Pharmacodynamics for Antifungal Drug Development: an Approach for Acceleration, Risk Minimisation and Demonstration of Causality

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ABSTRACT

The treatment of invasive fungal diseases constitutes a significant unmet medical need. There are relatively few antifungal agents in clinical development and paucity of novel targets. Morbidity and mortality remain high and clinical outcomes are compromised by submaximal efficacy, emergence of drug resistance and drug related toxicity. Thus, new antifungal agents are urgently required. A deep understanding of exposure response relationships underpins the development of safe and effective clinical regimens of any therapeutic agent. Pharmacokinetics and pharmacodynamics (PK-PD) is increasingly recognized as a vital tool in the development of new antimicrobial agents, and maximises the probability that the right dose will be studied the first time. There is currently no information or agreement as to what constitutes an adequate PK-PD package for the development of a new antifungal agent. This review provides a summary of the achievements of antifungal PK-PD for the treatment of invasive candidiasis, invasive aspergillosis and cryptococcal meningoencephalitis and outlines the necessary components of a PK-PD package for a new antifungal agent. Such information is critical for the accelerated and efficient development of new agents and enables improved clinical outcomes to be secured.

1. INTRODUCTION

 Invasive fungal diseases (IFDs) are a persistent clinical challenge. They predominantly occur in patients with significant abnormalities of underlying immunity, and in the setting of complex multisystem diseases that are themselves associated with excess morbidity and mortality. IFDs are usually rapidly progressive, diagnosed late in their clinical course, and are still lethal in many patients. A significant proportion of patients fail therapy with first-line agents and/or experience drug-related toxicity. Antifungal drug resistance poses ever-increasing therapeutic challenges and is associated with high mortality. There are relatively few licensed therapeutic agents and fewer still in development. Thus, a refreshed and sustainable pipeline of antifungal agents is urgently required.

 Developing new chemical entities is the first critical step in the refreshment of the antifungal pipeline. This is clearly not a trivial exercise, given the relative paucity of agents that are currently available or in late stages of clinical development. Fungi are eukaryotes with a relatively high degree of phylogenetic similarity to humans—therefore, they offer relatively few differential targets that can be exploited for antifungal drug development. This review considers the steps that are required to shepherd compounds from the laboratory to the clinic, across the “valley of death” where many promising compounds succumb to the pitfalls of the drug development process. From a societal perspective, the potential value of a new molecular entity is too high to risk sub-standard or inappropriate drug development, and this is the primary motivation for this review.

 Antifungal pharmacokinetics and pharmacodynamics is the discipline that describes, quantifies and enables control of drug-exposure-response relationships 1. For drug development, there are two major contributions that PK-PD can make to the drug development process. The first is the couplet of acceleration and de-risking of the steps that are required as compounds transit from the laboratory into the clinic. The second is the provision of supportive evidence for causality (i.e., evidence that the observed effects are a result of the drug, and that the drug exerts a known and predictable biological effect that can be harnessed for therapeutic benefit).2 The latter is especially important when causality cannot be readily established by more traditional approaches, such as multiple comparative Phase II and III clinical trials. The purpose of this review is to describe some of the key ideas for the use of PK-PD for antifungal drug development. It is not intended as a comprehensive review of antifungal PK-PD—examples of which can be found elsewhere. The key information that can be obtained from a preclinical PK-PD study is summarised in table 1. PK-PD is increasingly viewed as central to drug development programs. This review explains why this is the case and examines the constituents of a data package required for drug filing.

2. THREE CURRENT CHALLENGES IN ANTIFUNGAL DRUG DEVELOPMENT

*1. Invasive fungal Diseases constitute an unmet medical need*

 Invasive fungal diseases, in the most general of terms, constitute an unmet medical need as evidenced by the following: (1) current agents are not maximally effective (i.e. approximately 20-30% fail first-line agents and need to be switched to other agents; approximately 20% patients die at 6 weeks); (2) at least some of the current classes and agents are characterised by significant drug-related toxicity that has an impact on morbidity, mortality and mandates switching antifungal therapy; and (3) inherent (e.g. fluconazole resistance in *Candida krusei* or *C. glabrata*) and acquired drug resistance (e.g. triazole resistance in *Aspergillus fumigatus*) are both issues that are increasingly prevalent and compromise antifungal therapy.3 Stated simply, many patients fail antifungal therapy because there are too few agents with the necessary therapeutic index to consistently secure favourable clinical outcomes.

*2. There are few agents in developmental pipelines and approval for clinical use is a lengthy process*

 The paucity of new antifungal agents in developmental pipelines is in part a reflection of the phylogenetic relatedness of humans and fungi. The subsequent development process is also slow. This reflects a number of factors that include the relative scarcity of IFDs (at least compared with bacterial infections), complexity of antifungal trial design, and difficulties in enrolling complex patients who often have a multitude of clinical problems. New approaches to streamline and de-risk both preclinical development and early phase clinical studies could maximise the probability of a new drug ultimately succeeding in the clinic and shorten the time for this to occur.

*3. Traditional approaches for establishing optimal antifungal regimens are no longer fit for purpose*

 Unlike the situation in preclinical research where continuous endpoints such as reduction in organism load are possible, the endpoint measures that are usually required by regulatory agencies for clinical studies are generally dichotomous (e.g., all-cause mortality at 6 weeks, clinical response) and do not readily demonstrate graded responses. Clinical studies are a slow and inefficient way to identify optimal regimens, especially when there is uncertainty about the underlying dose-exposure-response relationships. A good example of this is liposomal amphotericin B, which was studied in multiple clinical studies in order to define a regimen suitable for clinical use. Multiple studies were conducted that studied regimens in the range of 1-15 mg/kg/day,4–6 with no clear clinical signal suggesting therapeutic benefit of a higher dose. In retrospect, almost all of these studies could have been avoided with the application of modern PK-PD techniques.7 A PK-PD model of invasive pulmonary aspergillosis suggests that 3 mg/kg is associated with near maximal antifungal activity.7 Thus, an earlier bridging study would have enabled the right regimen to be studied the first time, which in turn could have saved millions of dollars, many years of painstaking clinical research, and undoubtedly prevented morbidity and mortality directly attributable to use of suboptimal antifungal regimens.

3. PK-PD AND THE FUNDAMENTAL RELEVANCE OF CAUSALITY IN DRUG DEVELOPMENT

 As described by Peck *et al*2, the notion of causality in drug development refers to the idea that a drug exerts its actions via a sequence of events that can be observed, quantified and prospectively tested. Causality is central to proving that the drug is not sham and does not work by magic. Rather, its effects are well understood and largely predictable, and the drug can therefore be expected to behave in a similar way when administered to similar groups of patients in the future.

Two major approaches to proof of causality are possible. In settings where a detailed biological understanding of pharmacological action is lacking, causality may be difficult to demonstrate at a mechanistic level. Hence, development in many therapy areas relies on conduct of a second clinical trial that provides empirical confirmation of drug effect. In effect, the second trial minimises the probability that the effect observed in the first trial had merely arisen by chance and was independent of any drug action.

In other settings, Peck *et al*2 suggest that evidence for causality can be derived using more rational, efficient, and informative approaches than conducting repetitive costly clinical trials. In the particular case of antimicrobial agent development, PK-PD provides evidence of causality by providing the linkage between administration of the drug (dosage), concentrations at various sites within the body and the ultimate clinical effect(s) (e.g. clinical cure, survival). An understanding of exposure-response relationships enables the design of validation experiments that can be performed prospectively, which enable drug development to proceed on a rational basis.

 In an era of progressively rising antimicrobial resistance and changing epidemiology, there are increasing examples where it is simply infeasible to conduct any randomized comparative phase II or III clinical trials. Examples may include diseases caused by a rare fungus such as *Fusarium* spp., or cases in which there is no good standard of care (e.g. *Scedosporium prolificans*, triazole-resistant *Aspergillus fumigatus*). In these circumstances, causality cannot be established from a standard paradigm of large comparative clinical trials. An alternative approach is required. Antifungal PK-PD studies can potentially be used to establish causality, and enable antifungal agents to be developed for clinical diseases that are otherwise difficult to study in the clinic.

4. OVERVIEW OF ANTIFUNGAL PK-PD: A SUMMARY OF CURRENT KNOWLEDGE

 Much of the current understanding and knowledge of antifungal PK-PD has developed from antibacterial PK-PD. The central tenant of modern pharmacodynamics is that the shape of the antimicrobial drug concentration-time curve can be used to predict antimicrobial effect. These concepts have been elucidated in detail elsewhere.1,8 Briefly, information that is contained within the drug concentration time curve is extracted and condensed into three concise measures of drug exposure: the peak concentration, the area under the concentration-time curve and the percentage of the dosing interval that drug concentrations are above some threshold (often the MIC). This paradigm works reasonably well much of the time, but there are increasing examples where it does not, and this is a subject of ongoing research.

 The relevant pharmacodynamic index is determined using dose-fractionation studies in experimental models. Dose fractionation studies involve studying the effect of different schedules of drug administration where the same total daily dose is administered with different frequencies. If the least fractionated regimen (i.e. large infrequent dosages) has the most antifungal activity, Cmax:MIC is likely to be important. If the most fractionated regimen (i.e. small frequent dosages) has the most activity, T>MIC is likely to be important. If the effect is the same regardless of the regimen, then area under the concentration-time curve (AUC):MIC ratio is likely to be the dynamically linked variable. There are multiple nuances related to the appropriate design of these studies to ensure correct conclusions are obtained. Most importantly is the relationship of the schedule of administration relative to the plasma half-life. Stretching the dosing interval beyond multiples of the half-life will inevitably tend to make the pattern of antifungal activity appear time-dependent. Conversely, if the dosing interval is much shorter than the half-life, the peak concentration will tend to appear important. An important additional consideration is the post antifungal effect (PAFE; defined as the time for a 1-log growth after drug concentrations drop beneath the MIC). Agents that exhibit prolonged PAFE exhibit persistent antifungal effect even when serum drug concentrations are negligible.

 Elucidation of the relevant pharmacodynamic index for any given agent can generally only be established in preclinical models. Usually, only one schedule of drug administration is studied in clinical settings, which means there is no opportunity to examine the impact of different indices on clinical outcomes. While there may be substantial pharmacokinetic variability following the administration of a single schedule, this is insufficient to break the covariance that exists for the three different pharmacodynamic indices.

a. Invasive Candidiasis

1. Models of Invasive Candidiasis:

 The most commonly used models of invasive candidiasis are neutropenic and non-neutropenic murine models of disseminated infection. In both models, a suspension of *Candida* blastoconidia is injected i.v., thereby rapidly causing disseminated disease in the liver, spleen, brain and kidney. This model mimics disseminated infection in the context of an i.v. catheter. The most reliable effect site that is used quantify fungal burden is the kidney. The histopathological sequence for invasion within the murine kidney has been documented.9 Both neutropenic and non-neutropenic rabbit models of disseminated candidiasis have also been used to characterise the PK-PD of antifungal agents (see for example10,11). Rabbit models of hematogenous *Candida* meningoencephalitis (HCME), which is a commonly encountered disease in premature neonates, have been developed in which *Candida* invades from the bloodstream into various sub-compartments within the central nervous system and the eye.

 The pharmacodynamic endpoint used in all of these models of disseminated candidiasis is a reduction in fungal burden in the primary effect site (kidney, brain, eye). Several studies have also used circulating concentrations of 1,3 ß-D-glucan in serum and CSF as a biomarker to quantify the antifungal effect.12–14

1. Bridging from the laboratory to the clinic

 Information and insights have been derived using PK-PD models of disseminated candidiasis:

* Triazoles exhibit concentration-dependent killing with the AUC:MIC consistently identified as the dynamically linked variable.15
* A pharmacodynamic target *f*AUC:MIC of 25-50 is associated with near maximal antifungal efficacy with confirmation/ cross validation using human datasets.16–19
* Echinocandins exhibit concentration-dependent killing with the AUC:MIC and/or the peak concentration:MIC consistently identified as the dynamically linked variables.20,21 *C. glabrata* and *C. parapsilosis* have lower pharmacodynamic targets than *C. albicans*.22 Higher echinocandin dosages than are currently used may be required to achieve fungicidal activity against *C. glabrat*a in neutropenic hosts.23 PK-PD has been used to support innovative dosing regimens of micafungin.24 There has been an understanding of the pharmacodynamics of echinocandins for Fks1 mutations within *C. albicans* and *C. glabrata*,25,26 and an understanding of the use of these agents for infections in sanctuary sites.27,28 These insights have been used to help establish in vitro susceptibility breakpoints for these agents against *Candida* spp.25,26
* Flucytosine (5FC) exhibits time-dependent antifungal activity and the T>MIC of 45% is associated with logarithmic killing and prolongation of survival29,30
1. Strengths and limitations

 The murine model has been extensively used in a number of research groups and is well validated. The severity of the model and hence the dynamic range of the effects being measured can be usefully manipulated by moving from non-neutropenic to neutropenic animals. The model is relatively easy to perform and is both reliable and robust. These models have been used to characterize existing agents, but not yet to provide preclinical PK-PD support for developing new agents. There is some uncertainty regarding the endpoint in the models that are associated with “success” and are of potential clinical relevance, especially for the brain and eye. Stasis and various orders of logarithmic killing have been used and probably serve as reasonable endpoints for the assessment of new agents relative to active controls.

b. Invasive aspergillosis

1. Models of Invasive Aspergillosis

 Until recently, a detailed understanding of the pharmacodynamics of anti-*Aspergillus* agents was been limited by the need to overcome two obstacles before useful predictive models could be developed. First, there was a need to develop respiratory models of invasive pulmonary aspergillosis (IPA) as opposed to disseminated models where conidia are injected i.v. This was necessary because the lung is the primary infection site in a majority of patients with IPA and exposure response relationships are probably idiosyncratic for that site. Second, the filamentous nature of this fungus meant that non-culture-based methods to assess antifungal effect on fungal tissue burden were required. A contract for New Animal Models for Invasive Aspergillosis funded by the National Institute of Allergy and Infectious Diseases (NIAID) National Institutes of Health (NIH) in the USA to develop standardized models of invasive aspergillosis and to characterise novel diagnostic and therapeutic modalities addressed the former issue.31,32

 The identification of relevant and clinically tractable biomarkers for pharmacodynamic studies was particularly problematic for many years. Different investigators have used different approaches. *Aspergillus* spp. are filamentous multinucleated organisms that produce a syncytium. Homogenisation of tissue that is successfully used to quantify the bacterial burden in tissues is not appropriate for fungi because individual propagules (genome equivalents) are either inadequately separated or are irreparably damaged—either scenario results in an imprecise estimate of fungal burden.33 The development of qPCR and commercially available ELISA systems for galactomannan were pivotal to enabling PK-PD experimental models of anti-*Aspergillus* agents.34–36 Quantitative PCR provides a robust estimate of the amount of fungal DNA in tissues34 although may simultaneously amplify extracted DNA from conidia (inactive environmental forms) and hyphae (tissue invasive forms). Galactomannan is a soluble large molecular weight polysaccharide that is released from the surface of tissue-invasive forms of *Aspergillus* spp.37 Circulating concentrations of galactomannan provide a robust and reproducible measure of the fungal burden within tissue beds.33 A third approach is use survival as the primary pharmacodynamic endpoint.38,39

 A rabbit model of IPA has been used to characterize most of the anti-*Aspergillus* agents that are currently licensed for clinical use. More recently, this model has been used to characterize the PK-PD of the various formulations of amphotericin B, using galactomannan as the primary pharmacodynamic endpoint.7 The rabbit model has the advantage of enabling repeated sampling from the same animal as occurs in patients (as opposed to the murine model where a destructive design is required). A limitation of the rabbit model is the expense, meaning that it is generally not possible to perform extensive analyses with multiple strains and different experimental conditions. Rather, the model can be used to confirm critical findings obtained from other models to ensure that unanticipated idiosyncrasies in that model do not lead to biased and inappropriate estimates for patients.

 More recently, dynamic *in vitro* models of IPA have been developed that are an extension of the hollow fibre infection model that is used to characterise the PK-PD of many antibacterial agents against Gram-positive and Gram-negative pathogens 40,41. The model of Jeans *et al*40 uses a cellular bilayer of alveolar epithelial cells and endothelial cells grown on a semipermeable polyester membrane and galactomannan as the primary pharmacodynamic readout. An advantage of the model is that it mimics the structure and function of the human lung and the important events in the early stage of tissue invasion and pathogenesis.

1. Bridging from the laboratory to the clinic

 There are fewer dose fractionation studies with *Aspergillus* spp. compared with *Candida* spp. Caspofungin, amphotericin B and isavuconazole display concentration-dependent antifungal activity against *Aspergillus fumigatus*,36,42,43 which is consistent with their pattern of activity against *Candida albicans*. In other cases the relevant pharmacodynamic index derived in studies with one pathogen (e.g. *Candida*) is often assumed to apply to *Aspergillus* spp. without formally assessing whether this is the case. There is significantly less certainty about how to design and analyze dose fractionation studies for *Aspergillus* spp. compared with bacteria and yeasts, and this is an area of ongoing research. A recent study suggests that galactomannan may be used to distinguish different pharmacodynamic responses following dose fractionation experiments.44

The following has been achieved using pharmacodynamic models of IPA:

* Description of the PK-PD of posaconazole, including an insight into a pharmacodynamic target of clinical relevance and the predictive value of the MIC for determining the therapeutic response to posaconazole.45–47
* Description of the PK-PD of voriconazole with cross validation with clinical data and the extension these findings to provide decision support for establishing *in vitro* susceptibility breakpoints at EUCAST.16,40,48
* Description of the PK-PD of isavuconazole including the impact of the MIC on exposure response relationships34,43,49
* Description of the PK-PD of amphotericin B deoxycholate, amphotericin B lipid complex and liposomal amphotericin B.7
1. Strengths and limitations

 New models of IPA have enabled the PK-PD of triazoles to be determined including the impact of MIC on exposure response relationships. This understanding has been important for providing decision support for setting in vitro susceptibility breakpoints. Experimental models of IPA have also been important in the development of new anti-*Aspergillus* agents.44

 The principal limitation of anti-*Aspergillus* PK-PD models is a relative lack of experience with the relevance of magnitude of drop in the biomarker (qPCR signal, reduction in galactomannan or survival prolongation) and use for bridging to humans. *Aspergillus* models conducted on a neutropenic background are typically severe, rapidly progressive and uniformly lethal. Consequently, conservative endpoints such as 100% survival or complete suppression of a biomarker may not be realistic or appropriate for bridging to the clinic.

Given the relatively newer nature of work in this area, the use of comparative measures within a standardized model may be helpful. For example, the effect of a clinically relevant exposure of an agent with known efficacy could be used as an internal benchmark, with the experimental conditions (e.g. the inoculum, delay in administration of drug, duration of the model, degree of background immunosuppression) adjusted to produce ”on scale” readouts. A demonstration that the novel agent produced an effect in the same range as the known agent can then be used as evidence of having identified an appropriate model and endpoint. More work is required to better understand the endpoints used in these various models.

c. Cryptococcal meningitis

1. Models of cryptococcal meningoencephalitis

 Two models of cryptococcal meningoencephalitis have been developed. The first is a murine model where *Cryptococcus neoformans* is inoculated into the respiratory tract or administered i.v. (see for example50,51) In either case disseminated disease ensues with predictable and reproducible encephalitis. The histopathological sequence has been described.52 The volume of CSF in mice is too small to enable this compartment to be sampled. Mice are treated for 7-14 days and exposure-dependent antifungal activity of drugs alone and in combination can be generated. A rabbit model of cryptococcal meningoencephalitis has also been developed.53 Both the CSF and brain can be sampled to determine fungal burden. The ability to obtain serial CSF cultures mimics the clinical care of patients where repeated lumbar punctures are often performed to assess the response to therapy and to manage elevated intracranial pressure.

1. Bridging from the laboratory to the clinic

 The following has been achieved using experimental models of cryptococcal meningoencephalitis:

* Experimental basis for innovative induction regimens of amphotericin B deoxycholate52
* Insight into antifungal clinical regimens alone and in combination associated with near maximal antifungal effect50,54–56
* Potential breakpoints for fluconazole51
1. Strengths and limitations

One of the strengths of experimental models of cryptococcal meningoencephalitis is that they are extremely robust and reproducible. At least in rabbits, the primary model readout of fungal burden in the CSF is the same as is used in patients. The murine model enables burden in the cerebrum to be easily assessed and provides complementary evidence to that obtained within the CSF. There is significantly less experience with models of cryptococcal meningoencephalitis compared with invasive candidiasis and invasive aspergillosis and a degree of uncertainty as to the clinical relevance of various orders of logarithmic killing in the cerebrum or CSF. Nevertheless, there is a lot of clinical experience and data with fluconazole, amphotericin B deoxycholate and flucytosine alone and in combination, which provides the opportunity to benchmark new regimens and agents. Hence, various degrees of logarithmic killing (or other decline in fungal burden) induced by these standard agents can be used to define biological targets that are also likely to be relevant for new molecules.

(d) Future directions and requirements: a focus on rare fungal diseases

 There are a multitude of medically important fungal pathogens. Some of these constitute an unmet medical need because of a near complete absence of therapeutic modalities. Obvious examples include the Mucorales, *Scedosporium* spp. and *Fusarium* spp. To date, pharmacodynamics has not been used to help develop antifungal agents for these pathogens. There are two principal reasons for this. First, experimental pharmacodynamic models that are a faithful mimic of the pathogenesis in humans of these diverse infections have not been developed. This is in turn is a function of the relatively low virulence of many of these pathogens, which is a challenge for model development. Second, reliable study endpoints that are an accurate reflection of fungal burden and drug effect are not generally available—tools such as galactomannan for IPA (see above) would need to be developed on a fungus-by-fungus basis. The development of pharmacodynamic models for these less common fungal pathogens would enable PK-PD studies and a rational basis for the selection of regimens for new antifungal agents. Currently, the regimen is extrapolated from more commonly encountered pathogens such as *Candida* *albicans* and/or *Aspergillus fumigatus*, which may not necessarily be appropriate.

5. REGULATORY CONSIDERATIONS FOR DEVELOPMENT OF ANTIFUNGAL AGENTS

 This section describes the current regulation in the US and Europe for developing new agents. Both the EMA and FDA have been active in considering ways in which new drugs can be developed faster, especially to address unmet medical need. Studying the right dose the first time requires a comprehensive high quality PK-PD package and a deep understanding of dose-exposure-response relationships

2. Expedited programs

 The overall intention of expedited programs is to accelerate the approval of drugs and make them available to patients as soon as it is clear that the benefits most likely outweigh the risks. The FDA, for example have a policy on “Drugs Intended to Treat Life-threatening and Severely Debilitating Illnesses”,57 while the EMA has recently piloted an adaptive pathways approach that provides a mechanism to provide timely access for new drugs for niche indications followed later by a broader range of indications. A pilot program was launched in March 2014.58

1. Food and Drug Administration (FDA)

 The FDA has four expedited programs for drug development to address unmet medical need in the treatment of serious and life-threatening conditions (see reference59): (1) fast track designation; (2) breakthrough therapy; (3) accelerated approval; and (4) priority review designation. Of these programs, only fast track and priority review are likely to be useful to antifungal development: breakthrough therapy requires clinical demonstration of substantial improvement over available therapies (generally unlikely unless there are no available therapies) and accelerated approval focuses on use of a surrogate or intermediate clinical endpoint (not routinely appropriate as it is not generally possible to measure an actual endpoint with antifungal agents). A drug that is designated as a Qualified Infectious Diseases Product (QIDP) is eligible for fast track designation, will undergo priority review, and has the potential for an additional 5 years marketing exclusivity. A 2003 guidance discusses approaches to clinical exposure-response relationships, but there are no guidance documents that summarise the nature and extent of preclinical package that is required for fast track status.

1. European Medicines Agency

 The EMA has guidance on antifungal drug development (CHMP/EWP/1343/Rev.1) that was last updated in 2010.60 This document makes it clear that PK-PD is important, but provides no specific further guidance. EMA also has guidance documents on development of antibacterial agents.61 The last of these documents (the so-called 2013 Addendum) makes clear reference to the use of PK-PD to provide evidence of therapeutic efficacy, to provide a rationale for dosage selection and to establish a mechanistic understanding that underpins the use of new agents against multidrug resistant organisms. Finally, draft guidance that has been recently released on the use of PK-PD for antibacterial agents includes an explicit discussion of the applicability of the concepts to antifungal and antimycobacterial agents.62

6. SUMMARY OF APPROACHES FOR ANTIFUNGAL PK-PD

 There is an increasing recognition that antibacterial PK-PD is critical to the drug development process of new antibacterial agents. The necessary tools have been developed to apply similar techniques and concepts for antifungal agents. However, there is no consensus on exactly what is required or any standardisation of approaches for either antibacterial or antifungal agents. Each academic PK-PD laboratory has different expertise, different experimental models and different approaches to the same problem. There is no guidance on what constitutes a reasonable package to support licensing. Here, we list our own views on the key steps, the pitfalls and potential solutions to developing a PK-PD package for a new antifungal agent. While these steps are presented as a unidirectional process the reality is different. Steps may proceed in parallel and often loop backwards and forwards, as more and more information is obtained and each step is progressively refined.

The constituents of a necessary preclinical PK-PD data package are summarised in Table 2.

*1. MICs and other measures of in vitro potency*

 The MIC distribution should be defined using a standardised methodology (e.g., CLSI63–64 or EUCAST65,66) using a suitably large and diverse collection of clinical isolates to enable a robust estimate of the extent of variability in MICs. The testing methodology should be examined closely for reproducibility and QC isolates should be identified as early as possible. These studies help define the modal MIC and the degree of variability in MIC. An idea of the MICs of organisms harbouring resistance motifs is helpful, although the types of mechanisms that may be encountered in the clinic (as opposed to those generated by passaging in the presence of drug in the laboratory) are unlikely to be available in the early phases of drug development. If enough strains are studied, it may be possible to define an epidemiological cut-off value (termed ECV in CLSI or ECOFF at EUCAST), which is defined as the highest MIC of the organism that belongs to the wild-type population. ECVs and ECOFFs can be defined statistically67 (although the statistical value that defines the ECV or ECOFF is necessarily arbitrary) or on the basis of known molecular mechanism of resistance. Defining MICs of isolates resistant to other first-line agents (i.e. demonstration of the lack of cross resistance) is key and likely to provide evidence for points of differentiation with existing agents.

*2. Experimental Model(s) of Infection*

 Notwithstanding a commitment of all researchers to the principles of the 3Rs (reduction, replacement and refinement) in relation to the use of laboratory animals in medical research, *in vivo* models remain central to antifungal PK-PD. The complex biology (e.g. dimorphism and thermal dimorphism) of invasive fungal pathogens, the impact of tissue damage and destruction (haemorrhagic infarction, pyogranulomatous inflammation) on antifungal exposure response relationships and difficulties quantifying fungal biomass are all potential obstacles to the use of hollow fibre infection models that have been central to PK-PD studies of antibacterial and antiviral agents. *In vitro* static and dynamic models of invasive pulmonary aspergillosis have been developed,35,40 but they represent early invasive disease rather than established disease, which is often characterised by significant destruction of tissue. At the current time, disease models in mice and rabbits represent the state-of-the-art of preclinical models of clinical invasive fungal diseases.

 The choice of experimental model should reflect the disease that is being mimicked. Both the site of infection and the underlying immune status can have a significant impact on drug exposure-response relationships, and require careful consideration in study design. The lung is especially important for *Aspergillus* spp. and there have been considerable efforts to develop pulmonary models of IPA in the mouse and rabbit that serve as surrogates for human disease. The CNS is primarily important for *C. neoformans* with well-established models in the mouse and rabbit. Finally, disseminated blood-borne models of invasive candidiasis are well established and validated, with infectious burden in the kidney as the primary model readout.

*3. Establishing exposure-response relationships.*

 Establishing exposure response relationships is a first critical step in any PK-PD study, and if successful demonstrates the deployment of a considerable amount of knowledge. The questions that are important at this very early stage include: (1) whether the compound has any effect on a chosen panel of biomarkers that are likely to be biologically and clinically relevant, and if there is any discrepancy in the behaviour of these biomarkers; (2) relative potency compared with current standard agents/ positive controls; (3) the nature of the exposure-response relationship (e.g. is it steep, has the entire dose-response relationship been delineated, is there a biologically (and clinically) relevant decline in the biomarker?); and (4) whether the compound is well tolerated.

*4. Define the pharmacodynamic parameter (or index) that best links drug exposure to the observed antifungal effect.*

 The effect of antimicrobial agents can be classified using in terms of the three classical pharmacodynamic indices (or parameters): AUC:MIC, Peak:MIC and T>MIC. Each of these standard measures of drug exposure is an attempt to capture system information that is locked within the shape of the concentration-time curve. Analyses using free drug concentration are often preferred, but total drug concentration may be used if protein binding is similar across test species and humans and has the advantage of aligning with observed total plasma concentrations. If, however, protein binding is high then correction for free drug may be difficult as the T>MIC measure used for time-dependent drugs may show T>MIC of 100% of the dosing interval. In these circumstances, the fraction of the dosing interval that free drug concentrations are > MIC (*f*T>MIC), the fraction of the dosing interval that plasma concentrations are > MIC determined in the presence of serum, or the minimum (trough) concentration:MIC may be useful ways of quantifying time-dependent antifungal activity.

 In terms of processes of establishing the relevant PD variable (or index), the following are pertinent:

* The design of dose fractionation studies depends to a large extent on the half-life of the drug. For example, compounds with a long half-life need to have extended study period to ensure the co-linearity between Peak:MIC, AUC:MIC and Time>MIC can be broken and therefore assessed.
* Dose-fractionation studies are almost exclusively conducted in experimental models of infection—it is generally not possible to resolve these relationships in clinical studies because of uniformity of the antimicrobial regimen (especially the schedule of drug administration), and the relatively larger degree of imprecision in both defining and measuring clinical (as opposed to experimental) endpoints for assessing antimicrobial therapy.
* The most commonly used biomarker for these studies is a continuous variable (colony counts, galactomannan, PCR etc.), but it is possible to use a dichotomous endpoint, such as survival, or even a time-to-event analysis, such as a Cox regression model.
* While standard and well accepted, it is important to consider some of the limitations of classifying activity of any agent into one of the three pharmacodynamic indices T>MIC, AUC:MIC or Peak:MIC). On occasions, drugs do not neatly fall into one category or the other. The reasons for this are beyond the scope of this review, but may include inappropriate experimental design, tissue hysteresis and the emergence of drug resistance.
* Caution is advised when the PK in an experimental system is completely discordant from that observed (or predicted) in humans. Mice especially tend to have concentration-time profiles that are strikingly different from humans. In such a situation good experimental design is absolutely critical to prevent erroneous conclusions from bridging studies.
* Dose-fractionation studies can be performed with a single well-characterised wild-type strain, or a small collection of strains. Some investigators use multiple strains and perform a pooled analysis, which may be appropriate if intra-experimental variation does not swamp any biological signal. Our own experiences with *Aspergillus* spp. suggest that pooling large datasets may be difficult, although it is clearly possible for *C. albicans*.
* Dose-fractionation studies are relatively straightforward to perform with *Candida* spp., but more difficult with *Aspergillus* spp. because of larger inherent biological variability that is characteristic of the latter. Nevertheless, dose fractionation studies in *Aspergillus* are emerging.
* A largely unsubstantiated assumption that is frequently made in the anti-infective PK-PD field is that the relevant PD index is the same for a given agent regardless of the invading pathogen. Such an assumption is probably reasonable in the majority of cases with the proviso that the pharmacological target is common, and antifungal resistance does not confound the analysis.

*6. Defining the magnitude of drug exposure (quantified in terms of the pharmacodynamic parameter/ index) that is likely to be associated with an acceptably high probability of a favourable therapeutic response.*

 Arguably, this is the most difficult part of any bridging study because there may be little to objectively guide the process of selecting the appropriate endpoint. The usual way of performing these analyses is to split a continuous relationship into two groups each with a high and low probability of a “good” therapeutic outcome using techniques such as recursive portioning algorithms and logistic regression. The point at which the cut is made is necessarily arbitrary without being necessarily well grounded. Such a decision is especially difficult for a new model system, new approach, or a new drug because of the absence of a precedent. In this regard, the following are relevant:

* Stasis, and a 1- and 2-log fall in burden have been extensively used in both antibacterial and antifungal studies, but there has been relatively little work to rigorously justify any of these endpoints. Differences in experimental design (e.g. inoculum, time to therapeutic intervention, challenge strains) make direct comparisons between different models and laboratories somewhat difficult. Improved inter-laboratory quality control measures may help address this problem.
* There is a risk that a conservative, but erroneous choice will be made (e.g. 95-100% survival will be chosen as an endpoint in an experimental model rather than a 50% survival rate because that “feels better”). Experimental models can be made arbitrarily difficult such that the ability to achieve any particular endpoint is more a function of the model than the endpoint. See below for a more detailed discussion on this point.
* While it may be reasonable to investigate and define the pharmacodynamic index using a single wild-type stain, this is not ideal when trying to understand the importance of the magnitude of the index. Here, it is important to have studied a multitude of strains, ideally with a variety of MICs. It is reassuring to observe the MIC providing some information that explains system variance (i.e. helps to explain the observed biological variability).

7. BRIDGING TO THE CLINIC; ASSUMPTIONS AND PITFALLS

 A fundamental assumption that underpins all bridging studies is that the target for the drug is the microorganism, and the interaction between the two is independent of the host. In such a paradigm, the PD model is largely irrelevant to obtaining the “correct” result and merely serves as a vessel that enables the interaction to occur. Thus, the dose-effect-response relationships of an antifungal agent will be the same regardless of whether they are established in a mouse, rat, rabbit or human. While such an assumption is central to the ability to bridge from any experimental systems to the clinic there are in fact a number of threats to the validity of the bridging process that must be considered to ensure it yields the “right” answer. In this regard, the following represent potential threats to the bridging process and are highlighted to ensure the development of an otherwise effective agent is not inappropriately terminated.

*1. Is the model a faithful mimic of human infection and disease??*

 The site of infection, the underlying immune status, the stage of infection and the timing of therapeutic intervention will all affect exposure-response relationships, and yield a variety of potential pharmacodynamic targets. This immediately raises the question is what is the “right” relationship and endpoint to bring forward in the bridging study.

 One consideration is that PK-PD models conducted in profoundly immunosuppressed experimental models (e.g. persistent and profound neutropenia) can be used to predict the very worst clinical scenario where the clinical outcome is solely dependent on the activity of the antifungal drug killing without any contribution of immunological effectors. Such a position has the advantage of being conservative, but may not reasonably represent the majority of patients that will be encountered in clinical trials (e.g. non-neutropenic patients with candidemia or non-neutropenic hematopoietic stem cell transplant (HSCT) recipients with invasive pulmonary aspergillosis). It may also be reasonable to assess the activity of new drugs in models with less severe degrees of immunosuppression to assess the impact of host immunity on exposure response relationships.

 An alternative approach is to provide a range of pharmacodynamic targets that can be used in the bridging process. This has the advantage of reflecting the fact that patients are different and some will likely need more drug than others, but has the disadvantage of making things more complicated.

*2. Experimental conditions used to establish the model*

 Perhaps the most fundamental issue is that experimental models are established so that the readout following clinically relevant regimens of licensed antifungal agents is “on scale”, and does not result in a biological response that is negligible or maximal—neither scenario will result in useful information for the assessment of new agents. Experimental factors that are independent of the antifungal drug that may affect exposure response relationships and therefore affect whether the model yields useful (i.e. predictive) information include: (1) the challenge strain (e.g. its growth rate, virulence9); (2) the time between inoculation and the commencement of treatment;9 (3) the immunosuppression at the time of inoculation and throughout the experimental period; and (4) the duration of the experiment. Manipulation of these parameters is required to yield on scale readouts that in turn can be harnessed for assessment of new agents.

*3. Pharmacokinetics*

 Accurately describing the underlying of PK in an infected experimental model is key. Poor experimental design with sparse poorly informative sampling and extrapolation to points well beyond the experimental data will all result in biased results. If the PK is wrong, all else will be wrong. There is no consensus as to what constitutes adequate experimental design and analysis for PK, which is an area that could be improved.

*4*. *The study endpoint problem*

 Meticulous description and quantification of exposure response relationships in a validated useful experimental model still leaves the problem of deciding what constitutes a favourable or successful therapeutic outcome. An endpoint that is too draconian may lead to predictions of clinical regimens that are unrealistic and potentially toxic. Thus, a well-intentioned investigator wanting to adopt the most conservative position may inadvertently define drug exposure for which there is simply not adequate safety information as defined in preclinical GLP studies and early phase clinical trials.

 As a pragmatic way forward, a control agent with known preclinical-clinical relationships can be used as an internal benchmark. Such an approach will define the minimum effect in the model that is likely to provide clinically useful activity. All available endpoints (stasis, various orders of logarithmic killing) should be reported to help define candidate regimens that result in drug exposure that are effective and are known (or expected) to be safe from preclinical toxicology studies.

 A real problem occurs when there is considerable uncertainty, as is the case when a new model is being developed, the absence of precedent, or with an agent that is first in class. In this circumstance, it may just be reasonable to use the entire drug-exposure response relationship and show the predicted pharmacodynamic effect with different drug exposures that are known (or predicted) to come from the clinical pharmacokinetic studies.

*5. Protein Binding*

 Although it is generally accepted that only free drug exerts antibacterial activity,8 the effect of protein binding on antifungal agent activity is relatively poorly understood. It is especially difficult to know how to best handle highly bound agents. In general, PK-PD estimates that incorporate adjustments for protein binding do not provide a better understanding of drug effect or exposure response relationships, although it may facilitate comparison between agents within the same class, and be helpful for bridging from preclinical models to humans. One important issue for bridging studies is to ensure there are robust estimates for protein binding in both the experimental system and in humans and a number of different approaches have been used to establish these relationships.

*6. Active metabolites*

 Some antifungal agents have metabolites that are microbiologically active and it is imperative to understand the potency and extent of formation in both experimental systems and humans. The best current example of currently licensed antifungal agents is itraconazole.68

*7. Tissue site pharmacokinetics*

 There can be no doubt as to the biological relevance of drug concentrations at the effect site—even if it is possible to establish a direct relationship between serum concentrations and the observed antimicrobial effect. The important question, however, is whether an understanding of tissue concentrations enables a better understanding of drug effect and behaviour.69 For bridging studies, an assumption is frequently made that the trafficking of drug from the plasma to the effect site (i.e. tissue) is the same in the experimental model and humans. When considering how to handle tissue drug concentrations, the following are relevant:

* The demonstration of drug concentrations in tissue homogenates is of relatively limited value in the absence of pharmacodynamic data.
* A detailed understanding of tissue concentrations can be helpful in understanding if there is any disconnect between plasma concentrations and the observed antifungal effect).

*8. Study design, analysis and interpretation of data*

 There are a number of issues related to the design of studies that are required for a strong case that underpins confidence that the drug is effective and further clinical studies are justified.

* Studies should involve more than one challenge strain to capture biological variability and ensure a degree of geographical representation. The optimal number of strains is difficult to define. Such strains need to be identified appropriately, using state-of-the art microbiological techniques that may include molecular approaches. If antifungal resistance is part of the argument related to unmet medical need, then these strains must be included in the dataset.
* Ideally, more than one model system should be used (which may include more than one type of background immunosuppression), especially for an agent that is first-in-class for which there is no pre-existing information. It may be reasonable to do the majority of the work in one model system and check important conclusions about antifungal activity in another. An in-depth understanding of the strengths and limitations of each of the models is required. The use of more than one model provides an ability to cross check and internally validate key findings before proceeding to detailed clinical studies.
* The standards to which preclinical work are conducted is an important issue. Most (if not all) academic laboratories are not GLP-certified, and are unlikely to ever be so. That does not mean, however, that work cannot be done to a satisfactorily high standard. More discussion is required to address this issue and agree standards that can ensure the data and conclusions generated form PK-PD studies can remain in the drug development pathway. For example, it may be reasonable in the future to use a series of quality control measures to ensure the readouts from model systems are reliable (e.g. induction of logarithmic killing of *Candida albicans* within an specified range following administration of an echinocandin). Such an approach would also facilitate inter-laboratory comparisons.
* Orthogonal reasoning (i.e. getting the same answer by using a different approach or perspective) is critical. Other than using more than one strain and model (as above), other approaches that can be considered include: (1) changing the experimental conditions (e.g. background immunosuppression, length of model, delay in treatment initiation); and (2) using more than one readout or biomarker (e.g. survival, histopathology, a range of biomarkers, imaging).

7. CONCLUSIONS

 Antifungal PK-PD is rapidly maturing as a field of investigation. The past decade has witnessed the development of the experimental models and analytical techniques required to elucidate exposure response relationships. An ability to quantify exposure-response relationships enables control of those same relationships. The knowledge and experience that has been gained from studying currently available antifungal agents provides a platform for the development of new agents. The task remains difficult: mortality is still too high, drug toxicity leads to detrimental clinical outcomes and antifungal resistance erodes the number of agents that can be used. Antifungal PK-PD is now ready to be harnessed to accelerate and de-risk antifungal drug development.

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| --- | --- |
| Key Strategic Steps for Preclinical Antifungal Development | Comments |
| Identification of the dosage that is likely to be associated with near maximal antifungal activity | * Define drug exposure that produces effects that at least match or exceed those induced by positive controls (benchmarking)
* Drug exposure quantified in terms of the relevant pharmacodynamic index enables results to be bridged to humans
 |
| Identification of the schedule of drug administration  | * In later phase clinical studies only a small number of regimens are studied providing little opportunity to break the covariance that exists between the different pharmacodynamic indices
* Dose fractionation studies are performed in preclinical models
 |
| Demonstration of antifungal activity against isolates resistant to other antifungal classes | * A range of isolates with well defined resistance mechanisms to other agents should be studied in experimental models to demonstrate exposure response relationships are indistinguishable from the wild type and there is no evidence of cross resistance
 |
| Establishing a relationship between drug exposure and clinically relevant biomarkers that can be used in early clinical phases of antifungal drug development | * As biomarkers for invasive fungal disease become better defined they can be used to aid the design and potentially as endpoints for phase II clinical trials.
* Current examples include log10CFU/mL CSF for cryptococcal meningitis, galactomannan for invasive aspergillosis and 1,3-ß-D glucan for both disseminated candidiasis and invasive aspergillosis
 |
| Provide an understanding of the predictive value of the MIC | * Establishing in vitro susceptibility breakpoints is a regulatory requirement. An understanding of the predictive power of the MIC generally comes from preclinical models where the portion of observed variance that can be ascribed to MIC is determined
 |
| Demonstration of antifungal activity at the relevant effect site | * Demonstrating antifungal activity in a preclinical model at the same site of infection as is intended for patients (e.g. lung or central nervous system) is powerful evidence for likely effect in humans and minimises the chance of clinical failure because of unanticipated issues with drug penetration or activity at the effect site
 |

Table 1. Summary of the key ideas for use of pharmacokinetics-pharmacodynamics in antifungal drug development that substantially de-risk clinical studies

|  | Key Endpoints and Outputs | Relevant Considerations |
| --- | --- | --- |
| In vitro susceptibility testing | * Establishment of the test methodology and QCs
* Definition of the wild-type population
* Use both CLSI and EUCAST methodology
* Early engagement with experts/ laboratories with expertise in in vitro susceptibility testing
 | * Number of organisms required for each species is not defined, but >100 is reasonable to ensure the wild-type population is robustly defined
* In vitro susceptibility against laboratory generated or engineered mutants helpful
* In vitro susceptibility against panels of isolates that are resistant to other antifungal classes is required
 |
| Protein Binding | * An estimate of protein binding is required for confidence in bridging from experimental systems to humans
 | * Different methods yield different estimates of protein binding
 |
| Characterisation of Exposure-response relationships | * Often requires multiple experiments to define exposure response relationships with an adequate degree of resolution and statistical confidence
 | * Experiments generally begin with dosages that stretch over several orders of magnitude before refinement to relevant areas
* If exposure response relationships are steep it may be difficult to get robust estimates of EC50
 |
| Dose fractionation studies | * Elucidation of the relevant pharmacodynamic index
 | * Dose fractionation studies require a detailed understanding of experimental design and considerable expertise in PK-PD experimentation and modelling
 |
| Determination of the magnitude of relevant pharmacodynamic index that is relevant for bridging to humans | * Overall drug exposure value that can be used for bridging to patients and aid in the selection of the regimen for phase II clinical trials
 | * Requires analysis of multiple strains
* Requires justification of the model endpoint that defines success, which in turn requires the model outputs to be benchmarked against a positive control
 |
| Pharmacodynamic studies in isolates resistant to other antifungal classes | * Demonstration of lack of cross resistance is a critical component for development of a new agent
 | * Requires specific studies in isolates with well-defined resistance mechanisms (ideally at a molecular level).
* Incorporation of a positive control to show failure of the comparator compound is required
 |
| Confirmatory Studies | * A number of model readouts may be considered
* Consider studies in other model systems to explore
 | * Models and ideas need to be "stressed" to ensure they bear scrutiny and can therefore be relied upon for bridging to humans
 |
| Other points for consideration depending on the compound and disease in question | * Resistance studies, tissue PK, impact of immune function on antifungal activity
 | * Aid in a more complete understanding of drug activity
 |

Table 2. Proposed components of a preclinical antifungal PK-PD package