Population Pharmacokinetics and Pharmacodynamics of Piperacillin: Systematic
Comparison of Cystic Fibrosis Patients and Healthy Volunteers

J. B. Bulitta1,#, S. B. Duffull2, M. Kinzig-Schippers1, U. Holzgrabe3, U. Stephan1,4, G. L. Drusano5, F. Sörgel1,4

1: IBMP – Institute for Biomedical and Pharmaceutical Research, Nürnberg-Heroldsberg, Germany;
2: School of Pharmacy, University of Otago, Dunedin, New Zealand;
3: Institute of Pharmacy and Food Chemistry, University of Würzburg, Würzburg, Germany;
4: Dep. of Pharmacology, University of Duisburg – Essen, Essen, Germany;
5: Ordway Research Institute, Albany, NY, USA

Corresponding author: Fritz Sörgel, PhD, BSc, Professor, IBMP – Institute for Biomedical and Pharmaceutical Research, Paul-Ehrlich-Str. 19, D-90562 Nürnberg-Heroldsberg, Germany; Phone: ++49-911-518290, Fax: ++49-911-5182920, e-mail: ibmp@osn.de

Running title: Population PKPD in cystic fibrosis patients

Key words:
cystic fibrosis / healthy volunteers,
population pharmacokinetics / pharmacodynamics of piperacillin,
Monte Carlo simulation,
allometric scaling by lean body mass,
PKPD breakpoints

#Present address: Department of Pharmaceutical Sciences, State University of New York at Buffalo, NY 14260, USA.

This work was in part presented as part of a meta-analysis at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2005 (poster abstract A-12).
Abstract:

Respiratory tract infections cause 90% of premature mortality in patients with cystic fibrosis (CF). In the treatment of those infections, *Pseudomonas aeruginosa* is often very problematic.

Piperacillin/tazobactam has good activity against *P. aeruginosa*, but its pharmacokinetics (PK) in CF-patients has not been compared to healthy volunteers in a controlled clinical study. Therefore, we compared the population PK and pharmacodynamics (PD) of piperacillin between CF-patients and healthy volunteers.

We studied eight adult (median age: 20yr) CF-patients (total body weight WT=43.1±7.8kg) and 26 healthy volunteers (WT=71.1±11.8kg). Dose: 4g piperacillin as 5min intravenous infusion. We determined piperacillin by HPLC and used NONMEM for population PK and Monte Carlo simulation. We used a target time of non-protein bound concentration above MIC of 50% which represents near-maximal bacterial killing.

Unscaled total clearance was 25% lower and volume of distribution was 31% lower in CF-patients. Allometric scaling by lean body mass reduced the unexplained (random) between subject variability in clearance by 26% compared to linear scaling by WT.

A standard dosage regimen of 3g/70kg WT q4h as 30min infusion (daily dose: 18g) achieved robust (≥90%) PTAs for MICs ≤12 mg/L in CF-patients and ≤16 mg/L in healthy volunteers. Alternative modes of administration allowed a marked dose reduction to 9g daily, as prolonged (4h) infusion of 3g/70kg WT q8h (daily dose: 9g) and continuous infusion achieved robust PTAs for MICs ≤16 mg/L in both groups. Piperacillin achieved PTA expectation values of 64% and 89% in CF-patients against *P. aeruginosa* based on susceptibility data from two German CF-clinics.
Introduction

Cystic fibrosis (CF) is the most common inherited disease in the Caucasian population. Respiratory diseases are the primary reason for frequent hospitalization of CF-patients and a primary cause of mortality (2, 36, 57). About 30% of the children aged 2-5 years and 81% of adults (aged 26-30 years) with CF are infected by *P. aeruginosa* (15). Piperacillin in combination with the betalactamase inhibitor tazobactam has good bactericidal activity against gram-positive microorganisms and *Pseudomonas aeruginosa* also in CF-patients (39, 42, 52, 61). The combination is frequently used in the empirical treatment of hospital acquired infections and for treatment of pulmonary exacerbations in CF-patients (31). There are reports that *P. aeruginosa* isolates from non-CF-patients show a better susceptibility to antipseudomonal agents (26) than isolates from CF-patients (53, 67). As *P. aeruginosa* can only be eradicated in the early stage in therapy of CF-patients (8), early aggressive anti-infective treatment against this pathogen is vital (27). Therefore, the probability of successful microbiological outcome for the treatment of *P. aeruginosa* is important.

It is now generally agreed upon that the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics should be used jointly to predict the probability of successful antibiotic treatment. For this task, population PK in combination with Monte Carlo simulation (MCS) is the method of choice as it combines the variability in the bacterial susceptibility with the variability in PK. However, MCS has not yet been used to determine the PKPD profile of piperacillin in CF-patients.

The PKPD profile of piperacillin in CF-patients can be affected by an altered PK in this patient group (15, 72). Although pronounced differences in clearance and volume of distribution between CF-patients and healthy volunteers were reported by some authors (59, 72), other data show smaller differences (23, 44, 63). We are not aware of any PK study of piperacillin in CF-
patients which included a healthy volunteer control group. Such a comparison would help to explain a possibly altered PK in CF-patients. For example, higher clearances in CF-patients could diminish the probability of attaining a PKPD target. For beta-lactams, the duration of non-protein bound concentration above the minimal inhibitory concentration ($fT_{>\text{MIC}}$) best predicts the drug related response (12, 16). A target time of 50% $fT_{>\text{MIC}}$ was shown to be the target for near-maximal bacterial killing and a target time of 30% $fT_{>\text{MIC}}$ correlates best with bacteriostasis for penicillins (12, 16). Based on these targets, the probability of target attainment (PTA) can be calculated.

To optimize the PTA in empiric therapy, knowledge of the best descriptor for body size and body composition may be important. There is considerable evidence that body composition influences PK (9, 25, 45, 49, 69) which requires dose adjustment at least for some patient groups. CF-patients are often undernourished and have a paucity of adipose tissue. There are reports that lean body mass (LBM) better describes volume of distribution (43, 59, 69-71) and clearance in CF-patients than does WT (43). Consequently, Touw et al. (70, 71) proposed to calculate the initial daily dose of tobramycin in CF-patients based on LBM rather than on WT. LBM was also proposed to be used as size descriptor in CF-patients (54) and has been proposed to be a superior predictor of drug dosage compared to other measures of body size (e.g. WT or body surface area, BSA) (45). However, the superiority of dosing by a size descriptor which accounts for body composition (e.g. LBM) to dosing by WT still needs to be shown (45, 59).

As penicillins are hydrophilic molecules, we expect them to distribute primarily into lean body compartments. For piperacillin, we believed $a \text{ priori}$ that body size is better described by LBM than by WT, since WT does not account for body composition whereas LBM does. The two most desirable properties of a size descriptor are: 1) It should be able to describe body size (including body composition) and functional capacity for healthy volunteers as well as for
undernourished, obese, and “normal” patients. If this was true e.g. for LBM in CF-patients, a physician could administer the same dose in mg/kg LBM to normal patients and CF-patients. 2) A size descriptor should reduce the unexplained between subject variability (BSV) as much as possible. This allows one to select doses in empiric therapy more precisely.

Population PK can directly rank different size descriptors based on their performance for these two criteria and is also a powerful tool to compare the PKPD characteristics between CF-patients and healthy volunteers. Therefore, our first objective was to compare the PK of piperacillin between CF-patients and healthy volunteers via population PK. As our second objective, we studied whether the average differences and the variability in clearance and volume of distribution are better described by LBM than by WT. Our third objective was to study the influence of patient related differences in PK parameters on PD characteristics of piperacillin given as various dosage regimens.
Methods

Subjects: A total of 34 Caucasian volunteers (eight adult CF-patients and 26 adult healthy volunteers) of both sexes participated in the study after they had given their written informed consent. Table 1 shows the demographic data. The subjects’ health status was assessed by physical examination, electrocardiography and laboratory tests including urinalysis and screening for drugs of abuse. Cystic fibrosis had been confirmed for each patient by standard sweat test and clinical history (including Shwachman score) prior to inclusion of the patient in the study. Most patients had been treated in the center for more than a decade and the diagnosis and status were well known and repeatedly confirmed. Patients were studied during an infection-free period. Consumption of alcohol, methylxanthines, and tobacco in any form, or of other medication was forbidden from 24 h before the piperacillin dose until the last sample. The subjects took no antibiotics during this time period. The subjects fasted overnight and received a standard breakfast at one hour post dose and a standard lunch at four hours post dose. Sufficient fluid intake of mineral water was assured during the study. All volunteers were closely observed by physicians for the occurrence of adverse events during the period of drug administration. The study protocol had been approved by the local ethics committee.

Study design and drug administration: The study was a single dose, single-center, open, parallel group study. Each subject received a dose of 4g piperacillin as 5 min intravenous infusion except for one CF-patient who received a dose of 3g piperacillin. All infusions were administered with a BRAUN-Perfusor® (Braun, Melsungen, Germany). The instruments were checked on a daily basis by weighing defined volumes delivered by the perfusor.

Blood sampling: All blood samples were drawn from a forearm vein via an intravenous catheter contralateral to the one used for drug administration. Blood samples were drawn immediately before start of the infusion (0 min), at the end of the infusion (5 min), as well as at 5,
10, 15, 20, 30, 45, 60, 90 min and 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, and 24 h post end of infusion. The samples were cooled in an ice-water bath for 15 min before centrifugation. After centrifugation all plasma samples were immediately frozen and stored at -70°C until analysis.

**Drug analysis:** Piperacillin concentrations in plasma were determined by HPLC. For determination of piperacillin in plasma 100 µL of the sample were deproteinized with 200 µL acetonitrile containing the internal standard (mezlocillin). After mixing and centrifugation at 15,000 rpm, 40 µL were injected onto the HPLC-system. Piperacillin was determined using a reversed phase column [LiChrospher C18, 5µm (250 x 4.6 mm)], potassium dihydrogen phosphate (pH 6.2) / acetonitrile mobile phase with a flow of 2 mL/min. Piperacillin and the internal standard were detected at 220 nm.

The plasma samples were measured against a plasma calibration row. The calibration row in plasma was prepared by a 10:1 dilution of a tested drug-free plasma with a stock solution to obtain the highest calibration level. The other calibration levels were obtained by 1:1 dilution of the highest calibration level or a level of higher concentration with drug-free plasma.

For control of inter-assay variation spiked quality controls in plasma were prepared by adding defined volumes of the stock solution or the spiked control of higher concentration to defined volumes of tested drug-free plasma. No interferences were observed in plasma for piperacillin and the internal standard. Calibration was performed by linear regression. Linearity of piperacillin calibration curves in plasma was shown between 0.200 - 150 mg/L. The quantification limits were identical with the lowest calibration levels. The inter-day precision and analytical recovery of the spiked quality control standards of piperacillin in human plasma ranged from 3.5 to 9.2 % and from 95.0 to 106.9 %.

**Population PK analysis:**
**Population model:** We tested one, two, and three compartment disposition models. Drug elimination was described by 1) first-order, 2) mixed-order (Michaelis-Menten), or 3) parallel first-order and mixed-order elimination. We assessed these three elimination models a) for the whole dataset, b) for the data from CF-patients, and c) for the data from healthy volunteers separately. We discriminated competing models by their predictive performance assessed via visual predictive checks, NONMEM’s objective function, and standard diagnostic plots. For the visual predictive check, we simulated the plasma profiles for at least 2,000 CF-patients and 6,500 healthy volunteers for each competing model. We calculated the median, the nonparametric 80% prediction interval (10% to 90% percentile), and the nonparametric 50% prediction interval (25% to 75% percentile) from the simulated plasma concentrations. These prediction interval lines were then overlaid on the original raw data. If the model described the data correctly, then 20% of the observed data points should fall outside the 80% prediction interval and 50% of the data should fall outside the interquartile range at each time point. We compared the median predicted concentrations and the prediction intervals with the raw data and tested, whether the median and the prediction intervals mirrored the central tendency and the variability of the raw data for the respective model.

**Size:** We studied the following size models: 1) No size model, 2) linear scaling by WT, 3) allometric scaling by WT (29, 75, 76), 4) linear scaling by LBM (9, 32), and 5) allometric scaling by LBM. We compared the ability of the size models to describe the differences in the central tendency of PK parameters between CF-patients and healthy volunteers. Additionally we studied by how much the random (unexplained) BSV was reduced by the respective size model relative to linear scaling by WT.

Our allometric model assumes that volume of distribution scales linearly (allometric exponent 1.0) with body size (i.e. WT or LBM), whereas clearance scales slightly less than
linearly with body size (allometric exponent 0.75). The allometric exponent was fixed to 1.0 for all volume terms and fixed to 0.75 for all clearance terms. The $F_{Size,V,i}$ and $F_{Size,CL,i}$ are the fractional changes in volume of distribution and clearance for the $i$th subject (with $WT_i$) standardized to a weight $WT_{STD}$ of 70 kg.

$$F_{Size,V,i} = \frac{WT_i}{WT_{STD}}$$

$$F_{Size,CL,i} = \left(\frac{WT_i}{WT_{STD}}\right)^{0.75}$$

The same allometric size model was applied for LBM with a standard lean body mass ($LBM_{STD}$) of 53 kg.

$$F_{Size,V,i} = \frac{LBM_i}{LBM_{STD}}$$

$$F_{Size,CL,i} = \left(\frac{LBM_i}{LBM_{STD}}\right)^{0.75}$$

For linear scaling by WT or LBM all exponents were set to 1.0.

**Between-subject variability model:** We estimated the BSV for clearance and volume of distribution by assuming a log-normal distribution for the PK parameters. The $\eta_{BSVi}$ is the log scale difference of the individual PK parameter estimate from its mean for the $i$th subject. The $\eta_{BSV}$ is assumed to be a normally distributed random variable with mean zero and standard deviation BSV. The BSV was estimated as variance but we report the square root of the estimate. We expressed these values as a percentage, as this quantity is an approximation to the apparent coefficient of variation of a normal distribution on log-scale. The variance of the $\eta_{BSVi}$ is the
unexplained (random) BSV. The individual PK parameters were calculated as follows (parameters explained below):

\[
CL_i = CL \cdot F_{\text{Size},i} \cdot FCYF_{\text{CL}} \cdot \exp(\eta_{\text{BSVCLI}})
\]  

(5)

\(CL_i\) is the individual estimate for total clearance for the \(i\)th subject. The \(\eta_{\text{BSVCLI}}\) is \(\eta_{\text{BSV}}\) of CL for the \(i\)th subject. The CL is the group estimate for total clearance of a healthy volunteer with standard body size (i.e. \(WT_{\text{STD}} = 70\) kg or \(LBM_{\text{STD}} = 53\) kg). The FCYF\(_{\text{CL}}\) is the disease specific scale factor of CF-patients and is calculated as the average total clearance in CF-patients divided by the average total clearance in healthy volunteers after accounting for body size. If this scale factor is 1.0, CF-patients and healthy volunteers of the same body size have identical group estimates. We also used a disease specific scale factor for volume of distribution at steady-state (FCYF\(_{\text{VSS}}\)).

**Observation model and computation:** We described the residual unidentified variability by a combined additive and proportional error model for plasma concentrations. We used the first order conditional estimation (FOCE) method with the interaction estimation option in NONMEM version V release 1.1 (NONMEM Project Group, University of California, San Francisco, CA, USA) (5) for all population PK modeling.

**Non-compartmental analysis and descriptive statistics:** We used WinNonlin™ Professional (version 4.0.1, Pharsight Corp., Mountain View, CA, USA) for non-compartmental analysis and descriptive statistics.

**Non-parametric bootstrap:** We used non-parametric bootstrap resampling with 2,000 replicates for each size model (18, 51) to quantify the uncertainty in our PK parameter estimates. Each replicate contained observations from eight randomly selected CF-patients and 26 randomly selected healthy volunteers from the original raw dataset (subjects could be drawn multiple times).
We derived the median and non-parametric 90% confidence intervals (5% to 95% percentile) from the 2,000 estimated PK parameter sets for each parameter of our population PK model.

**Monte Carlo simulation:** We used a PKPD target of 50% $f_{\text{T} > \text{MIC}}$ for near-maximal bactericidal activity and 30% $f_{\text{T} > \text{MIC}}$ for bacteriostasis for piperacillin. A target of about 50% $f_{\text{T} > \text{MIC}}$ was required to achieve a reduction in bacterial counts by two $\log_{10}$ at 24 h and a target of 30% was required for bacteriostasis at 24 h for penicillins in the animal infection models studied by Craig et al. (12, 16). We studied a range of MICs from 0.5 to 128 mg/L and assumed a fixed protein binding of 30% for piperacillin (3, 62). We assumed the same protein binding of 30% for piperacillin in CF-patients and in healthy volunteers, as the protein binding is similar for CF-patients and healthy volunteers for most drugs including various beta-lactams (54, 59). An altered protein binding in CF-patients is not expected to affect the unbound plasma concentrations substantially for a drug with low protein binding like piperacillin.

We studied the following dosage regimens: 1) continuous infusion of 9g / 70kg WT per day, 2) prolonged (4 h) infusion of 3g / 70kg WT q8h (daily dose: 9g), and 3) short-term (30 min) infusion of 3g / 70kg WT q4h (daily dose: 18g). We used NONMEM to simulate each dosage regimen at steady-state in absence of residual error and predicted profiles for 9,600 CF-patients and 10,400 healthy volunteers with the same demographic data as the subjects in our study for each dosage regimen. The $f_{\text{T} > \text{MIC}}$ values and the PTA were calculated by linear interpolation between simulated data points (frequent sampling) with Perl scripts written by the first author. These Perl scripts were validated for all studied dosage regimens for this and several other studies in comparison to WinNonlin™ Pro (version 4.0.1). We used the population mean PK parameter estimates and the variance-covariance matrix representing the BSV provided by NONMEM for MCS and assumed that the dose, duration of infusion, and timing of infusion had no variability.
We used the clearance and volume notation of our population PK model both during estimation and MCS. The diagonal elements of the variance-covariance matrix are the variances (on natural log scale, see above) of the individual random terms $\eta_{BSVi}$ of the respective PK parameter. These variance terms can be obtained as the square of the apparent coefficient of variation for the BSV reported for our final population PK model. We included the covariance between the volume of the central and volume of the peripheral compartment in the variance-covariance matrix. We did not include the variability of the intercompartmental clearance, as this term was difficult to estimate.

The predictive performance of our final population PK model was assured via visual predictive checks. The PTA was derived by calculating the fraction of subjects who attained the PKPD target at each MIC. The PKPD breakpoint was defined as the highest MIC for which the PTA was at least 90%. We also provided the breakpoints for a PTA of at least 95% or 99%. Additionally, we ran simulations based on the individual PK parameter estimates and covariates (WT and LBM) from the eight CF-patients in our study to compare various dosing algorithms based on our studied size models (J. B. Bulitta, S. B. Duffull, C B Landersdorfer, et al., Abstr. AAPS Annual Meeting, abstr. W4056, 2006).

To determine the clinical relevance of the differences in the PTA between CF-patients and healthy volunteers, we calculated the expected PTA for various MIC distributions [i.e. the PTA expectation value, (47)]. We calculated the PTA expectation value by multiplying the PTA at each MIC with the frequency of how often this MIC occurred in the respective MIC distribution. The individual products at each MIC are summed up and the sum is the PTA expectation value for the respective MIC distribution. The PTA expectation value is the probability of target attainment in a specific target population (e.g. CF-patients) with infections caused by bacteria from a specific MIC distribution (ideally the MIC distribution of each local hospital). If achieving the PKPD target is
the primary determinant of successful treatment, the PTA expectation value can be interpreted as
the probability of successful treatment of a CF-patient at the local hospital in empiric therapy.

We calculated the PTA expectation value based on published MIC distributions. These
comprised susceptibility data on *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella
pneumoniae* from the 2002 MYSTIC program in North America (34) and in South America (33),
*Pseudomonas aeruginosa* data from the Hartford Hospital, CT, USA (35), as well as data on non-
mucoid and mucoid *Pseudomonas aeruginosa* isolates from CF-patients in Freiburg, Germany
(61), and in Leipzig, Germany (64).
Results

**Non-compartmental analysis:** Figure 1 shows the average plasma concentrations of CF-patients and healthy volunteers after a 5 min infusion of 4g piperacillin. Table 2 lists the results of the non-compartmental analysis. These parameters were not scaled by any size descriptor. Total clearance was 25% lower in CF-patients and volume of distribution at steady-state was 31% smaller in CF-patients than in healthy volunteers. CF-patients had higher peak concentrations. The terminal-half-life was 34% shorter and the mean residence time was 17% shorter in CF-patients.

**Population PK analysis:** The visual predictive check indicated a very good predictive performance for the two and for the three compartment model with first-order elimination. The one compartment model had insufficient predictive performance. The objective function was 253 points better for the two than for the one compartment model. All studied models with mixed-order elimination or with parallel first-order and mixed-order elimination provided no statistically significant (p>0.05, log-likelihood ratio test) improvement in the objective function. This was observed for the analysis of the whole dataset, for analysis of data in CF-patients, and for analysis of data in healthy volunteers separately. For all our studied models, the first-order elimination was estimated to account for more than 95% of total clearance indicating a limited degree of saturation. As the two compartment model with first-order elimination had highly sufficient predictive performance and as models with saturable elimination yielded no improvement, we selected a two compartment model with first-order elimination as our final structural model.

Figure 2 reveals the highly sufficient predictive performance for the final model, as the central tendency of the observations was well captured by the median of the simulated concentrations and as the 80% prediction interval [10 to 90% percentile] described the variability of the observations well. Ideally 10% of the observations should fall outside the 80% prediction
interval on each side and at each time point. The final estimates for this model are shown in Table 3. The disease specific scale factors FCYF<sub>CL</sub> and FCYF<sub>VSS</sub> shown in Table 4 are the ratios of group estimates between CF-patients and healthy volunteers after accounting for body size (functional capacity). A value of 1.0 for FCYF means that a CF-patient and a healthy volunteer of the same size have the same group estimate for the respective PK parameter. A value above (below) 1.0 means that CF-patients have a higher (lower) estimate for the respective PK parameter compared to healthy volunteers of the same size.

We distinguished the different size models 1) by their estimates for the disease specific scale factors FCYF and 2) by their ability to reduce the unexplained (random) BSV in clearance and volume of distribution. CF-patients had a 27% lower total clearance and a 40% lower volume of distribution relative to healthy volunteers, when no size descriptor was included (see size model 1, Table 4). For all other size models FCYF<sub>VSS</sub> was close to 1.0 (range: 0.924 to 0.999). However, total clearance was estimated to be 20% larger in CF-patients than in healthy volunteers for linear scaling by WT and to be 12% larger for linear scaling by LBM. For allometric scaling by WT or LBM, total clearance was estimated to be similar (range: 1.00 to 1.06) between both subject groups. Linear or allometric scaling by LBM reduced the unexplained BSV by about 30% for clearance and by about 14% for volume of distribution of the central compartment relative to linear scaling by WT (see Table 5). We selected allometric scaling by LBM as our final covariate model, as this model explained the differences in average clearance between both subject groups and as this model reduced the unexplained BSV similarly to linear scaling by LBM.

**Monte Carlo simulation:** CF-patients achieved slightly shorter $fT_{\geq \text{MIC}}$ values than healthy volunteers (see Figure 3). Therefore, CF-patients had slightly lower PTAs which resulted in similar PKPD breakpoints compared to healthy volunteers for the studied dosage regimens (see
The PKPD breakpoints for PTAs of at least 90%, 95%, or 99% are shown in Table 6 for the targets $f_{T>MIC} \geq 30\%$ and $f_{T>MIC} \geq 50\%$. Continuous infusion of 9g / 70kg WT per day and 4h infusion of 3g q8h (daily dose: 9g) achieved slightly higher or similar breakpoints for the $f_{T>MIC} \geq 50\%$ target compared to 30 min infusions at the much higher dose of 18g / day (3g q4h). Dosing based on allometric scaling by LBM or WT increased the PKPD breakpoints by about 10-30% compared to mg/kg WT dose selection for our three studied piperacillin dosage regimens (data not shown elsewhere).

The PTA expectation values for CF-patients and healthy volunteers with the same demographic data as the subjects in our study are shown in Table 7. Although the daily doses of 9g and 18g were studied in these dosage regimens, PTA expectation values differed only by 3.2% or less between the three dosage regimens in each subject group. The maximum difference in PTA expectation values between CF-patients and healthy volunteers was 5.5%. We also calculated the PTA expectation values shown in Table 7 for bacteriostasis (target: $f_{T>MIC} \geq 30\%$). For continuous infusion, we obtained the same PTA expectation values for the bacteriostasis target as for the near-maximal killing (target: $f_{T>MIC} \geq 50\%$). The PTA expectation values for prolonged and short term infusion were slightly higher (median [range]: 4.5% [0.2 to 14.8%]) for the bacteriostasis target than for the near-maximal killing target (comparison within the same dosage regimen).

PTA expectations values were lower for susceptibility data from South America (86% for *E. coli* and 71% for *K. pneumoniae*) compared to data from North America (97% for *E. coli* and 94% for *K. pneumoniae*, see Table 7). Differences in PTA expectation values between different regions were more pronounced for *P. aeruginosa*. The PTA expectation values were about 93% for the MIC distribution of the Hartford Hospital, 82% for the 2002 MYSTIC data from North America, and only 43% for the 2002 MYSTIC data from South America. For *P. aeruginosa* isolates from
CF-patients in two German CF-clinics, the PTA expectation values were about 89% for Leipzig and about 65% for Freiburg. The PTA expectation values for the non-mucoid and mucoid strains were similar.
**Discussion**

Medical advances have led to tremendous improvements in the life-expectancy and quality of life for CF-patients during the last 70 years. In the 1930s, the average life-expectancy of CF-patients was only a few months. The median survival age of CF-patients increased to 14 years in 1969 and to 31.3 years in 1996 in the US (2). The probability of surviving 40 years was even 83.3% in 1995 in Denmark for a diagnosed CF-patient (22). This improved life-expectancy seems to correlate with early treatment of *P. aeruginosa* (22) as it is almost impossible to eradicate this pathogen once a chronic infection is established. Early aggressive treatment was shown to prevent or delay chronic *P. aeruginosa* infection significantly (20, 28) and resulted in a probability of >80% for still not having developed a chronic *P. aeruginosa* infection 7 years after the first isolation of this pathogen in the respective patient (21).

European experts strongly recommend the use of local susceptibility patterns for treatment of *P. aeruginosa* (15). As the time required to identify *P. aeruginosa* and to assess its antibiotic susceptibility from a sputum culture is about 3-4 days, efficacious empiric therapy is very important. Data on the effectiveness of intravenous antibiotic treatment in CF-patients are rather sparse and large randomized controlled trials are required (11, 19, 40).

MCS offers a tool to overcome this shortage of data. A PKPD target like near-maximal bactericidal activity is used as surrogate marker for successful clinical outcome in a MCS. More specifically, MCS allows one to identify the dose and mode of administration with the highest PTA for treatment of a specific patient population against target pathogens with a specific susceptibility pattern. MCS has been shown to be a very useful tool in rational dose selection for phase II/III clinical trials (17). Mouton et al. (48) used MCS to compare the PKPD profile of ceftazidime between CF-patients, intensive care patients, and healthy volunteers.
Knowledge of the PK in the relevant patient population (e.g. in CF-patients) is a prerequisite to apply a MCS, since MCS combines the patient specific PK parameters and their variability with the specific susceptibility data from a local hospital. PK studies in CF-patients often comprise a very heterogeneous patient population with considerable differences in age, weight, body composition, and disease state. To determine the influence of those disease specific factors on the PK, it is important to compare the PK of healthy volunteers and CF-patients within the same study. Population PK is a powerful concept to study the influence of these factors on the PK in CF-patients quantitatively. Our population PK analysis aimed at estimating both the differences in average PK parameters between CF-patients and healthy volunteers as well as the PK parameter variability. Our final model had an excellent predictive performance for both the average plasma concentration time profiles of piperacillin as well as their variability (see Figure 2). Therefore, we used it to compare the PD characteristics between both subject groups via MCS. Our MCS was based on data from 34 subjects in total (eight CF-patients and 26 healthy volunteers). We had frequent plasma samples and used population PK to describe the PK of piperacillin in both subject groups.

Our results for the PK parameters of piperacillin in healthy volunteers were well within the range of those from other authors (4, 6, 38, 68). The literature data on the PK of piperacillin in CF-patients are sparse. The data reported show a rather wide variability in PK parameters. None of the piperacillin studies in CF-patients included a healthy volunteer control group which makes it difficult to interpret the differences in the observed results between studies. The average terminal half-life of piperacillin is between 0.54 and 0.89 h in children and adolescents (13, 30, 31, 41, 55) with CF and 1.2 ± 0.9 h in adult (age: 25.8 ± 3.6 yrs) CF-patients (74). We determined a terminal
half-life of 0.69 [0.34 - 1.19] h (median [range]) in our adult CF-patients (age: 21.1 ± 3.8 yrs) which is well within the range of half-lives reported by other authors.

For CF-patients volume of distribution was reported to be 0.4 L/kg (31) in children, 0.64 L/kg (13, 30) and 0.17 L/kg in juveniles and young adults (13, 55), and 0.296 ± 0.11 L/kg (74) in adult CF-patients. Differences by a factor of 3.8 (max/min) for volume of distribution are unlikely to be caused by an altered body composition (e.g. between young and adult CF-patients). This large range could in part be due to the use of non-compartmental analysis which is less powerful than population PK. We determined a volume of distribution at steady-state of 0.181 ± 0.034 L/kg WT and a volume of distribution during the terminal phase of 0.229 ± 0.100 L/kg WT both by non-compartmental analysis. Our estimates were at the lower end of the range of values reported in literature. Total clearance was reported to be about 0.43 L/h/kg in children (age: 5 yrs, (31)), 0.497 L/h/kg (age: 8-16 yrs, (13, 30)) and 0.188 L/h/kg (age: 12-21 yrs, (13, 55)) in juveniles and young adults. Although higher clearances in L/h/kg are often observed in younger (and smaller) patients compared to adults, a difference by a factor of 2.6 between the two juvenile-adult CF-patient groups seems large. Vinks et al. (74) found a clearance of 0.23 ± 0.04 L/h per kg LBM after intermittent treatment of adult CF-patients. Our total clearance of 0.24 ± 0.04 L/h per kg LBM (see Table 1 and Table 2) is in good agreement with the data from Vinks et al. (74).

The differences in PK parameters between CF-patient groups reported by various authors (13, 30, 55) underline the need to include a healthy volunteer control group to compare the PK of CF-patients to the PK in healthy volunteers. Some of those large differences might also be due to inadequate scaling of CF-patients for body size. Our CF-patients had a 39% lower WT than the healthy volunteers (see Table 1). The LBM was 34% lower in CF-patients. Our non-
compartmental analysis showed a 31% smaller unscaled volume of distribution at steady-state and a 25% lower unscaled total clearance in CF-patients relative to healthy volunteers (see Table 2).

Our population PK analysis aimed at ranking the different size models according to 1) their ability to describe the average differences between CF-patients and healthy volunteers and 2) their ability to reduce the unexplained BSV in clearance and volume of distribution. In absence of a size descriptor (size model 1, Table 4), CF-patients had a 27% lower clearance and a 40% lower volume of distribution, because they were smaller. Size models 2 to 5 estimated FCYF_{VSS} close to 1.0 (range: 0.924-0.999). Linear scaling of clearance by WT or LBM over-accounted for the fact that our CF-patients were smaller. CF-patients had a 20% higher clearance expressed as L/h/kg (linear scaling by WT), probably because WT ignores that our CF-patients were leaner than our healthy volunteers. For the allometric size models, the estimates were close to 1.0 (range: 1.00-1.06). Therefore, the two allometric size models explained the differences in average volume of distribution and average clearance between CF-patients and healthy volunteers.

Besides the ability of the various size models to describe the differences in average PK parameters, we studied the ability of these models to reduce the unexplained BSV. This is important in empiric therapy, as target concentrations can be attained more precisely, if the unexplained BSV is reduced by a body size descriptor. Linear and allometric scaling by LBM (see Table 5) reduced the unexplained BSV by about 30% for clearance and by about 14% for volume of the central compartment relative to linear scaling by WT. Thus, allometric scaling by LBM described the differences in average clearance and volume of distribution and reduced the unexplained BSV relative to linear scaling by WT. This result seems reasonable, because LBM accounts for body composition whereas WT does not.
We used the population PK model based on allometric scaling by LBM to calculate the PTA in CF-patients and healthy volunteers and to compare the PKPD breakpoints. The elimination in our final model was non-saturable, although there is data which support a saturable component of piperacillin elimination (6, 68, 74). We systematically compared models with first-order, mixed-order (Michaelis-Menten), and parallel first-order and mixed-order elimination for the whole dataset and for the data in each subject group separately. Inclusion of a saturable elimination did not result in a statistically significant improvement in the objective function. More importantly, the first-order (non-saturable) clearance component accounted for more than 95% of total clearance at low concentrations indicating a small degree of saturation for all studied models. However, our study only assessed the PK of piperacillin at one dose level after a short-term infusion. Based on the Michaelis-Menten constants reported by Vinks et al. (74) and Lodise et al. (38), plasma concentrations in our study remained above the average Michaelis-Menten constants for about two hours or less. This is probably the reason, why our study had a low power to detect the saturable elimination of piperacillin. Most (6, 68, 74) reports in literature showing a saturable elimination of piperacillin studied more than one dose level, collected serial urine samples, or studied more than one dosage regimen.

We could show by exhaustive MCS based on data in healthy volunteers that the differences between a saturable and non-saturable elimination model for piperacillin were small for the dosage regimens and MIC distributions reported in our study (data available on request). Therefore, the PKPD breakpoints and PTA expectation values from our linear (non-saturable) PK model are adequate for the studied dosage regimens and dose levels, since the PK of piperacillin appears as “pseudo-linear” for the studied dosage regimens although it is truly nonlinear. Vinks et al. (74) found a clearance of 24.4 ± 11.7 L/h in CF-patients for a continuous infusion of 16 g piperacillin /
2 g tazobactam over 24 h. Our clearance in CF-patients was 11.7 L/h with a range of 6.25 to 14.5 L/h (see Table 2). As the clearances reported by Vinks et al. are about twice as large and more variable than our clearances, we found PKPD breakpoints of about 4 to 6 mg/L for a continuous infusion of 9g / day in CF-patients, when we used the clearances from Vinks et al. for MCS (see Table 6). The CF-patients in the study from Vinks et al. (74) were treated for an acute exacerbation of their chronic *P. aeruginosa* infection by piperacillin-tazobactam. Our patients had no acute infection and received piperacillin without tazobactam. This may have contributed to the differences between our results and the study from Vinks et al. (74). The results for short-term infusion of piperacillin were similar between our study and the study by Vinks et al.

CF-patients had only slightly lower PTAs (see Figure 4) which resulted in similar PKPD breakpoints compared to healthy volunteers. Figure 4 contains no confidence intervals for the PTA. The expected width of confidence intervals for the PTA vs. MIC plot for MCS of beta-lactams was studied as a function of sample size by Bulitta et al. (7). We studied continuous infusion and prolonged (4 h) infusion q8h at a daily dose of 9g / 70kg WT as well as short-term (30 min) infusion q4h at a daily dose of 18g / 70kg WT. Remarkably, continuous and prolonged infusions achieved the same PKPD breakpoint for near-maximal killing of about 16 mg/L at a 50% lower daily dose compared to short-term infusions (see Table 6). Our PKPD breakpoint is in good agreement with the PKPD breakpoint determined via MCS by Lodise et al. (38), with the breakpoint of 16 mg/L for susceptibility specified by the BSAC (1), and with the DIN breakpoints (14) of ≤4 mg/L for susceptibility and >32 mg/L for resistance. Importantly, the breakpoint specified by the Clinical and Laboratory Standards Institute (CLSI) is ≤64 mg/L for susceptibility of *P. aeruginosa* to piperacillin and piperacillin-tazobactam (10). As chronic infection by *P. aeruginosa* in CF-patients causes a high risk for morbidity and mortality, the CLSI breakpoint
for piperacillin against *P. aeruginosa* seems inappropriately high for CF-patients. The CLSI breakpoint of $\leq 16$ mg/L for susceptibility of Enterobacteriaceae to piperacillin and piperacillin-tazobactam agrees more closely with our PKPD breakpoints.

The dose reduction from 18g (intermittent treatment) to 9g daily (prolonged and continuous infusion) underlines the benefit of prolonged and continuous infusion with regard to drug acquisition costs and a possibly lower risk for adverse events. There is also clinical data showing that continuous infusion yields comparable or superior clinical efficacy for piperacillin in non-CF-patients (24, 37, 56). Vinks et al. (73) showed the clinical effectiveness of continuous infusion of ceftazidime in CF-patients.

Early aggressive treatment of *P. aeruginosa* in CF-patients may require very high piperacillin doses which carry an increased risk for adverse events. Doses up to 900 mg/kg daily divided into six doses (equivalent to 63g for a 70kg patient) were administered to CF-patients aged 12 years or less (58). A distinct serum-sicknesslike adverse reaction was observed in 32% of these patients and its incidence appeared to be correlated with the piperacillin dose. Adverse events after piperacillin treatment in CF-patients were also reported by other authors (50, 60, 65, 66).

As the options for efficacious antipseudomonal treatment become increasingly fewer due to emergence of resistance, prolonged infusion of piperacillin seems to be an appealing option which can combine a high probability for clinical success and a possibly lower risk for adverse events secondary to considerably lower daily doses.

To determine the clinical relevance of the observed PTAs, we calculated the PTA expectation values for various MIC distributions. The PTA expectation values differed only slightly ($<5.5\%$) between CF-patients and healthy volunteers for the studied MIC distributions. The PTA expectation values for *E. coli* and *K. pneumoniae* from the 2002 MYSTIC data in North
America as well as for the *P. aeruginosa* data at the Hartford Hospital were robust (>92%) for all three studied dosage regimens (see Table 7). Secondary to a decreased susceptibility of the *P. aeruginosa* isolates in the 2002 MYSTIC data, PTA expectation values were only 82% in North America and 43% in South America. When we studied MIC distributions in German CF-patients, the PTA expectation values for *P. aeruginosa* were between 64 and 89%. These differences underline the importance to use the MIC distribution of each local hospital to calculate PTA expectation values for successful empiric treatment against each pathogen (15).

In conclusion we found a 25% lower unscaled total clearance and a 31% lower volume of distribution at steady-state in CF-patients than in healthy volunteers, because our CF-patients were smaller. Allometric scaling by WT or LBM explained the differences in average clearance and volume of distribution better than linear scaling by WT. Linear scaling by WT predicted clearance in CF-patients to be 20% higher than in healthy volunteers, probably because WT does not account for body composition. Linear or allometric scaling by LBM reduced the unexplained (random) BSV by about 30% for clearance and by about 14% for volume of the central compartment relative to linear scaling by WT. This is important to achieve target concentrations more precisely in empiric therapy. Large clinical studies are warranted to show a higher probability of successful clinical outcome for dose selection in CF-patients based on LBM. The PKPD breakpoint for near-maximal bactericidal activity was 16 mg/L in CF-patients and healthy volunteers for 3g / 70kg WT q8h given as prolonged (4 h) infusion. The PTA expectation values were between 64% and 89% for *P. aeruginosa* isolates from CF-patients in two Germany CF-clinics. Clinical trials are warranted to compare the clinical cure rates and adverse events between prolonged infusion at an about 50% lower daily dose and short-term infusion at the full daily dose.
Acknowledgement

We thank Dr. Cornelia Landersdorfer for her critical review of this manuscript.
References:


<table>
<thead>
<tr>
<th></th>
<th>CF-patients</th>
<th>Healthy volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>8 (5 / 3)</td>
<td>26 (13 / 13)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160 ± 11.9</td>
<td>174 ± 8.4</td>
</tr>
<tr>
<td>Body surface area$^S$ (m$^2$)</td>
<td>1.38 ± 0.18</td>
<td>1.85 ± 0.18</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>21 ± 4</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>Total body weight (kg)</td>
<td>43.1 ± 7.8</td>
<td>71.1 ± 11.8</td>
</tr>
<tr>
<td>Lean body mass$^o$ (kg)</td>
<td>37.2 ± 6.9</td>
<td>56.4 ± 7.2</td>
</tr>
<tr>
<td>Body mass index (kg m$^{-2}$)</td>
<td>16.7 ± 1.1</td>
<td>23.6 ± 3.7</td>
</tr>
</tbody>
</table>

$^S$: Calculated by the formula from Mosteller (46).

$^o$: Calculated by the formula from Cheymol and James (9, 32).
Table 2 Median [range] of the unscaled PK parameters derived via non-compartmental analysis for CF-patients and healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>CF-patients</th>
<th>Healthy volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total clearance (L h^{-1})</td>
<td>8.78 [6.39 - 12.1]</td>
<td>11.7 [6.25 - 14.5]</td>
</tr>
<tr>
<td>Volume of distribution at steady-state (L)</td>
<td>8.13 [5.16 - 10.8]</td>
<td>11.8 [9.06 - 30.6]</td>
</tr>
<tr>
<td>Peak concentration (mg L^{-1})</td>
<td>767 [408 - 1044]^{*,#}</td>
<td>446 [272 - 721]</td>
</tr>
<tr>
<td>Terminal half-life (L)</td>
<td>0.69 [0.34 - 1.19]</td>
<td>1.05 [0.49 - 7.52]</td>
</tr>
<tr>
<td>Mean residence time (h)</td>
<td>0.85 [0.66 - 1.03]</td>
<td>1.02 [0.79 - 3.49]</td>
</tr>
</tbody>
</table>

*: Normalized to a dose of 4g piperacillin, since one CF-patient received a dose of 3g instead of 4g.

#: Only four CF-patients had a blood sample drawn directly at the end of the infusion. The other four CF-patients had blood draws 5-12 min post end of infusion. The peak concentrations provided here are calculated based on the highest observed concentration in all eight CF-patients. Therefore, the true peak concentrations at the end of the infusion in CF-patients were probably higher than the value shown here.
Table 3  PK parameter estimates for the two compartment model based on allometric scaling by lean body mass (LBM)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Estimate for CF-patients</th>
<th>Estimate for healthy volunteers</th>
<th>Coefficient of variation (%)°</th>
<th>Estimate for CF-patients</th>
<th>Estimate for healthy volunteers</th>
<th>Coefficient of variation (%)°</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>L h⁻¹</td>
<td>11.3*</td>
<td>11.3*</td>
<td>10.4</td>
<td>11.4*</td>
<td>11.3*</td>
<td>9.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(10.5-12.3)</td>
<td>(10.9-11.7)</td>
<td>(5.99-12.8)</td>
</tr>
<tr>
<td>Vssᵃ</td>
<td>L</td>
<td>9.61*</td>
<td>10.4*</td>
<td></td>
<td>9.63*</td>
<td>10.4*</td>
<td>(8.60-10.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(9.88-11.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>L</td>
<td>6.49*</td>
<td>7.01*</td>
<td>26.0#</td>
<td>6.55*</td>
<td>7.07*</td>
<td>24.5#</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(5.60-7.66)</td>
<td>(6.36-8.02)</td>
<td>(16.0-31.6)</td>
</tr>
<tr>
<td>V2</td>
<td>L</td>
<td>3.12*</td>
<td>3.37*</td>
<td>34.2#</td>
<td>3.08*</td>
<td>3.33*</td>
<td>34.1#</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2.51-3.58)</td>
<td>(2.74-3.86)</td>
<td>(23.7-45.5)</td>
</tr>
<tr>
<td>CLic</td>
<td>L h⁻¹</td>
<td>12.8*</td>
<td></td>
<td></td>
<td>12.7*</td>
<td>(7.31-15.4)</td>
<td></td>
</tr>
<tr>
<td>TK0 (fixed)</td>
<td>min</td>
<td>5</td>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV_c</td>
<td>%</td>
<td>13.2</td>
<td></td>
<td></td>
<td>13.2</td>
<td>(10.5-15.8)</td>
<td></td>
</tr>
<tr>
<td>SD_c</td>
<td>mg/L</td>
<td>1.88</td>
<td></td>
<td></td>
<td>1.83</td>
<td>(1.00-2.42)</td>
<td></td>
</tr>
</tbody>
</table>

*: Medians and non-parametric 90% confidence intervals (5% - 95% percentile) from 2,000 non-parametric bootstrap replicates. Each replicate included data of eight CF-patients and 26 healthy volunteers who were randomly drawn from the original dataset (with replacement).

*: Group estimate for a subject of standard body size (LBM = 53 kg).

ᵃ: Derived from model estimates, not an estimated parameter.

°: Apparent coefficients of variation for between subject variability.

#: Coefficient of correlation for the random variability between V1 and V2, r = -0.80. The median and 90% confidence interval for r(V1, V2) from bootstrap was -0.81 (-0.59 to -0.94).

CL: total clearance, Vss: volume of distribution at steady-state, V1: volume of distribution for the central compartment, V2: volume of distribution for the peripheral compartment, CLic: intercompartmental clearance between the central and the peripheral compartment, TK0: duration of zero order input (not estimated), CV_c is the proportional and SD_c is the additive residual error component for the plasma concentrations.
Table 4  
Ratio of group estimates (CF-patients / healthy volunteers) for clearance and volume of distribution for different size models

<table>
<thead>
<tr>
<th>Size model</th>
<th>Estimates from original dataset</th>
<th>Median (90% confidence interval) from 2,000 non-parametric bootstrap replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FCYF_{CL}</td>
<td>FCYF_{VSS}</td>
</tr>
<tr>
<td>1) No size model</td>
<td>0.729</td>
<td>0.598</td>
</tr>
<tr>
<td>2) WT (linear scaling)</td>
<td>1.20</td>
<td>0.997</td>
</tr>
<tr>
<td>3) WT (allometric)</td>
<td>1.06</td>
<td>0.999</td>
</tr>
<tr>
<td>4) LBM (linear scaling)</td>
<td>1.12</td>
<td>0.924</td>
</tr>
<tr>
<td>5) LBM (allometric)</td>
<td>1.00</td>
<td>0.926</td>
</tr>
</tbody>
</table>

FCYF_{CL}: Ratio of group estimates for total clearance in CF-patients divided by total clearance in healthy volunteers.

FCYF_{VSS}: Ratio of group estimates for volume of distribution at steady-state in CF-patients divided by volume of distribution at steady-state in healthy volunteers.
Table 5  Comparison of between subject variability (variances) between different size models:
The table shows the variance for each size model divided by the variance for linear scaling by WT for the respective PK parameter (see Table 3 for parameter explanations).

<table>
<thead>
<tr>
<th>Size model</th>
<th>Estimates from original dataset</th>
<th>Relative between subject variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CL</td>
<td>V1</td>
</tr>
<tr>
<td>2) WT linear scaling</td>
<td>100%*</td>
<td>100%*</td>
</tr>
<tr>
<td>3) WT allometric</td>
<td>88%°</td>
<td>101%</td>
</tr>
<tr>
<td>4) LBM linear scaling</td>
<td>68%°</td>
<td>85%</td>
</tr>
<tr>
<td>5) LBM allometric</td>
<td>74%°</td>
<td>87%</td>
</tr>
</tbody>
</table>

*: The between subject variances were scaled to the variance for linear scaling by WT.

°: The lower this number, the more was the unexplained (random) variability reduced by the respective size model. These values mean that the between subject variability (variance) for total clearance was reduced by 12% for allometric scaling by WT, by 32% for linear scaling by LBM, and by 26% for allometric scaling by LBM, all relative to linear scaling by WT.
Table 6  PKPD breakpoints for various dosage regimens of piperacillin for PTA of at least 90%, 95%, or 99%

<table>
<thead>
<tr>
<th>Dosage regimens</th>
<th>Target: $f_{T&gt;MIC} \geq 30%$ representing bacteriostasis at 24 h</th>
<th>Target: $f_{T&gt;MIC} \geq 50%$ bacterial killing by $2 \log_{10}$ at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF-patients</td>
<td>Healthy volunteers</td>
</tr>
<tr>
<td><strong>Daily dose 9g / 70 kg WT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4h infusion of 3 g q8h</td>
<td>24 / 24 / 24$^5$</td>
<td>32 / 24 / 24</td>
</tr>
<tr>
<td>Continuous infusion</td>
<td>16 / 12 / 12$^5$</td>
<td>16 / 16 / 16</td>
</tr>
<tr>
<td>Continuous infusion based on the data from Vinks et al.$^#$</td>
<td>6 / 6 / 4$^{#,#}$</td>
<td>6 / 6 / 4$^{#,#}$</td>
</tr>
<tr>
<td><strong>Daily dose 18g / 70 kg WT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min infusion of 3 g q4h</td>
<td>32 / 32 / 24$^5$</td>
<td>32 / 32 / 32</td>
</tr>
</tbody>
</table>

$^#$: Importantly, Vinks et al. (74) found a clearance of 24.4 ± 11.7 L/h (average ± SD) in CF-patients for continuous infusion of 16 g piperacillin / 2 g tazobactam over 24 h. We found a clearance of 11.3 L/h (10.4%), geometric mean (CV for between subject variability).

We ran a MCS with 10,000 CF-patients for continuous infusion of 9g piperacillin per day based on the average ± SD clearance of 24.4 ± 11.7 L/h reported by Vinks et al. (74) for continuous infusion in CF-patients. We assumed an average protein binding of 30% (fixed) for piperacillin in CF-patients. The lower breakpoints for the data from Vinks et al. are a consequence of the fact that the clearances from Vinks et al. are about two times larger and more variable than our clearances.

$^5$: Please note that these PKPD breakpoints were derived based on data from eight CF-patients.
Table 7  Expectation values for the probability of target attainment for CF-patients and healthy volunteers based on the PKPD target for near-maximal killing ($f_{T > MIC} \geq 50\%$) and published MIC distributions (see method section for details)

<table>
<thead>
<tr>
<th>Source</th>
<th>Pathogen</th>
<th>Continuous infusion</th>
<th>Prolonged (4h) infusion</th>
<th>Short-term (30min) infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>9g/70kg WT / day</td>
<td>3g / 70kg WT q8h</td>
<td>3g / 70kg WT q4h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CF</td>
<td>H</td>
<td>CF</td>
</tr>
<tr>
<td>MYSTIC 2002, North America (34)</td>
<td><em>P. aeruginosa</em> (n=427)</td>
<td>82.2%</td>
<td>83.4%</td>
<td>82.2%</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> (n=433)</td>
<td>97.3%</td>
<td>97.4%</td>
<td>97.3%</td>
</tr>
<tr>
<td></td>
<td><em>K. pneumoniae</em> (n=288)</td>
<td>94.3%</td>
<td>94.8%</td>
<td>94.3%</td>
</tr>
<tr>
<td>MYSTIC 2002, South America (33)</td>
<td><em>P. aeruginosa</em> (n=233)</td>
<td>43.2%</td>
<td>44.2%</td>
<td>43.1%</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> (n=98)</td>
<td>85.6%</td>
<td>86.8%</td>
<td>85.6%</td>
</tr>
<tr>
<td></td>
<td><em>K. pneumoniae</em> (n=92)</td>
<td>71.3%</td>
<td>71.8%</td>
<td>71.3%</td>
</tr>
<tr>
<td>Hartford, CT, USA (35)</td>
<td><em>P. aeruginosa</em> (n=557)</td>
<td>92.7%</td>
<td>93.6%</td>
<td>92.7%</td>
</tr>
<tr>
<td><em>Isolates from CF-patients</em></td>
<td><em>P. aeruginosa</em>, non-mucoid strains (n=229)</td>
<td>64.5%</td>
<td>65.9%</td>
<td>64.4%</td>
</tr>
<tr>
<td>Freiburg, Germany (61)</td>
<td><em>P. aeruginosa</em>, mucoid strains (n=156)</td>
<td>65.5%</td>
<td>66.7%</td>
<td>65.4%</td>
</tr>
<tr>
<td>Leipzig, Germany (64)</td>
<td><em>P. aeruginosa</em> (n=38)</td>
<td>88.6%</td>
<td>89.5%</td>
<td>88.6%</td>
</tr>
</tbody>
</table>

Figure legends:

**Figure 1**  Average plasma concentrations of piperacillin for CF-patients and healthy volunteers after a single 5 min intravenous infusion of 4g piperacillin

**Footnote to Figure 1:** We did not calculate the average concentration, if less than three observations were quantifiable at the respective time point. Therefore, data for CF-patients are only shown up to 4 h post end of infusion (see also Figure 2).

**Figure 2**  Visual predictive check based on 8,000 simulated CF-patients and 26,000 simulated healthy volunteers for plasma concentrations for the two compartment model based on allometric scaling by LBM (see Table 3): The plots show the raw data, the 80% prediction interval [10 - 90% percentile] and the interquartile range [25 - 75% percentile]. Ideally, 50% of the raw data points should fall inside the interquartile range at each time point and 80% of the raw data should fall inside the 80% prediction interval.

**Figure 3**  Median and prediction interval [1 to 99% percentile] of the $f_{T_{>\text{MIC}}}$ at steady-state in CF-patients and healthy volunteers after continuous infusion of 9g / 70kg WT per day (dashed line, marker ▲), after 4h-infusion of 3g / 70kg WT q8h (continuous line, marker □), or after 30min-infusion of 3g / 70kg WT q4h (continuous line, marker ●)
Footnote to Figure 3: The curve for the 4h-infusion was shifted to the right by 6% in the MIC for easier identification of the corresponding prediction intervals [1 to 99% percentile]. The 1 and 99% percentiles for the continuous infusion were omitted for clarity. After continuous infusion all simulated CF-patients achieved a \( f_{T>\text{MIC}} \) of 100% at an MIC of 8 mg/L and all healthy volunteers had a \( f_{T>\text{MIC}} \) of 100% at an MIC of 12 mg/L.

Figure 4 Probability of target attainment for different dosage regimens of piperacillin at steady-state

Footnote to Figure 4: Importantly, Vinks et al. (74) found a clearance of 24.4 ± 11.7 L/h (average ± SD) in CF-patients for continuous infusion of 16 g piperacillin / 2 g tazobactam over 24 h. We found a clearance of 11.3 L/h (10.4% CV for between subject variability). The PTA vs. MIC profiles shown in this Figure are based on our population PK model (see Table 3). As the clearances from Vinks et al. (74) were about two times larger and more variable than our clearances, the PTA curves for continuous infusion in CF-patients would be shifted by a factor of about two to four towards lower concentrations in these plots, if one used the data from Vinks et al. for simulation.
Figure 2

Healthy volunteers

A) Plasma concentrations from 0 to 6.5 h

B) Plasma concentrations from 0 to 6.5 h

CF-patients

C) Plasma concentrations from 0 to 1.75 h

D) Plasma concentrations from 0 to 1.75 h
Figure 3

CF-patients

Healthy volunteers

FT>MIC

MIC (mg/L)

MIC (mg/L)
Figure 4

3g / 70kg WT q4h as 30min infusion
(daily dose: 18g)

3g / 70kg WT q8h as 4 h infusion
(daily dose: 9g)

9g / 70kg WT per day
as continuous infusion

\[\text{Probability of Target Attainment} \]

\[\text{MIC (mg/L)}\]

\[\begin{array}{c}
\text{0%} \\
\text{10%} \\
\text{20%} \\
\text{30%} \\
\text{40%} \\
\text{50%} \\
\text{60%} \\
\text{70%} \\
\text{80%} \\
\text{90%} \\
\text{100%}
\end{array}\]

\[\begin{array}{c}
8 \\
16 \\
32 \\
64
\end{array}\]

\[\text{\(\triangle\) 30% \(fT_{\text{MIC}}\): CF-patients}\]
\[\text{\(\bullet\) 30% \(fT_{\text{MIC}}\): Healthy volunteers}\]
\[\text{\(\diamondsuit\) 50% \(fT_{\text{MIC}}\): CF-patients}\]
\[\text{\(\blacksquare\) 50% \(fT_{\text{MIC}}\): Healthy volunteers}\]

30% \(fT_{\text{MIC}}\): target for bacteriostasis

50% \(fT_{\text{MIC}}\): target for near-maximal killing