Antifungal Pharmacokinetics and Pharmacodynamics

David R. Andes and Alex J. Lepak

The critical role of pharmacokinetics and pharmacodynamics in the selection and dosing of antimicrobial therapeutics, including antifungal agents, has gained increasing recognition [1–4]. The study of pharmacokinetics involves understanding the interaction of a drug with the host, including measurements of absorption, distribution, metabolism, and elimination. The study of antimicrobial pharmacodynamics provides insight into the link between drug pharmacokinetics, in vitro susceptibility, and treatment outcome. Knowledge of the pharmacokinetic/pharmacodynamic index and magnitude associated with efficacy can be helpful for clinicians to predict therapeutic success/failure, guide optimal dosing levels and intervals, aid in susceptibility breakpoint development, guide therapeutic drug monitoring, and limit potential adverse outcomes, including toxicity and the development of resistance [5–8]. Numerous in vitro, animal, and clinical studies have been instrumental in characterizing the pharmacodynamic activity of the clinically available antifungal drug classes, including triazoles, polyenes, flucytosine, and echinocandins [6–18]. The analyses of data with these antifungal drug classes have identified distinct pharmacodynamic characteristics that result in different optimal dosing strategies. Accumulating clinical data have also become available with several antifungals that allow pharmacodynamic data analyses [19–25]. Most often the results of these investigations have corroborated information from experimental models. The following chapter outlines the pharmacodynamic characteristics of antifungals and presents evidence of the clinical relevance of these concepts.

D.R. Andes (✉)
Department of Medicine, and Medical
Microbiology and Immunology, Section of Infectious Diseases,
Madison, WI, USA
e-mail: dra@medicine.wisc.edu

Pharmacokinetic Concepts

Pharmacokinetic studies describe how the body handles a drug, including absorption, distribution, binding to serum and tissue proteins, metabolism, and elimination [1]. Antifungal drug concentrations have been well characterized in numerous body fluids and tissues, including serum, urine, cerebrospinal fluid (CSF), vitreous body, epithelial lining fluid, bronchoalveolar lavage fluid, brain, lung, and kidney. The pharmacokinetic goal of antifungal therapy is to achieve adequate drug concentrations at the site of infection. This begs the rather simplistic question, where is the fungus relative to the antifungal drug? The site of infection for fungal pathogens can range from the bloodstream, where one would expect serum measurements to be of importance, to various tissue sites for which tissue drug concentrations may be of greater interest. Most pathogenic fungi exist primarily in extracellular tissue fluid; thus, even at tissue sites of infection serum measurements serve as a reliable tissue concentration surrogate.

The body sites for which tissue antifungal concentrations have been suggested to be most important include the brain parenchyma and the vitreous body [26]. Outcomes of infection at other tissue sites have correlated well with serum concentrations. For example, Groll and colleagues examined the relationship between efficacy and CSF and brain kinetics for several amphotericin B (AmB) preparations [27]. The CSF concentrations of four polyene compounds were remarkably similar. Brain tissue concentrations of liposomal AmB (LAmB), however, were from six- to tenfold higher than the other polyene preparations. The burden of *Candida* in the brains of rabbits following therapy correlated well with brain tissue penetration of the various drugs.

Another pharmacokinetic factor shown to impact the availability of antimicrobial compounds in tissue is binding to serum proteins such as albumin. In general it is accepted that only unbound (free) drug is pharmacologically active [28, 29]. This is related to the limited ability of protein-bound drug to diffuse across tissue and cellular membranes to reach the drug target. The relevance of protein binding has been

C.A. Kauffman et al. (eds.), *Essentials of Clinical Mycology*,
DOI 10.1007/978-1-4419-6640-7_8, © Springer Science+Business Media, LLC 2011

most clearly demonstrated for drugs from the triazole class, in which there are marked differences in degree of binding among the drugs [1, 11, 12, 15, 30]. The studies demonstrating these findings are discussed later.

Pharmacodynamic Concepts

Pharmacodynamics examines the relationship between pharmacokinetics and outcome. An added dimension of antimicrobial pharmacodynamics is consideration of the drug exposure relative to a measure of in vitro potency or the minimum inhibitory concentration (MIC) (Fig. 1). Three pharmacodynamic indices have been used to describe these relationships, including the peak concentration in relation to the MIC (Cmax/MIC), the area under the concentration curve in relation to the MIC (24 h area under the concentration curve, AUC/MIC), and the time that drug concentrations exceed the MIC expressed as a percentage of the dosing interval (%T > MIC). Knowledge of which of the three pharmacodynamic indices describes antifungal activity provides the basis for determining the frequency with which a drug is most efficaciously administered. For example, if the Cmax/MIC index relationship strongly correlates with activity of drug A, the optimal dosing schedule would provide large infrequent doses. Conversely, if the %T > MIC better describes drug activity, a dosing strategy may include smaller more frequent or even continuous drug administration to prolong the period of time that drug levels exceed the MIC.

Traditionally, three pharmacodynamic questions have been addressed in studies designed to define these concepts. First, what is the pharmacodynamic index associated with treatment efficacy? Second, what is the magnitude of the pharmacodynamic index needed for efficacy, or simply put, how much drug is needed for efficacy? Finally, do the pharmacodynamic results from experimental models predict outcome in patients?

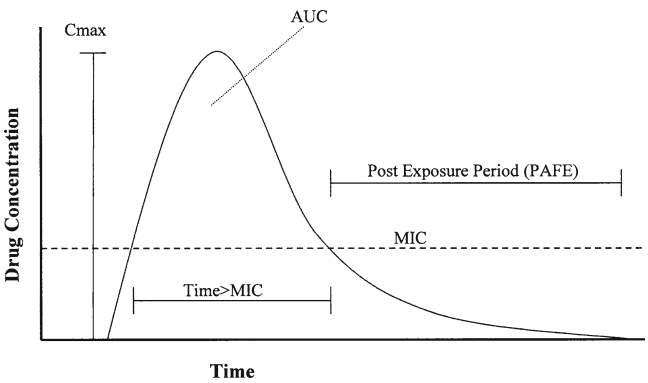

Fig. 1 Pharmacokinetic/pharmacodynamic relationship of antifungal drug concentration over time relative to organism minimum inhibitory concentration (MIC). Pharmacodynamic indices include the maximum or peak drug concentration relative to MIC (Cmax/MIC), the area under the drug concentration curve relative to MIC (AUC/MIC), and time that the concentration of drug exceeds the MIC (Time > MIC). Also represented is the post-exposure period, which represents the time period of drug exposure that is below the MIC in which many antifungals express continued antifungal effect, termed the post-antifungal effect (PAFE)

Concept 1: Impact of Antifungal Concentration on Activity over Time

Two observations have been made in examining the impact of escalating antifungal drug concentrations on fungal viability over time. First is the finding that for some drugs, increasing drug concentrations above the MIC enhances the rate and extent of organism death. When higher concentrations enhance killing, the pharmacodynamic pattern of activity is referred to as concentration-dependent killing. The second observation was noted during periods long after drug exposure (after the antimicrobial is no longer present or present at concentrations below the MIC). For some drugs there is a period of prolonged growth suppression following the initial supra-MIC exposure. This period of growth suppression is termed a post-antifungal effect (PAFE) [31, 32]. Three combinations of these time-kill end point characteristics have been described, and each combination is typically associated with one of the pharmacodynamic indices. The Cmax/MIC is associated with concentration-dependent killing and prolonged PAFEs. The %T > MIC is associated with concentration-independent killing and short PAFEs. The AUC/MIC is associated with prolonged PAFEs and either concentration-dependent or -independent killing.

Concept 2: Impact of Dosing Interval Variation or Fractionation

A second experimental design used to determine which pharmacodynamic index is predictive of efficacy is termed dose fractionation. Traditional dose escalation studies use a single dosing interval. With only a single dosing interval, escalating doses increase the values of all three indices. Dose fractionation studies examine efficacy of various dose levels that are administered by using three or more dosing intervals. In examining treatment results, if the regimens with shorter dosing intervals are more efficacious, the time-dependent index (T > MIC) is the more important index. If the large, infrequently administered dosing regimens are more active, the peak level in relation to the MIC is most predictive. Finally, if the outcome is similar with each of the dosing intervals, the outcome depends on the total dose or the AUC for the dosing regimen.

Concept 3: Pharmacodynamic Target

Knowledge of the pharmacodynamic characteristics of a compound allows one to better design a dosing interval strategy. This knowledge can also be useful to design studies to determine the amount of drug or index magnitude that is associated with treatment efficacy. For example, what pharmacodynamic magnitude of a drug is needed to treat a *Candida* infection? Is this pharmacodynamic magnitude the same as that needed to treat a drug-resistant *Candida* infection? Is the magnitude similar for other fungal species, for different infection sites, in different animal species? The answers to these questions have been explored and most times successfully addressed using a variety of infection models. The results of these studies have demonstrated that the magnitude of a pharmacodynamic index associated with efficacy is similar for drugs within the same class, provided that free drug levels are considered. The pharmacodynamic evaluation of each antifungal drug class and the clinical implications of these studies are detailed in subsequent sections.

Concept 4: Clinical Pharmacodynamics

The final and most important pharmacodynamic question involves determining if the results from the experimental model investigations are helpful for predicting efficacy in patients. The analysis needed for this correlation requires clinical data sets that include drug dose or drug concentration monitoring, organism MIC, and treatment efficacy. These data can be used to determine the pharmacodynamic exposure associated with an acceptable outcome and to determine which treatment end point from preclinical models (e.g., 50% maximal effect or the static dose) correlates with efficacy in patients.

Polyenes

Impact of Antifungal Concentration on Activity over Time

In vitro polyene time-kill studies have been undertaken with numerous yeast and filamentous fungal pathogens [5, 7, 13, 14, 18, 27, 32–37]. The majority of studies have been undertaken with AmB or one of the lipid formulations of this drug. Each of these studies has demonstrated marked concentration-dependent killing and maximal antifungal activity at concentrations exceeding the MIC from two- to tenfold. Several of these in vitro models have also demonstrated prolonged persistent growth suppression following drug exposure and removal (PAFE). The duration of these persistent effects was also linearly related to the concentration of the AmB exposure. For example, the longest periods of in vivo growth suppression were nearly an entire day (>20 h) following a single high dose of AmB in neutropenic mice [14]. For drugs displaying this pattern of activity the Cmax/MIC ratio has most often been the pharmacodynamic index predictive of efficacy.

Impact of Dosing Interval Variation or Fractionation

In vivo dose fractionation studies with AmB in an in vivo *Candida* model demonstrated optimal efficacy when large doses were administered infrequently, and pharmacodynamic analysis of the dose fractionation data illustrate that the Cmax/MIC index best predicts efficacy [14]. With each increase in length of the dosing interval from every 12 h to every 72 h, efficacy was enhanced, and the dose needed to achieve a net static effect was up to tenfold lower when administered with the most widely spaced dosing interval. A similar experimental approach was undertaken in an in vivo *Aspergillus* model [38]. Over a fourfold total dose range, the lung burden of *Aspergillus* was significantly lower when AmB was administered every 72 h compared to every 24 h or every 8 h. The results of these experiments corroborate the importance of the Cmax/MIC pharmacodynamic index and suggest that the pharmacodynamic driver of efficacy is similar among yeast and filamentous fungi.

Pharmacodynamic Target

In vivo study with AmB against multiple *Candida* species in a neutropenic disseminated candidiasis model observed a net static effect (growth inhibition) when the Cmax/MIC ratio approached values of 2–4 [14]. Maximal microbiologic efficacy against these strains in the same model was observed with ratios near 10. Similar investigation of efficacy in a murine pulmonary aspergillosis model demonstrated maximal efficacy with Cmax/MIC exposures in the range of 2–4 [38]. These most recent studies with *Aspergillus* address a critical gap in knowledge and suggest that at least for AmB, both the pharmacodynamic pattern of efficacy and the pharmacodynamic target are similar among fungal species.

It is generally accepted that the lipid formulations of AmB are not as potent in vivo as conventional AmB on a weight (mg/kg) basis. Each of the lipid formulations is complexed to

a different lipid and exhibits unique pharmacokinetic characteristics [39]. For example, LamB, which utilizes small unilamellar particles, liposomes, exhibits both high serum and CNS concentrations that are hypothesized to be due to the large serum: CNS gradient relative to the other AmB preparations [27]. Conversely, amphotericin B lipid complex (ABLC) and amphotericin B colloid dispersion (ABCD) achieve higher concentrations in the intracellular space and in organs of the reticuloendothelial system. Several studies have also suggested that ABLC attains higher concentrations in the lung than other formulations [40, 41].

Recent investigations have explored the impact of these pharmacokinetic differences on pharmacodynamic outcomes. For example, a study in an in vivo candidiasis model demonstrated that the difference in potency among the lipid preparations in the lungs, kidneys, and liver were congruent with tissue kinetics in these organs [13]. A novel study in a CNS candidiasis model examined the relationship between kinetics in serum, CSF, and brain parenchyma [27]. The kinetic studies demonstrated no significant difference in CSF concentrations, but higher brain concentrations of LAmB. The brain parenchymal differences in kinetics correlated closely with treatment efficacy in the model for which LAmB appeared to hold an advantage.

Similar investigations in *Aspergillus* pneumonia models have included assessment of lung tissue concentrations [41]. These studies have also suggested a relationship between these lung tissue site concentrations and efficacy. In this case, these pharmacodynamic investigations appear to favor ABLC. Recent studies have also begun to consider compartmental pharmacokinetics in the lung [40]. Specifically, a study in a murine model examined total lung, epithelial lining fluid, and pulmonary macrophage concentrations of each of the AmB preparations. As in previous studies, ABLC produced higher lung concentrations (70-fold higher than serum concentrations); however, a large amount of the compound appeared to reside in the pulmonary alveolar macrophages. The highest epithelial lining fluid concentrations were noted in LAmB-treated animals. Determination of the impact of these pharmacokinetic differences has not yet been reported.

Clinical Relevance

The pharmacokinetics of conventional AmB and the various lipid formulations have been carefully characterized in serum and tissues for several patient populations. Several investigations have attempted to demonstrate a correlation between AmB MIC and outcome [42, 43]. Most of these studies have found it difficult to discern MIC impact. We hypothesize that this is related to the narrow MIC and dose range in these studies, making it difficult to have enough Cmax/MIC or AUC/MIC variation to correlate with outcome. We are aware of only a single investigation that has attempted to correlate individual patient pharmacokinetics, MIC, and outcome with polyenes [44]. The study examined LAmB kinetics and outcome of invasive fungal infections in pediatric patients. In this small study, data from a subset of patients provided detailed kinetics, MIC, and outcome. The results demonstrate a statistically significant relationship between Cmax/MIC ratio and outcome. Maximal efficacy was observed with LAmB serum Cmax/MIC ratios greater than 40. This value is similar to that observed in the animal model studies described earlier when using serum LAmB measurements. This small study demonstrates that pharmacodynamic investigation with a drug from the polyene class can produce meaningful results that are congruent with those from preclinical infection models.

One large clinical study with LAmB tested the impact of dose escalation and observed conflicting results. Cornely et al. compared standard dosing of LAmB (3–5 mg/kg/day) to higher initial doses (10 mg/kg/day) for initial treatment of invasive mold infections [45]. The study was a multicenter, prospective, randomized, double-blinded, trial comparing LAmB administration at 3 mg/kg/day to that of 10 mg/kg/day for the first 14 days of a proven or probable invasive mold infection. After 14 days all patients continued with regular dosing of 3 mg/kg/day. The patient population was overwhelmingly represented by hematologic malignancy (93%), neutropenia (73% at baseline and 90% within 60 days of enrollment), pulmonary site of infection (90%), and aspergillosis as the infecting agent (97%). There was no statistically significant difference in outcomes between the two groups in regard to response rates; however, there were significant differences in renal toxicity, with 31% doubling of creatinine in the high-loading-dose arm versus 14% in the conventional dose arm. In addition, discontinuation of treatment prior to completion of the initial 14 days was higher in the high-dose group (24% vs. 13%). The conclusions from this study are that administration of higher dosages of LAmB for the first 14 days does not improve outcomes and leads to increased risks of toxicity and cost in patients primarily with hematologic malignancy, neutropenia, and pulmonary aspergillosis. From a pharmacodynamic perspective one may speculate (1) that the concentration-effect relationship is either maximal at the 5 mg/kg dose level, (2) that the threefold change in dose level was not enough to discern an efficacy difference, or (3) perhaps more likely, the toxicity of the drug at high concentration outweighed any efficacy benefit.

One additional exploration of AmB dosing regimens has been in the area of toxicodynamics. Investigators have theorized that toxicity, like efficacy, is related to high AmB concentrations. It follows that administration of the total daily dose by continuous infusion would result in lower peak

concentrations and thus reduced toxicity. Several small clinical studies have compared toxicity of the continuous infusion dosing strategy with a conventional once-daily regimen [46–49]. For example, Eriksson et al. compared AmB 0.97 mg/kg/day given as a continuous infusion to once-daily administration of the same dosage over 4 h [46]. The continuous regimen resulted in fewer infusion-associated side effects and instances of renal insufficiency. Several case series have reported use of continuous infusion in patients with hematologic malignancies and refractory fever. The majority of these reports note less rise in creatinine than has been reported in historic controls. However, other published experiences have reported conflicting results. Hall et al. observed a similar rate of nephrotoxicity in their use of the continuous-infusion regimen in a cohort of hematology patients with suspected or proven invasive fungal infection [47]. Unfortunately, studies examining the treatment efficacy of this strategy have not been undertaken. Studies from preclinical infection models would predict this strategy would be less effective.

Flucytosine

Impact of Antifungal Concentration on Activity over Time

Several concentration ranging, time-kill investigations have identified a pharmacodynamic pattern of activity distinct from that seen with the polyenes [16–18, 32, 50]. The antifungal activity of flucytosine (5FC) in both in vitro and in vivo *Candida* infection models has been shown to be maximal at concentrations not far above the MIC. Additional exposure to higher concentrations does not impact the extent of organism killing. This pattern of activity is termed time-dependent killing as opposed to the concentration-dependent activity described for amphotericin B. In addition, examination of *Candida* growth following 5FC exposure over a wide concentration range demonstrated organism recovery soon after exposure; thus there were no short or no post-antifungal effects.

Impact of Dosing Interval Variation or Fractionation

5FC in vivo dose fractionation studies in an in vivo candidiasis model similar to those described for AmB demonstrated that efficacy was optimal when drug was administrated in smaller dose levels more frequently [16]. Tenfold less drug was needed for efficacy when administered using the most fractionated dosing strategy by prolonging the time of the antifungal exposure. This time course and dose fractionation result suggests the %T>MIC would be the most predictive index. Consideration of each of the pharmacodynamic indices further demonstrates that the %T>MIC is most closely associated with efficacy.

Pharmacodynamic Target

The index magnitude for which optimal efficacy against *Candida albicans* was noted in a mouse infection model was a time above MIC magnitude of only 40% of the dosing interval (serum concentrations above the MIC for just less than one-half of the dosing interval) [16, 50]. However, as opposed to target studies with other antifungals, the 5FC studies were limited to two strains. However, these studies were corroborated in two independent laboratories. Unfortunately, there has not been a pharmacodynamic study with the most clinically relevant organism and infection site, *Cryptococcus neoformans* and meningitis. Studies using this model may offer critical dosing regimen strategies for this compound with a relatively narrow therapeutic index.

Clinical Relevance

There are no clinical data sets that allow pharmacodynamic analysis with 5FC in regard to treatment efficacy. However, one group of investigators have provided a model of human 5FC pharmacokinetics relative to the %T>MIC target (40–50%) against *Candida* species in a murine model [50]. The group considered the pharmacokinetics of a range of 5FC doses and the MIC distribution for *C. albicans.* Interestingly, doses as low as 25 mg/kg/day (sixfold lower than the currently recommended regimen) would be predicted to achieve the pharmacodynamic target against organisms in the current MIC distribution. Again, the major gap in knowledge for 5FC is characterization in a cryptococcal meningitis model to determine if the pharmacodynamic target is similar.

While pharmacodynamic studies linking 5FC exposure to efficacy have not been adequately explored, examination of toxicodynamic relationships are well established [51–55]. The primary toxicity of 5FC has been associated with high peak concentrations. These studies have shown that bone marrow toxicity is observed when levels in serum exceed 50–60 μg/mL. If one were to consider the human kinetics of the most frequently recommended 5FC dosing of 100 mg/kg/day divided into four doses, each dose of 37.5 mg/kg would remain higher than the MIC for 90% of *C. albicans*

isolates tested for more than 10 h. That the pharmacodynamic driver of success and toxicity are different provides an opportunity to design dosing strategies to both optimize treatment efficacy and reduce toxicity. Use of significantly smaller amounts of drug would allow 5FC administration with much less concern about related toxicities. Whether higher concentrations would be optimal for cryptococcal CNS infection remains an important unanswered question.

Triazoles

Impact of Antifungal Concentration on Activity over Time

In vitro and in vivo time-kill studies have been undertaken with all of the clinically available triazole compounds [7, 11, 12, 18, 30, 32, 56–60]. The observations have shown that triazoles exhibit growth inhibition at concentrations near the MIC, much like that observed with 5FC (concentration-independent or time-dependent activity). These investigations have shown that over a wide triazole concentration range (starting below the MIC [sub-MIC] to those more than 200-fold in excess of the MIC), growth of *Candida* organisms are similarly inhibited. In other words, increasing drug concentrations do not enhance antifungal effect.

Furthermore, in vitro studies demonstrated organism regrowth soon after drug removal (i.e., no in vitro post antifungal effect). In vivo studies, however, demonstrated prolonged growth suppression after levels in serum decreased to below the MIC. These prolonged in vivo PAFEs have been theorized to be caused by the profound sub-MIC activity of these drugs (i.e., effect of the triazoles after concentrations fall below the MIC in vivo, similar to those shown in vitro). The pharmacodynamic pattern combination of concentration-independent killing and prolonged PAFEs suggest that the 24 AUC/MIC index is most closely tied to treatment effect.

Impact of Dosing Interval Variation or Fractionation

Dose fractionation studies in several in vivo models with each of the triazole compounds have demonstrated that efficacy is dependent upon the dose, but independent of the dosing frequency. The earliest dose fractionation studies with fluconazole examined the impact of dividing four total dose levels into one, two, or four doses over a 24-h period [60]. The results clearly demonstrated that outcome depended on the total amount of drug or AUC rather than the dosing interval. Subsequent studies with fluconazole, posaconazole, ravuconazole, and voriconazole similarly demonstrated that outcome was independent of fractionation of the total drug exposure supporting the 24-h AUC/MIC as the pharmacodynamic index driving treatment efficacy [11, 12, 15, 30]. These later observations importantly suggest that the pharmacodynamic index associated with efficacy was similar among drugs with a similar mechanism of action, in this case inhibition of ergosterol synthesis.

Pharmacodynamic Target

The usefulness of knowing which index predicts efficacy is being able to then determine the magnitude of the index needed for successful outcome. The most efficient experimental way to define the magnitude of the predictive index is to examine treatment efficacy against organisms with widely varying MICs. These experiments have been difficult for AmB and 5FC, for which the MIC range is fairly narrow for the majority of isolates.

Resistance development has been a clinically relevant issue for the triazoles and *Candida* species. Thus, incorporation of MIC variation into experimental models has been more feasible. For example, the efficacy of posaconazole was examined over a more than 1,000-fold AUC range against 12 *C. albicans* with MICs varying nearly 100-fold [11]. Results from these studies showed that the AUC/MIC exposure associated with treatment efficacy was similar across the group of strains with widely varying MICs. For each of the triazoles examined in these animal model studies, the 24-h AUC/MIC necessary to produce the ED50 corresponds to a value near 25 [11, 12, 15, 30, 61]. This is essentially the same as averaging a drug concentration near the organism MIC for a 24-h period (1 X MIC X 24 h = AUC/MIC of 24) (Table 1).

Similar studies have been undertaken with four triazole compounds that include more than 100 drug/organism combinations for which MICs and dose levels varied more than 1,000-fold each. Two observations from these studies have been particularly relevant. First, for an individual drug, the AUC/MIC target for the triazole was independent of the MIC or the drug resistance mechanism. The second observation was initially less clear. Analysis of treatment outcome among the triazoles with similar strains demonstrated a wide range of dose/MIC and AUC/MIC relationships. However, one major difference among the triazoles is the degree of protein binding with lower values for fluconazole, intermediate values for voriconazole, and high binding with the remaining compounds.

A second look at these relationships, taking into account only free drug concentrations, identified very congruent data plots.

Table 1 Antifungal pharmacodynamic characteristics

	PD characteristic				PD target	
Antifungal class	Concentration dependence	Time dependence	Prolonged PAFE	PD index predictive of efficacy	Experimental	Clinical
Polyenes	X		X	Cmax/MIC	2–4	
5-FC		X		Time above MIC	≥40%	
Azoles		X	X	AUC/MIC	25	25
Echinocandins	X		X	AUC/MIC	3–5[a]	3–5[a]
					10–20	10–20

[a]PD target magnitude calculated using free drug concentration (i.e.,% drug not protein bound). For the echinocandins, the area under the drug concentration curve(AUC) / minimum inhibitory concentration (MIC) magnitude is different when comparing *C. albicans* to *C. glabrata* or *C. parapsilosis*, with the lower magnitude correlating with the latter two organisms and higher magnitude correlating with *C. albicans*

Calculation of the pharmacodynamic target among triazoles was indeed similar as long as free drug concentrations were considered. The consistency of data with the triazoles demonstrates that when protein binding and hence free drug concentrations are considered, the antifungal pharmacodynamic target is similar among drugs within a mechanistic class, such as triazoles.

The majority of antifungal pharmacodynamic target investigations have been undertaken in *Candida* models. More recently, a model of disseminated aspergillosis has been utilized in these investigations. Mavridou et al. investigated the relationship between posaconazole and voriconazole AUC/MIC and survival in neutropenic mice with disseminated infection with strains of *A. fumigatus* [62, 63]. The group made several important observations. Similar to what has been reported for *Candida* species, the treatment target was similar among four *Aspergillus* strains, which included one wild-type drug-susceptible strain and three strains with reduced azole susceptibility, suggesting that the target is similar among susceptible and resistant strains. Second, the AUC/MIC exposure associated with survival was nearly identical for both of the triazoles when free drug concentrations were considered. Perhaps most interesting, the drug exposure associated with efficacy in these *Aspergillus* models was similar to that described for these drugs in disseminated candidiasis models.

It is clear from multiple epidemiologic investigations that factors other than drug choice or dose impact patient outcome. It has been hypothesized that any of these factors may impact the pharmacodynamic exposure-response relationship. For example, one may intuitively posit that nonneutropenic patients may require less antifungal exposure than neutropenic patients. Several host, pathogen, and infection site factors have begun to be included in pharmacodynamic magnitude studies. For example, a recent study with an investigational triazole (isavuconazole) measured survival rates in mice with and without neutropenia [58]. The dose of isavuconazole needed to produce maximal survival rates was twofold higher in the neutropenic model.

Another host factor of importance for interpretation of preclinical antifungal pharmaocdynamic studies is the impact of the infected animal species. One may expect differences in pharmacokinetics in different animal species to impact the pharmacodynamic target. Consideration of drug exposures in pharmacodynamic terms, relative to the MIC of the organism, however, corrects for interspecies kinetic differences. Simply put, the drug target is in the organism and not in the host and thus host pharmacokinetic differences should not change the antimicrobial exposure the organism needs to see for effect. Studies with fluconazole in mice, rats, and rabbits allow testing of this hypothesis. Results from these treatment studies have shown that the fluconazole AUC/MIC needed to achieve 50% of the maximal microbiologic effect was near 25 and remarkably similar among the mammalian models [15, 60, 64, 65]. This knowledge allows one to hypothesize that results from preclinical animal pharmacodynamic target studies could be used to estimate antifungal dosing efficacy in humans.

Clinical Relevance

The logical next step is to determine if and how the experimental pharmacodynamic studies relate to outcome in patients. Data from antibacterial pharmacodynamics provide a compelling precedence for the predictive value of animal model pharmacodynamics and clinical therapeutic efficacy [1, 4]. The complexities surrounding patients who have fungal disease are well known and undoubtedly contribute to outcomes independent of antifungal pharmacodynamics. The most important confounding host variable is underlying host immune deficiency, which has been shown to be extremely important in influencing patient survival. Despite this limitation, there are several data sets that allow one to consider the relationship between antifungal dose, organism MIC, and clinical outcome [19, 20, 23, 25, 66].

For fluconazole there have been numerous large clinical studies that have provided sufficient data on drug dose, MIC, and outcomes for pharmacodynamic analysis. The earliest and largest (more than 1,000 patients) of these data sets emanated from studies of oropharyngeal candidiasis [20]. Analysis demonstrated that treatment efficacy was maximal with fluconazole exposures relative to the MIC of the infecting *Candida* species near a 24-h AUC/MIC value of 25, congruent with data from the in vivo models. In the largest single analysis, when the fluconazole dose/MIC (AUC/MIC) exceeded 25, clinical success was noted in 91–100% of patients. However, when AUC/MIC was less than 25, clinical failure was noted in 27–35% of patients.

A more contemporary analysis corroborates these findings showing a clinical efficacy of 92% with an AUC/MIC greater than 25, and 9% with values below 25 [23]. These results have been used in the development of in vitro susceptibility breakpoints for fluconazole and other triazoles by the Clinical Laboratory Standards Institute (CLSI). A similar analysis using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) susceptibility method resulted in a twofold higher MIC breakpoint [23]. This difference is due in part to the lower MIC values that are observed using the EUCAST susceptibility media.

A number of data sets from studies of candidemia have allowed similar pharmacodynamic investigation. There is now detailed information on fluconazole pharmacodynamics for more than 600 episodes of candidemia [19, 20, 24, 67, 68]. These data have identified a remarkably similar triazole exposure–clinical response relationship, with both clinical efficacy and patient survival associated with a fluconazole 24-h AUC/MIC ranging from 25 to 50. For example, in study of nearly 90 episodes of candidemia, CART analysis of dose and MIC found that the critical AUC/MIC value associated with patient survival (80% vs. 50%) was the 24-h AUC/MIC value of 25 [19].

A similar clinical analysis is now also available for voriconazole [66]. The dataset includes 1,681 isolates of 16 different *Candida* species from more than 400 subjects during six phase III clinical trials. Analysis demonstrated a strong relationship between MIC and outcome. If one estimates the free drug AUC for the voriconazole regimen used in these trials, the AUC/MIC can then be calculated using the geometric mean MIC for each of the species. Based on this analysis, therapeutic success was observed in 72–85% of cases with 24-h AUC/MIC greater than 25, whereas when AUC/MIC was less than 25, clinical failures were noted in 45% of patients.

Unfortunately, to date there has not been a published dataset for aspergillosis that allows similar analysis. However, there have been several recent voriconazole therapeutic drug monitoring publications that do allow pharmacodynamic estimations for aspergillosis [52, 69–71]. Two studies observed clinical success and patient survival, respectively, with voriconazole serum trough concentrations ranging from 1 to 2 μg/mL for these patients with invasive aspergillosis. If one considers the free drug AUC associated with these trough concentrations and the MIC90 for voriconazole and Aspergillus, the resulting 24-h AUC/MIC value is near 25.

A similar concentration/outcome relationship has also been recently reported for the triazole posaconazole in patients with invasive aspergillosis [72]. In 67 patients with invasive aspergillosis and serum concentration monitoring, the posaconazole average concentration at steady state ranged from 0.13 to 1.25 μg/mL. Maximal clinical response (75%) was observed in the cohort with the highest posaconazole concentration (1.25 μg/mL), while the least successful group (24% success) were found to have the lowest posaconazole serum levels.

Thus, one can use drug monitoring information to examine efficacy from the pharmacodynamic standpoint. It will be critical to include therapeutic drug monitoring in future studies and to include MIC testing when an organism is available.

Echinocandins

Impact of Antifungal Concentration on Activity over Time

Numerous in vitro and in vivo studies with compounds from the echinocandin drug class have been undertaken using *Candida* models [10, 73–81]. Results from these investigations have been consistent. Each of the agents have exhibited pronounced concentration-dependent killing effects and prolonged PAFEs. In vitro time course studies with each of the available echinocandin drugs have demonstrated concentration-dependent killing and prolonged PAFEs similar to those observed with the polyenes. Ernst et al. found that the extent of caspofungin killing of *C. albicans* varied more than 10,000-fold over only a 16-fold rise in concentrations [6]. Over this same range of drug concentrations the investigators observed an increase in the rate of killing and suppression of regrowth that exceeded the 12-h period of study.

Several in vivo studies have confirmed these pharmacodynamic characteristics [10, 74–80]. For example, following single escalating doses of the new echinocandin, aminocandin, marked killing of *C. albicans* was observed when drug levels in serum were more than four times the MIC. The extent of killing increased as concentrations relative to the MIC approached a factor of 10. However, there are a number of reports detailing a concentration-effect ceiling, above which reduced activity is observed. This phenomenon

is termed the paradoxical effect [82–85]. Mechanistic evaluation has identified elevated chitin concentrations in strains surviving very high echinocandin concentrations. These findings appear to be strain dependent and occur at concentrations far above those that would occur in patients with current clinical regimens. The clinical relevance of this phenomenon remains unclear.

Impact of Dosing Interval Variation or Fractionation

The earliest dose fractionation studies with the first echinocandin derivative, cilofungin, also demonstrated enhanced efficacy by maximizing serum and tissue concentrations [86]. Subsequent investigations in vivo with newer derivatives against *Candida* species and *A. fumigatus* found that efficacy was maximized by providing large, infrequently administered doses [10, 73, 75, 87]. The total amount of drug necessary to achieve various microbiologic end points over the treatment period was 4.8–7.6-fold smaller when the dosing schedule called for large single doses than when the same amount of total drug was administered in two to six doses. The concentration-dependent killing pattern and results from dose fractionation studies would suggest that either the Cmax/MIC or AUC/MIC would best represent the driving pharmacodynamic index. In vivo studies using serum kinetics suggest that the Cmax/MIC is a better predictor of efficacy. These pharmacodynamic studies with these compounds utilized serum pharmacokinetics.

Recent studies have examined the impact of tissue concentrations at the site of infection. The dose–response relationships were similar in these investigations and support a dosing strategy that involves administration of large doses given infrequently. A recent clinical study with micafungin explored this dosing strategy in an esophageal candidiasis trial [88]. The two micafungin dosing regimens examined included the standard regimen of 150 mg given daily in comparison to a regimen of 300 mg every other day. The total drug exposure or AUC would be similar for the two regimens. Interestingly, clinical and microbiologic efficacy was similar for both regimens, consistent with results from the preclinical models. It will be interesting to see if additional lengthening of the dosing interval can be explored in clinical trials. In animal models studies, the dosing interval has been successfully lengthened to every 7 days while maintaining efficacy [10, 75, 76]. A similar approach has also been undertaken with caspofungin in an in vivo model of aspergillosis [87]. In these dose fractionation studies as well, outcome was optimal with regimens that maximized the drug exposure. Studies in this aspergillosis model further support the contention that antifungal pharmacodynamic relationships for a drug class are similar among fungal organisms.

Pharmacodynamic Target

Recent studies have begun to explore the magnitude of the Cmax/MIC and AUC/MIC indices needed for treatment efficacy. Experiments with anidulafungin against five *C. albicans* isolates demonstrated similar exposure-response relationships when expressed as either the 24-h AUC/MIC or Cmax/MIC indices [10]. The pharmacodynamic target associated with achievement of a static end point corresponded to an anidulafungin-free drug (non-protein bound), 24 h AUC/MIC from 10 to 20. The Cmax/MIC needed to produce this degree of treatment success was a value near 1. Experiments with micafungin and caspofungin with this same group of organisms identified very similar AUC/MIC targets [75]. This compilation of data again supports the premise that the pharmacodynamic target is similar for compounds within a drug class when protein binding is taken into account.

Additional studies were undertaken with groups of organisms from the *C. glabrata* and *C. parapsilosis* species [89]. Susceptibility studies for these two groups demonstrated higher MICs than the *C. albicans* group. One unexpected finding was observed from the in vivo treatment studies. The AUC/MIC pharmacodynamic target for echinocandins against these two *Candida* species was two- to threefold lower than for *C. albicans*.

The approved steady-state regimens for treating invasive candidiasis with these drugs includes 100 mg/day of both anidulafungin and micafungin and 50 mg/day of caspofungin. These regimens produce total and free drug 24-h AUC values in healthy volunteers of 112 mg·h/mL and 1.12 mg·h/mL for anidulafungin, 98 mg·h/mL and 2.94 mg·h/mL for caspofungin, and 126 mg·h/mL and 0.38 mg·h/mL for micafungin. If one considers the pharmacokinetics of the echinocandins and the presented pharmacodynamic targets, the highest MICs for the three *Candida* species that would allow the pharmacodynamic free drug 24-h AUC/MIC (fAUC/MIC) to be met can be estimated. The MIC ceiling based on fAUC/MIC ranging from 5 to 20 would place the susceptibility breakpoint lower than the current CLSI value of 2 μg/mL for each of the drugs. However, the MICs for nearly all of the wild-type strains from surveillance studies would be expected to fall within the "pharmacodynamically susceptible" category based upon the fAUC/MIC targets reported in this study, with the exception of a subset of *C. parapsilosis* isolates.

Pharmacodynamic target investigation against other fungal species has been limited. A single study examined the caspofungin target against a strain of *A. fumigatus* in a murine

pulmonary model [87]. Interestingly the caspofungin exposure associated with maximal reduction in organism burden based upon RT-PCR end points was quite similar to that described for efficacy against *C. albicans.*

Clinical Relevance

Most clinical studies with echinocandins have not been extensively examined from the pharmacodynamic standpoint. However, a recent evaluation of three micafungin candidemia trials provided the opportunity to explore the relationship among pharmacokinetics, MIC, and treatment outcome [90]. The dataset included pharmacokinetics from patients with candidemia and outcome in 507 patients. Successful outcome in the entire population was observed with a total drug AUC/MIC greater than 3,000 (success in 98% with AUC/MIC > 3,000 and 84% < 3,000). If one considers the degree of protein binding for micafungin (>99%), the value of 3,000 would be similar to that observed for *C. albicans* in animal model studies. Since the infection model investigations had demonstrated differences among the *Candida* species, subgroup analysis examined the impact of infecting species as well. Interestingly, the AUC/MIC target of patients infected with *C. parapsilosis* was tenfold lower than for the remaining cohort. This difference was even larger than that observed in the animal model experiments. The findings from both the animal model studies and these clinical studies suggest a re-evaluation of current echinocandin susceptibility breakpoints and the consideration of these values at the level of the fungal species.

Combination Therapy

Patient outcomes associated with invasive fungal infections remain less than acceptable. It has been theorized that the combination of two or more antifungal compounds with different mechanisms of action could improve efficacy. The success of the combination of AmB and 5FC for cryptococcal meningitis serves as a critical proof of principle [91]. Numerous in vitro and in vivo infection models have been used to investigate various combinations against *Candida* and *Aspergillus* [57, 92–116]. Combination antifungal therapy continues to be an area of high interest and active investigation. As of 2008 there were >60 in vitro studies, >50 in vivo animal model studies, and >20 case reports or small single-center trials in humans evaluating combination therapy for A*spergillus* infection. The results have been variable, ranging from reduced effects to enhanced effects.

Prior study of antibacterial combinations has demonstrated that consideration of pharmacodynamics can help to decipher these often complex relationships. Even if two drugs together can enhance outcome, it is possible or even likely that this positive interaction is not evident at all drug concentration combinations. Recent in vitro antifungal combination studies using pharmacodynamic analysis have shown this to be the case. Examination of a wide variety of concentration combinations in these studies provides a means to determine not only if drug A and drug B interact in a helpful way, but they allow estimation of the optimal concentrations of each compound. In vivo pharmacodynamic studies should be useful to design clinical trials investigating antifungal drug combination therapy.

An area of intense interest is combination therapy with an echinocandin and a triazole antifungal for *Aspergillus* infections. Numerous in vitro and in vivo studies have examined the impact of this combination. The interpretation of the efficacy data from these investigations have varied from suggesting an enhanced interaction to antagonism. Drug exposure from the standpoint of dose level has been considered in several of these studies. However, extensive pharmacokinetic and pharmacodynamic design and analysis remain, for the most part, unexplored in the area of antifungal combination. The analyses from a few studies suggest the importance of this additional level of investigation. For example, in a study of the combination of caspofungin and voriconazole, the impact of the interaction was dependent upon the dose of the echinocandin; there was enhanced activity with some dosages, but reduced activity with others [117]. Among the unanswered antifungal pharmacodynamic questions, detailed examination of combination therapy is among the most important.

References

1. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis. 1998;26:1–10.
2. Andes D. Pharmacokinetics and pharmacodynamics of antifungals. Infect Dis Clin North Am. 2006;20:679–97.
3. Andes D. Clinical utility of antifungal pharmacokinetics and pharmacodynamics. Curr Opin Infect Dis. 2004;17:533–40.
4. Ambrose PG, Bhavnani SM, Rubino CM, et al. Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's not just for mice anymore. Clin Infect Dis. 2007;44:79–86.
5. Lewis RE, Wiederhold NP, Klepser ME. In vitro pharmacodynamics of amphotericin B, itraconazole, and voriconazole against *Aspergillus*, *Fusarium*, and *Scedosporium* spp. Antimicrob Agents Chemother. 2005;49:945–51.
6. Ernst EJ, Klepser ME, Ernst ME, Messer SA, Pfaller MA. In vitro pharmacodynamic properties of MK-0991 determined by time-kill methods. Diagn Microbiol Infect Dis. 1999;33:75–80.
7. Ernst EJ, Klepser ME, Pfaller MA. Postantifungal effects of echinocandin, azole, and polyene antifungal agents against *Candida albicans* and *Cryptococcus neoformans*. Antimicrob Agents Chemother. 2000;44:1108–11.

8. Ernst EJ, Roling EE, Petzold CR, Keele DJ, Klepser ME. In vitro activity of micafungin (FK-463) against *Candida* spp.: microdilution, time-kill, and postantifungal-effect studies. Antimicrob Agents Chemother. 2002;46:3846–53.
9. Andes D. Use of an animal model of disseminated candidiasis in the evaluation of antifungal therapy. Methods Mol Med. 2005; 118:111–28.
10. Andes D, Diekema DJ, Pfaller MA, et al. In vivo pharmacodynamic characterization of anidulafungin in a neutropenic murine candidiasis model. Antimicrob Agents Chemother. 2008;52:539–50.
11. Andes D, Marchillo K, Conklin R, et al. Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis. Antimicrob Agents Chemother. 2004;48:137–42.
12. Andes D, Marchillo K, Stamstad T, Conklin R. In vivo pharmacokinetics and pharmacodynamics of a new triazole, voriconazole, in a murine candidiasis model. Antimicrob Agents Chemother. 2003;47:3165–9.
13. Andes D, Safdar N, Marchillo K, Conklin R. Pharmacokinetic-pharmacodynamic comparison of amphotericin B (AMB) and two lipid-associated AMB preparations, liposomal AMB and AMB lipid complex, in murine candidiasis models. Antimicrob Agents Chemother. 2006;50:674–84.
14. Andes D, Stamsted T, Conklin R. Pharmacodynamics of amphotericin B in a neutropenic-mouse disseminated-candidiasis model. Antimicrob Agents Chemother. 2001;45:922–6.
15. Andes D, van Ogtrop M. Characterization and quantitation of the pharmacodynamics of fluconazole in a neutropenic murine disseminated candidiasis infection model. Antimicrob Agents Chemother. 1999;43:2116–20.
16. Andes D, van Ogtrop M. In vivo characterization of the pharmacodynamics of flucytosine in a neutropenic murine disseminated candidiasis model. Antimicrob Agents Chemother. 2000;44: 938–42.
17. Lewis RE, Klepser ME, Pfaller MA. In vitro pharmacodynamic characteristics of flucytosine determined by time-kill methods. Diagn Microbiol Infect Dis. 2000;36:101–5.
18. Ernst EJ, Yodoi K, Roling EE, Klepser ME. Rates and extents of antifungal activities of amphotericin B, flucytosine, fluconazole, and voriconazole against *Candida lusitaniae* determined by microdilution, Etest, and time-kill methods. Antimicrob Agents Chemother. 2002;46:578–81.
19. Baddley JW, Patel M, Bhavnani SM, Moser SA, Andes DR. Association of fluconazole pharmacodynamics with mortality in patients with candidemia. Antimicrob Agents Chemother. 2008;52:3022–8.
20. Rex JH, Pfaller MA, Galgiani JN, et al. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of in vitro-in vivo correlation data for fluconazole, itraconazole, and candida infections. Subcommittee on Antifungal Susceptibility Testing of the National Committee for Clinical Laboratory Standards. Clin Infect Dis. 1997;24: 235–47.
21. Pfaller MA, Diekema DJ, Ostrosky-Zeichner L, et al. Correlation of MIC with outcome for *Candida* species tested against caspofungin, anidulafungin, and micafungin: analysis and proposal for interpretive MIC breakpoints. J Clin Microbiol. 2008;46:2620–9.
22. Cuesta I, Bielza C, Larranaga P, et al. Data mining validation of fluconazole breakpoints established by the European Committee on Antimicrobial Susceptibility Testing. Antimicrob Agents Chemother. 2009;53:2949–54.
23. Rodriguez-Tudela JL, Almirante B, Rodriguez-Pardo D, et al. Correlation of the MIC and dose/MIC ratio of fluconazole to the therapeutic response of patients with mucosal candidiasis and candidemia. Antimicrob Agents Chemother. 2007; 51:3599–604.
24. Clancy CJ, Yu VL, Morris AJ, Snydman DR, Nguyen MH. Fluconazole MIC and the fluconazole dose/MIC ratio correlate with therapeutic response among patients with candidemia. Antimicrob Agents Chemother. 2005;49:3171–7.
25. Pai MP, Turpin RS, Garey KW. Association of fluconazole area under the concentration-time curve/MIC and dose/MIC ratios with mortality in nonneutropenic patients with candidemia. Antimicrob Agents Chemother. 2007;51:35–9.
26. Kethireddy S, Andes D. CNS pharmacokinetics of antifungal agents. Expert Opin Drug Metab Toxicol. 2007;3:573–81.
27. Groll AH, Giri N, Petraitis V, et al. Comparative efficacy and distribution of lipid formulations of amphotericin B in experimental *Candida albicans* infection of the central nervous system. J Infect Dis. 2000;182:274–82.
28. Kunin CM. Protein binding of antibiotics does affect their antistaphyloccal activity. J Antimicrob Chemother. 1986;17:263.
29. Craig WA, Kunin CM. Significance of serum protein and tissue binding of antimicrobial agents. Annu Rev Med. 1976;27:287–300.
30. Andes D, Marchillo K, Stamstad T, Conklin R. In vivo pharmacodynamics of a new triazole, ravuconazole, in a murine candidiasis model. Antimicrob Agents Chemother. 2003;47:1193–9.
31. Vogelman B, Gudmundsson S, Turnidge J, Leggett J, Craig WA. In vivo postantibiotic effect in a thigh infection in neutropenic mice. J Infect Dis. 1988;157:287–98.
32. Turnidge JD, Gudmundsson S, Vogelman B, Craig WA. The postantibiotic effect of antifungal agents against common pathogenic yeasts. J Antimicrob Chemother. 1994;34:83–92.
33. Klepser ME, Wolfe EJ, Jones RN, Nightingale CH, Pfaller MA. Antifungal pharmacodynamic characteristics of fluconazole and amphotericin B tested against *Candida albicans*. Antimicrob Agents Chemother. 1997;41:1392–5.
34. Denning DW, Warn P. Dose range evaluation of liposomal nystatin and comparisons with amphotericin B and amphotericin B lipid complex in temporarily neutropenic mice infected with an isolate of *Aspergillus fumigatus* with reduced susceptibility to amphotericin B. Antimicrob Agents Chemother. 1999;43:2592–9.
35. Gavalda J, Martin T, Lopez P, et al. Efficacy of high loading doses of liposomal amphotericin B in the treatment of experimental invasive pulmonary aspergillosis. Clin Microbiol Infect. 2005;11:999–1004.
36. Vitale RG, Meis JF, Mouton JW, Verweij PE. Evaluation of the post-antifungal effect (PAFE) of amphotericin B and nystatin against 30 zygomycetes using two different media. J Antimicrob Chemother. 2003;52:65–70.
37. Warn PA, Morrissey J, Moore CB, Denning DW. In vivo activity of amphotericin B lipid complex in immunocompromised mice against fluconazole-resistant or fluconazole-susceptible *Candida tropicalis*. Antimicrob Agents Chemother. 2000;44:2664–71.
38. Wiederhold NP, Tam VH, Chi J, Prince RA, Kontoyiannis DP, Lewis RE. Pharmacodynamic activity of amphotericin B deoxycholate is associated with peak plasma concentrations in a neutropenic murine model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother. 2006;50:469–73.
39. Adler-Moore JP, Proffitt RT. Amphotericin B lipid preparations: what are the differences? Clin Microbiol Infect. 2008;14 Suppl 4:25–36.
40. Groll AH, Lyman CA, Petraitis V, et al. Compartmentalized intrapulmonary pharmacokinetics of amphotericin B and its lipid formulations. Antimicrob Agents Chemother. 2006;50:3418–23.
41. Lewis RE, Liao G, Hou J, Chamilos G, Prince RA, Kontoyiannis DP. Comparative analysis of amphotericin B lipid complex and liposomal amphotericin B kinetics of lung accumulation and fungal clearance in a murine model of acute invasive pulmonary aspergillosis. Antimicrob Agents Chemother. 2007;51:1253–8.

42. Baddley JW, Marr KA, Andes DR, et al. Patterns of susceptibility of Aspergillus isolates recovered from patients enrolled in the Transplant-Associated Infection Surveillance Network. J Clin Microbiol. 2009;47:3271–5.
43. Park BJ, Arthington-Skaggs BA, Hajjeh RA, et al. Evaluation of amphotericin B interpretive breakpoints for Candida bloodstream isolates by correlation with therapeutic outcome. Antimicrob Agents Chemother. 2006;50:1287–92.
44. Hong Y, Shaw PJ, Nath CE, et al. Population pharmacokinetics of liposomal amphotericin B in pediatric patients with malignant diseases. Antimicrob Agents Chemother. 2006;50:935–42.
45. Cornely OA, Maertens J, Bresnik M, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). Clin Infect Dis. 2007;44:1289–97.
46. Eriksson U, Seifert B, Schaffner A. Comparison of effects of amphotericin B deoxycholate infused over 4 or 24 hours: randomised controlled trial. BMJ. 2001;322:579–82.
47. Hall P, Kennedy G, Morton J, Hill GR, Durrant S. Twenty-four hour continuous infusion of amphotericin B for the treatment of suspected or proven fungal infection in haematology patients. Intern Med J. 2005;35:374.
48. Imhof A, Walter RB, Schaffner A. Continuous infusion of escalated doses of amphotericin B deoxycholate: an open-label observational study. Clin Infect Dis. 2003;36:943–51.
49. Peleg AY, Woods ML. Continuous and 4 h infusion of amphotericin B: a comparative study involving high-risk haematology patients. J Antimicrob Chemother. 2004;54:803–8.
50. Hope WW, Warn PA, Sharp A, et al. Derivation of an in vivo drug exposure breakpoint for flucytosine against *Candida albicans* and Impact of the MIC, growth rate, and resistance genotype on the antifungal effect. Antimicrob Agents Chemother. 2006; 50:3680–8.
51. Kauffman CA, Frame PT. Bone marrow toxicity associated with 5-fluorocytosine therapy. Antimicrob Agents Chemother. 1977;11:244–7.
52. Smith J, Andes D. Therapeutic drug monitoring of antifungals: pharmacokinetic and pharmacodynamic considerations. Ther Drug Monit. 2008;30:167–72.
53. Francis P, Walsh TJ. Evolving role of flucytosine in immunocompromised patients: new insights into safety, pharmacokinetics, and antifungal therapy. Clin Infect Dis. 1992;15:1003–18.
54. Stamm AM, Diasio RB, Dismukes WE, et al. Toxicity of amphotericin B plus flucytosine in 194 patients with cryptococcal meningitis. Am J Med. 1987;83:236–42.
55. Pasqualotto AC, Howard SJ, Moore CB, Denning DW. Flucytosine therapeutic monitoring: 15 years experience from the UK. J Antimicrob Chemother. 2007;59:791–3.
56. Andes D, Forrest A, Lepak A, Nett J, Marchillo K, Lincoln L. Impact of antimicrobial dosing regimen on evolution of drug resistance in vivo: fluconazole and *Candida albicans*. Antimicrob Agents Chemother. 2006;50:2374–83.
57. Ernst EJ, Klepser ME, Pfaller MA. In vitro interaction of fluconazole and amphotericin B administered sequentially against *Candida albicans*: effect of concentration and exposure time. Diagn Microbiol Infect Dis. 1998;32:205–10.
58. Warn PA, Sharp A, Parmar A, Majithiya J, Denning DW, Hope WW. Pharmacokinetics and pharmacodynamics of a novel triazole, isavuconazole: mathematical modeling, importance of tissue concentrations, and impact of immune status on antifungal effect. Antimicrob Agents Chemother. 2009;53:3453–61.
59. Hope WW, Drusano GL, Moore CB, et al. Effect of neutropenia and treatment delay on the response to antifungal agents in experimental disseminated candidiasis. Antimicrob Agents Chemother. 2007;51:285–95.
60. Louie A, Drusano GL, Banerjee P, et al. Pharmacodynamics of fluconazole in a murine model of systemic candidiasis. Antimicrob Agents Chemother. 1998;42:1105–9.
61. Andes D, Lepak A, Nett J, Lincoln L, Marchillo K. In vivo fluconazole pharmacodynamics and resistance development in a previously susceptible *Candida albicans* population examined by microbiologic and transcriptional profiling. Antimicrob Agents Chemother. 2006;50:2384–94.
62. Bruggemann RJ, Mavridou E, Burger DM, Melchers WJG, Verweij P, Mouton JW. Pharmacokinetics/pharmacodynamics of posaconazole in a murine model of disseminated aspergillosis. In: 19th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Helsinki, Finland, 16–19 May 2009.
63. Mavridou E, Mouton JW, Mellado E, Melchers WJG, Verweij P. Efficacy of voriconazole against three clinical *Aspergillus fumigatus* isolates with mutations in cyp51A gene. In: 19th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Helsinki, Finland, 16–19 May 2009.
64. Rogers TE, Galgiani JN. Activity of fluconazole (UK 49, 858) and ketoconazole against *Candida albicans* in vitro and in vivo. Antimicrob Agents Chemother. 1986;30:418–22.
65. Van t Wout JW, Mattie H, van Furth R. Comparison of the efficacies of amphotericin B, fluconazole, and itraconazole against a systemic *Candida albicans* infection in normal and neutropenic mice. Antimicrob Agents Chemother. 1989;33:147–51.
66. Pfaller MA, Diekema DJ, Rex JH, et al. Correlation of MIC with outcome for Candida species tested against voriconazole: analysis and proposal for interpretive breakpoints. J Clin Microbiol. 2006;44:819–26.
67. Takakura S, Fujihara N, Saito T, Kudo T, Iinuma Y, Ichiyama S. Clinical factors associated with fluconazole resistance and short-term survival in patients with Candida bloodstream infection. Eur J Clin Microbiol Infect Dis. 2004;23:380–8.
68. Lee SC, Fung CP, Huang JS, et al. Clinical correlates of antifungal macrodilution susceptibility test results for non-AIDS patients with severe Candida infections treated with fluconazole. Antimicrob Agents Chemother. 2000;44:2715–8.
69. Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. Antimicrob Agents Chemother. 2009;53:24–34.
70. Smith J, Safdar N, Knasinski V, et al. Voriconazole therapeutic drug monitoring. Antimicrob Agents Chemother. 2006;50:1570–2.
71. Pascual A, Calandra T, Bolay S, Buclin T, Bille J, Marchetti O. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. Clin Infect Dis. 2008;46:201–11.
72. Walsh TJ, Raad I, Patterson TF, et al. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. Clin Infect Dis. 2007;44:2–12.
73. Andes D, Marchillo K, Lowther J, Bryskier A, Stamstad T, Conklin R. In vivo pharmacodynamics of HMR 3270, a glucan synthase inhibitor, in a murine candidiasis model. Antimicrob Agents Chemother. 2003;47:1187–92.
74. Andes D, Safdar N. Efficacy of micafungin for the treatment of candidemia. Eur J Clin Microbiol Infect Dis. 2005;24:662–4.
75. Andes DR, Diekema DJ, Pfaller MA, Marchillo K, Bohrmueller J. In vivo pharmacodynamic target investigation for micafungin against *Candida albicans* and *C. glabrata* in a neutropenic murine candidiasis model. Antimicrob Agents Chemother. 2008;52:3497–503.
76. Gumbo T, Drusano GL, Liu W, et al. Once-weekly micafungin therapy is as effective as daily therapy for disseminated candidiasis in mice with persistent neutropenia. Antimicrob Agents Chemother. 2007;51:968–74.

77. Gumbo T, Drusano GL, Liu W, et al. Anidulafungin pharmacokinetics and microbial response in neutropenic mice with disseminated candidiasis. Antimicrob Agents Chemother. 2006;50:3695–700.
78. Groll AH, Mickiene D, Petraitiene R, et al. Pharmacokinetic and pharmacodynamic modeling of anidulafungin (LY303366): reappraisal of its efficacy in neutropenic animal models of opportunistic mycoses using optimal plasma sampling. Antimicrob Agents Chemother. 2001;45:2845–55.
79. Groll AH, Mickiene D, Petraitis V, et al. Compartmental pharmacokinetics and tissue distribution of the antifungal echinocandin lipopeptide micafungin (FK463) in rabbits. Antimicrob Agents Chemother. 2001;45:3322–7.
80. Hope WW, Mickiene D, Petraitis V, et al. The pharmacokinetics and pharmacodynamics of micafungin in experimental hematogenous Candida meningoencephalitis: implications for echinocandin therapy in neonates. J Infect Dis. 2008;197:163–71.
81. Clancy CJ, Huang H, Cheng S, Derendorf H, Nguyen MH. Characterizing the effects of caspofungin on *Candida albicans*, *Candida parapsilosis*, and *Candida glabrata* isolates by simultaneous time-kill and postantifungal-effect experiments. Antimicrob Agents Chemother. 2006;50:2569–72.
82. Clemons KV, Espiritu M, Parmar R, Stevens DA. Assessment of the paradoxical effect of caspofungin in therapy of candidiasis. Antimicrob Agents Chemother. 2006;50:1293–7.
83. Lewis RE, Albert ND, Kontoyiannis DP. Comparison of the dose-dependent activity and paradoxical effect of caspofungin and micafungin in a neutropenic murine model of invasive pulmonary aspergillosis. J Antimicrob Chemother. 2008;61:1140–4.
84. Stevens DA, Ichinomiya M, Koshi Y, Horiuchi H. Escape of Candida from caspofungin inhibition at concentrations above the MIC (paradoxical effect) accomplished by increased cell wall chitin; evidence for beta-1, 6-glucan synthesis inhibition by caspofungin. Antimicrob Agents Chemother. 2006;50:3160–1.
85. Stevens DA, White TC, Perlin DS, Selitrennikoff CP. Studies of the paradoxical effect of caspofungin at high drug concentrations. Diagn Microbiol Infect Dis. 2005;51:173–8.
86. Walsh TJ, Lee JW, Kelly P, et al. Antifungal effects of the nonlinear pharmacokinetics of cilofungin, a 1, 3-beta-glucan synthetase inhibitor, during continuous and intermittent intravenous infusions in treatment of experimental disseminated candidiasis. Antimicrob Agents Chemother. 1991;35:1321–8.
87. Wiederhold NP, Kontoyiannis DP, Chi J, Prince RA, Tam VH, Lewis RE. Pharmacodynamics of caspofungin in a murine model of invasive pulmonary aspergillosis: evidence of concentration-dependent activity. J Infect Dis. 2004;190:1464–71.
88. Buell D, Kovanda L, Drake T, Fisco C. Alternative day dosing of micafungin in the treatment of esophageal candidiasis. In: 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2005.
89. Andes D, Diekema DJ, Lepak A, Pfaller M, Marchillo K, Bohrmuller J. Identification of the in vivo pharmacodynamic (pd) target for echinocandins against *C. albicans* (CA), *C. glabrata* (CG), and *C. parapsilosis* (CP) in the neutropenic murine disseminated candidiasis model. In: Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2009.
90. Andes D, Ambrose PG, Hammel JP, et al. Exposure-response relationships for efficacy of micafungin in patients with invasive candidiasis. In: 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2009.
91. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis. 2010;50:291–322.
92. Arikan S, Lozano-Chiu M, Paetznick V, Rex JH. In vitro synergy of caspofungin and amphotericin B against *Aspergillus* and *Fusarium* spp. Antimicrob Agents Chemother. 2002;46:245–7.
93. Chaturvedi V, Ramani R, Ghannoum MA, et al. Multilaboratory testing of antifungal combinations against a quality control isolate of *Candida krusei*. Antimicrob Agents Chemother. 2008;52:1500–2.
94. Clemons KV, Espiritu M, Parmar R, Stevens DA. Comparative efficacies of conventional amphotericin b, liposomal amphotericin B (AmBisome), caspofungin, micafungin, and voriconazole alone and in combination against experimental murine central nervous system aspergillosis. Antimicrob Agents Chemother. 2005;49:4867–75.
95. Clemons KV, Stevens DA. Efficacy of micafungin alone or in combination against experimental pulmonary aspergillosis. Med Mycol. 2006;44:69–73.
96. Clemons KV, Stevens DA. Animal models testing monotherapy versus combination antifungal therapy: lessons learned and future directions. Curr Opin Infect Dis. 2006;19:360–4.
97. Demchok JP, Meletiadis J, Roilides E, Walsh TJ. Comparative pharmacodynamic interaction analysis of triple combinations of caspofungin and voriconazole or ravuconazole with subinhibitory concentrations of amphotericin B against *Aspergillus* spp. Mycoses. 2009;53:239–45.
98. Johnson MD, MacDougall C, Ostrosky-Zeichner L, Perfect JR, Rex JH. Combination antifungal therapy. Antimicrob Agents Chemother. 2004;48:693–715.
99. Hope WW, Warn PA, Sharp A, et al. Surface response modeling to examine the combination of amphotericin B deoxycholate and 5-fluorocytosine for treatment of invasive candidiasis. J Infect Dis. 2005;192:673–80.
100. Hope WW, Warn PA, Sharp A, et al. Optimization of the dosage of flucytosine in combination with amphotericin B for disseminated candidiasis: a pharmacodynamic rationale for reduced dosing. Antimicrob Agents Chemother. 2007;51:3760–2.
101. Kirkpatrick WR, Perea S, Coco BJ, Patterson TF. Efficacy of caspofungin alone and in combination with voriconazole in a Guinea pig model of invasive aspergillosis. Antimicrob Agents Chemother. 2002;46:2564–8.
102. Kontoyiannis DP, Hachem R, Lewis RE, et al. Efficacy and toxicity of caspofungin in combination with liposomal amphotericin B as primary or salvage treatment of invasive aspergillosis in patients with hematologic malignancies. Cancer. 2003;98:292–9.
103. Lewis RE, Diekema DJ, Messer SA, Pfaller MA, Klepser ME. Comparison of Etest, chequerboard dilution and time-kill studies for the detection of synergy or antagonism between antifungal agents tested against Candida species. J Antimicrob Chemother. 2002;49:345–51.
104. Lewis RE, Kontoyiannis DP. Micafungin in combination with voriconazole in Aspergillus species: a pharmacodynamic approach for detection of combined antifungal activity in vitro. J Antimicrob Chemother. 2005;56:887–92.
105. Lewis RE, Lund BC, Klepser ME, Ernst EJ, Pfaller MA. Assessment of antifungal activities of fluconazole and amphotericin B administered alone and in combination against *Candida albicans* by using a dynamic in vitro mycotic infection model. Antimicrob Agents Chemother. 1998;42:1382–6.
106. Louie A, Banerjee P, Drusano GL, Shayegani M, Miller MH. Interaction between fluconazole and amphotericin B in mice with systemic infection due to fluconazole-susceptible or -resistant strains of *Candida albicans*. Antimicrob Agents Chemother. 1999;43:2841–7.
107. Louie A, Liu W, Miller DA, et al. Efficacies of high-dose fluconazole plus amphotericin B and high-dose fluconazole plus 5-fluorocytosine versus amphotericin B, fluconazole, and 5-fluorocytosine monotherapies in treatment of experimental endocarditis, endophthalmitis, and pyelonephritis due to *Candida albicans*. Antimicrob Agents Chemother. 1999;43:2831–40.

108. Marr KA, Boeckh M, Carter RA, Kim HW, Corey L. Combination antifungal therapy for invasive aspergillosis. Clin Infect Dis. 2004;39:797–802.
109. Meletiadis J, Mouton JW, Meis JF, Verweij PE. In vitro drug interaction modeling of combinations of azoles with terbinafine against clinical *Scedosporium prolificans* isolates. Antimicrob Agents Chemother. 2003;47:106–17.
110. Meletiadis J, Mouton JW, Meis JF, Verweij PE. Combination chemotherapy for the treatment of invasive infections by *Scedosporium prolificans*. Clin Microbiol Infect. 2000;6:336–7.
111. Meletiadis J, Mouton JW, Rodriguez-Tudela JL, Meis JF, Verweij PE. In vitro interaction of terbinafine with itraconazole against clinical isolates of *Scedosporium prolificans*. Antimicrob Agents Chemother. 2000;44:470–2.
112. Meletiadis J, Verweij PE, TeDorsthorst DT, Meis JF, Mouton JW. Assessing in vitro combinations of antifungal drugs against yeasts and filamentous fungi: comparison of different drug interaction models. Med Mycol. 2005;43:133–52.
113. Ostrosky-Zeichner L. Combination antifungal therapy: a critical review of the evidence. Clin Microbiol Infect. 2008;14 Suppl 4:65–70.
114. Petraitis V, Petraitiene R, Hope WW, et al. Combination therapy in treatment of experimental pulmonary aspergillosis: in vitro and in vivo correlations of the concentration- and dose- dependent interactions between anidulafungin and voriconazole by Bliss independence drug interaction analysis. Antimicrob Agents Chemother. 2009;53:2382–91.
115. Petraitis V, Petraitiene R, Sarafandi AA, et al. Combination therapy in treatment of experimental pulmonary aspergillosis: synergistic interaction between an antifungal triazole and an echinocandin. J Infect Dis. 2003;187:1834–43.
116. Te Dorsthorst DT, Verweij PE, Meis JF, Punt NC, Mouton JW. In vitro interactions between amphotericin B, itraconazole, and flucytosine against 21 clinical Aspergillus isolates determined by two drug interaction models. Antimicrob Agents Chemother. 2004;48:2007–13.
117. Meletiadis J, Stergiopoulou T, O'Shaughnessy EM, Peter J, Walsh TJ. Concentration-dependent synergy and antagonism within a triple antifungal drug combination against Aspergillus species: analysis by a new response surface model. Antimicrob Agents Chemother. 2007;51:2053–64.