Impact of Different Carbapenems and Regimens of Administration on Resistance
Emergence with Three Isogenic Pseudomonas aeruginosa Strains with Differing Mechanisms of Resistance

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Abstract:

Background: We compared drugs (imipenem; doripenem) doses (500 mg; 1g) and infusion times (0.5, 1.0 [imipenem], 1.0, 4.0 hr [doripenem]) in our hollow fiber model, examining cell kill and resistance suppression for 3 isogenic strains of *P. aeruginosa* PAO-1.

Methods: The experiments ran for 10 days. Serial samples were taken for total organism and resistant subpopulation counts. Drug concentrations were determined by LC/MS/MS. Free Time > MIC was calculated using ADAPT II. Time to resistance emergence was examined with Cox modeling.

Results: Cell kill and resistance emergence differences were explained, in the main, by differences in potency (MIC) between doripenem and imipenem. Prolonged infusion increased free drug Time > MIC and improved cell kill. For resistance suppression, the 1 g, 4 hour infusion was able to completely suppress resistance for the full period of observation for the Wild-Type isolate. For the mutants, control was ultimately lost, but in all cases, this was the best regimen.

Conclusion: Doripenem gave longer free Time > MIC relative to imipenem and, therefore, better cell kill and resistance suppression. For the Wild-Type organism the 1 g 4 hour infusion regimen is preferred. For organisms with resistance mutations, larger doses or addition of a second drug should be studied.
Introduction

*Pseudomonas aeruginosa* continues to be a major problem in the nosocomial setting. Increasing rates of resistance makes the development of effective therapeutic regimens problematic.

Doripenem is a new carbapenem antibiotic with potent activity against *Pseudomonas aeruginosa*. Pre-clinical studies have indicated that it is highly stable to the ampC enzyme seen in this pathogen and that it interacts differently with the pathogen regarding oprD down-regulation, resulting in lower MIC shifts, in at least 50% of instances (6). Clinically, the use of the prolonged infusion has been shown to have a salutary impact on Pseudomonas resistance emergence during therapy, relative to imipenem (1). We chose to study Imipenem because the doripenem clinical trial program employed Imipenem as a comparator, as meropenem does not have the breadth of FDA indications as is present for Imipenem (e.g. nosocomial pneumonia).

Previous work from our group has shown that the use of prolonged infusion optimizes Time > MIC target attainment and may have an impact on resistance emergence (3, 10). This leads to four major factors requiring exploration: 1) drug (potency) 2) dose 3) infusion schedule 4) difference between drugs regarding mechanism of resistance.

In order to ascertain the contribution of each we decided to study three different isogenic isolates: a wild-type isolate (PA01); an isolate with a stably derepressed chromosomal ampC enzyme (ampC β-lactamase production is markedly increased when a mutation in the repressor system occurs and the increase is stable and not
dependent upon the presence or absence of drug); an isolate with a defined down-
regulation of oprD (oprD is a carbapenem-specific transport porin; when down-
regulated, less drug is available per unit time in the periplasmic space). In addition, we
decided to examine both doripenem and imipenem to ascertain the impact of differing
potency and interaction with oprD downregulation. Finally, we hypothesized that
infusion time would have an impact. Therefore we studied doripenem at a 500 mg dose
with a one hour infusion, a 500 mg dose with a 4 hour infusion and a 1 g dose with a
four hour infusion. Imipenem’s stability is such that a 4 hour infusion cannot be
recommended clinically. We therefore decided to examine 500 mg every 6 hours with a
half hour infusion and 1 g every 8 hours with a 1 hour infusion. Both regimens are
consistent with the package insert for imipenem. The endpoints were cell kill at 24 hours
(before emergence of resistant clones would obfuscate the endpoint) and emergence of
resistance (both initial time when the number of resistant clones exceeded that at
baseline and the time to near-maximal number of resistant clones. “Near maximal” is
defined as being within 1 standard deviation of true maximal. This is approximately 0.3
Log\textsubscript{10} (CFU/ml).

**Methods**

**Microorganisms:** The *Pseudomonas aeruginosa* strain PA01, its oprD down-regulated
isogenic mutant (selected by exposure to Imipenem; ampC RNA levels were checked
by RT PCR to document the oprD selection did not stably de-repress ampC; expression
of mexAB, mexCD, mexEF and mexXY were also checked and shown not to differ from
wild-type) and a stably-derepressed (selected by exposure to ceftazidime) isogenic
mutant were the kind gift of Drs. Karen Bush and Brian Morrow). MIC values were
determined by CLSI macrobroth methodology for both doripenem and imipenem (7). This was done on at least three occasions. Imipenem was employed as the selecting agent. We had done an extensive stability evaluation of the drugs in MH II agar at incubator temperature that drove the choice of selecting agent (data not shown).

**Hollow fiber infection model.** A schematic diagram of the system is as shown in Reference 4. Doripenem or imipenem was directly injected into the central reservoir over a period of 0.5 to 4 hours on an appropriate schedule (every 6 or every 8 hours) to achieve the peak concentration desired. The experiments were carried out for 10 days or more. Methods specific to the operation of this model have been published previously (4).

**Duration-response studies.** Each bacterium was stored at -70°C in skim milk. Fresh isolates were grown on blood agar plates (BBL Microbiology Systems, Cockeysville, Md.) for 24 h at 35°C before each experiment. The bacterial inoculum was prepared by employing 3 medium-sized colonies grown overnight in Mueller-Hinton II broth at 35°C. Hollow fiber systems were maintained at 35°C in a humidified incubator. Fifteen ml of bacterial culture in late log phase growth (circa 1 x 10^8 CFU/ml) were infused into six hollow fiber cartridges, one each for nominal drug exposure (AUC_{24}/MIC) of 0 (control), doripenem dose of 500 mg every 8 hours, 1 hour infusion, doripenem 500 mg every 8 hours, 4 hour infusion, doripenem 1 g every 8 hours, 4 hour infusion, imipenem 500 mg every 6 hours, half hour infusion and imipenem 1 g every 8 hours 1 hour infusion. These regimens simulated steady-state human pharmacokinetics of unbound doripenem and imipenem. Experimentally attained doripenem and imipenem exposures (central compartment) were based upon drug concentrations quantified by a validated
LC/MS/MS method (see below). Samples were obtained 20-22 times (depending on regimen) over 47 hours. At 0, 0.17, 1, 2, 3, 4, 6, 8 and 10 days, samples of the bacterial cultures were obtained, centrifuged at 3200 x g for 15 minutes, re-suspended in normal saline in order to minimize drug carry-over effect and diluted ten-fold serially. The serially diluted samples were quantitatively cultured onto drug-free Mueller-Hinton II agar plates to enumerate the total bacterial population. The resistant bacterial subpopulation was quantified by culturing on media plates supplemented with imipenem at a concentration 2.5x baseline MIC, except for the oprD isogenic mutant, where 3x baseline MIC was employed because of the shift in Imipenem MIC. The media plates were incubated at 35°C for 24 and 48 hours to evaluate for the impact on the total and antibiotic-resistant subpopulation, respectively. MIC values of the resistant sub-populations to doripenem and imipenem at experiment end were determined to confirm resistance.

**LC/MS/MS assays for Doripenem and Imipenem.**

Doripenem: Mueller-Hinton II Broth PK simulation samples were diluted with HPLC water (0.050mL sample into 1.00mL water), and were analyzed by high pressure liquid chromatography tandem mass spectrometry (LC/MS/MS) for doripenem concentrations. The LC/MS/MS system was comprised of a Shimadzu Prominance HPLC system and an Applied Biosystems/MDS Sciex API5000 LC/MS/MS.

Chromatographic separation was performed using a Water Novapak C-18 column, 5 µm, 150 x 3.9 mm column and a mobile phase consisting of 85% 0.1% formic acid in water and 15% 0.1% formic acid in acetonitrile, at a flow rate of 1.0 mL/min.
Doripenem concentrations were obtained using LC/MS/MS monitoring the MS/MS transition m/z 421 → m/z 274. Analysis run time was 2.5 minutes. The assay was linear over a range of 0.100 – 25.0 µg/ml ($r^2 > 0.996$). The inter-day CVs for the quality control samples analyzed in replicates of three at three concentrations on each analysis day (0.500, 4.00, and 20.0 µg/mL) ranged from 2.04 to 6.38%, with accuracies (%REC) ranging between 98.2% to 102%.

Imipenem: Mueller-Hinton II Broth PK simulation samples were diluted with HPLC water (0.050mL sample into 1.00mL water), and were analyzed by high pressure liquid chromatography tandem mass spectrometry (LC/MS/MS) for imipenem concentrations. The LC/MS/MS system was comprised of a Shimadzu Prominence HPLC system and an Applied Biosystems/MDS Sciex API5000 LC/MS/MS.

Chromatographic separation was performed using a Water Novapak C-18 column, 5 µm, 150 x 3.9 mm column and a mobile phase consisting of 90% 0.1% formic acid in water and 10% 0.1% formic acid in acetonitrile, at a flow rate of 1.0 mL/min. Imipenem concentrations were obtained using LC/MS/MS monitoring the MS/MS transition m/z 300 → m/z 170. Analysis run time was 3.0 minutes. The assay was linear over a range of 0.100 – 50.0 µg/ml ($r^2 > 0.996$). The inter-day CVs for the quality control samples analyzed in replicates of three at three concentrations on each analysis day (0.500, 4.00, and 25.0 µg/mL) ranged from 1.50 to 7.45%, with accuracies (%REC) ranging between 98.2% to 108%.

**Β-lactamase Hydrolysis Assay to Document Degree of Stable Derepression.** The working solution of Nitrocefin (500 mg/L) is diluted 10-fold in buffer (0.1 Mphosphate; 1
mM EDTA, pH 7.0). Changes in absorbance were measured in the spectrophotometer (SpectraMax M5 plate reader, running SoftMax Pro-5 software; Molecular Devices, Sunnyvale, CA) at 486 nM. The molar extinction coefficient of hydrolyzed Nitrocefin at 486 nM is 20,500 M\(^{-1}\) cm\(^{-1}\). The wild-type isolate and its isogenic stably de-repressed mutant were examined with this method.

**Pharmacokinetic and Statistical Methods.** Concentration time profiles for each drug and each regimen for each isolate were analyzed employing Maximum Likelihood Estimation. The Identification Module of the ADAPT II Package of Programs of D’Argenio and Schumitzky (2) was employed. As computer-controlled infusion pumps drove the profile, a 1-compartment open model with zero-order input and first order elimination was employed.

Free drug Time > MIC was estimated by integrating the following differential equation, which was a system output.

\[
\text{IF } \left(\frac{X(1)}{Vol}\right) \geq \text{MIC} \quad \text{THEN} \\
\frac{dX}{dt}(3) = 1.0 \\
\text{ELSE} \\
\frac{dX}{dt}(3) = 0.0 \\
\text{END IF}
\]

Where \(X(1)\) is the amount of drug in the central compartment; \(Vol\) = volume of the central compartment; \(\text{MIC}\) is the Minimal Inhibitory Concentration of the appropriate drug for the pathogen being studied. \text{GE} is “greater than or equal to”.

This measure of the independent variable (free drug Time > MIC) was linked to cell kill at 24 hours using either a sigmoid-Emax model or an Emax model, depending
upon whether the Hill’s constant was statistically supportable (i.e. model expansion tested with Akaike’s Information Criterion).

Inhibitory Sigmoid $E_{\text{max}}$ Model:

\[ \text{Effect} = E_{\text{con}} - \left( E_{\text{max}} \cdot (\text{Time}>\text{MIC})^H / ((\text{Time}>\text{MIC})^H + EC_{50}^H) \right) \quad (2) \]

Inhibitory $E_{\text{max}}$ Model:

\[ \text{Effect} = E_{\text{con}} - \left( E_{\text{max}} \cdot (\text{Time}>\text{MIC}) / ((\text{Time}>\text{MIC}) + EC_{50}) \right) \quad (3) \]

Where Effect is the bacterial burden at 24 hours in treated animals (Log$_{10}$ (CFU/g)); $E_{\text{con}}$ is the bacterial burden at 24 hours in the no-treatment control animals (Log$_{10}$ (CFU/g)); $E_{\text{max}}$ (Log$_{10}$ (CFU/g)) is largest bacterial kill obtainable with the time frame employed; $E_{50}$ is the measure of the independent variable (fraction of the dosing interval that drug concentration exceeds the MIC) that drives half of the maximal effect; $H$ is Hill’s constant (also called the sigmoidicity constant).

These models recognize that exposure-responses are relatively flat at low values of the independent variable, are steep and relatively linear in the midrange (between approximately 20% of maximal effect and 80% of maximal effect) and again flatten out at higher values of the independent variable.

Time to Resistance and Time to Near Maximal Resistance were analyzed using Cox Proportional Hazards Regression (SYSTAT for Windows v 11.0).

**Results**
**MIC Values to Doripenem and Imipenem:** The MIC values were 1.0 and 2.0 mg/L for doripenem and imipenem, respectively for the wild-type PA01 isolate. For the stably de-repressed isogenic mutant, the values were 2.0 and 2.0 mg/L. For the Δ-oprD isolate, the MIC values were 2.0 mg/L for doripenem and 16.0 mg/L for imipenem.

**B-lactamase hydrolysis:** The hydrolysis of nitrocefin was statistically different for the wild-type PA01 isolate relative to the stably de-repressed mutant as would be expected (data not shown).

**Hollow Fiber System Evaluations for 10 or More Days:**

**Wild-Type Isolate of PA01** - The total CFU/ml and resistant colony counts for the control and different regimens of doripenem and imipenem are displayed in Figure 1, panels A-F. The high (1g) dose, 4 hour infusion arm is the only one to completely suppress resistance emergence.

**Stably De-repressed Mutant** - The total CFU/ml and resistant colony counts for the control and different regimens of doripenem and imipenem are displayed in Figure 2, panels A-F. The high (1g) dose, 4 hour infusion arm is the only one to hold the total population under some control and also limit, but not suppress completely, the amplification of resistant mutants. The doripenem 500 mg 4 hour infusion slightly underperformed at this dose, in contrast to the wild-type isolate.

**Δ-oprD Mutant** – This resistance mechanism had a greater impact on cell kill than stable de-repression of the AmpC β-lactamase for both drugs and all the regimens (Figure 3, panels A-F). As previously, doripenem had overall greater log kill relative to imipenem, which is likely related to the MIC of this mutant for the drugs (2 mg/L for doripenem and...
16 mg/L for imipenem. Only the doripenem 1 g, 4 hour infusion regimen administered every 8 hours achieved a 3 Log_{10} (CFU/ml) cell kill.

Time > MIC for the Various Isogenic Organisms and Regimens: The Time > MIC was calculated for each regimen and for each organism and is displayed in Table 1. Because of the previous observations of Tam et al (9), the Time > 6.2 x MIC was also calculated and these are displayed in Table 1.

These times were used to create an inhibitory sigmoid-Emax effect model. The parameters of this model for each organism are also listed in Table 1. The Curves are displayed in Figure 4, Panels A-C.

Time to Emergence of Resistance: We examined time to the first time that that resistant mutant isolates exceeded their time zero baseline values and also the time that they achieved near maximal colony counts. Tam and colleagues had indicated that for the carbapenem meropenem that trough concentrations greater than 6.2 times the baseline MIC were necessary to suppress resistance in *Pseudomonas aeruginosa*. Consequently we calculated Time > than this value and employed Cox Proportional Hazards Regression to ascertain whether the time to resistance for these agents and organisms was influenced by Time > 6.2 x MIC. We also used organisms and drug as stratification variables to determine if the time to resistance (first resistance and amplification to near-maximal numbers) was influenced by these factors. Neither drug nor organism significantly influenced the outcome as a stratification variable (Mantel test). Time > 6.2 x MIC significantly influenced time to first resistance (Estimate = -6.95; 95% Confidence Interval -12.93 to -0.976; p = 0.023) and time to amplification to near
maximal number of colony counts (Estimate -11.76; 95% Confidence Interval -19.979 to -3.533; p = 0.005). These survival curves are displayed in Figure 5, Panels A and B.

**Discussion**

A new, more potent weapon in the war against serious *Pseudomonas aeruginosa* infection would be most welcome. In order to determine whether doripenem had advantageous microbiological properties, we tested it in our hollow fiber infection model over a period of time (10-13 days) that is consistent with clinical usage. In addition, we also wished to examine the impact of differing mechanism of resistance on the microbiological effect of doripenem. Finally, we also wished to examine the impact of prolonged (4 hours) infusion on the observed microbiological effect. In order to place these findings into proper perspective, we also examined two regimens of imipenem against these isolates.

Doripenem was more potent (1 dilution) than imipenem in the wild-type isolate and was equivalent to imipenem in the stably de-repressed mutant. In the oprD mutant, however, the MIC was 2 mg/L for doripenem and 16 mg/L for imipenem, validating the previous observations of Mushtaq (6).

We examined both cell kill activity of the regimens and we looked at emergence of resistance. For cell kill, we first simply examined the colony counts at the end of 24 hours of drug exposure, as this time point will be least confounded by amplification of resistant mutant subpopulations.

For the wild-type PA01, all three doripenem regimens produced cell kill greater than either imipenem regimen, with the 1 g doripenem dose administered as a 4 hour
infusion every 8 hours producing the largest cell kill of 5.4 $\log_{10}$ (CFU/ml). The 500 mg doripenem regimens demonstrated an advantage for the 4 hour infusion, with the 1-hour infusion regimen having a kill of 3.71 $\log_{10}$ (CFU/ml), with the 4 hour infusion regimen of 500 mg producing a kill of 4.07 $\log_{10}$ (CFU/ml).

For the stably-derepressed isogenic mutant of PA01, the hierarchy of effect was the same, with all three doripenem regimens producing more 24 hour cell kill than either of the imipenem regimens. The advantage of the high (1 g) dose doripenem arm with the 4 hour infusion was also manifest here.

With the $\Delta$-OPRD isogenic mutant, essentially the same findings were seen. The only exception was that the 1g, 1 hour infusion imipenem regimen was slightly superior to the doripenem 500 mg, 1 hour infusion regimen with cell kill of 3.47 versus 3.12 $\log_{10}$ (CFU/ml), respectively. As in the other experiments, the best cell kill was seen with the doripenem 1g, 4 hour infusion regimen, at 4.24 $\log_{10}$ (CFU/ml).

When one examines all the regimens, it becomes apparent that the differences in cell kill activity seen are mediated through the MIC and through the mode of administration (prolonged infusion). In Figure 2, panels A-C, the cell kill for the three different regimens are displayed as a function of Time > MIC. Examination of the curves demonstrates that the fit of the model to the data was precise and unbiased. The 95% confidence intervals around the point estimates of the parameters were quite acceptable. The fit of the model to the data explained a significant amount of the variance (i.e. the fit was significant). In all instances, once the MIC is accounted for and the dose and mode of administration is accounted for by calculating Time>MIC, there is
little bias between drugs and modes of administration. As noted above, doripenem has
greater cell kill because it has lower MIC values and the mode of administration
generates longer T>MIC. The largest Emax is seen with the wild-type isolate, at 6.06
Log$_{10}$ (CFU/ml). The stably derepressed mutant and the Δ-oprD mutant had Emax
values of 5.05 and 4.56 Log$_{10}$ (CFU/ml), respectively. This suggests that the mutations
lead to less Log-kill than seen with the wild-type isolate and is not accounted for in the
Time>MIC calculation. One regimen in the OPRD experiment was an outlier, where
imipenem at 500 mg every 6 hours as a half hour infusion caused substantial cell kill,
but with a calculated Time>MIC of zero hours. This is likely because the imipenem MIC
for this isolate was 16 mg/L. We ran an arithmetic dilution MIC and the imipenem MIC
was 12 mg/L. For this value, the fraction of the dosing interval with Time > MIC (free
drug) was 0.3736. This makes the fit of the model to the data quite acceptable (see
Figure 4C; $r^2 = 0.949$; imipenem 500 mg every 6 hr regimen is the open triangle).

With respect to resistance emergence, Tam (9) has previously demonstrated that
for the carbapenem meropenem, a trough concentration 6.2 times the baseline MIC is
required to suppress resistance when that agent was administered alone.
Consequently, we calculated the time that drug concentrations remained above 6.2
times the MIC. As an endpoint we looked at the time until the regimen had resistance
emergence defined as having the resistant population increase to concentrations above
the baseline number. We also looked at the time until the resistant subpopulation was
amplified until near maximal values were obtained. Because we were looking at a time-
to-event, we employed Cox Proportional hazards regression as the analytical tool. For
both endpoints, Time > 6.2 x MIC was a statistically significant covariate in the analyses. For time till first emergence of resistance, the Time > 6.2 x MIC had a p-value of 0.023 (Figure 5-a). The endpoint of time till near-maximal resistance amplification was even more strongly influenced by Time > 6.2 x MIC (p = 0.005 – Figure 5-b).

It is important to note that of all the regimens for all the isogenic isolates, only the doripenem 1 g, 4 hour infusion every 8 hours suppressed amplification of the resistant subpopulation for the full 10 days of the experiment. The concentration-time profile here remained above 6.2 times the MIC for a fraction of 0.516 of the dosing interval. This is different from the Tam data, but is consistent with the idea that carbapenem regimens need to be above a multiple of the MIC for a considerable period of time to suppress resistance.

Given this, we wished to see if a larger doripenem dose would suppress resistance. We calculated that 1500 mg every 8 hr of doripenem as a 4 hr infusion would give a free drug Time > 6.2 x MIC as that seen with the wild-type isolate. When directly measured, we achieved a fraction of the dosing interval of free drug Time > 6.2 x MIC of 0.497 (versus 0.516 for the wild-type isolate). This did not suppress resistance for the full 10 days of the experiment (data not shown). We speculate that oprD mutants also have some Mex pump overexpression, which may make them more difficult to suppress (5, 8).

Other defined resistance mechanisms changed the MIC and shortened the time that concentrations remained above 6.2 times the MIC and, therefore, allowed ultimate
amplification of resistant subpopulations. It should be noted, however, that the doripenem 1 g, 4 hour infusion performed best with each of the isogenic mutants.

In summary, cell kill and suppression of resistance for these two carbapenem antibiotics was optimized by increasing the Time > Threshold. For resistance suppression, this threshold was substantially higher. This principle held, irrespective of the specific resistance mechanism. Doripenem, particularly at the 1 g dose with the 4 hour infusion performed best in all the evaluations. This is because of the increased potency of doripenem and because the prolonged infusion combined to maximize the Time > MIC and Time > 6.2 x MIC. Given these findings, the high dose, prolonged infusion of doripenem should be evaluated in clinical circumstances such as patients with ventilator-associated pneumonia, where risk of emergence of resistance is highest. Because resistance was suppressed for the full 10 days by the high dose 4 hr infusion only in the wild-type isolate, it is likely that Pseudomonal strains carrying a resistance mechanism should be treated by this regimen in combination with a second drug.

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Legends:

Figure 1: Effect on Wild-Type PAO-1 of multiple regimens of doripenem and imipenem
and a no-treatment control. A) No-treatment control B) Doripenem 500 mg 1 hr infusion
every 8 hrs  C) Doripenem 500 mg 4 hr infusion every 8 hrs  D) Doripenem 1 g 4 hr
infusion every 8 hrs  E) Imipenem 500 mg 0.5 hr infusion every 6 hr  F) Imipenem 1 g 1
hr infusion every 8 hr.

Figure 2: Effect on AmpC stably de-repressed PAO-1 of multiple regimens of doripenem
and imipenem and a no-treatment control. A) No-treatment control B) Doripenem 500
mg 1 hr infusion every 8 hrs  C) Doripenem 500 mg 4 hr infusion every 8 hrs  D)
Doripenem 1 g 4 hr infusion every 8 hrs  E) Imipenem 500 mg 0.5 hr infusion every 6 hr
F) Imipenem 1 g 1 hr infusion every 8 hr.

Figure 3: Effect on OPRD down-regulated PAO-1 of multiple regimens of doripenem
and imipenem and a no-treatment control. A) No-treatment control B) Doripenem 500
mg 1 hr infusion every 8 hrs  C) Doripenem 500 mg 4 hr infusion every 8 hrs  D)
Doripenem 1 g 4 hr infusion every 8 hrs  E) Imipenem 500 mg 0.5 hr infusion every 6 hr
F) Imipenem 1 g 1 hr infusion every 8 hr.

Figure 4: Relationship between free drug Time > MIC and cell kill for A) Wild-type PAO-
1  B) AmpC stably de-repressed PAO-1  C) OPRD down-regulated PAO-1.

Figure 5a: Cox Proportional Hazards regression examining time to initial resistance
emergence. Fraction sensitive indicates the fraction of regimens over time where
resistance has not emerged.
Figure 5b: Cox Proportional Hazards regression examining time to near-maximal resistant population amplification. Fraction sensitive indicates the fraction of regimens over time where resistance has not emerged.

Figure 1:
Log$_{10}$ (CFU/ml) = 9.65 - (6.06 x Time/MIC $^{1/2}$) / (Time/MIC $^{1/2}$ + 0.55$^{1/2}$)

$r^2 = 0.947; p < 0.001$

Time > MIC (Fraction of Dosing Interval)

- Control
- Imipenem 500 mg Q8h, 0.5h inf
- Doripenem 500 mg Q8h, 1h inf
- Imipenem 1 g Q8h, 1h inf
- Doripenem 500 mg Q8h, 4h inf
- Doripenem 1 g Q8h, 4h inf
**Figure 4B**

Log$_{10}$ (CFU/ml) vs. Time > MIC (Fraction of Dosing Interval)

- **Control**
- **Imipenem**
  - 500 mg Q6h, 0.5h inf
- **Doripenem**
  - 500 mg Q6h, 1h inf
- **Imipenem**
  - 1 g Q8h, 1h inf
- **Doripenem**
  - 500 mg Q8h, 4h inf
  - 1 g Q8h, 4h inf

Log$_{10}$ (CFU/ml) = 9.77 - (5.06 x T>MIC / (T>MIC + 0.20))

$r^2 = 0.969; p < 0.001$
\[
\log_{10}(\text{CFU/ml}) = 10.38 - (4.89 \times T > \text{MIC} / (T > \text{MIC} + 0.203))
\]

\[r^2 = 0.943; p < 0.001\]
Resistance to Carbapenems

Fraction Sensitive

Time (Days)
### Table 1: Measured Time > MIC for different regimens of Doripenem and Imipenem Against Three Isogenic Strains of *Pseudomonas aeruginosa* PA01 and Parameter Values for their Inhibitory Sigmoid-Emax Effect Models

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Time &gt; MIC</th>
<th>$r^2$ of Model Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild-Type PA01</strong></td>
<td></td>
<td></td>
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<tr>
<td>Doripenem 500 mg Q8h, 1 hr inf</td>
<td>0.571</td>
<td>0.999</td>
</tr>
<tr>
<td>Doripenem 500 mg Q8h, 4 hr inf</td>
<td>0.804</td>
<td>0.992</td>
</tr>
<tr>
<td>Doripenem 1 g Q8h, 4 hr inf</td>
<td>0.919</td>
<td>0.993</td>
</tr>
<tr>
<td>Imipenem 500 mg Q6h, 0.5 hr inf</td>
<td>0.620</td>
<td>0.939</td>
</tr>
<tr>
<td>Imipenem 1 g Q8h, 1 hr inf</td>
<td>0.561</td>
<td>0.986</td>
</tr>
<tr>
<td><strong>Stably De-repressed PA01</strong></td>
<td></td>
<td></td>
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<tr>
<td>Doripenem 500 mg Q8h, 1 hr inf</td>
<td>0.479</td>
<td>0.998</td>
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<tr>
<td>Doripenem 500 mg Q8h, 4 hr inf</td>
<td>0.692</td>
<td>0.992</td>
</tr>
<tr>
<td>Doripenem 1 g Q8h, 4 hr inf</td>
<td>0.854</td>
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</tr>
<tr>
<td>Imipenem 500 mg Q6h, 0.5 hr inf</td>
<td>0.528</td>
<td>0.997</td>
</tr>
<tr>
<td>Imipenem 1 g Q8h, 1 hr inf</td>
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</tr>
<tr>
<td><strong>Δ-OPRD PA01</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doripenem 500 mg Q8h, 1 hr inf</td>
<td>0.523</td>
<td>0.997</td>
</tr>
<tr>
<td>Doripenem 500 mg Q8h, 4 hr inf</td>
<td>0.712</td>
<td>0.913</td>
</tr>
<tr>
<td>Doripenem 1 g Q8h, 4 hr inf</td>
<td>0.847</td>
<td>0.895</td>
</tr>
<tr>
<td>Imipenem 500 mg Q6h, 0.5 hr inf</td>
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<td>0.993</td>
</tr>
<tr>
<td>Imipenem 1 g Q8h, 1 hr inf</td>
<td>0.316</td>
<td>0.998</td>
</tr>
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SigMoid-Emax Effect Models

<table>
<thead>
<tr>
<th>Organism</th>
<th>$E_{max}$</th>
<th>$E_{50}$</th>
<th>$H$</th>
<th>$E_{con}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-PA01</td>
<td>6.06</td>
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<td>2.71</td>
<td>9.65</td>
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<tr>
<td>Stably Derpressed PA01</td>
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<td>1.0</td>
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<td>0.126</td>
<td>1.0</td>
<td>10.38</td>
</tr>
</tbody>
</table>

$E_{max}$ = maximal log kill achievable by the regimen; $E_{50}$ = Fractional Time $> \text{MIC}$ resulting in 50% of the maximal cell kill; $H$ = Hill's Constant; $E_{con}$ = colony counts ($\log_{10} \text{CFU/ml}$) at 24 hours in the untreated control group.
References:


