In vivo infection models in the pre-clinical pharmacokinetic/pharmacodynamic evaluation of antimicrobial agents

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Animal infection models serve a critical role in the pre-clinical development of antimicrobials. Thoughtful use of these tools can be useful to design and de-risk subsequent clinical trials. Specifically, pharmacokinetic/pharmacodynamic (PK/PD) evaluation of antimicrobials can define the PK/PD driver and target magnitude. In doing so they provide guidance for dosing regimen design and forecast the likelihood of success against target pathogens at the infection site of interest. This review outlines the key design features to consider for successful assessment of experimental output.

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Introduction
The goal of both preclinical and clinical antimicrobial PK/PD investigation is to improve the probability of a positive therapeutic outcome. The premise underlying the PK/PD field of study is that there is an optimal drug exposure for efficacy and safety. Antimicrobial PK/PD traditionally links drug pharmacokinetics and a measure of potency in vivo (the minimum inhibitory concentration or MIC) to efficacy [1,2]. Animal infection models have been used to answer two key questions regarding pharmacokinetic optimization. First, which pharmacokinetic index is the strongest driver of efficacy, or simply put, how often do I need to administer the drug? Second, what is the PK/PD target, or how much drug do I need for effect? These PK/PD questions have been addressed using several animal infection models. However, the neutropenic mouse thigh and lung infection models are the traditional ‘work-horse’ models in the PK/PD field. The models represent relatively faithful mimics of soft tissue infection/sepsis and pneumonia, respectively [3]. When immunocompromised via neutropenia, most bacteria are pathogenic in the infection models. Organism burden, measured as number of colony forming units (CFU), at the site of infection provides a relatively reproducible measure of antibiotic effect that has accurately forecasted efficacy in patients. A variety of host, pharmacokinetic, and microorganism factors impact model performance and data interpretation. We discuss these study design variables and output assessment below.

Defining the PK/PD index
PK/PD studies have shown that antibiotics can be divided into two major groups: [1] those that exhibit concentration-dependent killing and prolonged persistent effects (e.g., aminoglycosides, fluoroquinolones), for which the area under the concentration–time curve (AUC) and peak concentration in relation to the MIC of the organism causing the infections (AUC/MIC and C_max/MIC, respectively) are the PK/PD indices correlating with efficacy; [2] those antibiotics that exhibit time-dependent killing and minimal-to-moderate persistent effects (e.g., beta-lactam and macroline classes), for which the time (expressed as a percentage of the dosing interval) that drug concentration exceed the MIC (%T > MIC) is the major driver of efficacy (Figure 1) [1]. Identification of the PK/PD indices that is most closely associated with efficacy requires dose-fractionation study [4]. In such studies, the same total drug exposure is administered using different dosing intervals. For example, a dose might be delivered as 400 mg once daily or in 4 equally divided doses of 100 mg throughout the day (i.e. every 6 hours). Regardless of dosing interval, each regimen would have nearly identical AUC/MIC values, but different %T > MIC and C_max/MIC values. Typically, when outcome is similar across dosing intervals the AUC/MIC is the associated PK/PD index. Conversely, when large doses administered infrequently result in the most efficacy, the C_max/MIC is commonly the PK/PD linked index. Finally, when the most frequently administered regimen produces the largest reduction in organism burden, the %T > MIC index represents the appropriate dosing parameter.

Identifying the PK/PD target
The goal of studies designed to discern the PK/PD target is to define the magnitude of PK measure relative to MIC needed for effect. We detail the approach to this question and highlight several experimental factors that can impact the determination of an accurate PK/PD target below.
Dose range and administration
The typical experiment will include a single dosing interval but wide dose range that brackets clinically relevant exposures in patients. Given the variation in clearance between mice and humans, it is common to attempt to 'humanize' the regimens to more closely mimic the shape of the exposure [5]. Typically, this includes reducing $C_{\text{max}}$ fluctuations associated with higher dose levels via more frequent administration of drug or continuous infusion pumps. The value of this practice is unclear from the standpoint of changes in the defined target, but is rational from the view of mimicking infection and treatment in patients.

Strain number
An additional component of importance for a robust PK/PD target study is incorporation of a sufficient number of strains, strain MIC variation (when possible), and inclusion of isolates with resistance genotypes and phenotypes of interest [6,7]. There is no widely agreed upon optimal number of isolates required for an adequate assessment. However, our group typically utilizes at least four strains per species. The major goal of a further increase in strain number is to account for the biological variation due to individual strains. As a 'rule of thumb', we suggest an increase in strain number above four until the median and mean target PK/PD values approach each other. There are two main reasons for model variability related to individual strains. The most common is a difference in organism fitness or growth in the model. In general, organisms with reduced fitness require a lower drug exposure for a given endpoint as shown in Figure 2. This variable is relatively simple to handle by requiring a certain level of control growth among strains. We typically require growth of 2–3 log$_{10}$ CFU/organ over the 24 hours treatment period for inclusion into a PK/PD target study. Additional reasons for variation among strains include differences in organism response to the antibiotic and human error. This problem can be addressed by increasing strain number in units of 1–3 per experiment.

Study endpoint
There is growing evidence linking microbiologic endpoints in these infection models to efficacy in patients [8,9,10]. The ability to translate results from one in vivo model to another is based upon the premise that the antimicrobial target is in the organism and not the host. Thus, despite difference in pharmacokinetics between mice and man, consideration of exposure relative to the MIC provides a normalization across host species. A generally accepted practice involves consideration of effect relative to the organism burden at the start of therapy. Exposure associated with both a stasis effect (eliminating outgrowth from the start of therapy) and killing endpoints have been evaluated for their predictive value in patients. There is not a consensus on which endpoint is most valuable for predicting efficacy in different infection types. However, there is general agreement that the endpoint chosen should be dependent upon the severity and expected burden of infection. For example, a net stasis (or no growth relative to the start of therapy) is likely sufficient for indications such as skin and soft tissue infection. Conversely, a more severe infection in the lung in hospitalized patients may be more closely linked to a killing target in the animal model.
Variable microbe fitness in the mouse model and PK/PD impact. The left panel shows variable growth of select bacterial species in the neutropenic murine lung model. The vertical bars represent the organism burden at the start and end of therapy. The right panel demonstrates the PK/PD treatment impact following study of two strains with different levels of fitness as shown by the red and blue symbols which represent control growth in the mouse lung infection model. SD is the static dose which differed 10-fold due to the less than 1 log difference in organism fitness.

[11,12]. There is growing but incomplete evidence to confirm these trends.

Infecting inoculum and timing of therapy
As the change in burden relative to the start of therapy is typically utilized, it is important to consider the quantity of the infecting inoculum. Typically, an inoculum of $10^9$ CFU/ml will result in a thigh or lung burden of $10^6$–$10^7$ CFU/tissue at the start of therapy. An additional variable that impacts the tissue organism load at the start of therapy is the time interval between infection and the start of antimicrobial therapy. Our group has traditionally utilized a 2 hour period from injection of microbes to first antimicrobial dose. The rationale for this design feature includes the absolute organism burden at therapy start and the time needed for organisms to return to early log phase growth.

A variety of investigations have explored the impact of a range of starting organism burden on the PK/PD target [13,14]. Typically, a lower burden at the start of therapy reduces the PK/PD magnitude. However, the degree of effect has been shown to vary among bacterial species and drug classes as shown in Figure 3. Importantly, the majority of animal model PK/PD study results that have been correlated with clinical outcomes have been based upon a standard $(10^6$–$10^7$ CFU/tissue) burden.

Neutrophils
A traditional component of the murine thigh and lung infection model is pharmacologic induction of neutropenia. The rationale for this study feature is to allow sufficient bacterial growth of organisms at the infection site and to remove control growth as a confounder in determination of antibiotic effect. In the presence of neutrophils, pathogenesis with individual bacterial isolates is variable and many strains are cleared from the tissues without antibiotic therapy. For the few

[High/Low Ratio](mg/kg)

Impact of inoculum on the PK/PD target. The data represent the difference in the drug dose needed to achieve a static effect in high $(10^9$–$10^7$ CFU/ml) compared to low inoculum $(10^2$ CFU/ml). The y-axis represents the ratio of the dose for high versus low inocula. The data represents a compilation from studies with four drugs and three bacterial species in the thigh infection model.
strains that do produce disease in non-neutropenic mice, investigations have explored the impact of this immune constituent on the PK/PD target. In general, the PK/PD magnitude is lower in the presence of neutrophils [15,16,17]. The quantitative impact of neutrophils on the treatment target varies among drug classes and also differs among bacterial species and can range from as little as 2-fold to more than 10-fold (Figure 4). It has been suggested by some that for patient scenarios without neutropenia, perhaps the non-neutropenic model would more effectively predict the PK/PD target. However, it is important to recognize that output from these animal models is not intended to determine the exposure needed for efficacy in neutropenic patients. In fact, the predominant translation of data from neutropenic animals to clinical outcome in patients has been in non-neutropenic patients.

Infection site and PK
A variety of host factors have been suggested to impact treatment efficacy. One of particularly profound influence is the infection site. Specifically, there are certain complex organ infection sites for which the preponderance of organism and drug vary relative to the interstitial space and plasma. The infection sites for which plasma concentrations can vary from the organ drug distribution include the urine, cerebral spinal fluid, some ocular compartments, and the epithelial lining fluid in the lung [18-20]. Emerging evidence suggests the relevance of PK in the lung as important for predicting efficacy. Adding complexity to this issue is the variable location of organisms within a complex organ for a given clinical scenario. For example, Streptococcus pneumoniae is primarily an extracellular pathogen and the majority of organisms are found in the epithelial space. In contrast, Legionella spp. reside in the intracellular space. Intuitively, for the former, antibiotic exposure in epithelial space would be expected to correlate closely with outcome. Importantly, penetration into the epithelial lining fluid (ELF) varies widely among antibiotics. This observation has been documented in both rodent models and patients. Thus, if pneumonia is a target for clinical development, assessment of effect in relation to ELF PK in murine models is imperative, but it is also essential to recognize the possibility of differential penetration of antibiotics into ELF in animals and humans [22-25]. Therefore, while measures of drug concentrations in ELF in animals and PD correlation to effect is useful, accurate translation to patients requires assessing ELF drug exposure in patients as well.

Protein binding
It is widely recognized that antimicrobial binding to host protein, especially albumin, can impact both pharmacokinetics and efficacy [26]. Most often, studies exploring the significance of protein binding have shown that only unbound antibiotic concentrations are available for microbiologic activity. This finding has led to the paradigm that antibiotics with a similar mechanism of action have similar PK/PD magnitude targets when free (non-protein bound) concentrations are considered. This observation is particularly critical for translation of animal PK/PD data to patients since the degree of binding can vary considerably between rodents and humans. Thus, similar to potential PK differences in ELF, measurement and consideration of protein binding differences in animal models and patients is needed for accurate translation to patients.

Human translation and conclusions
The PK/PD index and target can be used in drug development to select treatment regimens for select indications as well as for guidance in preliminary susceptibility breakpoints. The latter can be particularly important for determining the likelihood of treatment success against emerging drug-resistance pathogens that are difficult to study in randomized clinical trials [6,27-29]. As noted above, there are two general categories of caveats to guide successful translation of animal model PK/PD to patients. The first category relates to robust animal model study design, which includes selection of sufficient strain numbers with appropriate pathogenesis, MIC variation including those with resistance mechanisms, use of organism inocula that reflect the burden of disease in patients, and for most indications using the neutropenic form of these models. The second is to recognize the possibility of differences in tissue site pharmacokinetics (such as ELF) and protein binding. Finally, there are clinical scenarios that may not be well represented with these commonly used models. In particular, infection of the lower urinary tract and perhaps intra-abdominal abscesses.
may be poorly guided by the thigh model. Future model development with PK/PD assessment and clinical validation are needed. However, for many other common clinical infectious diseases, a robust animal model PK/PD package can significantly de-risk costly clinical antibiotic development programs.

Conflicts of interest
DA: Consultant and grant support — Astellas, Melinta, Actelion, Theravance, Zavante, Paratek, Cidara, Scynexis, Amplyx, Meiji, Geom.

AL: None.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:
• of special interest
•• of outstanding interest


Review of preclinical PK/PD translation to patients.


Study demonstrating the utility of the murine lung infection model for assessment of defined resistance mechanism on the PK/PD target.


Study highlights the importance of incorporating sufficient strain number to account for strain variability in PK/PD target identification studies.


Analysis of clinical trial results demonstrates the correlation between the preclinical model PK/PD target and treatment efficacy in patients.


Study explores the impact of organism burden on the PK/PD target.


This investigation demonstrates the impact of host neutrophils on the PK/PD target measure.


Study results underscore the relevance of infection site and ELF PK on delineation of the PK/PD target.


