method was the use of Mueller-Hinton broth supplemented with 50mg/L calcium for daptomycin testing. Three ATCC reference strains were tested. Vancomycin was the control antibiotic and as such, if one or more vancomycin MIC was out of established control limits, the testing was repeated.

Each of ten testing sites received frozen broth microdilution panels

with daptomycin diluted in six different lots of broth and vancomycin serially diluted in two lots of broth.

At the ten microbiology laboratories, testing was performed on 10 different test days using appropriate QC strains to produce 6 daptomycin and 2 vancomycin MIC values per panel. A total of 600 daptomycin MIC test values were obtained. The results for vancomycin were all within range except for one laboratory and the proposed limits for daptomycin MICs for the three ATCC strains are listed in Table 36.

Table 36: MIC limits proposed for broth microdilution tests of daptomycin

Organism	MIC Range (µg/ml)	No. of MICs Tested	No. of MICs in Range	% in Range
E. faecalis ATCC 29212		600		
S. aureus ATCC 29213		450		
S. pneumoniae ATCC 49619		600		

The Applicant has provided data demonstrating that the *in vitro* activity of daptomycin does not appear to be affected by the susceptibility testing methodology (i.e. agar versus broth) provided that the concentration of free (ionized) calcium is adequate (see section 8.1.5 of the Microbiology Section).

Quality Control

Daptomycin QC testing was performed at two central microbiology laboratories supporting the cSSSI studies and showed excellent agreement for appropriate QC strains utilized for both MIC and K-B disk diffusion methods. Both laboratories carried out MIC and Kirby-Bauer disk diffusion testing according to NCCLS standards. QC testing was performed using the appropriate ATCC organism on each day that clinical isolates were tested. For MIC testing, both laboratories utilized The 30 µg DAP disks produced by were used throughout the studies. Disk production lots were used by both Central laboratories and no variation in range of zone sizes was detected in side-by-side tests of different disk lots performed at . Standard daptomycin powder and 30 µg disks should provide the range of values noted in Table 37.

An independent 21-laboratory expanded QC study (NCCLS Tier 3 study) was performed [131]. Each of 21 participating laboratories performed disk diffusion and broth microdilution susceptibility tests with each of the QC strains *(S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 MICs only; *S. aureus* ATCC 25923, disk test only; *S. pneumoniae* ATCC 49619, MICs & disk tests).

Table 37: Acceptable Quality Control Ranges for Daptomycin to be Used in Validation of Susceptibility Test Results

QC Strain	Acceptable Quality Control Ranges	
	Minimum Inhibitory Concentration (MIC in µg/ml) [a]	Disk Diffusion (Zone Diameters in mm) [b]
Enterococcus faecalis ATCC 29212	1-8	Not applicable
Staphylococcus aureus ATCC 29213	0.25-1	Not applicable
Staphylococcus aureus ATCC 25923	Not applicable	18-23
Streptococcus pneumoniae ATCC 49619 [c]	0.06-0.5 [d]	19-26 [e]

[a] Quality Control ranges reflect MICs obtained when Mueller-Hinton broth is supplemented with calcium to a final concentration of 50µg/ml.
[b] Some lots of Mueller-Hinton Agar are deficient in calcium and give small zone diameters.
[c] This organism may be used for validation of susceptibility test results when testing *Streptococcus* spp. other than *S. pneumoniae*.
[d] This quality control range for *S. pneumoniae* is applicable only to tests performed by broth microdilution using cation adjusted Mueller-Hinton broth with 2-5% lysed horse blood inoculated with a direct colony suspension and incubated in ambient air at 35°C for 20 to 24 hours.
[e] This quality control zone diameter range is applicable only to tests performed using Mueller-Hinton agar supplemented with 5% defibrinated sheep blood inoculated with a direct colony suspension and incubated in 5% CO at 35°C for 20 to 24 hours.

An overview of the MIC and disk diffusion test results is provided in Table 38 and Table 39. The daptomycin MIC testing at all 21 sites was within range for >96.9% of all test values. The daptomycin QC K-B disk diffusion testing showed great variation, which was linked to the use of testing medium from different manufacturers. *S. aureus* QC was 100% and 92.7% within range with media A and B respectively. Similarly, the testing with *S. pneumoniae* QC strains was 100% and 99.1% within range using media from three (A, B and D) manufacturers. All test results with media from manufacturer C were unacceptable.

Table 38: Summary of QC MIC Testing

Drug	**No. of Labs Participating**	***S. aureus* ATCC 29213**	***E. faecalis* ATCC 29212**	***S. pneumoniae* ATCC 49619**
Daptomycin	21	424/425 (99.8%)	417/422 (98.8%)	409/422 (96.9%)
Vancomycin	21	425/425 (100%)	422/422 (100%)	406/422 (96.2%)

Table 39: Summary of QC Disk Testing Indicating Effect of Different Media

Drug	No. of Labs Participating	Multiple M-H Lots	Media Manufacturer	Media Manufacturer	Media Manufacturer
S. aureus					
ATCC 25923			**"A"**	**"B"**	**"C"**
Daptomycin	21	303/422 (71.8%)			
	3		60/60 (100%)		
	13			243/262 (92.7%)	
	5				0/100 (0%)
Vancomycin	21	402/422 (95.3%)			
S. pneumoniae					
ATCC 49619					
Daptomycin	21	350/422 (82.9%)			
	4		80/80 (100%)		
	11			220/222 (99.1%)	
	6				50/120 (41.7%)
Vancomycin	21	417/422 (98.8%)			

The Applicant has submitted data showing that broth microdilution susceptibility tests for QC standards *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Streptococcus pneumoniae* ATCC 49619 support the proposed QC range. These data can be found in Microbiology section 8.2.4.

As shown previously, disk diffusion testing of daptomycin can be problematic because the *in vitro* activity of this compound is affected by variations in the calcium content of the solid media [63]. While the calcium content of liquid media is generally controlled within the NCCLS suggested range of 20-25 µg/ml, no recommended calcium concentration ranges currently exist for solid media. Consequently, the calcium content of solid media from various manufacturers is known to vary widely. According to the Applicant, one supplier is known for producing solid media with calcium concentrations that are well below the industry mean and consequently, media from this supplier had significantly different disk diffusion results [63]. An NCCLS subcommittee is presently performing studies to select a new reference lot of Mueller-Hinton agar to be used as a reference standard by media manufacturers. Daptomycin is included in this study to provide a procedural control on calcium ion content.

The zone size limits for 30 µg daptomycin disks for *S. aureus* ATCC 25923 (QC strain) are currently 18 - 23 mm. Only 71.8% of the results reported in this survey fell within these limits [Figure 3].

The zones of inhibition were analyzed by manufacturer of the dehydrated powder. Dehydrated media from manufacturers "A" [redacted] and "B" [redacted] produced values that were 100% and 92.7% within proposed ranges for the QC organisms (see Figure 4 and Figure 10-15—not shown). However, none (0%) of the values obtained from media produced by manufacturer "C" [redacted] was within proposed zone size limits (see Figure 6). Previous studies have shown that the calcium content of media lots from manufacturers "A" and "B" are consistently higher than those of manufacturer "C". This would explain why the daptomycin test values obtained using media from manufacturer

"C" are consistently lower than the values obtained using media from the three other manufacturers. The majorities (95.3%) of the vancomycin values were within established ranges and did not show media dependent variations. Two laboratories reported the majority of out-of-range values.

Figure 3: Daptomycin vs. *Staphylococcus aureus* ATCC 25923 Using Multiple Mueller-Hinton Agar Lots

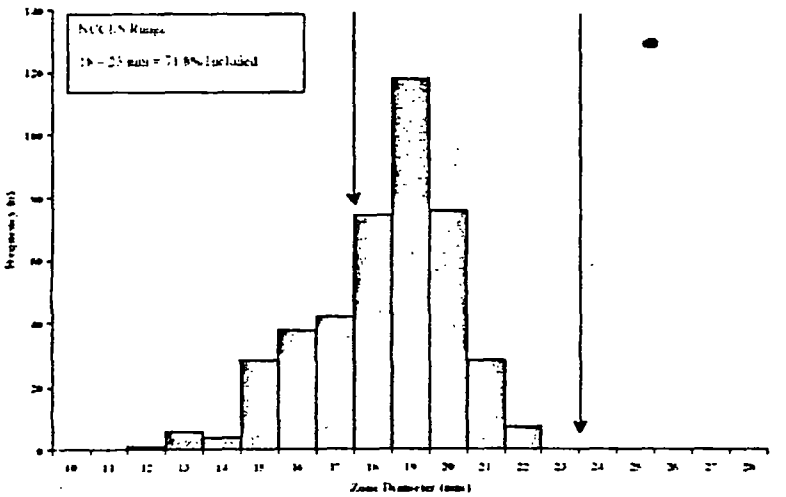


Figure 4: Daptomycin vs. *Staphylococcus aureus* ATCC 25923 Using Dehydrated Media Manufacturer "A"

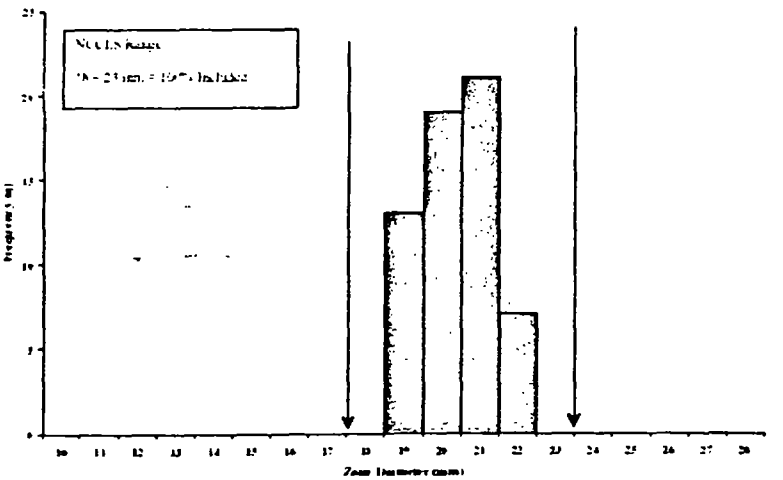
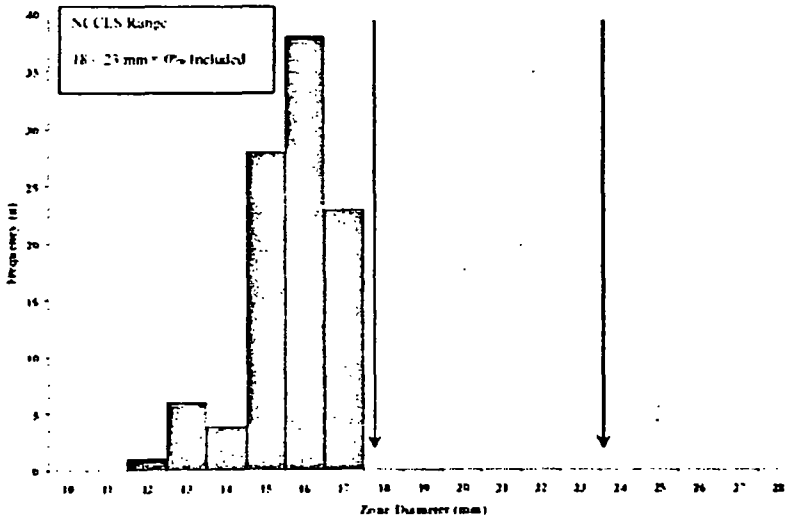


Figure 5: Daptomycin vs. *Staphylococcus aureus* ATCC 25923 Using Dehydrated Media Manufacturer "C"



The same media-related variations reported for *S. aureus* ATCC 25923 were also noted with *S. pneumoniae* ATCC 49619 (see Microbiology section 8.2.4). For daptomycin disks, the proposed *S. pneumoniae* ATCC 49619 (QC strain) limits are 19 - 26 mm. The data from this study were only 82.9% within range. Testing on media from manufacturers A, B and D were acceptable with >99% of zone sizes within the proposed range. However, on media room manufacturer C only 41.7% of QC was within the proposed range. These results were directly related to the calcium concentrations of the various media. The majority (98.8%) of the vancomycin control values were within established limits.

> ***Reviewer's Comments:*** Note that the Applicant did not test an *Enterococcus faecalis* QC strain for variability of daptomycin zone sizes.

PROVISIONAL SUSCEPTIBILITY INTERPRETIVE CRITERIA

Daptomycin is proposed as indicated for the treatment of complicated skin and skin structure infections,
strains of the following Gram-positive microorganisms:

- *S. aureus* (including methicillin-resistant strains)
- *S. pyogenes*
- *S. agalactiae*
- *S. dysgalactiae* subsp. *equisimilis*
- *E. faecalis* (vancomycin-susceptible strains only)

The provisional interpretive criteria for susceptibility are listed in Table 1.

Table 1: Provisional interpretive criteria for susceptibility to daptomycin

Pathogen	Minimal inhibitory concentration (µg/ml)[a]			Disk diffusion zone diameter (mm)[b]		
	S	I	R	S	I	R
Staphylococcus aureus (methicillin-susceptible and methicillin-resistant)	≤2	4	≥8	≥ 16	13-15	≤12
Streptococcus spp. other than *S. pneumoniae*[c]	≤2	(d)	(d)	≥ 13	(d)	(d)
Enterococcus faecalis (vancomycin susceptible only)	≤8	—	≥16	≥ 11	—	≤10

a. The MIC interpretive criteria for *S. aureus* and *E. faecalis* are applicable only to tests performed by broth microdilution using Mueller-Hinton broth adjusted to 50 mg/L calcium; the MIC interpretive criteria for *Streptococcus* spp. other than *S. pneumoniae* are applicable only to tests performed by broth microdilution using Mueller-Hinton broth adjusted to 50 mg/L calcium, supplemented with 2 to 5% lysed horse blood, inoculated with a direct colony suspension, and incubated in ambient air at 35°C for 20 to 24 hours.

b. The zone diameter interpretive criteria for *Streptococcus* spp. other than *S. pneumoniae* are applicable only to tests performed using Mueller-Hinton agar supplemented with 5% defibrinated sheep blood and incubated in 5% CO2 at 35°C for 20 to 24 hours.

c. *S. pyogenes, S. agalactiae, Streptococcus dysgalactiae equisimilis* ,

d. The current absence of data on resistant strains precludes defining any categories other than "Susceptible Strains" yielding test results suggestive of a "Non-susceptible" category should be retested, and if the result is confirmed, the isolate should be submitted to a reference laboratory for further testing.

The overall distribution of MIC and zone sizes for the pathogens listed in Table 1 is shown in Microbiology Section 8.3.1. Both the MIC and K-B disk diffusion tests performed in a reliable and predictable manner in relation to the proposed breakpoints. The MIC to zone size correlation also performs well in relation to the breakpoints, with a minimum of minor errors and no major errors. The MIC and zone size breakpoint standards proposed in Table 1 provide reliable testing criteria in relation to the population of *S. aureus*, the indicated *Streptococcus* spp., and *E. faecalis*.

The clinical support for the proposed breakpoints focused on the MIC and zone to outcome correlations. These analyses show no relationship between the MIC value or zone size and the clinical or microbiological outcome. Daptomycin was equally effective against Gram-positive pathogens across the MIC or zone size ranges. A clinical failure analysis also shows that the MIC and zone size values of isolates from subjects that failed therapy are similar to the MIC and zone size distribution of the total isolate population for each pathogen. The two clinical isolates that developed an increase in daptomycin MIC value during therapy (*S. aureus* MIC = 12.5 µg/ml and *E. faecalis* MIC = 32 µg/ml) would both be categorized as resistant under these criteria.

The provisional interpretive criteria for susceptibility are based on the following evaluations:

- Surveillance studies (SECURE-US and SECURE-EU) showing unimodal distribution curves for isolate MICs. The susceptibilities of the pathogens in the clinical trials were similar to the SECURE surveillance studies.

- Pharmacodynamic analysis demonstrating plasma level exposure (AUC/MIC) criteria for efficacy
- Monte Carlo simulations using human pharmacokinetic data (AUC) from phase 1 studies, exposure criteria for efficacy (AUC/MIC) based on animal pharmacodynamic studies, and distribution of isolate MICs based on surveillance studies.
- Clinical data demonstrating similar efficacy of daptomycin against the indicated Gram- positive pathogens across the susceptible MIC and zone size range. The efficacy of daptomycin against those pathogens was also comparable to conventional therapy (vancomycin or semi-synthetic penicillins).

For further inspection of the MIC distributions from the clinical studies and surveillance studies, consult Figures 10-23 to 10-40 from the Microbiology section (Clinical Efficacy) of the NDA submission.

The MIC distribution for all *S. aureus* isolates tested showed that the two populations from the clinical studies and the surveillance studies had the same unimodal MIC distribution pattern (see Figure 10-23). The distribution patterns were identical for both the MRSA and MSSA groupings (see Figures 10-24 and 10-25). However, the zone size distributions for the clinical isolates were shifted to the right of the surveillance isolates indicating that clinical isolates had a somewhat larger zone size than the surveillance isolates (see Figure 10-26). This was true for all regardless of methicillin-resistance (see Figures 10-27 and 10-28).

The MIC distribution for all Streptococci isolates tested showed that the two populations from the clinical studies and the surveillance studies had different modal distributions. The clinical studies tended to have a greater percentage of isolates with lower MICs than isolates from the surveillance studies (see Figure 10-29). Once again the zone size distributions for the clinical isolates were shifted to the right of the surveillance isolates indicating that clinical isolates had a somewhat larger zone size than the surveillance isolates (see Figure 10-30).

The MIC distributions of *Streptococcus pyogenes* for isolates from the clinical and surveillance studies were similar (see Figure 10-31). The zone size distributions for the surveillance isolates were shifted to the right of the clinical isolates indicating that surveillance isolates had a somewhat larger zone size than the clinical isolates (see Figure 10-32). This is in contrast to the zone size distributions for *S. aureus*.

The MIC and zone size distributions for *Streptococcus agalactiae* isolates from the clinical and surveillance studies were similar (see Figures 10-33 and 10-34).

The MIC distribution for *Streptococcus dysgalactiae equisimilis* isolates tested showed that the two populations from the clinical studies and the surveillance studies had different modal distributions (see Figure 10-35). The isolates from the clinical studies tended to have a greater percentage of isolates with MICs of 0.06 µg/ml while the isolates from the surveillance studies had a greater percentage of isolates with MICs of ≤0.03 µg/ml (see

Figure 10-35). The zone size distributions for the clinical isolates were shifted to the right of the surveillance isolates indicating that clinical isolates had a somewhat larger zone size than the surveillance isolates (see Figure 10-36).

The MIC and zone size distributions for Viridans Streptococci isolates from the clinical and surveillance studies were similar (see Figures 10-37 and 10-38).

The MIC distributions for *Enterococcus faecalis* isolates from the clinical and surveillance studies were similar (see Figures 10-39). However, zone size distributions for the clinical isolates were shifted to the right of the surveillance isolates indicating that clinical isolates had a somewhat larger zone size than the surveillance isolates (see Figure 10-40).

The Applicant states that these analyses show that the proposed breakpoint standards perform well for determining the daptomycin susceptibility of the indicated Gram-positive pathogens.

The scattergrams with regression analysis of daptomycin MICs versus zone sizes are presented for all Gram-positive pathogens for which an indication is being sought. Although an indication is not being sought for ——— were not considered pathogens in either cSSSI trial, they are included for completeness in accordance with FDA guidelines for drugs seeking an indication against *S. aureus*. For closer inspection of the scattergrams, see Figures 10-41 to 10-47, and 8-1 to 8-3 of the Microbiology Section.

The data analyzed is derived from testing results of the SECURE surveillance isolates with the 102 *S. dysgalactiae* subsp. *equisimilis* addition representing the Retrospective Surveillance set described above. The proposed breakpoints for MIC and K-B testing are indicated as dashed lines in the figures. These proposed breakpoints are discussed later and are based on bacterial population distributions, from *in vivo* daptomycin levels, and from therapeutic results. Overall, the scattergrams do not indicate a resistance subpopulation. This result is not unexpected as daptomycin represents a new class of antiinfectives, the lipopeptides, and no naturally resistant strains have been encountered.

The daptomycin dose being submitted for approval is 4 mg/kg i.v. This dosage produced a steady state mean C_{max} of 57.8 µg/ml and a mean AUC_{0-24} of 494 µg x hr/ml. The AUC value was used in pharmacodynamic calculations and Monte Carlo simulations to both estimate susceptibility criteria and to estimate probabilities of success. Using pharmacodynamic criteria established in animal models and human pharmacokinetic data generated at 4mg/kg, provisional susceptibility criteria were established (Table 20). A report of "Susceptible" indicates that the pathogen is likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable. A report of "Intermediate" indicates that the result should be considered equivocal, and, if the microorganism is not fully susceptible to alternative antimicrobial agents, clinically feasible drugs, the test should be repeated. The intermediate category implies possible clinical applicability in body sites where the drug is physiologically concentrated or in situations in which a high dosage of drug can be used. The intermediate category also provides a buffer zone, which prevents small uncontrolled technical factors from causing

major discrepancies in interpretation. A report of "Resistant" indicates that the pathogen is not likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable.

CORRELATION OF PROVISIONAL INTERPRETIVE CRITERIA WITH CLINICAL AND MICROBIOLOGICAL OUTCOMES

Overview of Primary Comparative cSSSI Studies

There were 1118 subjects enrolled in the two pivotal phase 3 trials; 535 subjects received at least one dose of daptomycin. The design, execution and analysis of these trials are presented in detail in the Integrated Summary of Efficacy (see ISE). In brief, both trials had the same basic design. Hospitalized subjects with cSSSI were randomized (1:1) to treatment with daptomycin or comparator for 7 to 14 days. Daptomycin was administered i.v. at 4 mg/kg q24h; comparators were semi-synthetic penicillins (oxacillin, nafcillin, cloxacillin or flucloxacillin) given as 4 to 14 grams i.v. daily in equal divided doses or vancomycin 1 g i.v. q12h. The two trials differed primarily in geographic areas represented. In Study 9801, ~80% of the subjects were enrolled in the USA and ~ 20% were enrolled in South Africa. In Study 9901, ~ 50% of the subjects were enrolled in South Africa, with the remainder primarily from Eastern and Western Europe.

The comparator agents in these trials -- vancomycin, oxacillin, nafcillin, cloxacillin, and flucloxacillin -- are currently approved for the treatment of cSSSI caused by susceptible pathogens. All four agents have very similar pharmacologic properties, including:

- •Resistance to staphylococcal beta-lactamase;
- •MIC_{90} for methicillin-susceptible *S. aureus*, <0.5 µg/ml;
- •Lack of activity against methicillin-resistant *S. aureus*;
- •Half-life in man, 0.5 to 1.0 hour;
- •93-94% bound to serum protein;
- •Approved dosing of cSSSI in the range of 4-14 g/day i.v. in divided doses as specified by the protocol.

Duration of intravenous therapy was 7 to 14 days for both regimens (daptomycin and comparator) as clinically indicated. If, in the investigator™s opinion, a subject required more than 14 days of therapy, then the duration could be extended by contacting the Medical Monitor.

Analysis of Outcomes by Specific Comparator Agents

The pivotal cSSSI trials (Study 9801 and Study 9901) were designed to compare daptomycin to currently available therapy. As noted above, two different classes of comparator (semi-synthetic penicillins and vancomycin) were included. Semi-synthetic penicillins are the drugs of choice for cSSSI; vancomycin is considered a second-line agent to be used for persons who are allergic to penicillins and/or infected with resistant organisms.

Clinical and microbiologic outcomes were based primarily on evaluations performed at the Test-of-Cure Evaluation, conducted between Day 6P to Day 20P (also referred to as the

Post-Therapy Evaluation). This included an evaluation of the clinical signs and symptoms of infection, the clinical response based on signs and symptoms, and the Gram stain and culture.

The clinical response at the Test-of-Cure evaluation was determined by the blinded Investigator based on comparison of the subject's signs and symptoms at that time to the signs and symptoms present at the Baseline Evaluation. Clinical Response was classified as follows:

- •Cure: Resolution of the clinically significant signs and symptoms associated with the skin infection present at Baseline Evaluation (Return to pre-infection condition).
- •Clinical Improvement: Partial resolution of clinical signs and symptoms of the skin infection such that no further antibiotic therapy is required.
- •Clinical failure: Inadequate response to therapy.
- •Unable to evaluate: Unable to determine response because the patient was lost to follow-up.

In brief, the primary requirements to be assessed a Sponsor-Defined Clinical Success were:

- •Subject received i.v. study drug as assigned and,
- •Subject was judged Cure or Clinical Improvement.

Subjects with a Sponsor-Defined Clinical Outcome of Failure or Non-evaluable were considered microbiologic failures for all Infecting Pathogens. For subjects with a Sponsor-Defined Clinical Outcome of Success, the Microbiological Response for each Infecting Pathogen isolated at Baseline was defined as follows:

- •Eradicated
- •Documented Eradicated: the pathogen was absent at the Test-of-Cure evaluation;
- •Presumed eradicated: the Test-of-Cure evaluation indicated "Nothing to culture".
- •Persistent
- •Documented Persistent: the pathogen was documented as present at the Test of Cure evaluation.;
- •Presumed Persistent (missing data): a culture was not obtained at the Test-of-Cure evaluation for any reason other than "Nothing to culture".

For purposes of analysis, the two subtypes of Eradicated were grouped together and represent "success" and the two subtypes of Persistent were grouped together and represent "failure".

All subjects with one or more Infecting Gram-positive Pathogens isolated at Baseline were assigned a Microbiological Response by subject using the following criteria.

- •Microbiologic Success: All Infecting Gram-positive Pathogens isolated at Baseline were Eradicated at the Test-of-Cure Evaluation **and** a Superinfecting Pathogen was **not** isolated either prior to or at the Test-of-Cure Evaluation.
- •Microbiologic Failure: Persistence of one or more Infecting Gram-positive Pathogens or isolation of a Superinfecting Pathogen prior to or at the Test-of-Cure Evaluation.