SUMMARY

NIAID Workshop 'Pharmacokinetics-Pharmacodynamics (PKPD) for Development of Therapeutics against Bacterial Pathogens'

June 14-15, 2017, Bethesda, MD

Note: This workshop summary reflects the scientific opinions of the workshop speakers and the associated discussion during the workshop. While this document provides scientific recommendations, it is not meant to be a regulatory guidance.

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9:20 AM	Evolution of Clinical Development Approaches for Antibacterial Agents - John Tomayko (Advisor, Spero Therapeutics) – 20 min
9:40 AM	Evolution of Regulatory Landscape, And Challenges in Conducting A "Harmonized" Clinical Program That Meets Standards Across Geographies – Ian Friedland (Achaogen) - 20 min
10:00 – 10:10 AM	BREAK
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9 AM - 12 PM	**Session 4: Clinical PKPD**
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1:30 – 2 PM	Discussion – 30 min
2 – 3:30 PM	**Session 5: Stakeholders feedback and discussion**
	Moderators John Rex (F2G, Ltd., CARB-X), George Drusano (Univ. of Florida), and Jane Knisely (NIAID)
3:30 PM	Workshop summary and next steps – Ann Eakin and Tina Guina (NIAID)

3 SESSION 1: CLINICAL DEVELOPMENT CHALLENGES AND UTILITY OF PKPD MODELS

AGENDA	
Moderators: John Tomayko (Pfizer) and Tina Guina (NIAID)	
John Tomayko, Pfizer	Evolution of clinical development approaches for antibacterial agents
Ian Friedland, Achaogen	Evolution of regulatory landscape, and challenges in conducting a harmonized clinical program that meets standards across geographies
Paul Ambrose, Institute for Clinical Pharmacodynamics (ICPD)	Forecasting effective antimicrobial dosing regimens: reducing risk through pharmacometrics
Patricia Bradford, Antimicrobial Development Specialists, LLC	Using the MIC as the foundation for setting breakpoints
Seong Jang, FDA/CDER	Application of PKPD for regulatory decisions in anti-infective drug development: perspectives and challenges

3.1 JOHN TOMAYKO (PFIZER) AND IAN FRIEDLAND (ACHAOGEN) - EVOLUTION OF CLINICAL DEVELOPMENT APPROACHES FOR ANTIBACTERIAL AGENTS AND CHALLENGES IN CONDUCTING CLINICAL TRIALS

The decades of the eighties and nineties were the era of abundant new antibiotic launches. Developers conducted large studies yielding multiple indications for agents that produced mainly small, incremental benefits over existing therapies. Non-inferiority trials comparing a test agent to a standard of care were the typical approach to registration. These trial designs were loose, often lumping in several body sites of infection with different expected outcomes, and less attention was given regarding trial eligibility criteria. During these times, antimicrobial resistance wasn't a major concern; several of the agents of this era remain gold standard treatments for infections today. However; paralleling this success, the field of pharmacodynamics was making great advances while clinical scientists and regulators were recognizing shortcomings in poorly designed non-inferiority trials. A new era was emerging, where the importance of pharmacodynamics was recognized early and its role in guiding dose selection aimed at a desired breakpoint was becoming a common practice. Regulators also began to tighten the parameters in non-inferiority trial design, essentially requiring larger studies with more discerning eligibility criteria.

By the middle of the first decade in the new millennium, most of the highly regarded "gold standard" antibacterial therapies such as the carbapenems and cephalosporins became generic. At the same time resistance was emerging to these classes, particularly in Gram-negative pathogens responsible for nosocomial infections in vulnerable patient populations. This progressive emergence of resistance was recognized as a major threat to both the public and to medical progress. Though developers recognized the unmet need for new agents active against emerging antibacterial resistance, the science of finding these agents remained difficult and the regulatory requirements were driving up costs of development. Even with regulatory approval of a novel antibiotic active against these resistant pathogens, use would be limited to settings where such resistance

was likely or confirmed, eliminating the developer's opportunity to recoup the investment made. Many large companies abandoned their effort in this area[1].

Recognizing these issues, remaining industry developers and regulators began to think differently about the requirements of clinical data to support new agents aimed at treating serious and life-threatening infections resulting from the growing numbers of highly resistant pathogens (1).

In 2013 both Food and Drug Administration (FDA)[2] and European Medicines Agency (EMA)[3] issued guidance documents enabling streamlined development programs, leading to approval with the caveat that agents should only be used in the setting of limited therapeutic options. These clinical programs are to be supported by a robust pharmacokinetic/pharmacodynamic (PKPD) package of studies. Since that time several antibiotics have been approved utilizing this approach.

Nonetheless, as FDA and EMA produced their guidance documents independently, many differences exist between them, adding complexity and expense to global development programs. Currently, for streamlined development programs, some areas of non-alignment between Agencies include the definition of unmet need, study design elements such as primary endpoints, acceptable noninferiority margins and patient population definitions. It is also not clear how the approval standards, especially acceptable patient database size, might differ between FDA and EMA. Fortunately, the FDA, EMA and Pharmaceuticals and Medical Devices Agency (PMDA) have recently begun a series of tripartite meetings to improve harmonization between agencies[4].

However, both FDA and EMA agree that robust PKPD data are central to streamlined development programs, but the exact scope of such data is only loosely defined. For example, the balance between animal versus in vitro model data and the number and types pathogens to include are not specified.

Conduct of clinical trials to demonstrate the efficacy against drug-resistant bacteria species is challenging, mainly because of lack of sufficient patients who are infected with target bacteria species, and well designed non-inferiority trials in patients infected with usual drug resistant pathogens provide the pivotal data (2). Thus, it is important to consider how other information, like PKPD, can support clinical effectiveness of new antibacterial drugs. Fortunately, the effect of an antibiotic in an animal model of infection can be translated to an anticipated similar effect in a human infection.

Any discussion of PKPD must begin with a minimum inhibitory concentration (MIC) of an antibiotic against a pathogen of interest. The MIC is the foundation on which we build our understanding of the relationship of the dose of antibiotic given to patients, the pharmacokinetics and the response of the infecting organism. Probability of target attainment (PTA) analysis, a specific analysis for antimicrobials has been used to support interpretive criteria for bacteria susceptibility and to determine doses to be evaluated in clinical studies. PTA analysis estimates percent of patients who achieve the magnitude of PKPD index [i.e., AUC:MIC ratio, Cmax:MIC ratio, or % time of dosing interval that drug concentrations are greater than MIC ($\%T_{Cf>MIC}/\tau$)] greater than a PKPD target at given MICs, using a PKPD target determined from nonclinical studies (i.e., in vitro Hollow-Fiber

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1 IDSA, Bad Bugs No Drugs: As Antibiotic Discovery Stagnates A Public Health Crisis Brews

2 https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm359184.pdf

3 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/11/WC500153953.pdf

4 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/11/WC500153953.pdf

system or infected animal studies) and PK distribution in simulated patient population (PK simulation)[5]. All this information is then used to set the interpretive criteria, or breakpoints, that are used to determine the susceptibility category reported following susceptibility testing.

References:

1. Rex, J.H., et al., A comprehensive regulatory framework to address the unmet need for new antibacterial treatments. Lancet Infect Dis, 2013. 13(3): p. 269-75.
2. Rex, J.H., et al., Progress in the Fight Against Multidrug-Resistant Bacteria 2005-2016: Modern Noninferiority Trial Designs Enable Antibiotic Development in Advance of Epidemic Bacterial Resistance. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 2017. 65(1): p. 141-146.

3.2 Paul Ambrose (ICPD) - Forecasting effective antimicrobial regimens: Reducing risk through pharmacometrics

The goal of the lecture was to review four topics associated with the forecasting of effective antimicrobial regimens. These included a review of important preclinical models and their simplifying assumptions, conditions under which these simplifying assumptions breakdown, PKPD of antibiotics in patients and how PKPD can be used to forecast effective antibody dosing. Emphasis was placed upon how a well orchestrated combination of preclinical infection model data in combination with human pharmacokinetic information should be integrated to reduce the risk of anti-infective agent development.

First, was a discussion surrounding the questions our basic PKPD models answer and don't answer. Focus was placed on our workhorse infection models, including the murine (1), one-compartment (2), and hollow-fiber in vitro infection models (3). Rather than present a cookbook of studies to do, a philosophy was presented where one uses all three models to answer different but sometimes overlapping questions.

The discussion also addressed the conditions where our traditional PKPD assumptions deviate from the norm, i.e., unusually long or short half-lives. On one end of the spectrum, a drug with an unusually long half-life with a concentration-dependent pattern of bactericidal activity may benefit from large single or infrequent dosing. This concept was illustrated with the case of azithromycin, oritavancin, and CD101, which is an antifungal agent currently in clinical development. On the other end of the spectrum, a drug with an unusually short half-life in animals presents a dose-forecasting challenge for the drug developer. In such a circumstance, use of a new PKPD index (AUC:MIC X 1/Tau) may be useful gain better certainty around human dose predictions (4). This concept was illustrated with the case of Geom-101, which is a siderophore cephalosporin currently undergoing development.

Subsequently focus shifted to a PKPD explanation of why some development programs were predictable failures and others were successes (5). The failures were most often associated with underestimations of drug clearance in subpopulations and a poor understanding of drug susceptibility in the target patient population. It was recommended that target patient pharmacokinetic information and susceptibility data be obtained prior to the conduct of clinical trials.

[5] http://www.ema.europa.eu/docs/en_GB/document_library/Other/2016/09/WC500212649.pdf

Finally, discussion of a dose selection paradigm for antibodies was discussed. This concept was illustrated with ASN101, an antibody that binds multiple staphylococcal toxins. The strategy involved the construct of an integrated minimal physiologic-based pharmacokinetic model integrated with pharmacodynamic model. This big model integrated multiple sub-models, including infected and non-infected rabbit pharmacokinetic models, a healthy human pharmacokinetic model, antibody toxin binding rates, and several other inputs, with the goal of predicting infected patient PK and subsequently effective human dosing regimens.

References:

1. Craig, W.A., Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis, 1998. 26(1): p. 1-10; quiz 11-2.
2. Ambrose, P.G., et al., Bacterial Replication Rate Modulation in Combination with Antimicrobial Therapy: Turning the Microbe against Itself. Antimicrob Agents Chemother, 2017. 61(1).
3. Tam, V.H., et al., Bacterial-population responses to drug-selective pressure: examination of garenoxacin's effect on Pseudomonas aeruginosa. J Infect Dis, 2005. 192(3): p. 420-8.
4. Lakota, E.A., et al., Traditional PKPD indices for efficacy - can we do better. ID Week, 2017.
5. Ambrose, P.G., Antibacterial drug development program successes and failures: a pharmacometric explanation. Curr Opin Pharmacol, 2017. 36: p. 1-7.

3.3 Patricia A. Bradford (Antimicrobial Development Specialists, LLC) - Using the MIC as the foundation for setting breakpoints

Any discussion of PKPD must begin and end with a minimum inhibitory concentration (MIC) of an antibiotic against a pathogen of interest. The MIC is the foundation on which we build our understanding of the relationship of the dose of antibiotic given to patients, the pharmacokinetics and the response of the infecting organism. All this information is then used to set the interpretive criteria, or breakpoints, that are used to determine the susceptibility category reported following susceptibility testing. The breakpoint is defined as a classification based on an in vitro response of an organism to an antimicrobial agent at concentrations corresponding to blood or tissue levels attainable with the most commonly prescribed dosing regimens. Using the breakpoints, a susceptibility test result for an antibiotic is reported to a treating physician as susceptible (S), intermediate (I), resistant (R), or non-susceptible (NS) .

Breakpoints are set after examining data from three sources; MIC distributions to determine epidemiologic cut off, PKPD including percent target attainment to determine the PKPD cutoff and the microbiologic outcomes of patients treated during the clinical trials to determine the clinical cutoff. MIC values of the antibiotic under study impact all three of these pieces of data. When considering the MIC distributions that are used in setting breakpoints, it is important to consider the characteristics and the source of the isolates. For example, Klebsiella pneumoniae may carry a variety of β-lactamases that respond very differently to cephalosporins or carbapenems. The MIC distribution could easily be skewed by the inclusion or exclusion of some of these strains. In addition, some genera are inherently resistant to a certain antibiotic. Proteeae test resistant with tigecycline, therefore MIC distributions with Enterobacteriaceae look markedly different if the Proteeae are included. Furthermore, isolates may display very different MIC patterns depending on the geographical location of isolation. Klebsiella pneumoniae isolated in Greece show a high percentage of resistance to carbapenems, whereas the incidence in the USA remains fairly low.

Breakpoints are also a tool that can be used in preclinical discovery programs for antibiotics. They should be considered early and used as a yardstick to measure program goals. These preliminary breakpoints should be reexamined in an ongoing iterative process during the life of the project (1-4).

References:

1. Turnidge, J. and D.L. Paterson, Setting and revising antibacterial susceptibility breakpoints. Clin Microbiol Rev, 2007. 20(3): p. 391-408, table of contents.
2. Andes, D. and W.A. Craig, Animal model pharmacokinetics and pharmacodynamics: a critical review. Int J Antimicrob Agents, 2002. 19(4): p. 261-8.
3. CLSI, M23 Development of in vitro susceptibility testing criteria and quality control parameters. Wayne, PA: Clinical and Laboratory Standards Institute, 2016.
4. EUCAST, Standard Operating Procedure - Setting breakpoints for new antimicrobial agents. 2016.

3.4 SEONG JANG (FDA/CDER) - APPLICATION OF PKPD IN ANTIBACTERIAL DRUG DEVELOPMENT: CURRENT CHALLENGES AND FUTURE PERSPECTIVES

Although many antibacterial drugs are approved and available, there are unmet medical needs for new antibacterial drugs targeting narrow spectrum of bacteria, including drug-resistant bacteria species. However, conduct of clinical trials to demonstrate the efficacy against drug-resistant bacteria species is challenging, mainly because of lack of sufficient patients who are infected with target bacteria species. Thus, it is important to consider how other information, like pharmacokinetics and pharmacodynamics, can support clinical effectiveness of new antibacterial drugs. Probability of target attainment (PTA) analysis, a specific analysis used for antimicrobials (see below for further details) has been used to support interpretive criteria for bacteria susceptibility and to determine doses to be evaluated in clinical studies. Currently, it is challenging to use the results of PTA analysis as supportive evidence for clinical effectiveness when sufficient clinical trials to demonstrate the efficacy are not conducted.

Briefly, PTA analysis estimates percent of patients who achieve the magnitude of PKPD index [i.e., AUC:MIC ratio, Cmax:MIC ratio, or % time of dosing interval that drug concentrations are greater than MIC ($\%T_{Cf>MIC}/\tau$)] greater than a PKPD target at given MICs, using a PKPD target determined from nonclinical studies (i.e., in vitro Hollow-Fiber system or infected animal studies) and PK distribution in simulated patient population (PK simulation). The robustness of PKPD target and PK simulation determines the uncertainty level of the results of PTA analysis. First, PKPD targets determined from nonclinical studies often vary with animal infection model (e.g., thigh infection model or lung infection model) and bacteria species (and number of isolates) used in animal infection model. It is preferable to determine a PKPD target in an animal infection model that mimics target indication (e.g., lung infection model for pneumonia) with sufficient number of isolates of target bacteria species. Second, PK simulation, mainly based on population PK models, is dependent upon the quality and quantity of PK data for simulation. PK and its variability are often different from indication to indication, as well as between healthy subjects and infected patients. Ideally, conducting PTA analysis based on sufficient PK data obtained from patient populations with the target indication(s) and a PKPD target determined in an animal infection model that mimics target indication with sufficient number of isolates of target bacteria species reduces uncertainty (or increases robustness) of PTA analysis. However, such data for an ideal PTA analysis are not always available, especially during development of new antibacterial drugs. In such cases, some degree of uncertainty in PTA analysis may need to be accepted, depending on the purpose of the PTA analysis in drug development stages. For example, the results of PTA analysis based on PK data obtained from healthy subjects

and a PKPD target determined in a mouse thigh infection model with a limited number of bacteria species may be acceptable to determine the doses to be evaluated in a Phase 2 dose-ranging study. These data, however, are not sufficient, to determine the dose to be evaluated in a pivotal Phase 3 study when a Phase 2 trial is skipped. Likewise, in order to use the results of PTA analysis as supportive evidence for clinical effectiveness, a high level of robustness of a PKPD target and PK simulation is essential. Last, in general, a PKPD target for PTA analysis is determined based on changes in bacterial loads from the baseline (e.g., net-stasis, 1-log or 2-log reduction in bacterial burden (colony forming units, CFU, per gram of tissue)) from animal infection models, but not based on efficacy endpoints from clinical studies. Currently, the changes in bacterial loads to determine PKPD targets for different infections are empirically selected (e.g., net-stasis for complicated urinary tract infection or 1-log reduction for bacterial pneumonia) without fully understanding how much reduction in bacterial loads in an animal infection model is needed for clinical efficacy. Selecting different target bacterial load reduction in animal studies results in a different PKPD target and, in turn, different results of PTA analysis. Thus, understanding the relationship between reduction in bacterial loads in an animal model and clinical effectiveness for different infections is also essential.

Decision-making based on the results of PTA analysis becomes more important and critical for the development of new antibacterial drugs targeting narrow spectrum of bacteria. However, one should take into consideration its potential risks and benefits, which vary with the purpose of PTA analysis in each development stage and the data quality/quantity for PKPD target determination and PK simulation. If the potential risks are not addressed, decisions made based on the results of PTA analysis may lead to erroneous conclusions. It should be noted that there were clinical trials that failed to demonstrate clinical efficacy although successful clinical outcome was predicted based on previous information including the results of PTA analysis (1-3).

References:

1. DORIBAX® (doripenem for injection) [Package Insert]. Florham Park (NJ): Shionogi, Inc, 2015. Available from https://www.shionogi.com/pdf/pi/doribax.pdf

2. TYGACYL® (tigacycline) [Package Insert]. Philadeliphia (PA): Pfizer Injectables, 2016. Available from http://labeling.pfizer.com/ShowLabeling.aspx?id=491

3. Freire AT, et al. Comparison of tigecycline with imipenem/cilastatin for the treatment of hospital-acquired pneumonia. *Diagn Microbiol Infect Dis* (2010) **68**: 140-151.

4 SESSION 2: IN VITRO PKPD MODELS

AGENDA	
Moderators: Dan Pevear (VenatoRx) and Francois Franceschi (NIAID)	
Vincent Tam, University of Houston	Overview of in vitro pharmacodynamics (PD) methods, their advantages, limitations and best practices
Arnold Louie, University of Florida	
Alasdair McGowan, University of Bristol	

4.1 SESSION 2 SUMMARY

Session 2 focused on the overview of in vitro pharmacodynamic (PD) methods, their advantages, limitations and best practices that were discussed by Vincent Tam (University of Houston), Arnold Louie (University of Florida) and Alasdair McGowan (University of Bristol). The best practices (A. Louie) discussion reviewed approaches to bacterial isolate selection, inoculum and isolate mutation frequency, PK measurements, drug solubility and stability, duration of therapy, and evaluation of possible causes of regrowth. Considerations for bacterial strain selection, impact of heterogeneous drug biodistribution and protein binding (V. Tam), and bactericidal vs. bacteriostatic targets, timing of endpoints and combination therapy considerations (A. MacGowan) were also discussed.

Widely used *in vitro* models that evaluate drug exposure-response relationships include time-kill models, 1-compartment systems (chemostat), and 2-compartment systems (hollow fiber infection models). The summary of the characteristics of three in vitro models and comparison to the mouse models is shown in **Table 1**. Time-kill exposure-range studies use static concentrations of antibiotics to evaluate bacterial survival upon exposure to varied drug concentrations. The advantages of using time-kill assays are the low cost and minimal equipment needed. These assays can evaluate the effect of bacterial inoculum on drug activity, help define whether microbial killing is drug concentration-dependent or time-dependent, evaluate drug interactions in combination studies, and identify drug exposures that maximize killing. Plating on media +/- drug should be utilized to determine impact of a drug exposure on both the total bacterial population in the culture as well as the less-susceptible population(s). These data reveal the optimal dosing of drug which could prevent growth of the less-susceptible populations that may lead to development of resistance. Time-kill assays are good screening tools for assessing drug structure-activity relationship (SAR) and for choosing drug exposures suitable for evaluation in longer duration, more complex 1- and 2-compartment systems.

Time-kill assay limitations are static drug exposures and typically short duration (24 hr) of the assay, although timing of the study can be extended with replacement of the media and drug (e.g., each 24 hrs). Care must be taken to monitor risk of depletion of nutrients and change in medium pH in these experiments that may result in an alteration of antibiotic potency, accelerated drug degradation, change in bacterial growth, metabolic state, or bacterial expression of resistance mechanisms.

An advantage of the ***in vitro* 1-compartment/chemostat pharmacodynamic (PD) model** over the time-kill studies is the ability to simulate in vivo PK profiles and fluctuations that are observed in animal efficacy models or humans. This flexibility enables the investigation of the effects of different drug dosing regimens and simulated drug half-lives on bacterial killing. With the continuous replenishment of growth medium and

nutrients, the 1-compartment system allows performance of longer term dose-range and dose fractionation studies, and can be used to evaluate antibiotic combination therapy. The chemostat is an improved model system for studies of emergence of drug resistance and resistance suppression studies. Chemostat limitations include the potential for the washout of bacterial cells and contamination of the media, particularly over longer duration studies (for this reason most published studies last 1 – 3 days). Simulating short half-lives can result in the wash out of a considerable number of the parental strain and less-susceptible bacteria, resulting in an underestimate of the drug dose or exposure needed for bacterial killing and resistance prevention.

In the *in vitro* 2-compartment hollow fiber infection model (HFIM), bacteria are contained within the peripheral compartment of hollow fiber cartridges. Hence, with the HFIM it is possible to simulate PK profiles with no bacterial cell washout and is suitable for simulated dose-ranging and dose fractionation studies to determine resistance prevention exposure for a range of simulated PK profiles. HFIM experiments can run for 10 or 14 days to simulate the durations of antibiotic(s) typically prescribed to human patients for the treatment of serious bacterial infections. If needed, studies can run for >6 months. Speakers agreed that the HFIM is the preferred in vitro PD model for dosing determination, and for establishment of PD indices for maximal bacterial killing and drug resistance prevention. HFIM is also a better predictor of efficacy for drug combination regimens. Since bacteria are not washed out of the HFIM it is also the best *in vitro* pharmacodynamic system for studies with highly communicable or virulent BSL-3 pathogens (e.g., Tier 1 select agent bacteria, or *Mycobacterium tuberculosis*). HFIM limitations include relatively high cost when compared to time-kill and chemostat studies and they are more difficult to set-up and run. Some drugs bind to HFIM components which hinders their testing in this model.

Importantly, none of the *in vitro* systems are suitable for pharmacodynamic evaluation of aminoglycosides as single agents because this drug class readily generate bacterial small colony variants that may not be seen *in vivo*. The *in vitro* systems may overstate the aminoglycoside dose intensity needed to kill the drug-susceptible parent strain and to prevent resistance emergence. However, the *in vitro* systems are excellent in evaluating the efficacy of aminoglycosides as part of combination regimens. Furthermore, for drugs that have a biologically active metabolite which contributes to the overall bacterial killing activity of the antibiotic, both the parent compound and the metabolite should be evaluated individually and together at the ratios found at the infection site in order to most accurately quantify the bacterial killing and resistance prevention potential of the antibiotic. Also, for drugs which are administered to people as prodrugs, such as tedizolid, ceftaroline or colistin methanesulfonate, the biologically active compound should be used in the *in vitro* pharmacodynamic systems (1).

Best practices for *in vitro* PD models need to take into consideration all model components. The experimental design is critical as PKPD data may guide the selection of the MIC range to support a proposed antibiotic breakpoint value. Bacterial strain MIC, antibiotic resistance profile/mechanism, and inoculum size in the PD studies need to be relevant to clinical indication and infection site. For example, high bacterial inoculum (>=1E8) is typically used in studies that target hospital-acquired and ventilator-associated bacterial pneumonia (HABP/VABP) indications, and in resistance prevention studies. Studies that use an inoculum of <1E5 are not relevant to most clinical indications and should not be used. The in vitro PD systems enable testing of multiple bacterial strains and the most robust PKPD analysis requires examination of multiple strains. Care must be taken when selecting the most relevant strains for these experiments to ensure they fully represent the range of target pathogens expected in the clinic. Bacterial strains which display the lowest resistance mutation frequency of resistance should be avoided in dose-ranging studies; instead strains which best represent the most commonly observed resistance rates are preferred. Investigators should include appropriate reference strains

into each study to control the quality and reproducibility of baseline results throughout the program. Strains with various resistance mechanisms and enzyme variants, and wide range of susceptibility to drug of interest also should be included. Investigating a range of PK exposures against bacterial stains with different MICs, an extended range of PKPD exposures could be attended, which would facilitate optimal target selection.

Table 1. Experiments which can be performed with widely used pharmacodynamic (PD) models.

Task	Time-kill assay	1-compartment system	2-compartment hollow fiber system	Mouse
1. Dose-range (kill of parent strain)	Yes	Yes	Yes	Yes
2. Dose-range (resistance prevention)	+/-	+/-	Yes	+/-
3. Dose-fractionation (kill of parent strain)	No	Yes	Yes	Yes
4. Dose-fractionation (resistance suppression)	+/-	+/-	Yes	+/-
5. Combination therapy	Yes	Yes (short term)	Yes	Yes
6. Combination therapy for resistance suppression	No	+/-	Yes	No
7. Toxin suppression by drugs	Yes	+/-	Yes	Yes
8. Dissect interaction of parent drug and metabolites on antimicrobial effect	+/-	+/-	Yes	No
9. Bacterial physiologic state & drug activity	+/-	+/-	Yes	?
10. PD index for drug toxicity	No	No (unless toxicity is acute)	Yes	Yes, murine PK

The importance of measuring drug concentrations in the model systems was also highlighted as a best practice, rather than relying on modeling of expected drug exposures. The measured exposures can be correlated with biological effect, whether it is the amount of bacterial killing associated with a drug exposure or resistance suppression or amplification. Measured drug exposures may explain unexpected results if the simulated PK profiles are higher or lower than targeted. Data points that meet a predetermined statistical definition of "outlier" (e.g >1 standard deviation, SD, from mean) should not be automatically discarded from analysis, as these outliers may be an indication of unexpected but important behaviors of the drug. Drug solubility and stability in the matrix (solvents and medium), at the environmental temperatures that will be used in *in vitro* PD studies for the given period of time expected for the assay to last, need to be established. Some drugs have limited solubility and their concentrations may decrease over time due to slow precipitation.

Ideally, the duration of the *in vitro* 1- and 2-compartment studies should mimic the durations of antibiotic therapy used in human patients for the treatment of infections with specific bacterial pathogens that cause, for example, a complicated urinary tract infection, a soft tissue infection, a community-associated pneumonia or VABP. Furthermore, to evaluate for drug dosages and regimens which minimize resistance amplification, the duration of these studies should be at least 5-7 days since this amount of time may be required for some drugs before resistance amplification is observed.

Simulation of systemic exposures in any *in vitro* system should **reflect exposures at the site of infection specific to clinical indication** (e.g., lung vs. bladder) whenever possible. Some drugs have heterogeneous PK distribution between major tissue/organ systems. For example, polymyxin B accumulates mostly in the mouse kidney (2) with serum exposures being typically several fold higher than in epithelial lining fluid (ELF) (3).

In pharmacodynamic studies, the effect of an antibiotic regimen on the killing of a parent bacterium is determined by quantitative culturing bacterial suspensions serially taken from the pharmacodynamic system onto drug free agar. In contrast, the effect of the drug regimen on the amplification or killing of the less-susceptible bacterial subpopulations is assessed by quantitative culture in an aliquot of the same bacterial suspensions onto agar supplemented with the antibiotic administered to that experimental arm. Determination of the mutation frequency (MF) of the parent bacterium to the administered antibiotic prior to conducting the pharmacodynamic experiment provides guidance to the size of the bacterial inoculum which should be evaluated for treatment effect and in the concentration of antibiotic to add to the drug-supplemented agar to assess the effect of the antibiotic regimen on the less-susceptible bacterial subpopulation(s). For most antibiotics, one day of incubation of the cultures before enumerating colonies on drug-supplemented agar is too short and may provide an underestimation of the bacterial population with reduced susceptibilities to the test antibiotic. Additional colonies may become visible on the culture after another 24 to 48 hours of incubation. The duration of incubation of antibiotic-supplemented agar prior to enumerating colonies on the agar should consider whether the drug is structurally stable or degrades. If the drugs, (such as the beta-lactam antibiotics) degrade, the duration of incubation should be guided by the rate of degradation of the antibiotic in agar when incubated at 35 ^{0}C, limiting the incubation time to when the concentration of the drug is expected to be above the MIC of the parent strain. It is important to perform antibiotic susceptibility studies for a subset of colonies which grow on antibiotic-supplemented agar to validate that the MICs of these isolates are indeed higher than the MIC for the parent isolate.

MF studies quantify the prevalence of pre-existing bacterial subpopulation(s) with reduced susceptibilities (higher MICs) to a drug that are already within the larger wild-type, parent bacterial population prior to the start of antibiotic treatment. The MF is calculated by dividing the number of colonies that grow on drug-free agar that is quantitatively cultured for the total bacterial population by the number of colonies that grown on agar supplemented with a multiple of the MIC of the antibiotic of the parent bacterial isolate. There is no standard method for conducting an MF study as it pertains to the multiple of MIC for the parent isolate which is evaluated nor the duration of time the agar plate is incubated before the MF value is calculated. For pharmacodynamic studies assessing the effect of drug regimens for resistance prevention, the *total number of bacteria* inoculated into each experimental arm should be ideally at least 1 log CFU higher than the MF value to ensure each arm contains pre-existing mutants with higher MICs than the parent isolate. Typically, the MF values for the first-step mutants with reduced susceptibility to an antibiotic are between -5 and -8.7 log CFU. Also, for pharmacodynamic studies evaluating the effect of an antibiotic regimen on the killing or amplification of the less-susceptible bacterial subpopulation, the concentration of drug added to agar plates should be between the MICs of the parent isolate and the first-step mutant. Bacterial samples collected from the pharmacodynamic

systems can also be quantitatively cultured onto agar containing drug concentrations equal to or higher than the MIC of the first-step mutant to evaluate for bacterial subpopulatons with second and third step mutations.

But evaluation of the effect of antimicrobial regimens on the amplification of killing of pre-existing first step mutations is requisite because the exposures of some drugs required to prevent the amplification of bacterial subpopulations expressing two or more resistance mechanisms may not be achievable with proposed or prescribed doses or may be toxic to humans. Also, for some drugs, killing the first-step mutants may prevent the generation of bacteria which acquire a second resistance mutation. An example study (4) evaluated emergence of resistance to fluoroquinolones in *Streptococcus pneumoniae* using a 1-compartment chemostat PD model. Bacterial cultures were exposed to free (non-protein bound) concentration-time profiles simulating those in humans treated with ciprofloxacin and levofloxacin to determine dosing regimens that result in prevention of resistance emergence. Mutations leading to resistance to levofloxacin identified were the first-step mutation efflux pump overexpression, which increased levofloxacin MICs by 2-fold. Second step mutations in *gyrA* or *parC* resulted in increase in levofloxacin MICs by 4-fold. Administering an efflux pump inhibitor in combination with ciprofloxacin and levofloxacin prevented the amplification of the bacteria expressing the efflux pump first-step mutation which, in turn, stopped the bacteria from acquiring *gyrA* or *parC* second-step mutations.

Crucial questions in the course of designing *in vitro* PD studies to provide results that translate to clinical efficacy include: Is the drug bactericidal or bacteriostatic and which observed endpoint (e.g stasis, 1-log kill, 2-log kill) is the most relevant target for the clinic? Which bacterial species and strains best define the PKPD target for each indication? What duration of experiment best predicts efficacy and likely resistance development in the clinic?

A number of in vitro studies showed that PKPD index (PDI) values vary among bacterial species and strains, which may have implication on translation to broad coverage and clinical utility of antibiotics (5-7).

The impact of **protein binding** on PDI is significant because the free fraction of drug is typically considered to be pharmacologically active. Binding saturation may result in exaggerated estimates of free drug fraction at high concentrations, and atypical binding kinetics have implications on dose escalation. Protein binding may result in over-inflation of PDI when AUC is very low, e.g., when protein binding is at ~99%, there is a large standard deviation that affects PDI calculations.

In vitro PD models are valuable in evaluation of **drug combinations**. When testing beta-lactam/beta lactamase inhibitor (BL/BLI) combinations, fixed concentrations of beta lactam are combined with varying concentrations of BLI and tested against multiple beta-lactam sensitive and resistant bacterial species and strains with different mechanisms of resistance (6-7, 9-11). Drug combinations studies in in vitro PD models are invaluable for testing ability of the combination to suppress resistance emergence (12-13).

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5 SESSION 3: ANIMAL PKPD MODELS

AGENDA	
Moderators: Jennifer Hoover (GlaxoSmithKline), Eileen Kim (Achaogen), and Ann Eakin (NIAID)	
David Andes, University of Wisconsin	Mouse Models for Antibacterial PKPD: Validation, History & Performance Variables
Jürgen Bulitta, University of Florida	Quality PK Data to Support PKPD and Translational Analyses
William Hope, University of Liverpool	Translation of Animal Model Data to Clinical Efficacy
Jennifer Hoover, GlaxoSmithKline	Basing Compound Progression Decisions on Animal Model Data

5.1 BACKGROUND

Goals of animal infection models: The purpose of conducting pharmacokinetic / pharmacodynamic (PKPD) studies in laboratory animal models is to identify effective dosing regimens for clinical trials. Although dosages, drug clearance (including metabolism), and other factors often differ considerably between animals and humans, in vivo models have a critical role for characterizing the PKPD for antibacterial agents. There are several reasons why our field relies heavily on these models: 1) contrary to other therapeutic areas, the antimicrobial drug target is the pathogen rather than the host; 2) PKPD targets are not isolate-specific since the drug exposure required for efficacy is normalized according to the MIC of the infecting pathogen; 3) animal models provide an in vivo infection environment and anatomical barriers which are difficult to reproduce in vitro; and 4) drug exposure profiles in animals can be matched to mirror those in humans. All these factors contribute to the value of animal infection models for antimicrobial drug development (**Figure 1**). It has been shown that PKPD infection models do forecast success in patients, and the probability of regulatory approval increases with the probability of PKPD target attainment (1, 2).

Common animal models: The most widely used models for anti¬bacterial PKPD are the murine thigh and lung infection models that are mimics of soft tissue infections and pneumonia, respectively. These models typically use immunocompro¬mised (neutropenic) mice to allow growth of a range of bacterial pathogens. Select isolates will also produce robust infections in normal (i.e., non-neutropenic) mice, which provide additional context regarding the contribution of the immune response to the efficacy of the drug. The primary endpoint is reduction of the bacterial burden in the infected tissue, which is typically assessed 24 or 48 h after initiation of antibiotic therapy. This endpoint in mice correlates with outcomes in patients (2-4).

Utility to support clinical drug development: In conjunction with safety considerations, animal infection models support the selection of clinical dosing regimens and the determination of in vitro susceptibility breakpoints. Although other animal models may also be used to characterize PKPD relationships, one particular benefit of the murine neutropenic thigh and lung models is that data are publicly available for a number of antibacterial agents that can be used as positive controls.

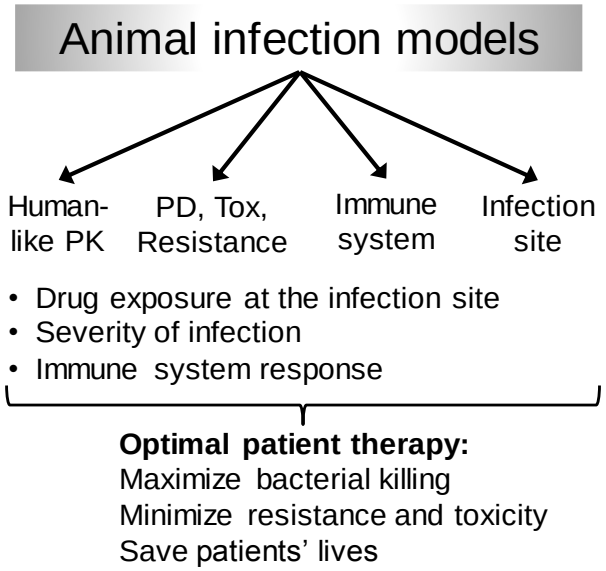

Figure 1. **Overview of important variables which contribute to the outcome of animal infection models.** These factors may need to be considered for study design and execution as well as for the data analysis and ultimate translation of rationally optimized regimens to patients.

5.2 RECOMMENDATIONS FOR STUDY CONDUCT AND ANALYSIS

5.2.1 Pharmacodynamic studies

There is considerable variation among laboratories in the design and conduct of PKPD models. However, "best practice" recommendations have already been developed based on experiments that have been shown to predict clinical success (2-4). Andes and Lepak have thoroughly reviewed this topic (5), and a summary is provided in **Table 2**. It should be noted that some of these recommendations may need adaptation to accommodate specific characteristics of any drug-pathogen combination and/or alternative animal models. Benchmarking studies and the inclusion of comparator control therapies to establish appropriate experimental conditions will enhance the utility of animal infection models and the robustness of predictions for translation to patients.

Table 2. Best practice recommendations for murine neutropenic thigh and lung infection models.

Study component	Recommendation	Comments
Mouse strain	Outbred (e.g., CD-1 or ICR)	Historically female, but studies in both sexes have been strongly encouraged recently, if feasible, and should be considered
Induction of neutropenia	Cyclophosphamide administered IP or SC at 150 mg/kg at 4 days prior to infection and 100 mg/kg at 1 day prior to infection	Results in neutrophils $< 100/mm^3$ for at least 2 days
Inoculum preparation	Culture should be in log growth phase [#]	Subculture aliquot from an overnight broth culture in fresh media for several hours prior to study start
Mouse inoculation	Infect thigh via IM injection of 100 µL and lung via intranasal inhalation of 50 µL (i.e., 25 µL per nare) [&]	Culture for inoculation should be 10^6 to 10^7 CFU/mL
Baseline bacterial burden	10^6 to 10^7 CFU/tissue (may differ by pathogen and strain)	Burden at the time therapy begins
Start of therapy	2 h post infection	Delay may be necessary for baseline tissue burden to reach 10^6 to 10^7 CFU
Study duration	24 h (sometimes 48 h)	Post inoculation
Bacterial growth over study period	Tissue burden should increase by 2-3 $\log_{10}$ CFU in untreated mice compared to baseline at initiation of therapy, assuming that the initial inoculum is sufficiently below the plateau	Less virulent isolates may underestimate the required drug exposure PKPD target
Number of isolates	At least 4 strains of each target pathogen (including a reference strain), if possible, with relevant resistance profiles and mechanisms	Include enough strains to assess strain-to-strain variability; mean and median PKPD target values should converge
Isolate phenotypes	Cover MIC range of compound, include clinically relevant resistant phenotypes	Consider *in vivo* virulence when choosing isolates
Control therapies	Inclusion of active comparator control (e.g., standard of care) may be beneficial	Especially important for evaluation of combination therapies against multidrug-resistant isolates

CD-1, outbred strain of albino mice; ICR, outbred strain of albino mice; IP, intraperitoneal; SC, subcutaneous; IM, intramuscular; CFU, colony forming units.

[#]Studies using other bacterial phenotypes (including growth stages) may be indicated, depending on the goal of the experiment.

[&]The maximum volume of the bacterial suspension which can be given per nare will depend on the mouse weight. This volume may affect the regional deposition of bacteria in the lung.

5.2.2 Pharmacokinetic studies

Generating high quality PK data is of utmost importance as this underpins all conclusions. The goal of PK experiments is to define the time course of drug concentrations in plasma, serum or blood, and potentially at the primary infection site. There are a number of factors that need to be considered in study design; these include terminal vs. serial PK sampling, determination of systemic and/or infection site concentrations, the type of blood matrix to measure (plasma, serum or whole blood), the number of dosage levels (to assess potential nonlinear PK over the targeted dose range), sampling times, use of infected or non-infected animals, and performing a satellite PK study vs. determining PK and PD in the same animals.

Determining the PK at the infection site becomes comparatively more important for deep (i.e., slowly or poorly equilibrating) infection sites which may additionally be sequestered due to the infection (6-8). Of note, infection may alter the PK parameters (e.g., clearance and volume) and it is critical to collect PK data from animals using the same infection model that is used in the PD studies. PK studies with drug combinations can be more complex, and drug-drug interactions may have an impact on the systemic and/or tissue exposure for one or both agents in the combination.

As discussed by the participants of this NIAID workshop, when studying agents for potential use in patients with bacterial pneumonia, it is recommended to utilize lung infection models for both PK and PD, and to collect tissue concentration data. The latter is important since penetration characteristics of the agent may result in significantly more or less drug at the site of infection compared to that in blood or plasma. The gold standard approach of measuring lung concentrations in both clinical and nonclinical studies is collection and measurement of drug concentrations in epithelial lining fluid (ELF). Briefly, a bronchoalveolar lavage (BAL) is performed. The BAL fluid is centrifuged to remove alveolar macrophages and other cells (which can bias ELF results). Drug concentrations in the supernatant are measured and adjusted for dilution using the urea correction method (9) to determine drug concentrations in the ELF. The cell pellet may also be utilized to determine concentrations within alveolar macrophages, as intracellular drug concentrations can be particularly important for some drugs and infections.

Systemic and/or tissue PK data are usually obtained at three or four dosage levels in a separate satellite PK experiment. A sufficient number of dosage levels are needed to identify and characterize non-linear PK, if present. Additionally, PK dosage levels should include the minimum and maximum dosage levels used in the PD studies, and extrapolation outside that range should be minimized. Performing satellite PK experiment(s) in infected mice (as opposed to determining the PK and PD in the same mice) may be required for logistical reasons.

The PK samples are almost invariably collected via terminal procedures; thus, each animal usually contributes one concentration measurement at a single time point (especially in mice). Collecting serial samples from the same animal throughout the study period better informs the PK parameters and allows one to separate between animal variability from residual error noise (e.g., bioanalytical noise). For example, multiple retro-orbital bleeds or multiple tail vein blood samples have been used previously. While serial sampling methods (e.g., for rats and sometimes for mice) have been developed and are routinely employed by some investigators (10-14), destructive sampling with one PK sample per mouse remains the most common approach.

5.2.2.1 Selecting sampling times

One of the challenges in PK study design is selecting the time points for sample collection. There is a practical limit of approximately 6 to 8 time points that can be chosen during any given experiment. This is based on

technical limitations, welfare considerations (including animal blood volume), and appropriate animal use. Informed by PK data which may already be available, PK sampling times should be carefully chosen to provide the maximal amount of information within these experimental constraints. Studies should be designed and repeated, if necessary and feasible, to adequately capture information related to the absorption phase, peak concentration, as well as the potentially multiple phases of drug distribution and elimination (including the terminal phase). If present, enterohepatic recirculation may greatly prolong the terminal half-lives and increase drug exposures (i.e., area under the curve) both in animals and patients, and may thus need to be considered when scaling from animals to humans (15).

Ideally, the final sampling times should be chosen to allow a reasonably accurate assessment of the time that drug concentrations fell below the limit of quantification and/or the lowest MIC of interest. Measuring drug concentrations to these limits can usually be accomplished via LC-MS/MS, which is highly recommended over older bioanalytical methods (such as bioassays). If present at relevant concentrations, bio-active metabolites should be measured and accounted for. In some situations, only limited (or no) prior PK information (e.g., on mean clearance, volume of distribution and half-life) may be available when the first animal PK study is being conducted. While advanced modeling methods to handle this uncertainty in the PK parameter values exist and are implemented in software packages, they are rarely utilized. In this case, a pilot PK study may be warranted.

If the first iteration of an animal study design and the associated results are suboptimal, even the most sophisticated PK modeling and simulation system will not compensate for poorly informative data. Modeling approaches (i.e., optimal design methods) can be prospectively applied to rationally support the selection of the most informative PK time points (16-21). This methodology seeks to improve the robustness of PK parameter estimates and is applicable for studies with one or multiple PK samples per animal. One drawback of this iterative process is that it may require multiple, sequential experiments. Although this stepwise approach is recommended as a scientifically sound practice, it may not always be possible due to time constraints, financial considerations, and/or limited drug supply.

While this review focuses on evaluating monotherapies, these points are also applicable for studying drug combinations. It is worth noting that combination studies can be complex and require special considerations to consider potential drug-drug or drug-vehicle (e.g., for dimethyl sulfoxide, DMSO) interactions. Furthermore, it is likely important to assure that both drugs of a combination regimen are present at the primary infection site at the same time both in animals and ultimately in patients. The design of combination PK and PD studies benefits greatly from prospective application of mathematical modeling approaches to rationally translate optimal dosage regimens to studies in patients. Additional, advanced considerations for designing and interpreting combination data is beyond the scope of this review.

5.2.2.2 Designing human-like exposure profiles in animals

Identifying the PKPD parameter (e.g., Cmax, AUC or %T>MIC) and the magnitude of that parameter required for efficacy is typically done using the cornerstone murine models described above. Significant value can be gained during clinical development by studying the efficacy of recreated human-like exposure profiles (also called 'humanized' regimens) in animal infection models. As predicted by allometric theory (22), drug half-lives are on average considerably shorter in smaller animals (e.g., mice) compared those in humans. This results in concentration-time profiles with different shapes for animals and humans, even if both profiles are matched in the AUC, for example.

This has been shown by Deziel et al. (23) for levofloxacin, where different approaches to achieve human-like exposure profiles in animals (guided by the AUC24h/MIC ratios) did not result in equivalent efficacy. This highlights the potential limitations of bridging from animals to humans solely based on achieving a single numerical value of a PKPD index (e.g., AUC24h/MIC) in animals and patients. Evaluating humanized PK profiles provides complementary information to traditional PKPD indices and should be considered during drug development programs. Additional guidance for these types of studies is provided in the Supplementary Materials.

5.3 Data Analysis

5.3.1 Viable counts

It is common practice to use a single time point Hill model to analyze dose- or exposure-response data (1); exposure-response analyses (e.g., AUC/MIC vs. effect) are strongly preferred, since they consider PK in contrast to dose-response analyses. This basic PD approach is often useful for optimizing antibacterial monotherapy based on single time-point data (e.g., viable counts at 24 h). If the time-course of bacterial viable counts is studied using multiple groups of mice, population PKPD modeling can characterize the time-course of bacterial killing and regrowth. In contrast to single time-point exposure response analyses, both empirical or mechanism-based PKPD time-course models can be used to rationally optimize innovative dosage regimens (e.g., front-loading) and combination regimens. Based on the time-course of drug concentrations at the target site, these models can describe and predict the drug effect over time. Mechanism-based PKPD models additionally offer the advantage that they can incorporate insights on the mechanism(s) of antibiotic action and, if determined, resistance (1, 24, 25).

5.3.2 Traditional pharmacokinetic approaches

A variety of methods are available to model PK drug exposure profiles (26, 27). The choice depends in large part on the type of experimental data that was collected, the complexity of the results (e.g., linear vs. nonlinear PK), and the skillset of the PK modeler. For a typical PK dataset that contains one measurement per animal at a single time point, a naïve pooling approach is often used. In this case, all observations for a given dose are combined (i.e., assumed to come from one animal) by calculating the average concentration at each time point; thus, between subject variability is ignored and only one estimate for clearance and volume of distribution is available based on these data pooled over all animals. Estimates tend to be biased unless variability is small (e.g., coefficients of variation [CV] are less than approximately 15%) (26-28). To obtain standard errors (e.g., for the AUC) for datasets with one sample per animal, the Bailer method (29, 30) and bootstrap re-sampling techniques have been developed (31-33). The latter method is more flexible and provides information on the between animal variability.

If serial samples are obtained from the same animal, a standard two-stage method can be used where the data from each animal is fit separately. Provided each profile contains sufficient information to estimate all PK parameters, reasonable average PK parameter estimates can be obtained via the two-stage approach; however, this method may substantially over-estimate the variability between subjects (26, 27) if each individual profile is not well-characterized across all phases of absorption, distribution and elimination.

5.3.3 **Popu**lation pharmacokinetic modeling

In contrast to the standard two-stage approach, population modeling can borrow information across all subjects (i.e., one subject is fit in context of all other subjects) and can simultaneously describe and predict plasma and

ELF concentrations, for example (34). Moreover, the population approach can estimate between subject variability which allows one to predict the range of expected plasma concentration time profiles via Monte Carlo simulations (35). Population estimation algorithms have proven useful and robust to estimate PK parameters both for frequently sampled and sparse datasets (28, 36). For drugs that show considerable non-linearity in PK in animals, humans or both, population PK modeling is the method of choice for data analysis and matching exposure profiles across different species.

From a practical perspective, fitting the average plasma concentration profile via naïve pooling or using a standard two-stage approach may be adequate to describe and predict the mean concentration profile for a dataset with small (approximately <15% CV) between subject variability; this will allow a broader range of scientists to perform the modeling analyses and progress a drug development program efficiently. For datasets with larger between subject variability, nonlinear PK, or multiple different types of observations (e.g., plasma and ELF concentrations), population modeling offers substantial benefits to accurately predict the mean and between subject variability of drug concentrations in plasma and at the target site.

Population PK modeling borrows information across all subjects (i.e., animals), accounts for between subject variability, and can handle datasets with sparse and frequent sampling (28); this is particularly true if advanced estimation algorithms which are based on the exact log-likelihood equation are employed. A variety of different population modeling algorithms and software packages are available. Compared to the time for performing experiments, population PK analyses rarely present the rate limiting step for translational PK and PKPD modeling within the overall project. However, time for regular discussions between experimental and modeling scientists and joint planning of study designs is essential.

Population PK modeling using exact log-likelihood methods is often the most suitable choice as it balances unbiased and precise estimation results with project timelines (**Table 3**) (28, 36-38). While full Bayesian approaches are appealing and powerful, they tend to require more time (e.g., for sensitivity analyses) and additional skills by the modeler (28, 39).

Table 3. Comparison of PK modeling and simulation approaches in increasing order of complexity.

Approach	Between Subject Variability	Accuracy of Predictions	Comments
Naïve pooling	Ignored (i.e., assumed to be zero or very small)	Only mean profiles can be predicted	Can be adequate to simulate mean concentration profiles, if variability is small. Yields biased predictions if variability is moderate or large. Cannot simulate between subject variability.
Standard two-stage	Often overestimated	Predicted concentration range may be too broad.	Can be adequate to simulate mean concentration profiles, if variability is small. Requires serial sampling which may be problematic for mouse PK studies.
Population modeling (approximate log-likelihood)	Bias can be large for sparse data	Can simulate variability, but may be considerably biased	Can simulate mean concentration profiles and between subject variability, but may yield biased results for sparse data.
Population modeling (exact log-likelihood)	Often most suitable choice	Often most reasonable choice	Can simulate mean concentration profiles and between subject variability with no (or less) bias. Can handle complex PK models with multiple dependent variables (e.g., PK, PD and resistance).
Population modeling (advanced three-stage methods)	Very powerful, can leverage prior information via a Bayesian approach	Can account for uncertainty as well as between subject variability.	Powerful, but more complex; requires more expertise and modeling time (e.g., for sensitivity analyses).

5.4 CHALLENGES OF STUDY CONDUCT AND INTERPRETATION

The success of determining PKPD in animal models depends largely on the ability to control variance, a sound experimental design, and suitable data analysis. Characterizing PKPD for a new drug is a process that involves learning and refining to progressively understand the sources of variability and then to minimize variance until the data converges around a final target. This process benefits greatly from being executed by close knit, highly functional teams of experts who regularly discuss experimental designs, results and interpretation.

5.4.1 **Potential** challenges for drug developers

Although the PKPD process for antibacterial agents has been clearly defined (e.g., by the EMA), it is not always simple and straightforward for a new drug. Drug developers may face one or more of challenges below.

5.4.1.1 Pharmacokinetic considerations

- Short drug half-lives (e.g., in mice) can complicate the achievement of PKPD parameter values (e.g., in dose fractionation studies).
- Species specific toxicities and/or PK profiles may impose experimental limits and hinder the ability to understand the full dose response (e.g., inability to use sufficiently high doses to observe near-maximal effect).
- Incorporating tissue concentration data may be complicated, yet it should not be assumed that penetration is the same across animal species. For pneumonia, for example, there are technical challenges associated with collecting BAL / ELF data; however, experimental approaches to determine drug concentrations in ELF have been established and widely applied in animals and humans (8, 40). And population PK modeling and Monte Carlo simulation strategies have been employed to design optimal dosage regimens based on ELF penetration data for patients (9, 41-43).
- The time-course of antibiotic penetration at the target site may not mirror circulating drug levels, and the rate of penetration may differ between drugs and target sites across species (e.g., for oritavancin). This may be particularly critical for synergistic drug combinations.
- Plasma protein binding of drugs may differ between animals and humans, as well as between 'normal' and critically-ill patients. Such protein binding differences may need to be considered when matching unbound drug concentration profiles (44).

5.4.1.2 Pharmacokinetic / pharmacodynamic considerations

- PKPD models are acute and a high degree of severity of infection is required for model stability and minimizing variability (these factors may need to be considered with regard to applicability to the clinic).
- Different PD targets can be obtained from different models, studies and isolates, as well as from different infection sites and/or test conditions (choice of the "right" models and conditions may be challenging).
- Some studies and strains may not perform the same as others, even in well characterized animal models; while between strain variability is expected, it may complicate the establishment of PKPD targets and subsequently human dose predictions.
- Opinions vary on which endpoints should be used to establish PD targets (i.e., stasis vs. 1- or 2-$\log_{10}$ reduction in CFU; ED_{50}, ED_{90}, etc.). Different endpoints may be required for various types of infections and patient groups (e.g., for immuno-compromised patients or those with serious infections such as ventilator associated bacterial pneumonia [VABP]). While this can be a contentious discussion point for monotherapies, the situation is even more complex when defining targets for combination therapies.

5.4.2 Sources of variability

Variability associated with the conduct of animal infection models can be largely addressed via careful planning. These controllable sources of variability and the types of data to be collected are outlined at the left part of **Figure 2**. However, unidentified components of variability associated with the PK, PD, infection site and immune response will remain; these random components are difficult or impossible to control. Sometimes, these sources of variability may lead to one or more extreme data points and it can be tempting to remove such presumed "outliers". However, with the exception of *a priori* documented experimental reasons (such as a missed dose), removal of outliers from a dataset is not generally appropriate, since this likely yields biased conclusions. Performing and presenting the results of a data analysis with and without a 'suspected' outlier is good practice.

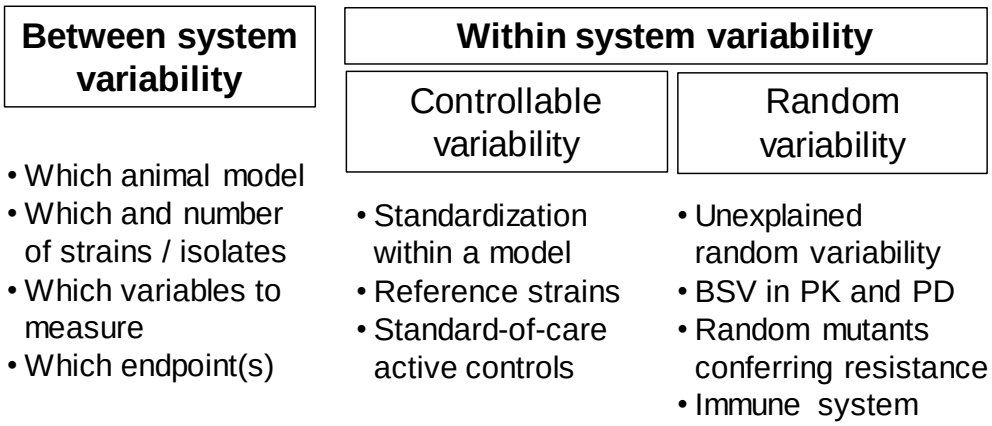

Figure 2. Different sources of variability which may affect the results of animal infection models. The between system variability can be handled by appropriate choices for and the selection of experiments to be performed. The within system variability can be split into a controllable portion and a random (i.e., usually not-controllable) part. Experimental design choices and careful execution of animal infection model studies can minimize the controllable variability. The random, unexplained variability will necessarily include components such as between subject variability (BSV) in pharmacokinetics, pharmacodynamics, the infection site, and the immune system.

Between study differences: One of the most concerning of these challenges is that results from studies conducted in different models or by different labs may vary widely. In fact, one set of results may support termination of a new drug candidate while another set of results from a different laboratory but on the same compound supports progression. It is highly likely that differences in study design, conduct and analyses, even for the 'workhorse' PKPD models, contribute to this issue. Careful experiment conduct is critical, and standardizing certain components (including those summarized in **Table 2**) may be helpful.

Standardization and active comparator controls: Experimental variables such as inoculum, strain fitness, timing of infection, infection site, inoculation method (including potential bacterial washing steps), and the status of the mouse immune system can have a large impact on results (5). For this reason, it is important to benchmark PKPD models and methods using positive controls (i.e., an effective reference antibiotic or reference antibiotic combination; **Table 2** and **Figure 2**). These controls should be licensed for the target indication (at least in some countries), have an established effective clinical dosage regimen, and be supported by nonclinical PKPD, clinical

PKPD and clinical outcome data. Using such active controls, a collection of data under a standardized test methodology could be developed to guide future drug development and, at a later stage, regulatory review. This could include evaluation of the performance of a new drug compared to benchmarked control(s). It would serve to solidify "best practice" study recommendations into "validated" experimental conditions. Importantly, this collection of data would also inform appropriate endpoints and support decision-making based on benchmarks rather than individual expert opinions.

5.5 GUIDELINES AND ENDPOINTS

Guidelines have been published (e.g., by EMA) which recommend specific efficacy endpoints for different clinical indications. The general belief is that the more serious the infection, the more antibacterial effect is required, since immunological effectors may only contribute marginally to the overall antibacterial effect. Thus, PD targets based on stasis (no change compared to pre-treatment baseline) or a 1-log10 reduction in CFU from initial inoculum have been recommended for less severe infections such as skin and soft tissue and complicated urinary tract infections (cUTI); in contrast, 2-log10 reductions in CFU have been suggested for more severe infections such as pneumonia (45). The rationale for a higher hurdle serves two goals. These are reducing the bacterial burden to a density that can be controlled by the immune system, and below mutational frequency in order to prevent emergence of resistance (2, 24). Although these are laudable goals, the use of absolute effect sizes requires highly standardized and codified model systems; with benchmarking based on positive controls. Also, the maximum achievable effect size may differ between pathogens and infection sites.

5.6 TRANSLATIONAL PHARMACOKINETICS / PHARMACODYNAMICS

Interpreting and translating PKPD results into clinically relevant dosage regimens requires careful planning and may be challenging. In the early stages of clinical drug development, sponsors usually aim for a high PKPD target and/or the maximum tolerated dose. The rationale is that a higher drug exposure enhances antibacterial efficacy and thus such high doses can successfully treat more severe infections. High doses may help mitigate against future emergence of bacterial resistance. They may also protect against the larger PK variability seen in severely ill patients, the potentially altered PK in special patient populations, and low drug concentrations at the primary infection site. Thereby, high doses can minimize the possibility of treatment failure due to under-dosing.

However, almost invariably, the amount of drug that can be given is limited (i.e., by good laboratory practice [GLP] nonclinical safety coverage, clinical adverse events, lack of therapeutic index, cost-of-goods, and other factors). When this happens, there are typically two options. First, sponsors can keep the same PD target and risk not covering the encountered MIC range; or second, sponsors can lower the PD target by using a stasis or 1-$\log_{10}$ endpoint (instead of 2-$\log_{10}$). The latter choice is the more likely path, as not being able to cover the full MIC range is a poor start for a new drug and creates problems for establishment of susceptibility breakpoints. However, lowering the target increases the risk of therapy failure for more severe infections, can accelerate the development of resistance, and may result in breakpoints which are higher than appropriate.

5.7 APPROACHES TO ADDRESS CHALLENGES

Despite potential complexities in interpretation and translation, there are steps that can be taken to provide additional confidence in the chosen PD target. Different strains, study endpoints and model systems can all be used to help provide confidence in conclusions of PKPD data. Data can be generated in more than one model system (i.e., another animal model, or the dynamic *in vitro* hollow fiber infection model, for example); however,

discordant results should be actively managed, and explanations for the differences sought. Study readouts (i.e., dependent variables) can be enriched by capturing additional information. While bacterial burden (i.e., CFU) is the primary endpoint, additional secondary readouts such as viable counts of resistant bacteria, biomarkers, survival, histopathology, inflammatory markers, radiology, bioluminescence, and others may also be useful.

5.8 FUTURE PERSPECTIVES

While PKPD is an evolving discipline, the antibacterial field is fortunate to have a considerable armamentarium of established and new PKPD tools and expertise available. However, challenges and open questions remain. The commonly used murine neutropenic thigh and lung models have provided a sound basis for PKPD to-date. As highlighted in this review, they are, however, not without issues and require careful attention to details.

Future steps: These models can be further optimized, or a set of experimental conditions can be identified, to provide more reliable, consistent and adequate model performance (**Figure 3**). This would also ensure better reproducibility from study-to-study and lab-to-lab, and enhance our ability to interpret the results for different types of infections and different classes of antibacterials. While standardization of methodologies will likely improve reproducibility, a non-controllable portion of variability will remain. By collecting and publishing benchmarking data for both model performance as well as exposure-response relationships for control / reference compounds, a standard set of methods for study conduct, analysis and interpretation could be identified for optimal translation to the clinic.

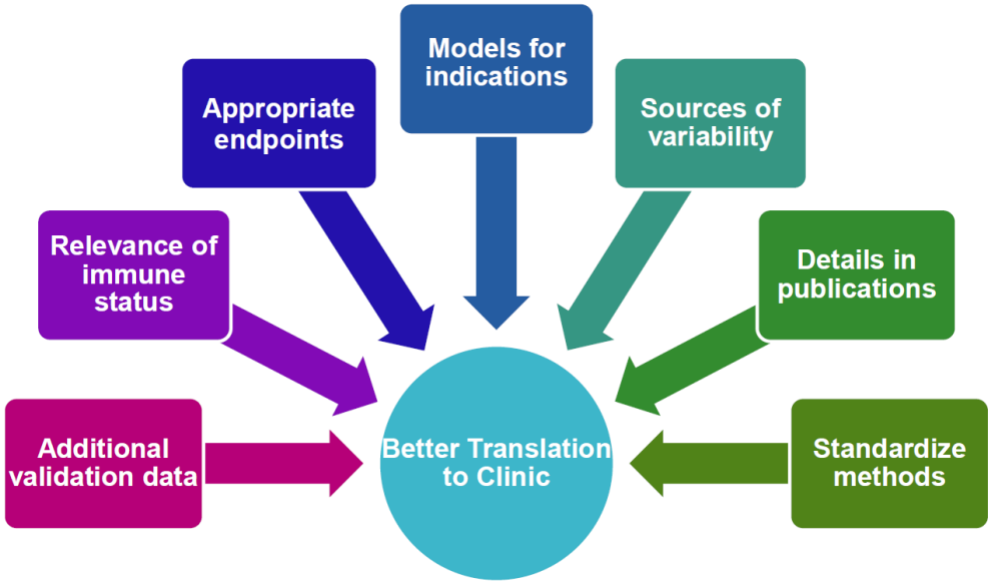

Figure 3. Considerations and perspectives to enhance the robustness of animal infection models and ultimately better translate efficacious and reliable dosage regimens to patients.

5.8.1 Latest modeling approaches

An integrated experimental and mathematical modeling approach can be valuable to determine the most informative dosage regimens for PD testing and the most informative sampling times for PK studies. Leveraging latest modeling and simulation approaches and in-depth discussions of study designs and clinical goals among team-members go a long way to enhance the utility of such a translation approach.

To further enhance the PKPD understanding for new drugs, mechanism-based modeling can be employed to integrate information about organ penetration of antibacterials and their receptor binding at the bacterial target site. Furthermore, these translational PKPD models can describe and predict the time course of bacterial killing, prevention of resistance (if studied), and the impact of the immune system (24, 42, 46-48).

5.8.2 **Choice and l**ack of validated infection model

Murine thigh and lung infection models provide a reasonable mimic of soft tissue infection and pneumonia, respectively. However, neither model may be ideal for characterization of the PKPD at other infection sites. The neutropenic thigh model is reflective of outcomes for pyelonephritis, where intra-kidney concentrations (i.e., a rapidly equilibrating PK compartment) are important. Unfortunately, animal infection models which mimic complicated lower urinary tract infections have not yet been validated.

There is a need for reliable, validated PKPD models representing complicated intra-abdominal infections (cIAI) and cUTI, especially since these are common target indications for Phase 2 studies. Consideration should also be given to developing models which better mimic human disease (e.g., more natural disease progression). Such models are likely to be low throughput and less practical for routine PKPD characterization. However, informed by commonly used murine infection models, these more complex studies could provide additional supporting information for new drugs and play an increasingly important role during drug development.

5.8.3 Dissemination of data

A final point for consideration is publication of PKPD data. It is important that sufficiently detailed information be provided to allow readers to assess the validity of the work and resulting PKPD targets and to reproduce the methods employed. Authors should include all pertinent details of the experiments and associated analyses (including the enabling equations of the final mathematical model[s]), at least within the supplementary materials. Variability in PD response should be reported, including performance of individual isolates tested (e.g., growth in untreated control animals, variability of drug effect, etc.) and their individual PD targets. PK data should be adequately described, and a thorough assessment of the quality of modeling and simulation methods should be provided (including assessment of accuracy and precision). It is suggested that editors consider both the ARRIVE guidelines (27) to ensure adequate reporting of *in vivo* data, as well as a set of extended criteria specifically for PKPD studies to improve the quality of these publications.

5.9 CONCLUSIONS

While much of this review provides a perspective on current challenges and potential issues, it is important to remember that animal infection models provide powerful PKPD information and have been shown to predict clinical outcomes (3). It is a healthy evolutionary process to critique current methods and seek ways to continuously improve the models, study designs, conduct, analyses, interpretation and communication. Optimizing our translational PKPD tools has become increasingly important as we rely more and more on this approach to predict successful clinical treatment regimens; often to combat serious infections by multidrug-resistant bacteria.

Optimization and standardization of nonclinical models are meant to improve this process, not to stifle innovation or eliminate the need for rational thought. Regular discussions within multi-disciplinary project teams are essential to maximize the utility and value of our translational tools. It is expected that future studies will identify scenarios where the recommendations in this review will need to be modified for special infection models, bacterial isolates, novel-acting therapies, and other situations. Some therapies may require special

considerations, and PKPD work packages should be tailored to the specific needs of the individual compound and ultimately to the target patient population.

5.10 SUPPLEMENTAL MATERIAL

Designing human-like exposure profiles in animals:

Bridging antibiotic exposures in animals to humans has generally been performed using the relevant PKPD index (23). The example of levofloxacin (**Supplementary Figure S1**) shows, however, that the partially humanized PK profiles with dosing every 12 h (**Fig. S1**, panel A) or every 8 h (**Fig. S1**, panel B) differed in their achieved time-course of antibacterial effects (**Fig. S1**, panel C).

Humanized PK profiles can be achieved by giving multiple dosages to animals. The 2.5th and 97.5th percentiles of plasma or target site concentration profiles in humans can be predicted via population PK modeling and Monte Carlo simulations. These percentiles characterize the 95% prediction interval of concentrations expected in patients. Dosages for lab animals can then be defined to provide concentration-time profiles within these limits.

For antibiotics with short half-lives such as β-lactam antibiotics in mice, for example, ethical and logistic reasons may prevent drug concentrations in animals to fall between the 2.5th and 97.5th percentiles in humans at all times. Animal welfare considerations may limit the maximum number of subcutaneous, inter-peritoneal, or intra-muscular doses which can be given to animals per 24 h. The use of computerized infusion pumps may address this limitation (12-14), however, this advanced technique is not widely available. Alternatively, translational, PKPD modeling can support a rational choice of humanized dosage regimens for animals; these regimens can be optimized via modeling to match the drug concentrations and receptor occupancy profiles in humans as close as possible given logistical constrains. Ultimately, both efficacy (i.e., dropping below the 2.5th percentile) and safety considerations (due to concentrations in animals above the 97.5th percentile) will need to be acknowledged. It seems likely though that an active discussion among team members of how to achieve clinically relevant human-like exposure profiles in animals will improve the design of nonclinical infection model studies and support more robust translation to humans.

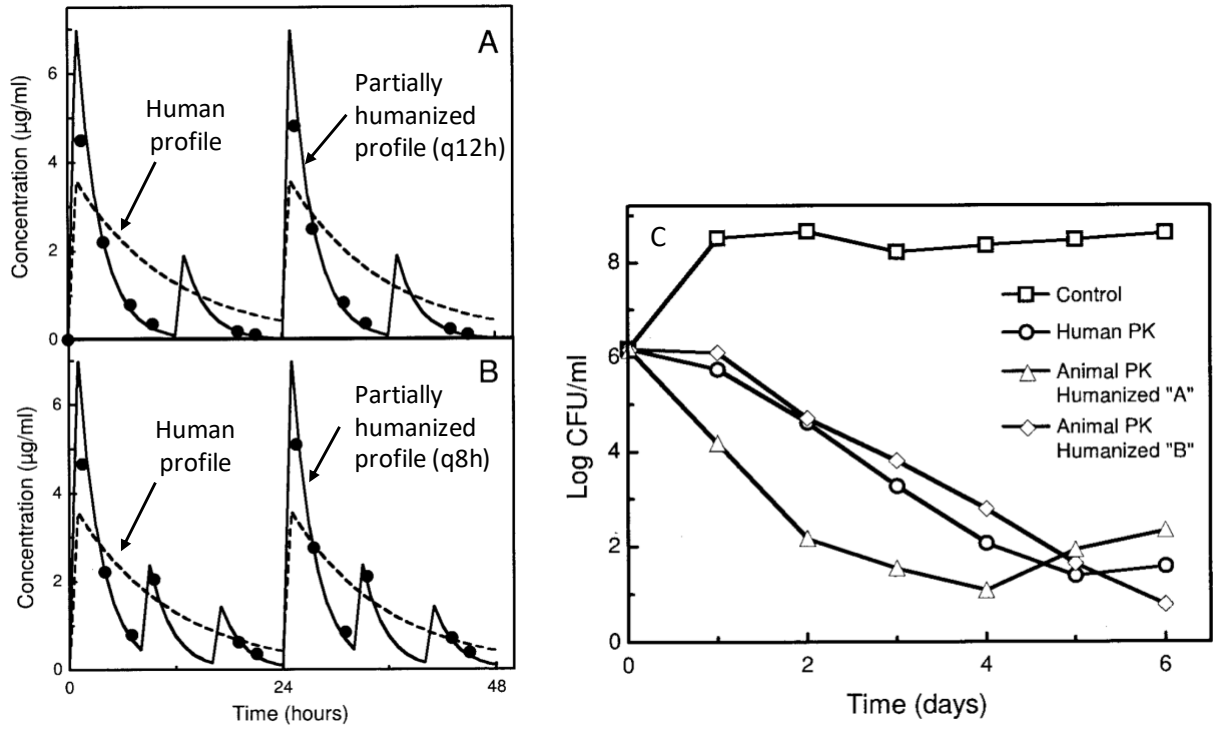

Figure S1. Observed (markers) and model fitted (continuous lines) plasma concentrations (panels A and B) and efficacy (panel C) of levofloxacin against *Bacillus anthracis* under "partially humanized" animal pharmacokinetic profiles. Panels A and B show treatment regimens in which levofloxacin was dosed at the beginning of each 24-h dosing interval (AUC = 23 mg · h/liter) and a smaller dose at 12 h (AUC = 6.1 mg · h/liter; partially humanized [A]) or in which levofloxacin was given in three decreasing doses at 8-h intervals (AUCs = 22, 7.5, and 4.5 mg · h/liter, respectively; partially humanized [B]). The broken line shows an equivalent human exposure (AUC_{24} = 36 mg · h/liter; equivalent to AUC_{24}/MIC = 300). Panel C shown the effect of the human exposure profiles and of the "partially humanized" animal exposures profiles against *B. anthracis*. Adapted from Deziel M *et al.* Antimicrob Agents Chemother. 2005; 49: 5099-106.

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6 SESSION 4: CLINICAL PKPD

AGENDA	
Moderators: Sumathi Nambiar (FDA/CDER) and Ian Friedland (Achaogen)	
Aaron Dane, Danestat Consulting	Factors for defining the robustness of the PKPD package
Luning (Ada) Zhuang, FDA/CDER	Application of PKPD Modeling and Simulation in Antibacterial Drug Development: FDA Perspective
George Drusano, University of Florida	Why ELF?
Sujata Bhavnani, ICPD	Clinical PKPD: A Strategy to Reduce Risk
Matthew Rizk, Merck	PKPD Considerations for Beta-Lactam/Beta-Lactamase Inhibitor Combinations
Jian Wang, FDA/CDER	Utility of PKPD in Pediatric Antibacterial Drug Development
Thomas Lodise, Albany College of Pharmacy	Clinical Applications of PKPD in Phase 3 Trials and Beyond: Dosing Considerations in Specialized Populations

6.1 AARON DANE (DANESTAT CONSULTING) - FACTORS FOR DEFINING THE ROBUSTNESS OF THE PKPD PACKAGE

PKPD information is highly informative for antibiotics and has been used prominently to set dose levels and to give confidence ahead of larger clinical trials. Due to feasibility challenges there are proposals to skip Phase 2 studies and/or reduce the size of Phase 3 programs when a development program is supported by "robust" PKPD information. This talk intended to prompt discussion of what is meant by robust PKPD, how this differs from a traditional PKPD package, what additional components can make a package more robust, and how this may change depending upon the additional supportive data that are available (such as clinical data using high MIC isolates, results regarding breakpoint evaluation and support from humanized exposure experiments at the predicted dose). This is not intended to define a set of rules, but rather a framework for what is needed to support smaller development programs. An additional question is then whether the "degree of robustness" would change depending on the degree of unmet need, the size of development program or in the situation of a new class of antibiotic.

In prompting the discussion, the key areas of focus related to the PKPD target, which is taken from a range of non-clinical models and species and a number of isolates in order to assess a range of scenarios. As such, there are questions as to which PD target should be used (stasis, 1-log, 2-log drop in CFU), how the uncertainty in parameter estimates from each model should be accounted for (use of the mean value or a more conservative measure) and how to interpret a variety of values across targets and experiments. These factors may be particularly relevant when looking for more robust PKPD information, and questions relate to whether more experiments are needed or whether we simply need to agree what is required from the experiments currently conducted. From these

questions, an example was presented to prompt discussion on what is reasonable in terms of considering the strength and consistency of PD target value and the PTA values to target for robust PKPD information.

In summary, the questions to be addressed relate to what constitutes "robust" and when do we know this has been achieved? Further discussion should occur to agree upon these principles, and relate to the confidence in PD index, consistency of PKPD target across experiments and defining what is a high enough rate of probability of target attainment. The role of the uncertainty in parameter estimates from the PKPD modelling, the role of tissue penetration and the use of human simulated dose studies should also be considered. Finally, although the definition of what is robust is important, this also needs to be considered alongside biological and clinical knowledge, the available clinical data, and should balance the efficacy requirements of a new antibiotic with a potential to address an unmet need and the toxicity risks of that antibiotic.

6.2 Luning (Ada) Zhuang (FDA/CDER) – Application of PKPD Modeling and Simulation in Antibacterial Drug Development: FDA Perspective

A total of 13 antibacterial new molecular entities (NMEs) have been approved by FDA between 2009 and 2016 (**Table 4**). The relatively slow discovery and development is attributed to the limited incentives for pharmaceutical industries in this therapeutic area and a high benefit-risk ratio of available treatment options (1). One of the critical challenges for antibacterial drug development is to identify optimum dosing to increase the success rate of clinical trials and decrease the probability of dose-related adverse events. Pharmacokinetic/pharmacodynamic (PKPD) modeling and simulation is an important group of techniques to characterize the relationship between dose, exposure and treatment outcome to determine the most suitable dosing regimen. Since bacteria are the direct target of antibacterial treatment, in vitro and animal data with a wide range of dose regimens can serve as the foundation to inform human dose selection. This unique nature of antibacterial drug development necessitates the use of translational PKPD modeling and simulation to streamline drug development.

Three PKPD modeling and simulation approaches were used to promote drug development and support drug approval of antibacterial NMEs between 2009 and 2016: population PK (Pop PK) analysis, exposure-response (E-R) analysis, and probability of target attainment (PTA) analysis.

Population PK (Pop PK) analysis is a well-accepted pharmacometrics methodology to predict the PK characteristics of drugs in patients where intensive PK sampling is not practical. A total of 11 antibacterial NME applications included Pop PK analysis. Pop PK analysis can provide the exposure information used in E-R analysis and PTA analysis. The covariate analysis within a Pop PK model evaluates the impact of demographic parameters on exposure and determines the need of dose adjustment in specific populations, such as obese patients, geriatric patients, or patients with renal/liver impairment. Specifically, Pop PK model analysis can inform and refine the dosing strategy in pediatric patients because the same disease progression and response to intervention as adults are assumed for many antibacterial indications.

E-R analysis evaluates the relationship between drug exposure and favorable and unfavorable outcomes. The exposure can be dose, area under the concentration versus time curve (AUC), maximum concentration (Cmax), or minimum concentration (Cmin) while the response can be clinical outcomes such as safety, efficacy, or a biomarker. E-R analysis plays a key role in dose selection through all phases of drug development by providing evidence of effectiveness and safety and supporting dose individualization. Of 13 antibacterial NMEs, 7 NME submissions had E-R analysis included. The E-R analysis for efficacy may not be informative for some antibacterial NMEs because Phase 2 and 3 trials did not include a wide enough range of exposures to avoid treatment failure.

Table 4. FDA-approved antibacterial new molecular entities between 2009 and 2016.

Year	Drug Name	Pharmacometrics analysis	Indication
2009	Telavancin	Pop PK	cSSSI, HABP/VABP
	Besifloxacin	N/A*	Bacterial conjunctivitis
2010	Ceftaroline fosamil	Pop PK, E-R, PTA	ABSSSI, CABP
2011	Fidaxomicin	N/A	C. difficile infection
2012	Raxibacumab	Pop PK	Anthrax
	Bedaquiline	Pop PK, E-R	TB
2014	Dalbavancin	Pop PK, E-R	ABSSSI
	Oritavancin	Pop PK, E-R, PTA	ABSSSI
	Tedizolid Phosphate	Pop PK, E-R, PTA	ABSSSI
	Ceftolozane and tazobactam	Pop PK, PTA	cIAI, cUTI
2015	Ceftazidime and avibactam	Pop PK, E-R, PTA	cIAI, cUTI
2016	Obiltoxaximab	Pop PK	Anthrax
	Bezlotoxumab	Pop PK, E-R	C. difficile infection

N/A: Not applicable due to local antibacterial treatment

An alternative E-R analysis for efficacy of antibacterial NMEs is to assess the relationship between PKPD indices (e.g., *f*AUC/MIC, *f*Cmax/MIC, and *f*T>MIC) that combine drug exposure and susceptibility of organisms and clinical outcomes. For antibacterial drugs, PKPD indices are considered more associated with efficacy than exposure measures alone. E-R analysis for safety can provide a fundamental rationale of dose adjustment for the scenario in which the amount of risk clearly outweighs the amount of benefit. Overall, both E-R analyses for efficacy and safety are taken into consideration for evaluation of the appropriateness of dosing regimens of antibacterial drugs.

PTA analysis is an assessment of the probability of attaining a PKPD target with a specific studied dosing regimen that is correlated with satisfying efficacy during preclinical studies. It is a tool to support dose selection in general and specific populations for a given dose and organism in antibacterial drug development. PTA analysis was included in 5 of 13 antibacterial NME applications as an essential component by integrating the information from population PK predictions in healthy volunteers and/or patients, PKPD index and target from in vitro microbiological studies and in vivo animal of infection studies.

Physiological-based pharmacokinetic (PBPK) analysis is a strategy to predict the effect of intrinsic and extrinsic factors on drug exposure to support dosing recommendations under specific clinical situations. Although PBPK analysis was not included in any of 13 antibacterial NME submissions, it is increasingly used during the assessment of drug-drug interaction and dose individualization in subpopulations. A FDA guidance regarding format and

content of PBPK analysis became publicly available in December of 2016 to facilitate the incorporation of this analysis tool into NME submissions to support decision making during drug development.

One limitation of PKPD modeling and simulation is the large uncertainty and variability of information. The uncertainty and variability surrounding in vitro and animal studies come from the limited number of organisms evaluated with narrow MIC ranges as well as the lack of standardization of experimental procedures. The uncertainty and variability of clinical studies are attributed to inadequate PK sampling from patients, restrictive inclusion and exclusion criteria of patients, and limited number of patients with resistant bacterial strains. The uncertainty and variability may make it challenging to inform dosing and facilitate clinical study design.

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6.3 George Drusano (University of Florida) – Why ELF?

Epithelial lining fluid (ELF) concentrations are a surrogate for the drug concentrations at the effect site in the case of pneumonia. In some instances (e.g., some β-lactams), the infection site acts as a deep pharmacokinetic compartment, with lower penetration relative to plasma (AUC_{ELF}/AUC_{Plasma}) and there may be substantial delays in attaining therapeutic concentrations. In others (e.g., oxazolidinones such as linezolid or tedizolid), penetration results in AUC_{ELF} values in excess of Plasma AUC.

To identify proper doses and schedules for a pneumonia trial, it is prudent to identify ELF concentration-time profile in an animal model. This allows calculation of a drug exposure that is linked to the desired microbiological effect (e.g., 2 Log_{10}(CFU/g) bacterial kill relative to stasis). Prior to the clinical trial, having human ELF penetration data is optimal. The profiles in animal and man need not be similar and are often discordant. Nonetheless, using the animal model desired exposure target allows calculation of the dose and schedule in man that will attain the desired exposure relative to the MIC of target organisms. Monte Carlo simulation provides insight into how the proposed dose/schedule will work for a population of patients.

Why not use plasma targets from animal models? First, translating the PK (not the PD) from animals to man is most often significantly different. As an example, a cephalosporin had 69% penetration into ELF in a murine model, but 15% penetration in man. Second, plasma targets function as targets for clearing bacteremia in deep compartment infections, but do not guarantee source control in these instances. It is quite possible to make improper conclusions about the efficacy of dose and schedule in this circumstance with the use of plasma targets.

What are some critical questions for the future? Two (among many) include "what are the physicochemical determinants of ELF concentration-time profiles in ELF (role for pumps?) and "what is the impact of protein binding in ELF?" Performing proper deep compartment penetration studies in animals and man provides the highest probability of identifying doses and schedules of drug in pneumonia and other deep-tissue compartment infections that are efficacious.

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6.4 SUJATA BHAVNANI (ICPD) – CLINICAL PKPD: A STRATEGY TO REDUCE RISK

During the course of this presentation on clinical pharmacokinetics-pharmacodynamics (PKPD), the following three topics were reviewed: 1) requirements for sufficient preclinical data prior to initiating Phase 2/3 studies; 2) the adequacy of Phase 2 data to discriminate among dosing regimens; and 3) considerations for studying PKPD relationships for efficacy based on data from Phase 3 studies.

Many different sources of data are integrated during early and late stages of development of an antimicrobial agent to support dose selection. These include inputs include preclinical PKPD targets, parameter estimates from a population pharmacokinetic (PK) model, *in vitro* surveillance data, and when available in late stage development, clinical PKPD data. Using simulation, PKPD target attainment analyses can be conducted, the data for which are useful to support dose selection and selection of susceptibility breakpoints. While previous new drug application (NDA) submissions have been based on non-clinical PKPD packages with limited numbers of isolates, regulators are increasingly seeking more robust preclinical PKPD packages for antimicrobial agents. This requirement is of even greater importance for the development of antimicrobial agents for the treatment of patients with infections arising from resistant pathogens, the clinical data packages for which will be limited.

The consequences of basing dose selection decisions on one isolate was examined using the example of gepotidacin, a novel triazaacenaphthylene bacterial topoisomerase inhibitor with in vitro activity against Staphylococcus aureus, and data for this agent against six S. aureus isolates studied in a neutropenic murine-thigh infection model (1). The danger in studying one isolate is that the PKPD target based on such a dataset may not be reflective of the central tendency of a larger collection of isolates. If PKPD targets for the single isolate studied are higher or lower than the central tendency, this could lead to dose selection of higher or lower doses than warranted (2). A sufficient number of isolates that allow for the variability among isolates to be characterized should be studied. The process to determine this number may need to be iterative and should be driven by the results analyses of such pre-clinical PKPD data. In addition to considering PK variability and MIC distributions, future efforts to utilize such preclinical PKPD data for dose selection should consider inter-isolate variability rather than a measure of central tendency (2).

Using results analyses based on preclinical data to support dose selection, Phase 2 studies can then be conducted to evaluate the safety and efficacy of two or more dosing regimens. However, the value of typical Phase 2 study designs needs to be considered in the context of the current paradigm for developing antimicrobial agents. With the increased certainty that comes from using preclinical PKPD and Phase 1 PK data to support dose selection, the utility of Phase 2 dose-ranging studies to discriminate efficacy between two dosing regimens that have overlapping distributions of drug exposures is questionable. Unless there are concerns about safety endpoints, Phase 2 studies could potentially be avoided (3). Instead, a PKPD optimized regimen could be chosen for study in a Phase 3 randomized-controlled trial. However, if a Phase 2 study is conducted, PKPD relationships for both efficacy and safety endpoints should be investigated. Such analyses were carried out using data from briacidin-treated patients with ABSSSI that were enrolled from two Phase 2 studies (4). Brilacidin is a defensin-mimetic, that disrupts cell membrane integrity and which has activity against Gram-

positive and Gram-negative organisms, including methicillin-resistant Staphylococcus aureus (MRSA). Pooled data from the two Phase 2 studies, the first of which provided active drug for 5 days and the second of which provided active drug for 1 or 3 days, allowed for the formation of a rich dataset that consisted of six different dose levels and three different therapy durations. PKPD relationships were explored for efficacy endpoints assessed early in therapy and at traditional time points, at the end-of-therapy (EOT) or test-of-cure (TOC)/short term follow-up visits. Relationships between briladicin exposure and two safety endpoints, systolic blood pressure and numbness/tingling, were assessed. PKPD relationships for ≥20% reduction from baseline in lesion area on Day 2 and clinical success at EOT and TOC/STFU and each of the two latter safety endpoints were identified (4). The application of these PKPD relationships to simulated data generated using a population PK model was carried out with the objective of discriminating among candidate brilacidin dosing regimens (5). As illustrated by this example, PKPD relationships for efficacy and safety, when identified, can be used to assess risk versus benefit and the value proposition for further clinical development. When PKPD analyses for efficacy and safety are conducted in late stage development using Phase 3 data, the results of such analyses can be used to support the identification of susceptibility breakpoints and patient populations at increased risk of safety events. Such data can then be used to inform labeling and/or clinical practice guidelines.

The last topic centered around considerations for the evaluation of PKPD analyses for efficacy. These considerations include approaches for evaluating different efficacy endpoints. The lack of identification of a PKPD relationship for efficacy is an expected outcome when evaluating data from patients treated with a PKPD optimized dosing regimen. However, if relationships are identified, these are usually based on PKPD indices that were evaluated as dichotomized variables based on optimally-determined thresholds. Thresholds may be determined using the first split of a classification or regression tree, a receiver operating characteristic curve, or may be based on a model-predicted threshold for achieving a target response. Relationships based on dichotomized variables for PKPD indices allow for patients with both lower PKPD indices and percentages of successful response to be distinguished from those with higher PKPD indices and percentages of successful response (3). As demonstrated by the results of clinical PKPD analyses based on Phase 3 data for dalbavancin and oritavancin (6,7), the difference in the percentage of successful responses between patients in the lower and higher AUC:MIC ratio groups is unlikely to be impressive when based on the evaluation of a PKPD optimized dosing regimen. When PKPD relationships based on clinical data are not found, assessments of distributions of PKPD indices achieved for patients relative to non-clinical PKPD targets for efficacy represent a useful assessment to confirm the original basis for dose selection.

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6.5 Matthew Rizk (Merck) – PKPD Considerations for Beta-Lactam/Beta-Lactamase Inhibitor Combinations

The assessment of antibacterial PKPD has followed a well-accepted workflow for decades: 1) generate robust preclinical data, 2) rigorously analyze this data to obtain the PK target, and then (3) optimize dosing using clinical PK data to ensure the majority of patients meet these targets. However, in the era of beta-lactam/beta-lactamase inhibitor (BL/BLI) combinations, these assessments can be increasingly complex. Illustrative examples from ceftolozane/tazobactam and imipenem/relebactam showcase how this can be approached and some of the challenges.

Ceftolozane/tazobactam is a combination of a novel cephalosporin antibiotic in combination with a marketed BLI, and is approved for treatment of complicated intra-abdominal infections (cIAI) and complicated urinary tract infections (cUTI) in adults at a dose of 1.5 g (1.0 g ceftolozane/0.5 g tazobactam) every 8 hours via intravenous (IV) infusion. In contrast, imipenem/relebactam is a combination of a marketed carbapenem antibiotic in combination with a novel BLI, and is in Phase 3 development, dosed as 500 mg imipenem/250 mg relebactam every 6 hours via IV infusion. In this talk, the data and analyses supporting the above workflow was reviewed for both drug regimens. For both combinations, robust preclinical data packages were generated from a combination of *in vitro* hollow fiber time-kill studies and *in vivo* murine infection models. These data were then analyzed to obtain PK targets for each component. Both antibacterial agents (ceftolozane and imipenem) were identified as being %T>MIC driven, while the BLI components differed – tazobactam being %T>C_t (time above threshold concentration) driven and relebactam being AUC (exposure) driven. In addition, in the case of imipenem/relebactam, a dynamic pharmacometric model was fit to the time courses of the in vitro time kill data, which enabled insight to be gained from the full dataset of PK and PD data, avoiding the loss of information that comes from summarizing both PK and PD into a single metric.

Following target definition for both regimens, dosing was optimized and justified using population PK approaches developed from PK data collected in patients in Phase 2 and 3 studies. It was emphasized that it is imperative to collect this information in the patient population, both to understand how disposition and elimination may differ in patients (as compared to healthy volunteers), and to also accurately estimate the degree of variability in the intended patient population. The results of this analysis provide dose justification for the drug regimens, ensuring that the majority of simulated patients (in excess of 90%) achieve the defined targets (the probability of target attainment, or PTA).

Additional discussion was centered around considerations for the selection of dosing regimens for the treatment of pneumonia. The selection of optimal doses requires consideration of the drug's penetration into the lung compartment,and can be incorporated via one of two approaches as shown below. In the case of the illustrative examples, method 1 was utilized for ceftolozane/tazobactam, while method 2 was leveraged for imipenem/relebactam (**Figure 4**).

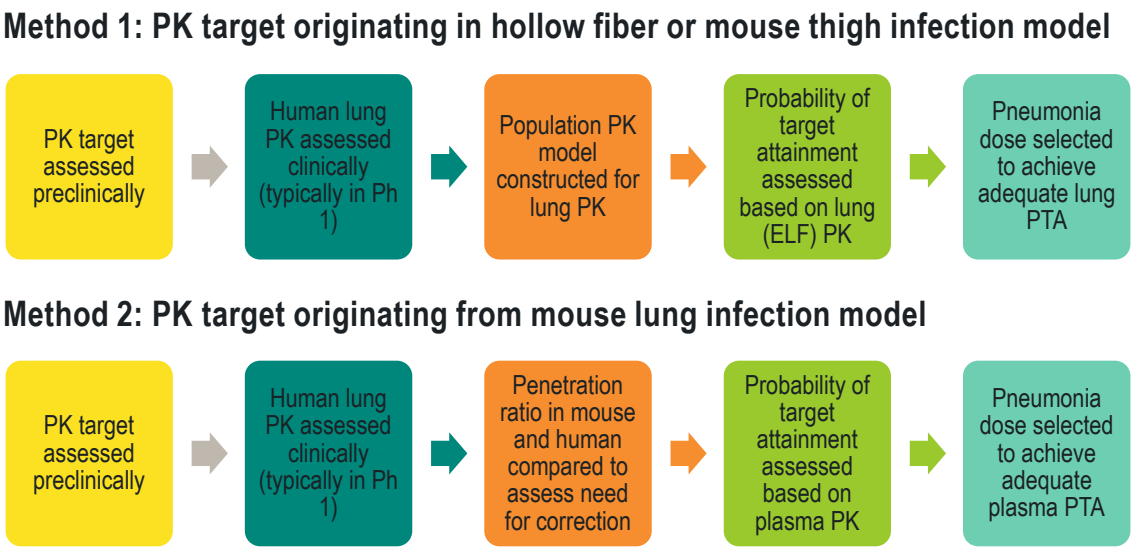

Figure 4. Examples of methods for selection of dosing regimens for the treatment of pneumonia.

In conclusion, the PKPD characterization of BL/BLI combinations can be complex, with dosing for each BLI dependent on its partner β-lactam, and vice versa. Thus, care must be taken in experimental design to ensure an appropriate assessment that interrogates the system dynamics with human simulated PK. We do currently have the experimental systems, quantitative tools and accumulated experience to make more robust assessments of antimicrobial PKPD, and need to consider the totality of the generated data in making dosing recommendations, as both in vitro and in vivo preclinical models each provide unique insight. Additionally, the discipline of quantitative pharmacology has substantially evolved and pharmacometrics should be leveraged to a greater degree for antibacterial PKPD through construction of dynamic models to utilize the entirety of the time-kill data set. Successful application of such approaches, together with continued assessment of how such PKPD assessments can be predictive of clinical outcome can potentially enable qualification of PKPD approaches to allow for therapies to be delivered to patients in a more streamlined and efficient manner.

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6.6 JIAN WANG (FDA/CDER) - UTILITY OF PKPD IN PEDIATRIC ANTIBACTERIAL DRUG DEVELOPMENT

The full extrapolation of efficacy may be appropriate if it is reasonable to assume that the two populations have: (a) similar to disease progression (b) similar response to intervention, and (c) similar exposure-response. A decision tree illustrating the use of an E-R relationship for bridging efficacy data in an adult population to a pediatric population is presented in the FDA Draft Guidance for Industry: *General Clinical Pharmacology Considerations for Pediatric Studies for Drugs and Biologic Products*[6]. PKPD or exposure-response is one of the decision tools and key elements in determining whether extrapolation of efficacy is possible from adult populations or from one pediatric age group to another pediatric age group.

As for antibacterial therapeutics, full extrapolation for efficacy is applicable for many antibacterial products. When efficacy in pediatric patients can be fully extrapolated from adult studies, then only pediatric PK and safety studies are required to establish the right dose. Establishing the pediatric dose can be performed by exposure matching in the case of full extrapolation, and occasionally applies to partial extrapolation.

For antibacterial drugs, a priori standards (key exposure metric) for matching have been pre-defined for anti-infective drug products, which include: 1) Target exposure metric (e.g., AUC:MIC, Cmax:MIC, and/or %Time>MIC); 2) Specific target values or range; and 3) Acceptable percentage of adult exposure.

Pharmacokinetic exposure is the major clinical outcome measures for many of the ongoing pediatric trials for antibacterial drugs, however, among them only few studies are conducted in neonates, and few studies are CSF penetrations (**Table 5**). These represent the two most challenging fields for pediatric drug development.

Modeling and simulation (M&S) can facilitate the development of exposure matching studies and may be able to optimize the clinical PK study design in pediatric patients. In recent years, the most widely used tools for dose determination include population pharmacokinetic modeling (Pop PK), and physiologically-based pharmacokinetic (PBPK) modeling. These tools are used by industry and highly recommended by regulators to design the studies and sampling strategies to obtain maximal information with a limited burden on all individual pediatric study participants (1). When using M&S as a tool for dose selection in pediatric trials, its application for neonatal dose selection is particularly challenging (2). The considerable inter- and intra-neonatal variability driven by growth and maturation will influence the outcomes of all types of models.

Physiologically-Based PK Modeling: PBPK models are mechanistic in nature such that organisms are comprised of organs/tissues that are interconnected through blood flow and are anatomically and physiologically reasonable representations. Mass balance equations describe drug compound movement through the system and aim to define the absorption, distribution, metabolism and excretion (ADME) of the compound. Within these equations, both organism-specific parameters (e.g., quantity of metabolizing enzymes in liver) and compound-specific parameters (e.g., affinity of compound for these enzymes) are incorporated into the model.

When in vivo pediatric pharmacokinetic data is lacking, dosing in children is difficult to predict. A pediatric PBPK model that provides a reasonable understanding of dosing integrates multiple forms of prior information about the compound (3). For neonates, fewer PBPK model applications have been developed (4). Preterm neonates are

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[6] U.S. Food and Drug Administration: Draft Guidance for Industry: General Clinical Pharmacology Considerations for Pediatric Studies for Drugs and Biological Products.
http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm425885.pdf Accessed January 27, 2015.

particularly challenging due to rapid changes in physiology and maturation of ADME processes. These limited results suggest that pharmacokinetics in full term neonates have the potential to be predicted by PBPK (5). While PBPK is a promising approach, we need better understanding of the ontogeny of enzymes/transporters, physiological parameters of CNS system properties, and pathophysiological changes.

Population PK Modeling: The Population PK (Pop PK) approach has been the most commonly used approach in neonatal drug development studies (6). Pop PK uses non-linear mixed effect modelling and allows for the analyses of sparse (limited number of blood samples per individual) and unbalanced data (unequal distribution of blood samples in various parts of the concentration- time profile in the individuals). This is particularly important when it comes to neonates as both scenarios are typically present. The influence of developmental changes in childhood can be explored primarily by using size and/or age as covariates.

Once internal and external evaluations of the model are performed to ensure that good PK parameter estimates have been made, a Pop PK model can be used to simulate dosing scenarios in the population for which the model was developed.

Table 5. Ongoing Pediatric Studies for Antibacterial Drugs.

Drug	Age Range	Outcome Measures	Disease/Indications
Telavancin	3 Months to 17 Years	PK	Gram-Positive Bacterial Infections
Ceftaroline fosamil	6 Months to 17 Year	Diffusion Into CSF	Cerebrospinal Fluid Shunts Ventriculoperitoneal Shunt (VPS)
Ceftaroline fosamil	up to 59 Days	Safety, Tolerability, PK, and Efficacy	Late-onset Sepsis
Fidaxomicin (and vancomycin)	up to 17 Years	Safety and Efficacy	Clostridium difficile-associated diarrhea (CDAD)
Dalbavancin hydrochloride	up to 28 Days	PK	Bacterial Infection
Tedizolid phosphate	2 to <12 Years,	PK	Gram-Positive bacterial Infection
Oritavancin diphosphate	<18 years old	PK, safety and tolerability	Gram Positive bacterial infection
Ceftolozane; tazobactam	up to 17 Years	PK, safety	Gram-negative Bacterial Infection or Peri-operative Prophylaxis
Daptomycin	3 Months to 16 Years	PK in CSF and plasma	Meningitis
Ceftazidime; avibactam	3 Months to 18 Years	Safety, PK and Efficacy	Complicated urinary tract infections (cUTIs)
Ceftazidime; avibactam	3 Months to 18 Years	Safety, PK and Efficacy	Complicated intra-abdominal Infections (cIAIs)

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6.7 Thomas Lodise (Albany College of Pharmacy) – Clinical Applications of PKPD in Phase 3 Trials and Beyond: Dosing Considerations in Specialized Populations

6.7.1 Background

Understanding exposure–response relationships is critical when designing antibiotic dosing schemes for use in clinical trials. To make the most informed dose selection decision, knowledge of inter-patient pharmacokinetic variability across target patient populations, the pharmacokinetic/pharmacodynamic (PKPD) index associated with maximal response, the relationship between antibiotic exposure and toxicity, and the exposure threshold associated with the on-treatment resistance emergence is required. For many antibiotics in development, the exposure target associated with maximal response can be elucidated through use of preclinical PKPD infection models, which have been shown to predict clinical efficacy. Furthermore, population PK modeling and Monte Carlo simulation can be used to select a dose for Phase 1-3 trials that has a high likelihood of achieving the exposure target associated with maximal response in preclinical studies.

Integration of PKPD analyses into the drug development process has contributed significantly to a number of successful NDAs. A recent analysis of 20 New Drug Applications (NDA) for pneumonia (17 different antibiotics) showed that the likelihood of NDA approval increased as a function of the predicted probability of achieving the PKPD target associated with response of the dose selected for the Phase 3 trial. Only one antibiotic, garenoxacin, had a highly favorably PKPD target attainment profile but had an unsuccessful NDA. This failure, however, was largely related to toxicity concerns not efficacy reasons.

While the success of a number of programs has been attributed to optimal dose selection through PKPD systems analyses, several recently approved agents were found to have lower response rates relative to the comparator across important patient population subpopulations, primarily patients with renal impairment. It is important to note that these studies were only powered to assess non-inferiority between treatments overall, and were not powered to make inferences across patient subpopulations. Although treatment differences across subpopulations in non-inferiority Phase 3 trials should be interpreted with extreme caution, post hoc analyses of these trials offered several potential explanations for the discordant response rates between treatment arms across patient subpopulations. It is difficult to precisely identify the reason for the lower response rates with new agents relative to comparator across important patient subpopulations, though there are several potential PKPD-related dose selection reasons that merit consideration in the future development of antibiotics. Some potential causes include: inappropriate dose selections for kidney disease, lack of prompt dose adjustment with improving kidney function, poor estimation of renal function among patients with acutely changing kidney

function, inappropriate dose extrapolation for body size, and presence of different PKPD driver in patients with altered PK profiles.

6.7.2 Dose Selection for Patients with Renal Impairment

In the US and Europe, specific guidance and criteria for PK analyses to promote optimal dosing in patients with renal impairment are available. It is currently recommended that studies are conducted during the development phase to assess the effects of renal impairment on the pharmacokinetic properties of the investigational drug. Both the Cockcroft-Gault and Modification of Diet in Renal Disease (MDRD) equations are considered suitable options to characterize patients' renal function for purposes of drug dose adjustment. Guidance for determining dose adjustments are derived from renal function categories based in chronic kidney disease stages. Appropriate antibiotic dosing in patients with acute renal impairment is not described, and the document acknowledges the limited utility of common renal function estimation calculators such as Cockcroft-Gault and MDRD in this setting. Therefore, dose adjustment recommendations that are employed in practice are determined on the basis of chronic kidney disease, and as such not designed with considerations for acute renal impairment.

Dosing becomes challenging in the setting of acute changes in renal function when relying on individual serum creatinine values. Any estimated creatinine clearance (CLCR) or glomerular filtration rate (GFR) equation that relies on a single point estimate requires a fundamental expectation of homeostasis, which is often not the case in acutely ill patients. There are alternative equations that may more accurately characterize renal function in the setting of rapidly changing serum creatinine. Rather than relying on a point estimate of the serum creatinine to estimate renal function like most traditional equation-based approaches, these equations quantify renal function by considering the magnitude to which serum creatinine is increased or decreased compared to its steady state value and the rapidity of the change. While this is a more intuitive approach, these have not been validated to guide drug dosing. Future antibiotic development should consider the evaluation and validation of these equations in determining optimal drug dosing, especially for patients with rapidly changing function.

It is also important to realize that acute renal impairment can be associated with alterations in a number of other physiologic processes and these should be considered when determining optimal dose adjustments. Though renal impairment most commonly affects excretion of renally eliminated drugs and metabolites, alterations in hepatic/gut metabolism, protein binding, and tissue distribution may also occur. Distribution may be substantially altered in the setting of volume shifts (e.g., due to capillary leakage, administration of large volume intravenous fluids in the setting of sepsis), or due to decreased protein binding. Altered volume of distribution would be particularly pertinent for hydrophilic agents such as β-lactams, as well as highly protein bound agents. Compensatory non-renal elimination may also be stimulated.

One of the more notable example in which non-inferiority was met but discordant clinical response rates were observed in patients with moderate renal impairment was the Phase 3 complicated intra-abdominal infections (cIAI) non-inferiority trials that compared ceftazidime-avibactam (CAZ-AVI) with metronidazole to meropenem (RECLAIM 1 & 2). In the RECLAIM trials, the clinical cure rates between the patients who received CAZ-AVI plus metronidazole or meropenem were nearly identical. For both treatment groups, the clinical cure rates decreased with worsening renal function. However, clinical cure was lower in the CAZ-AVI plus metronidazole group relative to the meropenem-treated group among patients moderate renal impairment at baseline. Additionally, more deaths among patients with moderate renal impairment at baseline were observed in the CAZ-AVI plus metronidazole group compared to the meropenem group. Based on these observations, the CAZ-AVI prescribing information has a warning for decreased efficacy in patients with moderate renal impairment (CLCR 30- 50 ml/min).

From a PKPD modeling perspective, this was somewhat of an unanticipated finding. Monte Carlo simulation studies indicated that that joint probability of target attainment (50% fT>MIC for ceftazidime and 50% fT>CT of 1 mg/L for avibactam) for the CAZ-AVI dosing regimen selected for its Phase 3 trials exceeded 90% for patients with infections with MIC values ≤ 16 mg/L. While the joint probability target attainment (PTA) profile was highly favorable, it is important to recognize that these overall PTA analyses provide an expectation of efficacy across all patient types. For antibiotics where there is no clinically significantly relationships between PK parameters and patient covariates, this is not an issue. When PK parameters, mainly clearance, vary as a function of well-defined patient covariates, it is important to assess the PTA profile across all important patient covariate patterns (i.e., patient subpopulations). Since the clearance of many antibiotics varies as a function of a patient's renal function, the role of the kidneys necessitates careful consideration in the candidate dose selection process.

Although the lower response rate with CAZ-AVI plus metronidazole relative to meropenem among patients with renal impairment in RECLAIM may have been a random finding (only 8% of study population had renal impairment), several factors could have contributed to the observed outcomes. In RECLAIM, patients with moderate renal impairment (CLCR 30- 50 ml/min) received a 66% total daily dose (TDD) reduction for CAZ-AVI (2.5 grams IV every 8 hours to 1.25 grams every 12 hours) and a 33% TDD reduction for meropenem (1 g IV Q8H to 1 G IV Q12). Secondly, 67.9% of study patients with a baseline CLCR <50 ml/min experienced improvement of renal function to >50 ml/min within 72 hours of study drug dosing initiation with variable timing for the corresponding dose correction. Therefore, potential of under-dosing, paired with lack of proper dose increase in the setting of improved renal function, may have resulted in deleterious patient outcomes with CAZ-AVI due to suboptimal drug exposure.

When evaluating the PTA profile of the moderate renal impairment dose (MRID) of CAZ-AVI selected for RECLAIM in a patient whose renal function improves to mild renal impairment or normal renal function, the potential for under-dosing in this patient is readily apparent. As shown in **Figure 5**, the PTA is approximately 60% for the MRID dose for patients whose renal function improves to the mild renal impairment range. The PTA drops to less than 20% for the MRID dose for patients whose renal function improves to the normal range. To mitigate the potential for this under-dosing, the recommended dose of CAZ-AVI for patients with moderate renal impairment was increased from 1.25 grams every 12 hours to 1.25g every 8 hours. As shown in **Figure 6**, the PTA with 1.25 grams every 8 hours is greater than 95% for patients with mild renal impairment and ~80% for patients with normal renal function. Furthermore, this newly proposed MRID dosing scheme did not result in excess accumulation, as measured by the by concentration-time curve at steady-state (AUCSS). The AUCSS were similar for both ceftazidime and avibactam for indicated doses across patients with varying degrees of renal dysfunction.

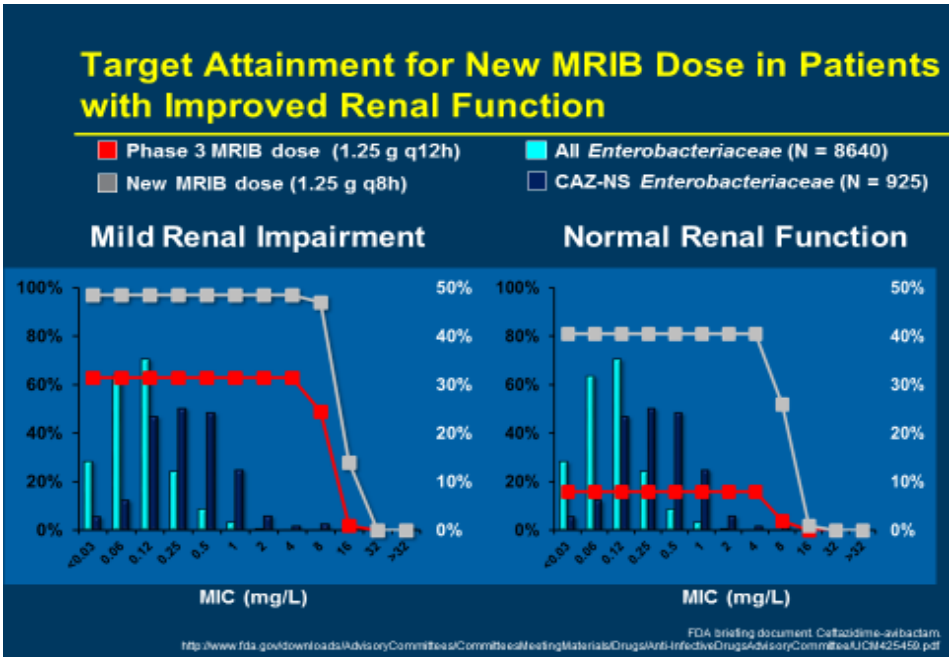


Figure 5. Target attainment for the new moderate renal impairment dose (MRID) CAZ-AVI dose in patients with improved renal function.

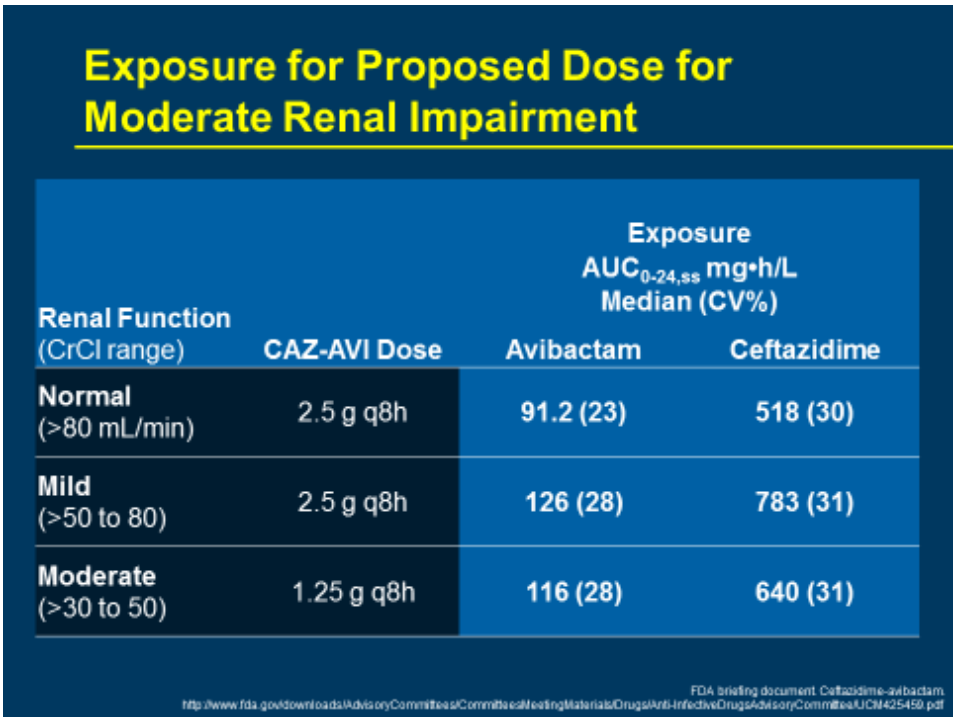


Figure 6. Exposure for proposed CAZ-AVI dose for moderate renal impairment.

6.7.3 Patients with Augmented Renal Function

The need for appropriate dose modifications for patients with renal impairment also applies to patients with augmented renal clearance (ARC). Augmented renal clearance, often defined as a CLCR >130 mL/min/173 m^2, is being increasingly described in subsets of critically ill patients. It is estimated that approximately 30-65% of patients in the intensive care unit (ICU) have ARC despite the presence of a normal serum creatinine concentration. Patient populations with the highest reported incidence of ARC include those with major trauma, sepsis, traumatic brain injury, subarachnoid hemorrhage, and central nervous system infections. Critically ill trauma patients are often hypermetabolic, and frequently require aggressive fluid resuscitation. This may result in increased renal clearance of drugs and larger volumes of distribution. Published data suggests these patients often require more intensive dosing schemes for antibiotics that are renally eliminated, due to their altered physiology. As an illustrative example, recent PTA analyses indicate that a more intensive cefepime regimen of 2 g every 6 h was required when CLCR exceeded 120 mL/min to optimize fT>MIC for MIC values ≤ 8 mg/L. Despite the larger daily dose, the resulting AUC24-48h values were not substantially different from those of the parent regimen (2 g every 8 hours at a CLCR of 120 ml/min). This phenomenon has been increasingly reported and indicates that dose supplementation may be required in patients with augmented renal function. The relevance of these findings are further under-scored by a recent multi-center study by Roberts et al. which found that ICU patients receiving β-lactams who failed to achieve critical PKPD targets were more like to experience negative outcomes.

Similar to patients with rapidly improving renal function, estimated CLCR or GFR equations that rely on serum creatinine concentrations do not accurately identify patients who exhibit ARC. Studies have shown that a substantial proportion of critically ill patients with normal serum creatinine concentrations and estimated CLCR or GFR values exhibit ARC. Due to the high incidence of ARC in patients with a normal serum creatinine concentration, it is recommended that an 8-hour continuous urine collection be collected in patients at high risk for ARC to assess CLCR versus empiric CLCR/GFR estimation equations. Alternatively, therapeutic drug monitoring (TDM) should be considered, although best practices for TDM merit further delineation.

6.7.4 Appropriate Extrapolation of Dose for Body Size

Another point consideration when selecting a dosing regimen is to determine if an antibiotic should be fixed dosed or weight-based dosed. Dosing on body surface area is another consideration but this is not frequently done with antibiotics in adult patients. When the decision is made to dose an antibiotic on weight, the assumptions are that key PK parameters (i.e., clearance and volume of distribution), change proportionately with weight and weight-based dosing is necessary to achieve isometric exposure distributions across the continuum of weights. Conversely, the lack of association between weight and key PK parameters permits use of a fixed dosing regimen as it is more likely to result in bioequivalent exposures across the weight continuum.

The decision to select a fixed or weight-based dosing schemes typically occurs in the early phases of clinical drug development. Unfortunately, the first human trials for a new antimicrobial entity typically only involve adults within a narrow range of body size. This practice hinders the ability to fully evaluate the association between weight and key PK parameters across the current weight distribution in the United States. It is now estimated more than one-third of adults in the US are obese, defined as a body surface area ≥ 30 kg/m^2. As a consequence, a weight-based or body surface area–based dosing regimen defined during drug development may not be applicable to all intended populations for use. Therefore, "early-phase clinical trials must include individuals at the extremes of the weight continuum to permit appropriate dose extrapolation for body size." As part of these evaluation, alternate body size descriptions such as body surface area, ideal body weight, adjusted

body weight, and lean body weight (LBW) should be considered as scalars to ensure isometric exposures across the distribution of weights observed in clinical practice.

Daptomycin is an illustrative example of an antibiotic that is currently dosed on total body weight (TBW) which could have been potentially fixed dosed or dosed according to another body size scalar besides TBW. While no dose adjustments or dosing caps are currently recommended for daptomycin in patients that are either overweight or obese, a matched study of morbidly obese (BMI > 40 kg/m^2) and non-obese (BMI 18-25 kg/m^2) subjects who received daptomycin 4 mg/kg demonstrated that estimates of clearance were nearly identical between morbidity obese and non-obese study subjects (0.82 ± 0.21 L/hr vs. 0.73 ± 0.14 L/hr, respectively, p-value=0.34). Not surprisingly, as AUC0-∞ equals Dose/clearance, the reported AUC0-∞ were nearly doubled among morbidity obese subjects vs. normal weight subjects (581 ± 104 vs. 346 ± 63 mg*h/L, respectively, p-value=0.003). The population PK model that was derived from patients who received daptomycin across nine Phase 1 (n = 153) and six Phase 2/3 (n = 129) clinical trials also failed to identify an association between daptomycin CL and Vc with total body weight (TBW).

Simulations of AUC0-∞ in subjects given 4 mg/kg and 6 mg/kg of TBW and LBW using this previously published population PK model further highlight the consequences of dosing an antibiotic on TBW when there is no relationship between TBW and key pharmacokinetic parameters (**Figure 7**). First, exaggerated exposures will be observed among subjects with higher weights and this may increase the risk of non-immunologic exposure-related toxicities in patients when dosed on TBW relative to fixed dosing or dosing on an alternative body size descriptor like LBW. Interestingly, most of the patients who experienced a CPK elevation in the daptomycin S. aureus bacteremia and infective endocarditis trial were obese, and this finding was substantiated by a number of real-world usage studies which found higher rates of CPK elevation among obese vs. non-obese patients who received daptomycin. Second, patients with lower weights who are dosed on TBW may receive a dose that results in suboptimal exposures that may potentially lead to higher rates of clinical failure.

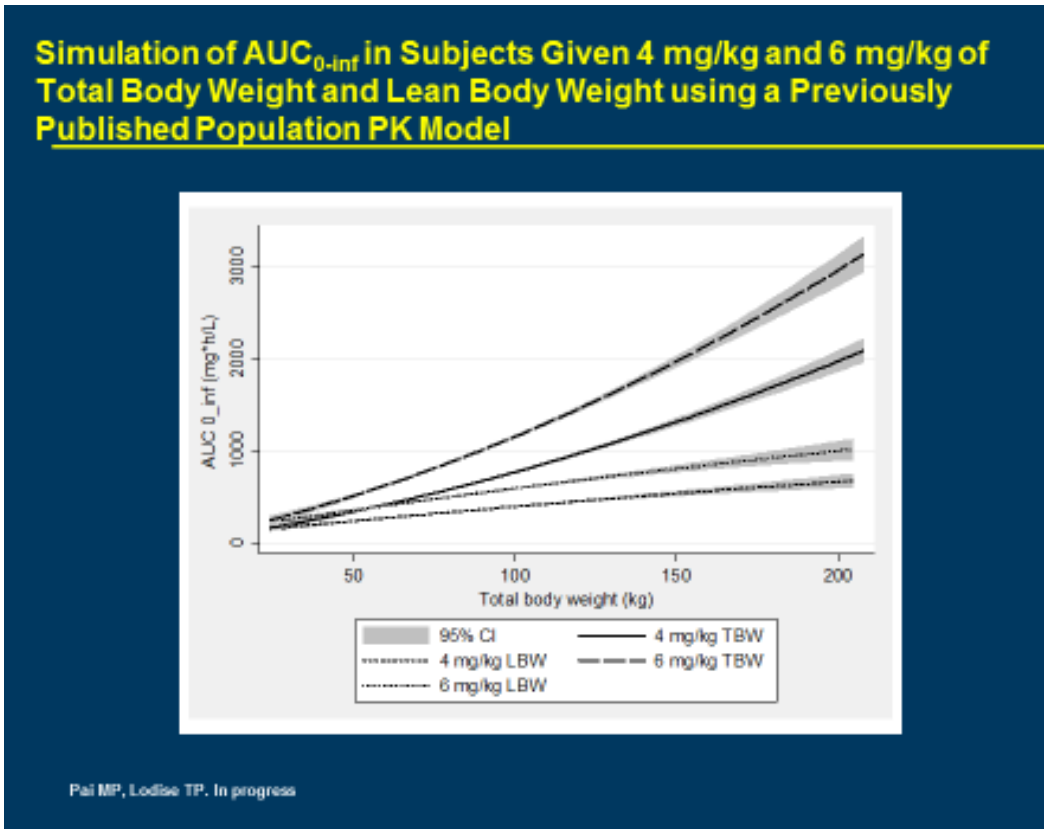
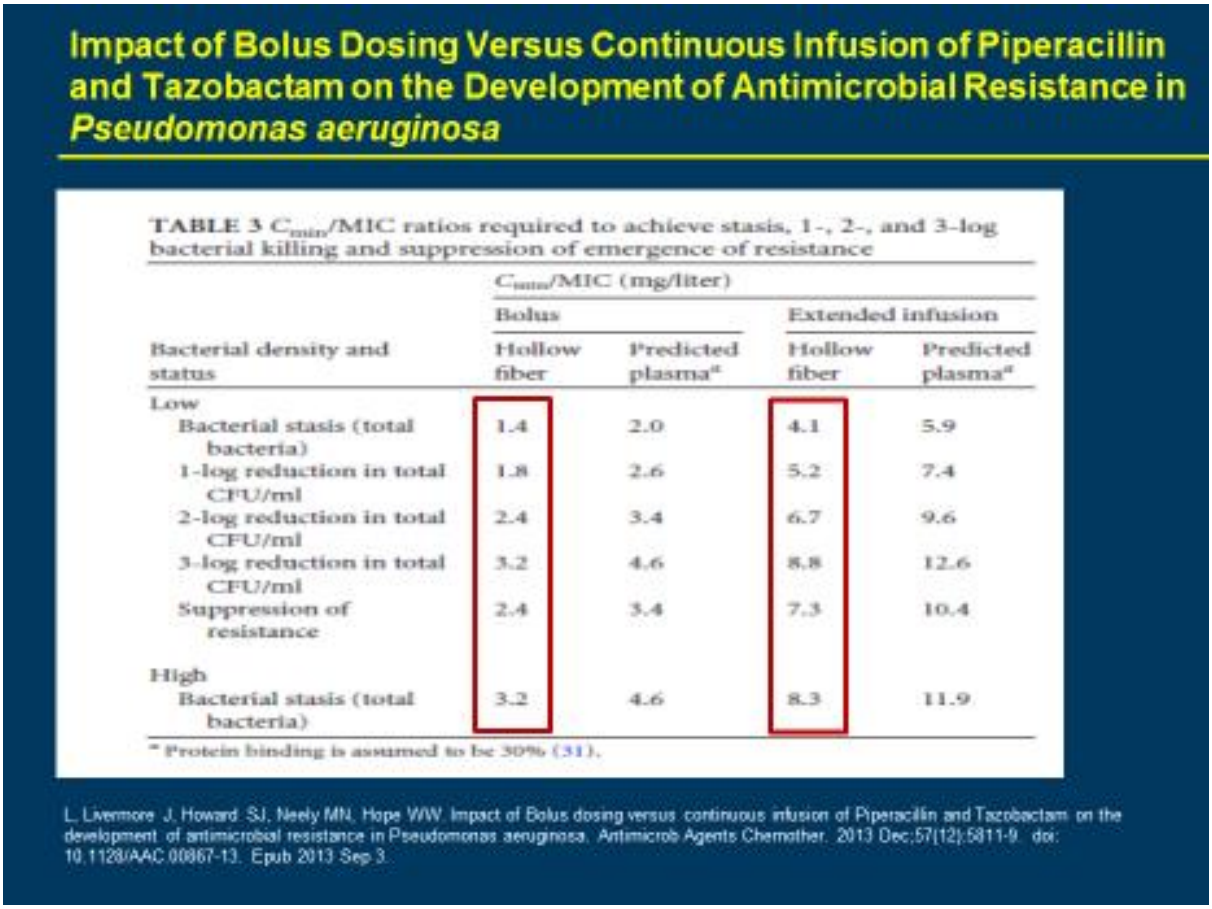


Figure 7. Simulations of AUC0-∞ in subjects given 4 mg/kg and 6 mg/kg of total body weight (TBW) and lean body weight (LBW) using a previously published population PK model.

6.7.5 Preclinical PKPD Models that Mimic the PK and Protein Binding Profiles of Important Patient Subpopulations

A critical consideration when developing antibiotics is understanding and appropriately identifying the target population for use. In addition to enriching for these target patient subpopulations in clinical studies, it may also be advantageous to conduct animal and in vitro PKPD infection model studies that mimic the altered PK profiles of the target patient populations. Currently, preclinical PKPD model studies are largely conducted using the PK profiles of healthy participants with normal renal function. It is important to recognize that the concentration-time curve for healthy participants is not always reflective of the targeted patient population. Although current thinking is that a single magnitude of exposure is needed to achieve a certain effect (e.g., stasis, 1 log10 CFU killing from baseline, etc.) with a given drug, recent studies suggests that the PKPD target required for a designated effect may vary by the shape of the concentration-time curve. In a recent study by Felton et al., the fCmin/MIC ratio required to achieve stasis, 1-, 2-, and 3-log bacterial killing and suppression of emergence of resistance varied between bolus and continuous infusions of piperacillin/tazobactam against Pseudomonas aeruginosa. The fCmin/MIC ratio threshold was higher with continuous infusion vs. bolus infusion dosing, highlighting the critical relationship between the shape of the concentration-time curve and associated effect (**Figure 8**).



Impact of Bolus Dosing Versus Continuous Infusion of Piperacillin and Tazobactam on the Development of Antimicrobial Resistance in *Pseudomonas aeruginosa*

TABLE 3 C_{min}/MIC ratios required to achieve stasis, 1-, 2-, and 3-log bacterial killing and suppression of emergence of resistance

Bacterial density and status	C_{min}/MIC (mg/liter)			
	Bolus		Extended infusion	
	Hollow fiber	Predicted plasma[a]	Hollow fiber	Predicted plasma[a]
Low				
Bacterial stasis (total bacteria)	1.4	2.0	4.1	5.9
1-log reduction in total CFU/ml	1.8	2.6	5.2	7.4
2-log reduction in total CFU/ml	2.4	3.4	6.7	9.6
3-log reduction in total CFU/ml	3.2	4.6	8.8	12.6
Suppression of resistance	2.4	3.4	7.3	10.4
High				
Bacterial stasis (total bacteria)	3.2	4.6	8.3	11.9

[a] Protein binding is assumed to be 30% (31).

L, Livermore J, Howard SJ, Neely MN, Hope WW. Impact of Bolus dosing versus continuous infusion of Piperacillin and Tazobactam on the development of antimicrobial resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2013 Dec;57(12):5811-9. doi: 10.1128/AAC.00867-13. Epub 2013 Sep 3.

Figure 8. Impact of bolus dosing vs. continuous infusion of Piperacillin and tazobactam on the development of antibiotic resistance in *Pseudomonas aeruginosa*.

It is also important to recognize that protein binding was assumed to be 30% in this hollow fiber infection model study. Protein binding is also an important factor in determining appropriate drug dosing and evaluating PKPD targets. As free drug is assumed to be the microbiologic active fraction of an antibiotic, it is important to conduct protein binding studies in target populations. The extent of protein binding varies across populations and it not always consistent with that observed in healthy participants.

6.7.6 Recommendations (loose recommendations for discussion)

- Conduct animal and in vitro PKPD studies mimicking altered PK profiles that are reflective of target patient populations.
- Conduct PK and protein-binding studies in target patient populations.
- Conduct PK studies in infected target patients vs. healthy volunteers.
- Enrich clinical trials for target patient populations.
- Determination of appropriate dose modifications for patients with renal impairment, including patients with rapidly changing renal function.

- Determination of appropriate dose modifications for patients with augmented renal function.
- Determine the most appropriate body size descriptor, if any, for drug dosing across the continuum of weight in target patient populations.

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