Pharmacodynamics of early, high-dose linezolid against vancomycin-resistant enterococci with elevated MICs and pre-existing genetic mutations

Brian T. Tsuji1,2*, Jurgen B. Bulitta1,3†, Tanya Brown1,2, Alan Forrest1, Pamela A. Kelchlin1,2, Patty N. Holden1,2, Charles A. Peloquin4, Laura Skerlos5 and Debra Hanna5,6

1Laboratory for Antimicrobial Pharmacodynamics, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, NY, USA; 2New York State Center of Excellence in Bioinformatics & Life Sciences, University at Buffalo, State University of New York, Buffalo, NY, USA; 3Centre for Medicine Use and Safety, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Melbourne, Australia; 4College of Pharmacy, University of Florida, Gainesville, FL, USA; 5Pfizer Global Research and Development, Groton, CT, USA; 6Critical Path Institute, Tucson, AZ, USA

*Corresponding author. Tel: +1-716-881-7543; Fax: +1-716-849-6890; E-mail: btsuji@buffalo.edu
†These authors contributed equally.

Received 3 August 2011; returned 29 September 2011; revised 12 April 2012; accepted 23 April 2012

Objectives: Vancomycin-resistant enterococci (VRE) have emerged as an important nosocomial pathogen in medical centres worldwide. This study evaluated the impact of front-loading of linezolid on bacterial killing and suppression of resistance against VRE strains with defined genetic mutations.

Methods: Time–killing experiments over 48 h assessed the concentration effect relationship of linezolid against eight strains of vancomycin-resistant Enterococcus faecalis. A hollow fibre infection model (HFIM) simulated traditional and front-loaded human therapeutic linezolid regimens against VRE strains at 10^6 cfu/mL over 240 h. Translational modelling was performed using S-ADAPT and NONMEM.

Results: Over 48 h in time–kill experiments, linezolid displayed bacteriostatic activity with \(2 \log_{10} \text{cfu/mL}\) killing for all strains with an MIC of 4 and minimal activity against VRE with MICs of 16 and 64 mg/L. Against one strain with no resistant alleles (MIC 4 mg/L), 600 mg of linezolid every 12 h achieved maximal reductions of 0.96 \(\log_{10} \text{cfu/mL}\) over 240 h in the HFIM, whereas front-loaded 1200 mg of linezolid every 12 h \(\times 10\) doses or 2400 mg of linezolid every 12 h \(\times 10\) doses followed by 600 mg of linezolid every 12 h provided significantly improved killing with maximal reductions of 3.02 and 3.46 \(\log_{10} \text{cfu/mL}\). Front-loaded regimens suppressed amplification of resistant subpopulations against VRE strains with no resistant alleles (MIC 4 mg/L) and postponed regrowth of resistant subpopulations against a VRE with 3.2 resistant alleles (MIC 4 mg/L). Modelling yielded excellent population fits \((r=0.934)\) and identified the number of sensitive alleles as a critical covariate.

Conclusions: Early, high-dose regimens of linezolid provided promising killing against selected susceptible strains and may be clinically beneficial if early bactericidal activity is necessary.

Keywords: oxazolidinones, pharmacokinetics, resistance, pharmacogenomics

Introduction
Vancomycin-resistant enterococci (VRE) have become a prevalent nosocomial pathogen in medical centres throughout the USA.\(^1\) Over the last decade, the incidence of VRE infections has been increasing, and nearly 50% of enterococci isolated from patients in intensive care units are vancomycin resistant.\(^1,2\) In addition, these organisms are persistent nosocomial pathogens capable of prolonged survival on environmental surfaces and frequently colonize the intestinal and genital tract in humans.\(^3,4\) Additionally, VRE bloodstream infections typically occur in critically ill patients, which highlights the need to focus on optimal treatment for these aggressive and persistent pathogens.\(^2\)

Linezolid represents the first marketed drug belonging to the oxazolidinone class of antimicrobials. Linezolid displays activity against aerobic and facultative Gram-positive microorganisms, including VRE. Although resistance to linezolid occurs in vitro at
a low frequency of $1 \times 10^{-9}$ to $1 \times 10^{-11}$, it has been attributed to a single nucleotide change in 23S rDNA at bp 2576. There have been few studies directed toward defining the pharmacokinetic-pharmacodynamic relationship of linezolid against VRE as it relates to amplification of resistance during therapy.\textsuperscript{5-8} Additionally, there has been limited information regarding whether higher exposure regimens of linezolid display activity against intermediate (MIC 4.0 mg/L) or resistant strains (MIC $\geq 8$ mg/L) of VRE with pre-defined, pre-existing genetic mutations.

We hypothesized that increasing the dose and exposure of linezolid during the first 5 days of therapy may have potential utility to rapidly reduce bacterial burden and suppress or post-pone resistance against these difficult-to-treat strains. Indeed, this proposed ‘front-loading’ strategy significantly differs from the loading dose approach. The loading dose approach seeks to achieve a steady state more rapidly by giving a larger dose on day 1, whereas the front-loading approach explored in this study uses significantly higher doses for multiple days to achieve more bacterial killing and prevent the emergence of resistance.

The hollow fibre infection model (HFIM) presents a sophisticated system that can simulate the time course of linezolid concentrations in humans for normal and front-loaded regimens over 10 days. We are not aware of a published translational, mechanism-based model that simultaneously described the pharmacokinetic-pharmacodynamic relationship of linezolid and was published reports that quantitatively implemented the time–kill and HFIM data. To our knowledge, there are also no published reports that quantitatively implemented the effect of sensitive and resistant alleles on bacterial killing by oxazolidinones.

Based on the time–kill experiments, bacterial strains that demonstrated adequate response to linezolid (maximal reductions of at least $2 \log_{10}$ cfu/mL) at 48 h were selected for further evaluation of front-loaded regimens in the HFIM. These strains were selected since they were responsive to higher concentrations of linezolid and were expected to benefit from a front-loading strategy. Therefore E. faecalis 4407, 4408 and 4422 were subsequently evaluated in the HFIM. This allowed a comparison of killing and prevention of resistance for strains with the same linezolid MIC (4 mg/L), but with different numbers of resistant alleles. Strains that exhibited a high level of resistance (such as 4393, with a linezolid MIC of 64 mg/L) were not evaluated in the HFIM, as linezolid monotherapy would not be considered as a treatment option against these strains in clinical practice. However, we considered it important to gain insight into the concentration response relationship against these highly resistant strains using time–kill studies. ATCC 29212 E. faecalis (linezolid MIC of 1.0 mg/L) was utilized as a vancomycin-susceptible control.

**Antibiotic, susceptibility testing and medium**

Linezolid analytical grade powder was obtained from Pfizer Global Research and Development, Groton, CT, USA. MIC values were determined by broth microdilution according to the CLSI. Brain heart infusion (BHI) broth (Difco, Detroit, MI, USA) and tryptic soy agar (TSA) with 5% sheep blood were used for all time–kill experiments and hollow fibre experiments.

**Time–kill experiments**

To first evaluate the full concentration–effect relationship, time–kill experiments were performed as previously described against a starting inoculum of $10^6$ cfu/mL over 48 h.\textsuperscript{10} In brief, for bacterial inocula preparation, fresh bacterial colonies from overnight growth were added to Brain heart infusion broth (Difco, Detroit, MI, USA) and tryptic soy agar with 5% sheep blood were used for all time–kill experiments and hollow fibre experiments.

**Materials and methods**

### Bacterial isolates

Eight clinical vancomycin-resistant Enterococcus faecalis isolates with varying susceptibility and genetically defined resistant alleles were utilized and obtained from Focus Technologies' clinical surveillance programme Phase IV studies. The number of resistant alleles was quantified by quantitative real-time PCR, by amplification of 72 bp of 23S rDNA by 5' nucleic acid real-time PCR, as previously described.\textsuperscript{11} E. faecalis strains utilized in time–killing experiments included: (i) 4408 (linezolid MIC of 6.0 mg/L, 4.3 sensitive alleles, 0 resistant alleles); (ii) 4407 (linezolid MIC of 4.0 mg/L, 3.5 sensitive alleles, 0 resistant alleles); (iii) 4422 (linezolid MIC of 4 mg/L, 2.4 sensitive alleles, 3.2 resistant alleles); (iv) 4412 (linezolid MIC of 16 mg/L, 1.2 sensitive alleles, 3.2 resistant alleles); (v) 4424 (linezolid MIC of 16 mg/L, 1.0 sensitive alleles, 3.6 resistant alleles); (vi) 4397 (linezolid MIC of 32 mg/L, 1.3 sensitive alleles, 3.5 resistant alleles); (vii) 4405 (linezolid MIC of 32 mg/L, 0 sensitive alleles, 3.5 resistant alleles); and (viii) 4393 (linezolid MIC of 64 mg/L, 0 sensitive alleles, 4.0 resistant alleles).

High-dose linezolid versus enterococci

**HFIM**

An HFIM, adapted from Louie et al.,\textsuperscript{11} was used to evaluate the effect of selected linezolid dosing regimens on the change in bacterial burden and suppression of resistance of Enterococcus faecalis over 240 h as previously described. In brief, a cellulose cartridge (C3008, FiberCell Systems Inc., Frederick, MD, USA) was utilized for all experiments. The determination of bacterial counts for each experiment was performed by obtaining samples at 0, 24, 48, 72, 96, 144, 192 and 240 h. Samples quantified the total population, and aliquots of the diluted sample were plated in quintuplicate on BHI plates containing linezolid at 2, 4 and 8 times the MIC in order to quantify the resistant subpopulations.

**Experimental design and simulated linezolid regimens**

The experimental design consisted of a no-treatment control arm and three simulated regimens including one traditional regimen and two front-loaded regimens. The following linezolid regimens were administered using an apparent half-life of 4.8 h and a protein binding of...
31%,\textsuperscript{12} resulting in a free (i.e., non-protein bound) maximal concentration (\( fC_{\text{prot}} \)) and a free AUC at steady state over 24 h (\( fAUC_{0-24} \)):

Traditional regimen: 600 mg every 12 h (\( fC_{\text{prot}} \) 10.4 mg/L, \( fAUC_{0-24} \) 244).
Front-loaded regimen: 1200 mg every 12 h \times 10 doses on days 0–5 (\( fC_{\text{prot}} \) 20.8, \( fAUC_{0-24} \) 248) followed by 600 mg every 12 h \times 10 doses on days 5–10 (\( fC_{\text{prot}} \) 10.4, \( fAUC_{0-24} \) 124)

\( \frac{dP}{dt} = k_{\text{prot}} \cdot \left[1 - \left(\frac{C_{\text{Drug}}}{IC_{50} + C_{\text{Drug}}}\right) - P\right] \quad \text{Initial condition (IC): 100\%} \quad (1) \)

where \( P \) is the protein pool, \( IC_{50} \) is the lincosamide concentration (\( C_{\text{Drug}} \)) associated with half-maximal inhibition of protein synthesis and \( k_{\text{prot}} \) is the turnover rate constant of the protein pool. The parameterization of equation (1) yields a steady-state of 1 for the protein pool (in the absence of lincosamide), representing 100\% of its hypothetical baseline. The lack of proteins (\( \text{Lack} \)) was calculated as (1 – \( P \)) and the probability of death during replication (\( \text{Prob}_{\text{death}} \)) was described via a maximal probability of death (\( \text{Imax}_{\text{Rep}} \)) at a pronounced lack of the protein pool:

\[ \text{Prob}_{\text{death}} = \text{Imax}_{\text{Rep}} \cdot \text{Lack} \quad (2) \]

\( \frac{dS1}{dt} = 2 \cdot \text{PLAT} \cdot (1 - \text{Prob}_{\text{death}}) \cdot k_{S2} \cdot S2 - k_{S1} \cdot S1 \quad (3) \)

\( \frac{dS2}{dt} = -k_{S1} \cdot S2 + k_{S2} \cdot S1 \quad \text{IC}: 0 \quad (4) \)

\( k_{S1} \) and \( k_{S2} \) are the first-order transfer rate constants between both states and \( \log \text{cfu} \) represents the \( \log_{10} \) of the initial inoculum. The total population (\( \text{cfu}_{\text{tot}} \)) is the sum of \( S1 \) and \( S2 \). The plateau factor (\( \text{PLAT} \)) is defined as 1 – (\( \text{cfu}_{\text{tot}}/(\text{cfu}_{\text{tot}}+\text{cfu}_{\text{prot}}) \)), with \( \text{cfu}_{\text{prot}} \) representing the maximum population size. The factor 2 represents the doubling of cells during the replication process.

\[ \text{IC}_{50} = \text{IC}_{50_{\text{Sen0}}} \cdot \left(1 - \frac{\text{Imax}_{\text{Sen}} \cdot \text{Nsen}_{\text{Sen}}}{\text{N50}_{\text{Sen}} + \text{Nsen}_{\text{Sen}}} \right) \cdot f_{\text{HFIM}} \quad (5) \]

Thus \( \text{IC}_{50} \) is affected by \( \text{Nsen} \) as defined by equation (5). \( \text{IC}_{50_{\text{Sen0}}} \) represents the typical \( \text{IC}_{50} \) for a strain with no sensitive alleles, \( \text{Imax}_{\text{Sen}} \) is the maximum fractional decline of \( \text{IC}_{50} \) and \( H_{\text{Sen}} \) is the Hill coefficient. \( f_{\text{HFIM}} \) represents the estimated ratio of \( \text{IC}_{50} \) in the HFIM compared with the static time-kill model. The number of resistant alleles (\( \text{Nres} \)) was used as a covariate for the mean generation time (\( \text{MGT}_{12} \)) for the transfer of bacteria from state \( S1 \) to \( S2 \). \( k_{S2} \) was calculated as 1/\( \text{MGT}_{12} \); see Table 1 for further parameter explanations. An effect of lincosamide to further prolong the \( \text{MGT}_{12} \) potentially due to a lack of proteins was explored:

\[ \text{MGT}_{12} = \text{MGT}_{0} \cdot \left(1 + \frac{\text{Imax}_{\text{Res}} \cdot \text{Nres}_{\text{Res}}}{\text{N50}_{\text{Res}} + \text{Nres}_{\text{Res}}} \right) \quad (6) \]

Parameter variability model
The inter-strain variability of parameters estimated on a log scale was described by a normal distribution. The \( \text{Imax}_{\text{Res}} \) and \( \text{Imax}_{\text{Sen}} \) were constrained between 0 and 1 using a logistic transformation as described previously.\textsuperscript{15} All other parameters were described by a log-normal distribution.

Residual error model and computation
Candidate models were fit simultaneously to: (i) all viable count profiles from eight strains studied in time-kill experiments; or (ii) all viable count profiles of the total population from the time-kill and hollow fibre studies using an additive residual error model on a log_{10} scale. Estimation in NONMEM\textsuperscript{VI} (level 6.2) used the first-order conditional estimation method with the interaction option and estimation in parallelized S-ADAPT (version 1.57) used the importance sampling Monte Carlo parametric expectation maximization algorithm.\textsuperscript{16} The SADAPT-TRAN facilitator tool was used to support model building and evaluated in S-ADAPT.\textsuperscript{15,17}

Model selection was based on the maximum likelihood objective function, plausibility of parameter estimates, standard diagnostic plots, visual predictive checks and the reduction in the unexplained (random) inter-strain variability due to inclusion of a covariate effect.\textsuperscript{18}
Results

Killing profile of linezolid against VRE with defined genetic mutations

Linezolid time–kill experiments (Figure 1) were first conducted to characterize the pharmacodynamic profile of linezolid against eight VRE strains to evaluate the full concentration response and select potential strains and regimens to be evaluated in the HFIM. Linezolid displayed bacteriostatic activity, with bacterial killing for all strains <1.5 log10 cfu/mL over 48 h. A concentration-dependent response was evident, with increasing concentrations resulting in additional killing. Selected time–kill...

Table 1. Estimates of the population pharmacodynamic model based on data from eight strains in the time–kill experiments and four strains in the HFIM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Unit</th>
<th>Mean (% relative standard error)</th>
<th>Inter-strain variabilityb (SE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial inoculum in time–kill</td>
<td>log10 [cfu0 (TK)]</td>
<td>—</td>
<td>6.07 (0.4%)</td>
<td>0.19 (20%)</td>
</tr>
<tr>
<td>Initial inoculum in HFIM</td>
<td>log10 [cfu0 (HFIM)]</td>
<td>—</td>
<td>6.47 (1.3%)</td>
<td>0.25 (44%)</td>
</tr>
<tr>
<td>Maximum inoculum in time–kill</td>
<td>log10 [cfu_max (TK)]</td>
<td>—</td>
<td>8.44 (0.6%)</td>
<td>0.29 (27%)</td>
</tr>
<tr>
<td>Maximum inoculum in HFIM</td>
<td>log10 [cfu_max (HFIM)]</td>
<td>—</td>
<td>10.8 (1.5%)</td>
<td>0.18 (215%)</td>
</tr>
<tr>
<td>Mean generation time in time–kill</td>
<td>MGT12 (TK)</td>
<td>min</td>
<td>79.8 (5.9%)</td>
<td>0.29 (66%)</td>
</tr>
<tr>
<td>Mean generation time in HFIM</td>
<td>MGT12 (HFIM)</td>
<td>min</td>
<td>144 (15%)</td>
<td>0.25 (71%)</td>
</tr>
<tr>
<td>Mean turnover time of protein pool</td>
<td>(t_{prot}=\frac{1}{k_{prot}})</td>
<td>min</td>
<td>6.50 (9.5%)</td>
<td>0.99 (22%)</td>
</tr>
<tr>
<td>Maximal extent of inhibition of successful replication</td>
<td>Imax_{rep}</td>
<td>—</td>
<td>0.561 (12%)</td>
<td>range 0.552–0.571</td>
</tr>
<tr>
<td>Linezolid concentration causing 50% of Imax_{rep} for a strain with no sensitive alleles</td>
<td>IC_{50Sen}</td>
<td>mg/L</td>
<td>10.8 (16%)</td>
<td>49 (26%)</td>
</tr>
</tbody>
</table>

Covariate effects

- Maximal fractional decrease in IC_{50} in the presence of \(\sim 3.6\) or more sensitive alleles\(a\):
  \(Imax_{Sen}\)

- Number of sensitive alleles associated with 50% of Imax_{Sen}:
  \(N50_{Sen}\)

- Hill coefficient for N50_{Sen}:
  \(H_{Sen}\)

- IC_{50} in the hollow fibre divided by IC_{50} in time–kill for strain 4422:
  \(f_{HFIM} (4422)\)

- IC_{50} in the hollow fibre divided by IC_{50} in time–kill for strains 4407 and 4408:
  \(f_{HFIM} (4407 and 4408)\)

- Maximal fractional increase in MGT_{12} due to resistant alleles:
  \(Smax_{Res}\)

- Number of resistant alleles causing 50% of Smax_{Res}:
  \(N50_{Res}\)

- Hill coefficient for N50_{Res}:
  \(H_{Res}\)

- SD of additive residual error on log10 scale:
  \(SDC\)

\(a\) This estimate means that the typical IC_{50} for a strain with \(\sim 3.6\) or more sensitive alleles was 1.04 mg/L.

\(b\) The variability estimates include both the variability between different strains and the variability between different viable count profiles within the same strain.

Figure 1. Observed and individual fitted viable counts (in NONMEM\(b\)) from the time–kill experiments for linezolid versus three VRE strains with an MIC of 4 mg/L.
experiments are shown in Figure 1(a–c). Against increasingly resistant strains with an MIC ≥16, linezolid generally displayed minimal killing with <1 log10 cfu/mL maximal activity over 48 h (data not shown).

**Modelling bacterial growth and killing as a function of the numbers of sensitive and resistant alleles as covariates**

Figure 2 shows the structure of the mechanism-based model with linezolid inhibiting protein synthesis. The subsequent depletion of the protein pool stimulated the probability of death during replication. The presence of ~3.6 or more sensitive alleles was estimated to decrease the IC50 of linezolid from 10.8 mg/L for strains with no sensitive allele to 1.04 mg/L for strains with at least 3.6 sensitive alleles (Table 1).

The number of sensitive alleles (Nsen) was assumed to affect the binding affinity of linezolid to its target, which is represented by the IC50 as a drug-related parameter. Inclusion of the covariate effect of Nsen on the IC50 of linezolid explained approximately 80% of the variance in IC50. To consider a potentially decreased biofitness of strains with resistant alleles (Nres) compared with strains without resistant alleles, an effect of Nres on the mean generation time (MGT12; a drug-independent system parameter) was additionally considered. Strains with resistant alleles were estimated to have an up to 23% longer mean generation time compared with strains without resistant alleles. These covariate effects underlined the benefits of using genetic information in a quantitative model to predict the bacterial susceptibility (IC50) and the rate of bacterial growth and killing.

The maximum extent of inhibition of successful replication (equal to the maximum probability of death) was 0.561 (Table 1), in agreement with the slow killing by linezolid. A probability of death of 50% would result in net stasis (if two parent cells try to replicate, one cell successfully doubles and the other dies; i.e. two parent cells generate two daughter cells). A probability of death of 100% would result in all replicating cells dying and cause the maximum rate of killing to be equal to –k12, as described previously.

Figure 1 shows the individual curve fits for the time–kill data for the intermediary model that was estimated in NONMEM based on the time–kill data. The characterization of the pharmacokinetic-pharmacodynamic relationship between linezolid concentrations and killing allowed for the selection of clinical regimens of linezolid to be simulated in the HFIM.

**Impact of linezolid front-loaded regimens on the total and resistant bacterial population**

Traditional and front-loaded regimens for linezolid in humans were subsequently evaluated in an HFIM against four strains of VRE as shown in Figure 3(a–d). Against VRE strains with a linezolid MIC of 4.0 mg/L, the greatest benefit of front-loading was evident in strain 4408 (Figure 3a). Against this strain the traditional regimen of linezolid 600 mg every 12 h demonstrated a gradual reduction in bacterial counts over the study duration: maximal reductions were 0.96 log10 cfu/mL. There was a
Significant improvement in bacterial killing for front-loaded regimens over a short duration, which demonstrated bactericidal activity at the 240 h study endpoint: 1200 mg every 12 h ×10 doses or 2400 mg every 12 h ×10 doses followed by 600 mg every 12 h with reductions of 3.02 and 3.46 log10 cfu/mL. All regimens suppressed the amplification of resistant subpopulations against VRE strains with an MIC of 4.0 mg/L. Interestingly, against strain 4422, which displayed an MIC of 4.0 mg/L and carried 3.2 resistant alleles, both front-loaded regimens displayed limited activity, resulting in eventual regrowth, and amplified the development of resistance, as shown in Figure 4(a–c).

**Simultaneous modelling of the time–kill and hollow fibre data**

The model developed in NONMEM based on the time–kill data over 48 h yielded unbiased and precise individual and population fits for the time–kill study (Figure 5a). This model was used to provide in silico predictions of the hollow fibre study without using any of the hollow fibre data. The in silico predictions showed that the model based on the time–kill data could excellently predict the first 48 h of therapy in the hollow fibre (Figure 5b, closed symbols), yielded reasonable predictions for days 3 and 4, but could only well predict counts in the hollow fibre for one of four strains on days 6–10. This was expected, since this model was only based on time–kill data over 48 h.

Upon re-estimation of the parameter values in S-ADAPT based on all data, the final model yielded excellent individual and population fits for both the time–kill and hollow fibre datasets (Figures 3 and 5c). The observed versus individual fitted log (cfu/mL) yielded a slope of 0.986 and the r of 0.999 and an r of 0.934. Interestingly, to optimally translate between the time–kill and the hollow fibre experiments, the IC50 estimate in the hollow fibre model was similar (0.895 times) to the estimate in the time–kill for strain 4422 carrying resistant alleles. However, the IC50 for strains 4407 and 4408 carrying no resistant alleles was only 0.205 times the IC50 estimate for the time–kill experiments for the respective strain (Table 1). This suggested some strain-to-strain variability for the translation from time–kill to hollow fibre experiments.

**Discussion**

VRE continues to be a persistent, difficult-to-treat pathogen posing significant challenges for clinicians as it relates to optimal treatment. Linezolid displays a unique mechanism of action by inhibiting bacterial protein synthesis through binding of 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex. Linezolid resistance in enterococci has been reported, attributed to a single conserved site in the 23S rDNA (G2576T). This was demonstrated in early clinical trials where resistance to linezolid developed in patients infected with VRE who received a dose of 600 mg every 12 h.

Therefore, to combat the increasing resistance, we evaluated the potential benefit of ‘front-loading’, a strategy to optimize the pharmacodynamic profile of an antibiotic through the administration of high doses early in therapy for a short duration. The

![Figure 4](https://academic.oup.com/jac/article-abstract/67/9/2182/879366)
effect of administering linezolid in this fashion had not been examined. We first determined in time–kill experiments that although linezolid has long been considered a bacteriostatic, concentration-dependent antimicrobial agent, there was a concentration–dependent response, with increasing concentrations resulting in more killing. Concentration-dependent pharmacodynamics of linezolid in enterococci and staphylococci have been previously reported.\(^6\)–\(^8\) All tested standard and front-loaded regimens with \(\text{AUC/MIC}\)s between 31 and 495 on days 1–5 and an \(\text{AUC/MIC}\) of 31 or 124 on days 6–10 prevented emergence of resistance over 10 days for strains 4408, 4407 and ATCC 29212. Strains 4408, 4407 and presumably also ATCC 29212 carried no resistant allele. The \(\text{AUC/MIC}\)s of 31–495 that prevented resistance were in part lower than the previously identified optimal \(\text{AUC}_{0–24}/\text{MIC}\) target of 230 assessed by Zinner et al.\(^8\) Further studies are required to elucidate these differences.

Our results for \(E.\) faecalis strain 4422 (MIC 4 mg/L) showed that very high linezolid doses of 2400 mg every 12 h achieving an \(\text{AUC}_{0–24}/\text{MIC}\) of 495 during the first 5 days could not prevent emergence of resistance. The latter results were in agreement with the \(\text{AUC}_{0–24}/\text{MIC}\) target of 230 identified by Zinner et al.\(^8\) Overall, these results suggest that there was notable strain–to–strain variability, with an \(\text{AUC/MIC}\) of 31 preventing resistance for some strains whereas other strains required an \(\text{AUC}_{0–24}/\text{MIC}\) of 230 (or >124) to prevent resistance.

Front-loading yielded the most pronounced benefit in the rate and extent of killing for strain 4408 (MIC 4 mg/L) carrying 4.3 sensitive alleles and no resistant allele. However, this benefit in killing was less pronounced for strains 4407 and ATCC 29212. For strain 4422 carrying 3.2 resistant alleles at baseline, emergence of resistance was observed for all tested regimens resulting in regrowth over 240 h. Strains 4408, 4407 and 4422 all had the same MIC of 4 mg/L to linezolid. The presence of resistant alleles was associated with the emergence of resistance for strain 4422, and a lack of resistant alleles successfully predicted no emergence of resistance for strains 4407 and 4408. While all studied dosage regimens failed with resistance against strain 4422, the time to emergence of resistance was notably delayed by high-intensity front-loading (Figure 4).

Such front-loading may leave the immune system or a combination antibiotic more time to kill the bacteria less susceptible to linezolid. Although resistance to linezolid has been uncommon in clinical trials, a low spontaneous mutation rate has been reported in VRE.\(^5\) The current study provides further evidence that suboptimal dosing leads to selection of linezolid-resistant VRE. Our study suggested that front-loading may provide additional killing for some, but not all, strains that lack resistant alleles and that high-intensity front-loading may delay, but not prevent, emergence of resistance for strains with an MIC of 4 mg/L carrying resistant alleles. To predict the impact of high-intensity front-loading on platelet-related toxicity of linezolid, the present model should be combined with a mechanism-based toxicodynamic model such as the model proposed by Sasaki et al.\(^19\) The toxicodynamic model should ideally account for the time course of linezolid concentrations (as opposed to steady-state AUCs) and for the time course of platelet counts. Such a simulation analysis has the capability to optimize both the intensity and duration of front-loading for future studies.

The population pharmacodynamic model estimated in the present study yielded excellent fits for the time–kill data and for a simultaneous analysis of all data (Figures 3 and 5c). Schmidt et al.\(^20\) modelled the effect of two oxazolidinones against methicillin-resistant \(S.\) staphylococcus aureus in a static time–kill and dynamic one-compartment model over 24 h. We chose to assess a longer duration of therapy and developed a translational model that can translate between 48 h time–kill and 10 day HFIMs. The present model additionally proposed an approach to use the number of sensitive alleles as an important covariate to predict the IC\(_{50}\) of linezolid. This covariate explained approximately 80% of the variance in IC\(_{50}\), which may be quite beneficial to predict the treatment response to linezolid if the number of alleles is available. This combined genomic and mathematical modelling approach offers the possibility for dose individualization and warrants further investigation.

---

**Figure 5.** Observed versus individual fitted (a1 and c1), population fitted (a2 and c2) and *in silico* predicted (b) viable counts for the total bacterial population. (a1 and a2) NONMEM results for estimation of eight strains in the 48 h time–kill studies. (b) *In silico* predictions of the hollow fibre study based on the time–kill results with filled symbols representing the first 48 h and open symbols referring to observations at 72–240 h. (c1 and c2) Final model estimated using S-ADAPT (see Table 1) that simultaneously described all data from nine strains in total.
These findings on front-loading may have significant implications for the optimal treatment of VRE infections. First, they provide evidence that the pharmacodynamic profile of linezolid is concentration dependent at higher exposures and that increased dosage regimens of linezolid may be useful to achieve additional killing in difficult-to-treat strains. Therefore we propose that front-loading may have clinical utility in selected infections that are deep seated, comprise high bacterial inocula, which may be subject to penetration barriers, or require longer durations of treatment (>10 days). In these specific clinical situations, such as the case in bi-lobar pneumonia, where early aggressive therapy is necessary and therapeutic options are limited, exploring novel strategies such as front-loading is warranted. On the other hand, for less severe infections such as uncomplicated skin and soft tissue infections, which have been proven to have high response rates to traditional doses (600 mg every 12 h), there may be limited utility in such an approach. Second, although front-loaded regimens demonstrated increased killing activity, this is balanced with the potential for toxicity, as increasing the cumulative exposure (AUC), primarily driven by duration, has been associated with haematologic toxicities including myelosuppression, anaemia and neutropenia. Our data suggest that front-loading linezolid regimens against VRE to achieve greater efficacy may be considered as a means to decrease the total duration of therapy. Additional lower exposure regimens that follow initial high-intensity regimens or shortening the course of therapy due to front-loading requires additional study and may be promising. We acknowledge a number of potential limitations in the current study. First, these results may not be translatable to other strains that display lower MICs, as only VRE with elevated MICs were selected for analysis in the current study. Second, there have been no human studies evaluating linezolid at such high exposure levels. Therefore additional animal and human studies are necessary before these results are considered in clinical practice. Overall, high-dose regimens of linezolid administered in a front-loading fashion are promising from a pharmacodynamic standpoint for early bactericidal activity and to postpone and potentially suppress resistance.

Acknowledgements
This work was presented in part at the Forty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, USA, 2009 (Abstract A1 – 549).

We are grateful to Arnold Louie, David Brown and George Drusano for providing insight into the HFIM.

Funding
This study was funded by Pfizer Global Research and Development, Groton, CT, USA.

Transparency declarations
B. T. T., J. B. B. and A. F. have received grant support from Pfizer, New York, NY, USA. J. B. B. has received grant support from Trius Therapeutics. At the time of study execution, L. S. and D. H. were employees at Pfizer Global Research and Development. All other authors: none to declare.

References
18. Bulitta JB, Duffull SB, Kinzig-Schippers M et al. Systematic comparison of the population pharmacokinetics and pharmacodynamics of


