Published in final edited form as:

Future Microbiol. 2011 April ; 6(4): 441–457. doi:10.2217/fmb.11.19.

Current perspectives on echinocandin class drugs

David S Perlin

Public Health Research Institute, New Jersey Medical School-UMDNJ, Newark, NJ 07103, USA

Abstract

It has been nearly a decade since caspofungin was approved for clinical use as the first echinocandin class antifungal agent, followed by micafungin and anidulafungin. The echinocandin drugs target the fungal cell wall by inhibiting the synthesis of α -1,3- β -glucan, a critical cell wall component of many pathogenic fungi. They are fungicidal for *Candida* spp. and fungistatic for moulds, such as *Aspergillus fumigatus*, where they induce abnormal morphology and growth properties. The echinocandins have a limited antifungal spectrum but are highly active against most *Candida* spp., including azole-resistant strains and biofilms. As they target glucan synthase, an enzyme absent in mammalian cells, the echinocandins have a favorable safety profile. They show potent MIC and epidemiological cutoff values against susceptible *Candida* and *Aspergillus* isolates, and the frequency of resistance is low. When clinical breakthrough occurs, it is associated with high MIC values and mutations in Fks subunits of glucan synthase, which can reduce the sensitivity of the enzyme to the drug by several thousand-fold. Such strains were not adequately captured by an early clinical breakpoint for susceptibility prompting a revised lower value, which addresses the FKS resistance mechanism and new pharmacokinetic/pharmacodynamic studies. Elevated MIC values unlinked to therapeutic failure can occur and result from adaptive cell behavior, which is FKS-independent and involves the molecular chaperone Hsp90 and the calcineurin pathway. Mutations in *FKS1* and/or *FKS2* alter the kinetic properties of glucan synthase, which reduces the relative fitness of mutant strains causing them to be less pathogenic. The echinocandin drugs also modify the cell wall architecture exposing buried glucans, which in turn induce a variety of important host immune responses. Finally, the future for glucan synthase inhibitors looks bright with the development of new orally active compounds.

Keywords

anidulafungin; caspofungin; echinocandin; FKS; fungal infection; glucan synthase; MIC; micafungin

Echinocandin antifungal drugs

The echinocandin drugs anidulafungin (ANF), caspofungin (CSF) and micafungin (MCF) were the first members of the lipopeptide class of antifungal drugs. They are cyclic hexapeptides *N*-linked to a fatty acyl side chain (Figure 1) and are potent inhibitors of β -1,3- β -glucan synthase, which is responsible for biosynthesis of β -1,3- β -glucan, the major cell wall biopolymer [1]. These fungal-specific drugs show concentration-dependent antifungal

© 2011 Future Medicine Ltd

Tel.: +1 973 854 3200 Fax: +1 973 854 3101 perlinds@umdnj.edu.

Financial & competing interests disclosure

The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

activity against susceptible *Candida* spp. and *Aspergillus* spp. without cross-resistance to existing antifungal agents, which enables them to be effective against azole-resistant yeasts and moulds [2–4]. The echinocandins demonstrate fungicidal activity against most species of *Candida* [5,6] but they exhibit complex growth inhibitory behavior with moulds, such as *Aspergillus* where they induce a sea urchin-like multi-budded structure with slowed growth and lysis of rapidly growing bud tips (Figure 2) [7–9]. All three echinocandin drugs have been approved by the US FDA for the treatment of esophageal candidiasis and invasive candidiasis including candidemia. The echinocandins are now the preferred systemically active antifungal agents for the treatment of invasive candidiasis [10–12]. CSF is also approved for empirical therapy for presumed fungal infections in febrile neutropenic patients, while MCF is approved for the treatment of esophageal candidiasis and for prophylaxis of *Candida* infections in patients undergoing hematopoietic stem-cell transplantation during the period of neutropenia [13–19]. Echinocandin drugs are highly effective against azole-resistant strains of *Candida* [20–23] and *Candida*-forming biofilms [24–26], but they are less active against *Zygomycetes*, *Cryptococcus neoformans* or *Fusarium* spp. [3]. There is now a broad clinical experience using the echinocandins to treat both mucosal and invasive forms of candidiasis [13,14,27–37]. Overall, echinocandin drugs demonstrate a high therapeutic index with strong efficacy and excellent safety and tolerability profiles with few drug interactions and related adverse events [13,29,35,38–41]. Although reports of resistance to echinocandin treatment in *Candida* spp. are increasing, clinical failure remains relatively low, even in spite of the expanded use of these drugs [42–44].

Glucan synthase complex

The β -1,3-D-glucan synthase is a multisubunit enzyme complex that catalyzes the transfer of sugar moieties from activated donor molecules to specific acceptor molecules forming glycosidic bonds in the reaction $\text{UDP-glucose} + ([1,3]\text{-}\beta\text{-D-glucosyl}) (N) \rightarrow \text{UDP} + ([1,3]\text{-}\beta\text{-D-glucosyl}) (N + 1)$ [45,46]. Most of our understanding of the genetics of glucan synthase has come from biochemical studies in yeast [47–53] and more recently with kinetic studies of *FKS*-resistant mutants from *Candida* spp. [42,43,54]. The enzyme complex has at least two subunits, Fks and Rho [55,56]. Fks is the presumed catalytic subunit [57] and is encoded by three related genes, *FKS1*, *FKS2* and *FKS3*; it is the target of the echinocandin drugs. *FKS1* is essential in *Candida albicans* [58,59] but in *Saccharomyces cerevisiae* and *Candida glabrata*, *FKS1* genetic disruptants remain viable due to paralog *FKS2*. These genes are calcineurin dependent [60] and are downregulated by the immunosuppressive drug FK506. Rho, a GTP-binding protein in the Rho/Rac subfamily of Ras-like GTPases, regulates the activity of glucan synthase [61]. A variety of biochemical and genetic studies have pointed to the direct or indirect involvement of other proteins contributing to glucan synthase complex, although their firm association has not been established [62,63]. It is still not understood how the echinocandin drugs interact to inhibit glucan synthase. Enzymatic removal of the aliphatic tail of the drugs render them inactive [64]. Kinetic studies of drug transport suggest that both saturable high affinity and low affinity transport systems exist in yeast cells to move the echinocandin drugs into the cell in an energy-independent manner [65]. However, it is not clear whether transport into the cell is required for echinocandin action, which may explain why the drugs are not subject to the action of multidrug resistant transporters prominent in azole-resistant strains and biofilms [24,25,66]. It has been speculated that the tail of the drugs may intercalate into the bilayer resulting in inhibition. Such drug-target interactions would not require the drugs to enter the cell and they may act on the enzyme from the extracellular face of the cell membrane. Such behavior would be comparable to that of cardiac glycosides on the membrane-bound sodium pump [67]. Topology models for the location of resistance hot spots support such a model (Figure 3) [5,52]. The mechanistic nature of the interaction between echinocandins and glucan synthase

remains ambiguous and will not be fully resolved until a 3D structure with bound drug is obtained.

FKS resistance mechanism

Echinocandin resistance resulting in clinical failure from susceptible species such as *C. albicans*, *C. glabrata* and *Candida tropicalis* is uncommon [44,68,69]. Yet, as the clinical use of echinocandins expands, *Candida* spp. isolates with reduced susceptibility to these drugs are increasingly encountered [42,43,68,70–80]. Recently, a collection of 490 *C. glabrata* bloodstream isolates from a population-based surveillance study identified 16 isolates (3.3%) with an elevated MIC value to one or more of CSF, MCF or ANF [68]. In another study of 133 *Candida* strains covering six species, 2.9% of isolates were considered resistant based on mechanistic criterion [69]. Fungi possess adaptive mechanisms that help protect against cellular stresses, such as those encountered following inhibition of glucan synthase by echinocandin drugs. These stress adaptation responses may result in elevated echinocandin MIC values [44,81], as determined *in vitro*, but they are not typically associated with clinical failures [82–84]. Rather, echinocandin resistance resulting in clinical failure is associated with mutations in two highly conserved ‘hot spot’ regions of *FKS1* and/or *FKS2* [42–44,68,85]. These limited-spectrum amino acid substitutions in the Fks subunits of glucan synthase confer cross-resistance among the class of drugs [44]. In *C. albicans* and most other *Candida* spp., mutations occur in hot spot regions of *FKS1*, which is the most prominently expressed *FKS* gene [69]. In *C. glabrata*, comparable mutations are found in either *FKS1* or *FKS2*, which are both expressed and comprise the active subunit [42,71,74,79]. Typically, *FKS3* is weakly expressed or not at all [42,71,74,79]. Mutations in *FKS* result in elevated MIC values (0.5–2 logs depending on the drug) and reduce the sensitivity of glucan synthase (inhibition constant producing 50% reduction in activity [IC_{50}] or K_i) to the drug by 30- to more than 3000-fold (Figure 4) [42,43,85]. The majority of echinocandin clinical breakthrough infections have been observed with *C. albicans* and *C. glabrata*, as expected by their prevalence in causing invasive disease. Amino acid changes at Ser645 (S645P, S645F and S645Y) are the most abundant and cause the most pronounced resistance phenotype (Figures 4 & 5) [42,43]. Other mutations (*C. albicans*: F641, L642, T643, L644, A645, L646, R647, D648) account for smaller increases [43], which is apparent from their affects on glucan synthase drug sensitivity (Figure 4). While the most prominent *FKS* mutations are associated with clinical resistance, not all *FKS* mutations confer the same phenotype and thus, they are less likely to result in breakthrough disease. On the basis of phenotypic behavior in MIC testing and animal models, as well as kinetic inhibition of glucan synthase, the allele order of resistance associated with the *FKS* genotype is: S645, F641>>L642, T643, L644, L646, R647, D648>P649. This limited spectrum of *FKS* mutations in *Candida* spp. cause reduced susceptibility across the entire class of echinocandin agents, which makes this mechanism of resistance ideal for molecular-based testing [86].

Clinically important nonsusceptible fungi

The recently described species *Aspergillus lentulus* demonstrates reduced pleiotropic susceptibility to most systemic antifungal drugs [87]. *A. lentulus* isolates are overall less susceptible to CSF although they retain susceptibility to ANF and MCF [88]. In this way, they resemble resistant mutants selected following cell wall digestion and exposure to CSF [89]. Analysis of the *A. lentulus FKS1* sequence did not reveal a polymorphism at any of the known hotspot regions of the gene [90] indicating that this rare species [91] has an unknown resistance mechanism that is stable and confers clinical resistance [87]. One possibility is that this species produces excess glucans, which limit the effective concentration of antifungals. Increased resistance to ANF and other antifungal agents was observed for

Candida biofilms overexpressing *FKSI* [92]. It was surmized that excess glucans may act to sequester the hydrophobic drugs. In *Cryptococcus neoformans*, the echinocandins are active *in vitro* on glucan synthase yet fail to show highly active antifungal properties [93]. The mechanism of action is unclear, although in the case of *C. neoformans*, melanin may play a protective role [94]. In non-*Aspergillus* moulds, the echinocandins are weakly active as antifungal agents, although they are potent inhibitors of glucan synthase [95]. Inhibition of β -1,3 glucan formation may result in compensatory changes to the cell wall, such as other glucans or chitin being synthesized in these organism, but this has not been reported.

Paradoxical drug action

Growth of susceptible fungi at echinocandin concentrations much greater than the MIC is termed 'the paradoxical effect'. The effect was first noted by Stevens and colleagues in a subgroup of *C. albicans* isolates grown in the presence of high CSF concentrations [96]. The prevalence of the paradoxical effect for CSF has been reported at 90, 60, 40 and 10% in *Candida parapsilosis*, *C. albicans*, *C. tropicalis* and *Candida krusei* isolates, respectively [97]. It is rarely seen with *C. glabrata*. These strains demonstrated a normal susceptibility pattern with a typical low MIC, but then paradoxically showed break-through growth at high drug concentrations. This drug-insensitive growth was conditional, as strains undergoing paradoxical growth showed normal susceptibility properties when cultured and re-challenged with drug. The mechanism responsible for paradoxical growth remains elusive. It is not related to modification of glucan synthase (*FKS* mutations) complex or to its upregulation in the presence of drug [98]. The growth behavior is consistent with adaptive stress responses observed in *S. cerevisiae* and *C. albicans*, which may lead to reduced susceptibility [99,100]. Overproduction of chitin as a compensatory cell wall component may account for some cases of paradoxical behavior [101,102]. Seemingly paradoxical behavior was reported for CSF in a murine model of pulmonary aspergillosis [103], although it was not reproduced in a systemic candidiasis mouse model with MCF utilizing strains displaying paradoxical behavior [104]. A paradoxical increase in circulating *Aspergillus* antigen was observed during treatment with CSF in a patient with pulmonary aspergillosis [105] and with MCF and CSF in animal models [106]. The clinical significance of the paradoxical effect remains unclear.

Hsp90 & echinocandin action

Fungal cells possess an array of mechanisms that enable them to adapt to stresses imposed by their environment or from the action of antifungal agents [44]. The molecular chaperone Hsp90 helps protect fungal cells following exposure to antifungal drugs by enabling the function of its client protein calcineurin [107–109]. This Ca^{2+} /calmodulin-dependent serine threonine phosphatase helps regulate responses to membrane stress in yeasts and moulds [108,110]. Hsp90 is an essential chaperone responsible for folding and maturation of client proteins including signal transduction proteins with unstable conformations [111]. When stress conditions overwhelm the function of Hsp90, otherwise silent polymorphisms are expressed and can be selected if they provide a survival advantage [81]. Inhibition of glucan synthase following exposure to echinocandin drugs activates calcineurin-dependent stress responses and the downstream effector Crz1 [112,113]. The key cellular responses generated as a result of cell wall stress caused by echinocandins are influenced by Hsp90 to the extent that compromising Hsp90 function reduces echinocandin tolerance in wild-type (WT) strains and resistance in clinical isolates [81,109]. Chemical or genetic impairment of Hsp90 reduces the tolerance of *Candida* and *Aspergillus* to echinocandin drugs, as well as to other cell wall inhibitors such as nikkomycin Z [114].

MIC & breakpoints

The Clinical Laboratory Standards Institute (CLSI) has defined laboratory standards to determine MICs for WT strains and the MIC distributions from large-scale surveillance studies are used to define epidemiological cut-off values (ECVs). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) work to the principle that breakpoints for susceptibility testing should not divide WT distributions for clinically important microorganisms, thereby ensuring that all individuals of a species that lack drug-specific mechanisms of resistance can be categorized as either susceptible, intermediate or resistant [115]. A WT organism is defined as a strain that does not possess acquired resistance to a given antimicrobial agent [116–118]; its MIC distribution covers three–four twofold dilution steps around the modal MIC [115]. Since the introduction of CSF in 2001, extensive surveillance has been conducted on the susceptibility of clinical strains to the echinocandin drugs [119–121]. Recently, a total of 8271 clinical isolates, obtained from blood or other normally sterile sites from more 100 medical centers worldwide during the period 2003–2007 were evaluated, including strains of *C. albicans* (4283), *C. glabrata* (1236), *C. parapsilosis* (1238), *C. tropicalis* (996), *C. krusei* (270), *Candida lusitanae* (99), *Candida guilliermondii* (88) and *Candida kefyr* (61) [122,123]. WT MIC distributions demonstrate very low MICs typical of susceptible *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. kefyr* and higher values typical of *C. parapsilosis*, *C. guilliermondii* and *C. lusitanae* for all three echinocandins (Table 1).

Clinical breakpoints (CBPs) assist in determining whether an anti-infective is useful in the treatment of an infection and are typically set prior to a drug being used clinically. They require an integrated knowledge of WT distributions of MICs, an assessment of the pharmacokinetics/pharmacodynamics (PK/PD) and an evaluation of clinical outcome. Once established, breakpoints are reviewed when anti-infective agents have been in clinical use for some time, especially when new mechanisms of resistance are elucidated [117]. The CLSI Subcommittee for Antifungal Testing proposed a clinical interpretive MIC breakpoint (CBP) for echinocandin drugs against *Candida* spp. [124]. Conventionally, clinical breakpoints are used to indicate those isolates likely to respond to treatment with a given antimicrobial agent at the approved dosing regimen. It is different from the ECV, which provides the most sensitive measure of the emergence of strains with reduced susceptibility to a given agent. An initial CBP for susceptible isolates was established with MIC ≤ 2 mg/ml for all three echinocandin drugs and all species of *Candida*, which followed from an analysis of existing clinical, PK/PD and microbiological data [84]. A resistant breakpoint was not established because resistant isolates with known *FKS* genotypes were not included in the original database. The Committee recommended that isolates with a MIC more than 2 μ g/ml be called non-susceptible. An initial CBP value of ≤ 2 μ g/ml encompassed 99.9–100% of the five most susceptible *Candida* spp. (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. kefyr*) isolates, while the ECVs were eight- and 64-fold lower. The ECVs for the three less susceptible species, *C. lusitanae*, *C. parapsilosis* and *C. guilliermondii*, were similar to the CBPs for all three of the echinocandins [123].

FKS-mediated resistance helps redefine breakpoints

It is now well established that clinically resistant *Candida* infections are associated with strains carrying mutations in *FKS1* and/or *FKS2* [42,43]. As many of these strains were obtained from patients failing therapy, the MIC values were not captured by the proposed susceptibility breakpoint, especially with MCF and ANF [42,43,80,115,123,125,126]. The CLSI breakpoint was inclusive of all *Candida* species, but it became apparent that a single breakpoint (≤ 2.0 μ g/ml) for all species and echinocandin drugs did not adequately reflect the clinical experience with strains harboring *FKS* genotypes, as many of the strains were not

captured by the susceptibility breakpoint, especially for MCF and ANF (Figure 6). Kinetic studies of the glucan synthase enzyme complex from *FKS* mutants also raised questions about the proposed CBP and suggested that a lower MIC cutoff of 0.25–0.5 mg/ml would be more inclusive of strains failing therapy (Figure 6) [42,43,127]. These findings coupled with better PK/PD indices for the echinocandins [128–130] provided a new basis to develop species-specific CBPs for both susceptibility and resistance, which would encompass data on strains with *FKS* genotypes [131]. Revised echinocandin breakpoints of 0.25 µg/ml/ml (susceptible), 0.5 µg/ml (intermediate) and 1 (resistant) for *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei* were recently proposed [131]. The revised CBP is applicable to all echinocandin drugs; although, a caveat was noted for MCF and *C. glabrata* in which a lower susceptible breakpoint of 0.06 µg/ml was proposed to provide adequate clinical utility. The CBP was not appropriate for either *C. parapsilosis* or *C. guilliermondii*, which are inherently less susceptible due to polymorphisms in the hot spot one region of *FKS1* [54]. Unlike *FKS* mutants with strong resistance phenotypes (high MIC and IC₅₀), these strains typically respond well to standard therapy [132], presumably because the polymorphisms only weakly affect the sensitivity of glucan synthase (IC₅₀) for drug [54]. The moderate enzyme sensitivity maintains the observed clinical efficacy of echinocandin drugs with these strains [123]. However, they are shifted somewhat (1 log vs 3 logs) in susceptibility, which may account for sporadic reports of clinical failure [12,79]. Despite the good clinical efficacy, the current Infectious Diseases Society of America guidelines do not recommend the echinocandins as first-line therapy for *C. parapsilosis* fungemia [10]. To date, there are no clinical strains of *C. parapsilosis* or *C. guilliermondii* reported that contain characteristic hot spot mutations in *FKS1* (apart from naturally occurring polymorphisms) that would increase an already high MIC and confer clinical failure due to reduced sensitivity of the enzyme for drug. A CBP for resistant *C. parapsilosis* and *C. guilliermondii* has not been determined, although isolates of these two species with MICs ≥ 8 µg/ml are likely to be resistant to the echinocandin drugs [123].

Serum effect on drug potency & MIC testing

A wide range of animal and human studies indicate that echinocandin drugs are tightly bound to serum proteins CSF 80–96% [133,134], ANF more than 84% and MCF more than 99% protein bound [135]. The binding of serum to echinocandin drugs reduces their relative antifungal properties, up to 128-fold in some *Candida* spp. [135–137]. Comparable effects are also observed when evaluating the relative potency of the drugs directly on the glucan synthase target [135]. Relative potency differences for susceptible *Candida* and *Aspergillus* strains (20-fold or more) observed in standard MIC testing (e.g., CLSI M27-A3) are minimized in the presence of serum [135]. The effect of serum in neutralizing MIC differences was also consistent with a murine candidiasis model in which CSF and ANF were comparable in efficacy while MCF was only somewhat less effective over the dose range (0.05–1 mg/kg/day) in reducing kidney fungal burdens (ED₉₉) [135]. Serum protein binding influences the antifungal properties of echinocandin drugs and standardized MIC values obtained in the absence of serum may only partially reflect *in vivo* efficacy. Recently, it was noted that the addition of 50% human serum to the MIC assay improves the identification of echinocandin-resistant *Candida* strains containing *FKS* hot spot mutations by broadening the separation between WT and *FKS* mutant-bearing resistant populations [138]. Despite this promising observation, which could aid in detection of *bona fide* resistance, this modification cannot be routinely applied to testing due to safety and standardization difficulties involving human serum. To circumvent this problem, studies have been initiated to evaluate commercial bovine serum albumin (BSA) as a standardized alternative to human serum. In one study, 79 *Candida* spp. strains from seven species and including 40 *FKS* hot spot mutants were evaluated. The addition of BSA was found to mimic that of human serum and helped distinguish between susceptible and *FKS*-resistant

Candida strains [138]. A larger-scale study is in progress using CLSI and EUCAST protocols to evaluate the merits of adding BSA to obtain a clearer indication of resistant isolates from standardized susceptibility testing [139].

FKS mutations & virulence

The virulence of fungal cells is in part linked to their ability to rapidly divide and grow through cell extension. Glucan synthase is critical to growth and development as it must be able to produce glucan polymers necessary for cell wall deposition and remodeling with high efficiency. Like most critical cellular biosynthetic enzymes, it operates at a basal level that is displaced from maximum velocity ($V_{\max}$). This property affords glucan synthase the ability to rapidly increase its catalytic capacity based on cellular demand. Detailed kinetic studies of glucan synthase isolated from drug-resistant cells with associated *FKS* hot spot mutations revealed that resistance has a cost to the enzyme. Many of the *FKS* mutations result in reduced $V_{\max}$ for the mutant glucan synthase enzymes [42,43], which limits the biosynthetic capacity of cells. Depending on the mutation and its affect on glucan synthase, the cells regulate the relative expression of *FKS1* and *FKS2* [42] but the overall effect is reduced biosynthesis. Some cells show thicker cell walls attributable in part to compensatory increases in cell-wall chitin content [140]. The *FKS1* mutants with the highest chitin content had reduced growth rates in liquid medium and impaired capacity for yeast-to-hyphal transformation. They also show attenuated virulence in a *Drosophila* minihost model, as well as in a model of disseminated candidiasis in a competitive mixed (isogenic WT and *FKS* mutants) challenge infection [140]. Similar attenuated virulence associated with decreased $V_{\max}$ has been observed with *C. glabrata* [Perlin Ds, Unpublished Data]. Collectively, these results strongly suggest that *FKS1* mutations conferring echinocandin resistance have a likely fitness cost resulting in reduced virulence because the cells cannot keep up with normal cell wall expansion associated with growth demand. Such a fitness cost would be expected to limit the epidemiological and clinical impact of these strains, which may help account for their low frequency in the clinic.

PK/PD considerations

Echinocandin drugs are only suitable for intravenous administration, as they have poor oral bioavailability and have half-lives ranging from 10–24 h. The tissue distributions are greatest in target organs for fungal disease namely liver, kidneys, large intestine, spleen and lungs. Low or trace levels of drug are found in the urine and cerebrospinal and vitreous fluids [141]. CSF is metabolized by hydrolysis and *N*-acetylation with loss in the feces and urine as degradation products [142]. ANF undergoes a slow chemical degradation at physiologic temperature and pH to an inactive compound, with approximately 30% excreted in the feces and <1% in urine [143]. MCF is metabolized to M-1 (catechol form) by arylsulfatase, with further metabolism to the M-2 (methoxy form) by catechol-*o*-methyltransferase [142]. Pharmacodynamic studies using *in vivo* candidiasis models have demonstrated that the 24 h area under the concentration-time curve (AUC)/MIC is a good indicator of the echinocandin exposure–response relationship. The 24 h AUC/MIC target for a stasis end point identified free-drug 24 h AUC/MIC against *C. albicans* for all echinocandin drugs [128]. ANF, CSF and MCF show linear PK values following intravenous administration with peak serum concentrations of 10 µg/ml and AUC values of 110 mg·h/l [142]. The echinocandin drugs show a concentration-dependent killing and a prolonged (12–24 h) post-antifungal effect [129,130,144] and it suggests that escalating doses may have additional antifungal benefits. In an *in vivo* model, ANF redistributed from serum to tissue where it persisted for many days. Four days after a single drug dose of more than 4 mg/kg, the measured drug concentrations in the tissue were in excess of the MIC₉₀ for clinical isolates of *Candida*, which is similar to the tissue concentration of CSF. Murine

studies demonstrate that fungicidal activity of the drugs is maximized against *C. albicans* when the ratio of total serum drug concentration to organism MIC ($C_{\max}/\text{MIC}$) approaches 10 or if the 24 h AUC:MIC ratio exceeds 10–20 [128–130]. Caspofungin also shows concentration-dependent PD in the treatment of invasive pulmonary aspergillosis with the $C_{\max}$:minimum effective concentration (MEC) ratio most closely associated with reduction of pulmonary fungal burden [103].

Drug–bug–immune responses

The observation that the addition of low levels of CSF to monocyte cultures increased anti-fungal activity was noted early in echinocandin research [145]. Dectin-1 is a pattern recognition receptor for β -1,3/ β -1,6-linked glucans; it is a type II transmembrane protein belonging to the NK-like C-type lectin-like receptor family [146,147] and its activation results in phagocytic, proinflammatory and antimicrobial responses [148–150]. At sub-MIC concentrations, CSF was found to increase β -glucan exposure on *C. albicans* through cell wall remodeling which triggered enhanced macrophage inflammatory responses via interaction with dectin-1 [151]. In *A. fumigatus*, the conidial surface is composed of a hydrophobic layer of RodA protein covalently bound to the conidial cell wall. This layer insulates the spores making them immunologically inert. The removal or disruption of this surface layer results in conidial morphotypes inducing immune activation [152]. As the live conidia swell, they display β -glucans on their cell surface and with hyphal formation, they trigger dectin-1 recruitment to phagosomal membranes of alveolar macrophages [153,154]. Exposure of macrophages to germinating conidia reveals a direct correlation between surface β -glucan display and inflammatory cytokine/chemokine induction. The innate immune system ignores the less threatening dormant conidia, but responds vigorously to germinating conidia and hyphae [155]. Echinocandins do not completely inhibit *in vitro* growth of *A. fumigatus*, rather they induce morphological changes in fungal hyphae (Figure 2) [7]. Echinocandin-treated hyphae enhance antifungal properties in a macrophage system [145]. Echinocandin-induced morphological changes in *A. fumigatus* hyphae and other fungi are accompanied by increased β -glucan exposure resulting in dectin-1-mediated inflammatory responses caused by macrophages [156,157]. Echinocandins stimulate release of TNF and CXCL2, and TLR 9 is also upregulated [156]. Overall, the echinocandins compensate for their relatively weak growth inhibitory properties in *Aspergillus* and other molds by unmasking glucans, which can then prime and stimulate components of the innate immune system, even when compromised. Finally, it has been observed that biofilms are significantly more susceptible to the presence of human phagocytes in the presence of an echinocandin than in its absence suggesting a combined action of the drug and host defense [158].

Oral glucan synthase inhibitors

The echinocandins are available only in intravenous formulation, which limits their use in less severe infections or as oral step-down agents. The enfumafungins are natural products that are structurally different from the echinocandins, but like the echinocandins, they inhibit fungal β -1,3- β -glucan synthesis. Enfumafungin is a potent antifungal compound isolated from *Hormonema carpetanum* [159,160]. It is a natural triterpene glycoside that inhibits glucan synthase and shows highly potent antifungal activity *in vitro* against *Candida* and *Aspergillus*, although its chemical structure is distinct from the echinocandins. Recently, Merck described a semi-synthetic derivative of enfumafungin, MK-3118, which is being developed as an oral therapy for fungal infections [161,162]. MK-3118 is a potent inhibitor of glucan synthases from *C. albicans* (IC_{50} 0.6 ng/ml) and *A. fumigatus* (IC_{50} 1.7 ng/ml). Against 160 strains of seven *Candida* species, it had a MIC_{90} of no more than 1 $\mu\text{g}/\text{ml}$ and against 40 *Aspergillus* spp., it had a MEC_{90} of no more than 0.015 $\mu\text{g}/\text{ml}$ [163]. MK-3118

also shows promising *in vivo* efficacy in murine models of candidiasis [164] and aspergillosis [165]. MK-3118 was also active *in vitro* against echinocandin-resistant *Candida* spp. isolates containing *FKS* mutations. This latter observation suggests that this molecule either binds at a site separate from the echinocandins on *Fks1* or it is active on another component of glucan synthase. More detailed biochemical and genetic studies will be required to assess this molecular property. The spontaneous mutation frequency in *C. albicans* for conferring resistance to MK-3118 was less than 4.6×10^{-9} mutations per cell per generation, which is comparable to that of CSF at 2.6×10^{-9} [163]. This level of mutant generation portends a low frequency of resistance, except at high burdens. But this remains to be determined experimentally.

Future perspective

The clinical experience with echinocandin drugs has been high successful, as the class exhibits potent efficacy, especially with *Candida* spp., with minimal side effects and a low frequency of resistance. A revised break-point has been proposed to better guide treatment when resistant isolates are encountered. As clinical resistance is associated with a narrow spectrum of point mutations in the *FKS* genes, real-time molecular diagnostics may be of value in diagnosing resistant strains. However, not all *FKS* mutations have the same potential for therapeutic failure and a rigorous PK/PD approach is needed to better understand weak *FKS* mutants. It remains to be seen whether commonly encountered strains with reduced susceptibility but which respond to therapy, such as *C. parapsilosis* will begin to show higher-order breakthrough infections. The effect of echinocandin drugs in stimulating the immune system by exposing critical antigens is an important consideration in the use of these drugs with moulds, and this drug–bug–immune response needs to be better understood. Finally, there is much excitement as a new generation of oral glucan synthase inhibitors is being developed with great promise to benefit patients requiring potent step-down therapy. Hopefully, there will also be an emphasis on broader spectrum inhibitors to meet the challenges of other fungal pathogens.

Acknowledgments

The author would like to thank S Park for his contributions to enhance and improve the manuscript.

Studies on echinocandin drugs in the Perlin laboratory are supported by grants to D Perlin from the NIH (AI069397), Merck, Pfizer and Astellas. D Perlin serves on advisory and opinion leader panels for Merck, Pfizer and Astellas and he also receives grant support from these companies. He is also on the Scientific Advisory Board for Myconostica.

Bibliography

Papers of special note have been highlighted as:

■ of interest

1. Kurtz MB, Douglas CM. Lipopeptide inhibitors of fungal glucan synthase. *J. Med. Vet. Mycol.* 1997; 35(2):79–86. [PubMed: 9147267]
2. Denning DW. Echinocandins: a new class of antifungal. *J. Antimicrob. Chemother.* 2002; 49(6): 889–891. [PubMed: 12039879]
3. Denning DW. Echinocandin antifungal drugs. *Lancet.* 2003; 362(9390):1142–1151. [PubMed: 14550704]
4. Morrison VA. Echinocandin antifungals: review and update. *Expert Rev. Anti Infect. Ther.* 2006; 4(2):325–342. [PubMed: 16597212]
5. Douglas CM. Fungal $\beta(1,3)$ -d-glucan synthesis. *Med. Mycol.* 2001; 39(Suppl. 1):55–66. [PubMed: 11800269]

6. Kurtz MB, Rex JH. Glucan synthase inhibitors as antifungal agents. *Adv. Protein Chem.* 2001; 56:423–475. [PubMed: 11329859]
7. Kurtz MB, Heath IB, Marrinan J, Dreikorn S, Onishi J, Douglas C. Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)- β -d-glucan synthase. *Antimicrob. Agents Chemother.* 1994; 38(7):1480–1489. [PubMed: 7979276]
8. Bowman JC, Hicks PS, Kurtz MB, et al. The antifungal echinocandin caspofungin acetate kills growing cells of *Aspergillus fumigatus* *in vitro*. *Antimicrob. Agents Chemother.* 2002; 46(9):3001–3012. [PubMed: 12183260]
9. Douglas CM. Understanding the microbiology of the *Aspergillus* cell wall and the efficacy of caspofungin. *Med. Mycol.* 2006; 44(Suppl.):95–99.
10. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* 2009; 48(5):503–535. [PubMed: 19191635]
11. Pappas PG, Rotstein CM, Betts RF, et al. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. *Clin. Infect. Dis.* 2007; 45(7):883–893. [PubMed: 17806055]
12. Reboli AC, Rotstein C, Pappas PG, et al. Anidulafungin versus fluconazole for invasive candidiasis. *N. Engl. J. Med.* 2007; 356(24):2472–2482. [PubMed: 17568028] ■ Important clinical study highlighting the value of anidulafungin as an echinocandin class drug in treating invasive candidiasis relative to the azole fluconazole.
13. Bormann AM, Morrison VA. Review of the pharmacology and clinical studies of micafungin. *Drug Des. Devel. Ther.* 2009; 3:295–302.
14. Temesgen Z, Barreto J, Vento S. Micafungin – the newest echinocandin. *Drugs Today.* 2009; 45(6):469–478. [PubMed: 19856528]
15. Cross SA, Scott LJ. Micafungin: a review of its use in adults for the treatment of invasive and oesophageal candidiasis, and as prophylaxis against *Candida* infections. *Drugs.* 2008; 68(15): 2225–2255. [PubMed: 18840009]
16. Kim R, Khachikian D, Reboli AC. A comparative evaluation of properties and clinical efficacy of the echinocandins. *Expert Opin. Pharmacother.* 2007; 8(10):1479–1492. [PubMed: 17661730]
17. Morris MI, Villmann M. Echinocandins in the management of invasive fungal infections, Part 2. *Am. J. Health Syst. Pharm.* 2006; 63(19):1813–1820. [PubMed: 16990627]
18. Morris MI, Villmann M. Echinocandins in the management of invasive fungal infections, part 1. *Am. J. Health Syst. Pharm.* 2006; 63(18):1693–1703. [PubMed: 16960253]
19. Chandrasekar PH, Sobel JD. Micafungin: a new echinocandin. *Clin. Infect. Dis.* 2006; 42(8):1171–1178. [PubMed: 16575738]
20. Kartsonis N, DiNubile MJ, Bartizal K, Hicks PS, Ryan D, Sable CA. Efficacy of caspofungin in the treatment of esophageal candidiasis resistant to fluconazole. *J. Acquir. Immune Defic. Syndr.* 2002; 31(2):183–187. [PubMed: 12394797] ■ Early clinical study demonstrating that caspofungin therapy is efficacious for some patients with esophageal candidiasis who were clinically refractory to fluconazole or infected with strains with reduced *in vitro* susceptibility to fluconazole.
21. Posteraro B, Sanguinetti M, Fiori B, et al. Caspofungin activity against clinical isolates of azole cross-resistant *Candida glabrata* overexpressing efflux pump genes. *J. Antimicrob. Chemother.* 2006; 58(2):458–461. [PubMed: 16757500]
22. Pfaller MA, Diekema DJ, Messer SA, Hollis RJ, Jones RN. *In vitro* activities of caspofungin compared with those of fluconazole and itraconazole against 3,959 clinical isolates of *Candida* spp., including 157 fluconazole-resistant isolates. *Antimicrob. Agents Chemother.* 2003; 47(3): 1068–1071. [PubMed: 12604543]
23. Bachmann SP, Patterson TF, Lopez-Ribot JL. *In vitro* activity of caspofungin (MK-0991) against *Candida albicans* clinical isolates displaying different mechanisms of azole resistance. *J. Clin. Microbiol.* 2002; 40(6):2228–2230. [PubMed: 12037093]
24. Ferreira JA, Carr JH, Starling CE, de Resende MA, Donlan RM. Biofilm formation and effect of caspofungin on biofilm structure of *Candida* species bloodstream isolates. *Antimicrob. Agents Chemother.* 2009; 53(10):4377–4384. [PubMed: 19546368]

25. Ramage G, Van de Walle K, Bachmann SP, Wickes BL, Lopez-Ribot JL. *In vitro* pharmacodynamic properties of three antifungal agents against preformed *Candida albicans* biofilms determined by time-kill studies. *Antimicrob. Agents Chemother.* 2002; 46(11):3634–3636. [PubMed: 12384379]
26. Bachmann SP, Van de Walle K, Ramage G, et al. *In vitro* activity of caspofungin against *Candida albicans* biofilms. *Antimicrob. Agents Chemother.* 2002; 46(11):3591–3596. [PubMed: 12384370]
27. Zaas AK. Echinocandins: a wealth of choice – how clinically different are they? *Curr. Opin. Infect. Dis.* 2008; 21(4):426–432. [PubMed: 18594297]
28. Zaas AK, Dodds Ashley ES, Alexander BD, Johnson MD, Perfect JR. Caspofungin for invasive candidiasis at a tertiary care medical center. *Am. J. Med.* 2006; 119(11):993, E991–E996. [PubMed: 17071169]
29. Goto N, Hara T, Tsurumi H, et al. Efficacy and safety of micafungin for treating febrile neutropenia in hematological malignancies. *Am. J. Hematol.* 2010; 85(11):872–876. [PubMed: 20882524]
30. Lehrnbecher T, Groll AH. Micafungin: a brief review of pharmacology, safety, and antifungal efficacy in pediatric patients. *Pediatr. Blood Cancer.* 2010; 55(2):229–232. [PubMed: 20583216]
31. Manzoni P, Rizzollo S, Franco C, et al. Role of echinocandins in the management of fungal infections in neonates. *J. Matern. Fetal Neonatal Med.* 2010; 23(S3):49–52. [PubMed: 20858036]
32. Aikawa N, Kusachi S, Oda S, Takesue Y, Tanaka H. Clinical effects of micafungin, a novel echinocandin antifungal agent, on systemic fungal infections in surgery, emergency, and intensive-care medicine: evaluation using the AKOTT algorithm. *J. Infect. Chemother.* 2009; 15(4):219–227. [PubMed: 19688240]
33. Kuti EL, Kuti JL. Pharmacokinetics, antifungal activity and clinical efficacy of anidulafungin in the treatment of fungal infections. *Expert Opin. Drug Metab. Toxicol.* 2010; 6(10):1287–1300. [PubMed: 20822479]
34. Menichetti F. Anidulafungin, a new echinocandin: effectiveness and tolerability. *Drugs.* 2009; 69(Suppl. 1):95–97. [PubMed: 19877741]
35. Sucher AJ, Chahine EB, Balcer HE. Echinocandins: the newest class of antifungals. *Ann. Pharmacother.* 2009; 43(10):1647–1657. [PubMed: 19724014]
36. VandenBussche HL, Van Loo DA. A clinical review of echinocandins in pediatric patients. *Ann. Pharmacother.* 2010; 44(1):166–177. [PubMed: 20009006]
37. Venditti M. Clinical aspects of invasive candidiasis: endocarditis and other localized infections. *Drugs.* 2009; 69(Suppl. 1):39–43. [PubMed: 19877733]
38. Maschmeyer G, Glasmacher A. Pharmacological properties and clinical efficacy of a recently licensed systemic antifungal, caspofungin. *Mycoses.* 2005; 48(4):227–234. [PubMed: 15982202]
39. Walsh TJ, Adamson PC, Seibel NL, et al. Pharmacokinetics, safety, and tolerability of caspofungin in children and adolescents. *Antimicrob. Agents Chemother.* 2005; 49(11):4536–4545. [PubMed: 16251293]
40. Heresi GP, Gerstmann DR, Reed MD, et al. The pharmacokinetics and safety of micafungin, a novel echinocandin, in premature infants. *Pediatr. Infect. Dis. J.* 2006; 25(12):1110–1115. [PubMed: 17133155]
41. Vazquez JA. The safety of anidulafungin. *Expert Opin. Drug Saf.* 2006; 5(6):751–758. [PubMed: 17044802]
42. Garcia-Effron G, Lee S, Park S, Cleary JD, Perlin DS. Effect of *Candida glabrata* FKS1 and FKS2 mutations on echinocandin sensitivity and kinetics of 1,3- β -d-glucan synthase: implication for the existing susceptibility breakpoint. *Antimicrob. Agents Chemother.* 2009; 53(9):3690–3699. [PubMed: 19546367] ■ Study correlating MIC and the effects of *FKS* mutations in *C. glabrata* on glucan synthase inhibition kinetics and implications for susceptibility and resistance breakpoints.
43. Garcia-Effron G, Park S, Perlin DS. Correlating echinocandin MIC and kinetic inhibition of *fksl* mutant glucan synthases for *Candida albicans*: implications for interpretive breakpoints. *Antimicrob. Agents Chemother.* 2009; 53(1):112–122. [PubMed: 18955538] ■ Study correlating MIC and the effects of *FKS* mutations in *C. albicans* on glucan synthase inhibition kinetics and the need to re-evaluate breakpoints.

44. Perlin DS. Resistance to echinocandin-class antifungal drugs. *Drug Resist. Update.* 2007; 10(3): 121–130.
45. Orlean PA. (1,3)- β -d-glucan synthase from budding and filamentous cultures of the dimorphic fungus. *Candida albicans*. *Eur. J. Biochem.* 1982; 127(2):397–403.
46. Andaluz E, Guillen A, Larriba G. Preliminary evidence for a glucan acceptor in the yeast. *Candida albicans*. *Biochem. J.* 1986; 240(2):495–502.
47. Lopez-Romero E, Ruiz-Herrera J. Biosynthesis of β -glucans by cell-free extracts from *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta.* 1977; 500(2):372–384. [PubMed: 22355]
48. Shematek EM, Braatz JA, Cabib E. Biosynthesis of the yeast cell wall. I: preparation and properties of β -(1 leads to 3) glucan synthetase. *J. Biol. Chem.* 1980; 255(3):888–894. [PubMed: 6766139]
49. Shematek EM, Cabib E. Biosynthesis of the yeast cell wall. II: regulation of β -(1 leads to 3)glucan synthetase by ATP and GTP. *J. Biol. Chem.* 1980; 255(3):895–902. [PubMed: 6766140]
50. Cabib E, Roberts R, Bowers B. Synthesis of the yeast cell wall and its regulation. *Annu. Rev. Biochem.* 1982; 51:763–793. [PubMed: 7051965]
51. Cabib E, Kang MS. Fungal 1,3- β -glucan synthase. *Methods Enzymol.* 1987; 138:637–642. [PubMed: 2955197]
52. Inoue SB, Takewaki N, Takasuka T, et al. Characterization and gene cloning of 1,3- β -D-glucan synthase from *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 1995; 231(3):845–854. [PubMed: 7649185]
53. Inoue SB, Qadota H, Arisawa M, Anraku Y, Watanabe T, Ohya Y. Signaling toward yeast 1,3- β -glucan synthesis. *Cell Struct. Funct.* 1996; 21(5):395–402. [PubMed: 9118246]
54. Garcia-Effron G, Katiyar SK, Park S, Edlind TD, Perlin DS. A naturally occurring proline-to-alanine amino acid change in Fks1p in *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* accounts for reduced echinocandin susceptibility. *Antimicrob. Agents Chemother.* 2008; 52(7):2305–2312. [PubMed: 18443110]
55. Douglas CM, Foor F, Marrinan JA, et al. The *Saccharomyces cerevisiae* FKS1 (ETG1) gene encodes an integral membrane protein which is a subunit of 1,3- β -d-glucan synthase. *Proc. Natl Acad. Sci. USA.* 1994; 91(26):12907–12911. [PubMed: 7528927]
56. Kondoh O, Tachibana Y, Ohya Y, Arisawa M, Watanabe T. Cloning of the RHO1 gene from *Candida albicans* and its regulation of β -1,3-glucan synthesis. *J. Bacteriol.* 1997; 179(24):7734–7741. [PubMed: 9401032]
57. Schimoler-O'Rourke R, Renault S, Mo W, Selitrennikoff CP. *Neurospora crassa* FKS protein binds to the (1,3) β -glucan synthase substrate, UDP-glucose. *Curr. Microbiol.* 2003; 46(6):408–412. [PubMed: 12732946]
58. Mio T, Adachi-Shimizu M, Tachibana Y, et al. Cloning of the *Candida albicans* homolog of *Saccharomyces cerevisiae* GSC1/ FKS1 and its involvement in β -1,3-glucan synthesis. *J. Bacteriol.* 1997; 179(13):4096–4105. [PubMed: 9209021]
59. Thompson JR, Douglas CM, Li W, et al. A glucan synthase FKS1 homolog in *Cryptococcus neoformans* is single copy and encodes an essential function. *J. Bacteriol.* 1999; 181(2):444–453. [PubMed: 9882657]
60. Mazur P, Morin N, Baginsky W, et al. Differential expression and function of two homologous subunits of yeast 1,3- β -d-glucan synthase. *Mol. Cell Biol.* 1995; 15(10):5671–5681. [PubMed: 7565718]
61. Mazur P, Baginsky W. *In vitro* activity of 1,3- β -d-glucan synthase requires the GTP-binding protein Rho1. *J. Biol. Chem.* 1996; 271(24):14604–14609. [PubMed: 8662910]
62. Edlind TD, Katiyar SK. The echinocandin “target” identified by cross-linking is a homolog of Pil1 and Lsp1, sphingolipid-dependent regulators of cell wall integrity signaling. *Antimicrob. Agents Chemother.* 2004; 48(11):4491. [PubMed: 15504893]
63. Radding JA, Heidler SA, Turner WW. Photoaffinity analog of the semisynthetic echinocandin LY303366: identification of echinocandin targets in *Candida albicans*. *Antimicrob. Agents Chemother.* 1998; 42(5):1187–1194. [PubMed: 9593148]
64. Pirri G, Giuliani A, Nicoletto SF, Pizzuto L, Rinaldi AC. Lipopeptides as anti-infectives: a practical perspective. *Cent. Eur. J. Biol.* 2009; 4:258–273.

65. Paderu P, Park S, Perlin DS. Caspofungin uptake is mediated by a high-affinity transporter in *Candida albicans*. *Antimicrob. Agents Chemother.* 2004; 48(10):3845–3849. [PubMed: 15388444]
66. Niimi K, Maki K, Ikeda F, et al. Overexpression of *Candida albicans* CDR1, CDR2, or MDR1 does not produce significant changes in echinocandin susceptibility. *Antimicrob. Agents Chemother.* 2006; 50(4):1148–1155. [PubMed: 16569823]
67. Charlemagne D. Molecular and cellular level of action of digitalis. *Herz.* 1993; 18(2):79–85. [PubMed: 7684015]
68. Zimbeck AJ, Iqbal N, Ahlquist AM, et al. FKS mutations and elevated echinocandin MIC values among *Candida glabrata* isolates from US population-based surveillance. *Antimicrob. Agents Chemother.* 2010; 54(12):5042–5047. [PubMed: 20837754]
69. Castanheira M, Woosley LN, Diekema DJ, Messer SA, Jones RN, Pfaller MA. Low prevalence of *fksl* hot spot 1 mutations in a worldwide collection of *Candida* strains. *Antimicrob. Agents Chemother.* 2010; 54(6):2655–2659. [PubMed: 20368396]
70. Hakki M, Staab JF, Marr KA. Emergence of a *Candida krusei* isolate with reduced susceptibility to caspofungin during therapy. *Antimicrob. Agents Chemother.* 2006; 50(7):2522–2524. [PubMed: 16801435]
71. Katiyar S, Pfaller M, Edlind T. *Candida albicans* and *Candida glabrata* clinical isolates exhibiting reduced echinocandin susceptibility. *Antimicrob. Agents Chemother.* 2006; 50(8):2892–2894. [PubMed: 16870797]
72. Miller CD, Lomaestro BW, Park S, Perlin DS. Progressive esophagitis caused by *Candida albicans* with reduced susceptibility to caspofungin. *Pharmacotherapy.* 2006; 26(6):877–880. [PubMed: 16716141]
73. Kahn JN, Garcia-Effron G, Hsu MJ, Park S, Marr KA, Perlin DS. Acquired echinocandin resistance in a *Candida krusei* isolate due to modification of glucan synthase. *Antimicrob. Agents Chemother.* 2007; 51(5):1876–1878. [PubMed: 17325225]
74. Cleary JD, Garcia-Effron G, Chapman SW, Perlin DS. Reduced *Candida glabrata* susceptibility secondary to an *FKSI* mutation developed during candidemia treatment. *Antimicrob. Agents Chemother.* 2008; 52(6):2263–2265. [PubMed: 18378714]
75. Desnos-Ollivier M, Dromer F, Dannaoui E. Detection of caspofungin resistance in *Candida* spp. by Etest. *J. Clin. Microbiol.* 2008; 46(7):2389–2392. [PubMed: 18448691]
76. Garcia-Effron G, Kontoyiannis DP, Lewis RE, Perlin DS. Caspofungin-resistant *Candida tropicalis* strains causing breakthrough fungemia in patients at high risk for hematologic malignancies. *Antimicrob. Agents Chemother.* 2008; 52(11):4181–4183. [PubMed: 18794386]
77. Arendrup MC, Garcia-Effron G, Buzina W, et al. Breakthrough *Aspergillus fumigatus* and *Candida albicans* double infection during caspofungin treatment: laboratory characteristics and implication for susceptibility testing. *Antimicrob. Agents Chemother.* 2009; 53(3):1185–1193. [PubMed: 19104024]
78. Thompson GR 3rd, Wiederhold NP, Vallor AC, Villareal NC, Lewis JS 2nd, Patterson TF. Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to *Candida glabrata* infection. *Antimicrob. Agents Chemother.* 2008; 52(10):3783–3785. [PubMed: 18676885]
79. Pfeiffer CD, Garcia-Effron G, Zaas AK, Perfect JR, Perlin DS, Alexander BD. Breakthrough invasive candidiasis in patients on micafungin. *J. Clin. Microbiol.* 2010; 48(7):2373–2380. [PubMed: 20421445]
80. Desnos-Ollivier M, Bretagne S, Raoux D, Hoinard D, Dromer F, Dannaoui E. Mutations in the *fksl* gene in *Candida albicans*, *C. tropicalis*, and *C. krusei* correlate with elevated caspofungin MICs uncovered in AM3 medium using the method of the European Committee on Antibiotic Susceptibility Testing. *Antimicrob. Agents Chemother.* 2008; 52(9):3092–3098. [PubMed: 18591282]
81. Cowen LE. Hsp90 orchestrates stress response signaling governing fungal drug resistance. *PLoS Pathog.* 2009; 5(8):E1000471. [PubMed: 19714223]

82. Kartsonis N, Killar J, Mixson L, et al. Caspofungin susceptibility testing of isolates from patients with esophageal candidiasis or invasive candidiasis: relationship of MIC to treatment outcome. *Antimicrob. Agents Chemother.* 2005; 49(9):3616–3623. [PubMed: 16127030]
83. Pfaller MA, Diekema DJ, Boyken L, et al. Effectiveness of anidulafungin in eradicating *Candida* species in invasive candidiasis. *Antimicrob. Agents Chemother.* 2005; 49(11):4795–4797. [PubMed: 16251335]
84. 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(8):2620–2629. [PubMed: 18579718] ■ Proposal for initial susceptibility breakpoint for echinocandin class drugs.
85. Park S, Kelly R, Kahn JN, et al. Specific substitutions in the echinocandin target Fks1p account for reduced susceptibility of rare laboratory and clinical *Candida* sp. isolates. *Antimicrob. Agents Chemother.* 2005; 49(8):3264–3273. [PubMed: 16048935] ■ Comprehensive foundation study demonstrating the importance of *FKS1* mutations in conferring clinical resistance.
86. Perlin DS. Antifungal drug resistance: do molecular methods provide a way forward? *Curr. Opin. Infect. Dis.* 2009; 22(6):568–573. [PubMed: 19741524]
87. Balajee SA, Gribskov JL, Hanley E, Nickle D, Marr KA. *Aspergillus lentulus* sp. nov., a new sibling species of *A. fumigatus*. *Eukaryot. Cell.* 2005; 4(3):625–632. [PubMed: 15755924]
88. Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A, Cuenca-Estrella M, Rodriguez-Tudela JL. *Aspergillus* section Fumigati: antifungal susceptibility patterns and sequence-based identification. *Antimicrob. Agents Chemother.* 2008; 52(4):1244–1251. [PubMed: 18212093]
89. Gardiner RE, Souteropoulos P, Park S, Perlin DS. Characterization of *Aspergillus fumigatus* mutants with reduced susceptibility to caspofungin. *Med. Mycol.* 2005; 43(Suppl. 1):S299–S305. [PubMed: 16110824]
90. Staab JF, Kahn JN, Marr KA. Differential *Aspergillus lentulus* echinocandin susceptibilities are Fksp-independent. *Antimicrob. Agents Chemother.* 2010; 54(12):4992–4998. [PubMed: 20855747]
91. Malani AN, Kauffman CA. Changing epidemiology of rare mould infections implications for therapy. *Drugs.* 2007; 67(13):1803–1812. [PubMed: 17722951]
92. Nett JE, Crawford K, Marchillo K, Ande DR. Role of Fks1p and matrix glucan in *Candida albicans* biofilm resistance to an echinocandin, pyrimidine, and polyene. *Antimicrob. Agents Chemother.* 2010; 54(8):3505–3508. [PubMed: 20516280]
93. Maligie MA, Selitrennikoff CP. *Cryptococcus neoformans* resistance to echinocandins: (1,3)-glucan synthase activity is sensitive to echinocandins. *Antimicrob. Agents Chemother.* 2005; 49(7):2851–2856. [PubMed: 15980360]
94. Ikeda R, Sugita T, Jacobson ES, Shinoda T. Effects of melanin upon susceptibility of *Cryptococcus* to antifungals. *Microbiol. Immunol.* 2003; 47(4):271–277. [PubMed: 12801064]
95. Kahn JN, Hsu MJ, Racine F, Giacobbe R, Motyl M. Caspofungin susceptibility in *Aspergillus* and non-*Aspergillus* molds: inhibition of glucan synthase and reduction of β -D-1,3 glucan levels in culture. *Antimicrob. Agents Chemother.* 2006; 50(6):2214–2216. [PubMed: 16723587]
96. Stevens DA, Espiritu M, Parmar R. Paradoxical effect of caspofungin: reduced activity against *Candida albicans* at high drug concentrations. *Antimicrob. Agents Chemother.* 2004; 48(9):3407–3411. [PubMed: 15328104]
97. Chamilos G, Lewis RE, Albert N, Kontoyiannis DP. Paradoxical effect of echinocandins across *Candida* species *in vitro*: evidence for echinocandin-specific and candida species-related differences. *Antimicrob. Agents Chemother.* 2007; 51(6):2257–2259. [PubMed: 17438060]
98. 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(3):173–178. [PubMed: 15766602]
99. Lesage G, Sdicu AM, Menard P, Shapiro J, Hussein S, Bussey H. Analysis of β -1,3-glucan assembly in *Saccharomyces cerevisiae* using a synthetic interaction network and altered sensitivity to caspofungin. *Genetics.* 2004; 167(1):35–49. [PubMed: 15166135]
100. Wiederhold NP, Kontoyiannis DP, Prince RA, Lewis RE. Attenuation of the activity of caspofungin at high concentrations against *Candida albicans*: possible role of cell wall integrity

- and calcineurin pathways. *Antimicrob. Agents Chemother.* 2005; 49(12):5146–5148. [PubMed: 16304189]
101. 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 β -1,6-glucan synthesis inhibition by caspofungin. *Antimicrob. Agents Chemother.* 2006; 50(9):3160–3161. [PubMed: 16940118]
 102. Fortwendel JR, Zhao W, Bhabhra R, et al. A fungus-specific ras homolog contributes to the hyphal growth and virulence of *Aspergillus fumigatus*. *Eukaryot. Cell.* 2005; 4(12):1982–1989. [PubMed: 16339716]
 103. 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(8):1464–1471. [PubMed: 15378439]
 104. Clemons KV, Stevens DA. Efficacy of micafungin alone or in combination against experimental pulmonary aspergillosis. *Med. Mycol.* 2006; 44(1):69–73. [PubMed: 16805095]
 105. Klont RR, Mennink-Kersten MA, Ruegebrink D, et al. Paradoxical increase in circulating *Aspergillus* antigen during treatment with caspofungin in a patient with pulmonary aspergillosis. *Clin. Infect. Dis.* 2006; 43(3):E23–E25. [PubMed: 16804836]
 106. 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(5):1140–1144. [PubMed: 18305201]
 107. Cowen LE, Lindquist S. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. *Science.* 2005; 309(5744):2185–2189. [PubMed: 16195452] ■ Role of stress response in altering drug susceptibility in fungi.
 108. Cowen LE, Carpenter AE, Matangkasombut O, Fink GR, Lindquist S. Genetic architecture of Hsp90-dependent drug resistance. *Eukaryot. Cell.* 2006; 5(12):2184–2188. [PubMed: 17056742]
 109. Cowen LE. The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. *Nat. Rev. Microbiol.* 2008; 6(3):187–198. [PubMed: 18246082]
 110. Steinbach WJ, Cramer RA Jr, Perfect BZ, et al. Calcineurin controls growth, morphology, and pathogenicity in *Aspergillus fumigatus*. *Eukaryot. Cell.* 2006; 5(7):1091–1103. [PubMed: 16835453]
 111. Semighini CP, Heitman J. Dynamic duo takes down fungal villains. *Proc. Natl Acad. Sci. USA.* 2009; 106(9):2971–2972. [PubMed: 19251661]
 112. Steinbach WJ, Singh N, Miller JL, et al. *In vitro* interactions between antifungals and immunosuppressants against *Aspergillus fumigatus* isolates from transplant and nontransplant patients. *Antimicrob. Agents Chemother.* 2004; 48(12):4922–4925. [PubMed: 15561883]
 113. Cowen LE, Steinbach WJ. Stress, drugs, and evolution: the role of cellular signaling in fungal drug resistance. *Eukaryot. Cell.* 2008; 7(5):747–764. [PubMed: 18375617]
 114. Cramer RA Jr, Perfect BZ, Pinchai N, et al. Calcineurin target CrzA regulates conidial germination, hyphal growth, and pathogenesis of *Aspergillus fumigatus*. *Eukaryot. Cell.* 2008; 7(7):1085–1097. [PubMed: 18456861]
 115. Arendrup MC, Kahlmeter G, Rodriguez-Tudela JL, Donnelly JP. Breakpoints for susceptibility testing should not divide wild-type distributions of important target species. *Antimicrob. Agents Chemother.* 2009; 53(4):1628–1629. [PubMed: 19188378]
 116. Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clin. Microbiol. Infect.* 2006; 12(5):418–425. [PubMed: 16643517]
 117. Turnidge J, Paterson DL. Setting and revising antibacterial susceptibility breakpoints. *Clin. Microbiol. Rev.* 2007; 20(3):391–408. [PubMed: 17630331]
 118. Rodriguez-Tudela JL, Arendrup MC, Cuenca-Estrella M, Donnelly JP, Lass-Flörl C. EUCAST breakpoints for antifungals. *Drug News Perspect.* 2010; 23(2):93–97. [PubMed: 20369073]
 119. Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S, Diekema DJ. Global surveillance of *in vitro* activity of micafungin against *Candida*: a comparison with caspofungin by CLSI-recommended methods. *J. Clin. Microbiol.* 2006; 44(10):3533–3538. [PubMed: 17021079]

120. Pfaller MA, Boyken L, Hollis RJ, et al. *In vitro* susceptibility of invasive isolates of *Candida* spp. to anidulafungin, caspofungin, and micafungin: six years of global surveillance. *J. Clin. Microbiol.* 2008; 46(1):150–156. [PubMed: 18032613] ■ Large-scale surveillance study looking at *in vitro* susceptibility to echinocandin drugs.
121. Rautemaa R, Richardson M, Pfaller MA, Perheentupa J, Saxen H. Activity of amphotericin B, anidulafungin, caspofungin, micafungin, posaconazole, and voriconazole against *Candida albicans* with decreased susceptibility to fluconazole from APECED patients on long-term azole treatment of chronic mucocutaneous candidiasis. *Diagn. Microbiol. Infect. Dis.* 2008; 62(2):182–185. [PubMed: 18597968]
122. Pfaller MA, Boyken L, Hollis RJ, et al. Wild-type minimum effective concentration distributions and epidemiologic cutoff values for caspofungin and *Aspergillus* spp. as determined by Clinical and Laboratory Standards Institute broth microdilution methods. *Diagn. Microbiol. Infect. Dis.* 2010; 67(1):56–60. [PubMed: 20207095]
123. Pfaller MA, Boyken L, Hollis RJ, et al. Wild-type MIC distributions and epidemiological cutoff values for the echinocandins and *Candida* spp. *J. Clin. Microbiol.* 2009; 48(1):52–56. [PubMed: 19923478]
124. 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(8):2620–2629. [PubMed: 18579718]
125. Arendrup MC, Garcia-Effron G, Lass-Flörl C, et al. Echinocandin susceptibility testing of *Candida* species: comparison of EUCAST EDef 7.1, CLSI M27-A3, Etest, disk diffusion, and agar dilution methods with RPMI and isosensitest media. *Antimicrob. Agents Chemother.* 2010; 54(1):426–439. [PubMed: 19884370]
126. Stein GE, El-Mortada M, Smith C, Dybas L, Prince R, Havlicek D. Fungicidal activity of anidulafungin in serum from patients does not correlate to its susceptible breakpoint against *Candida* spp. *J. Antimicrob. Chemother.* 2009; 65(2):374–376. [PubMed: 19996140]
127. Wiederhold NP, Grabinski JL, Garcia-Effron G, Perlin DS, Lee SA. Pyrosequencing to detect mutations in *FKS1* that confer reduced echinocandin susceptibility in *Candida albicans*. *Antimicrob. Agents Chemother.* 2008; 52(11):4145–4148. [PubMed: 18794385]
128. Andes D, Diekema DJ, Pfaller MA, Bohrmüller J, Marchillo K, Lepak A. *In vivo* comparison of the pharmacodynamic targets for echinocandin drugs against *Candida* species. *Antimicrob. Agents Chemother.* 2010; 54(6):2497–2506. [PubMed: 20385855] ■ Pharmacodynamic study using *in vivo* candidiasis models suggesting that current susceptibility breakpoints should be re-explored.
129. 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(2):539–550. [PubMed: 18070979]
130. Andes DR, Diekema DJ, Pfaller MA, Marchillo K, Bohrmüller 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(10):3497–3503. [PubMed: 18625768]
131. Pfaller, MA.; Diekema, D.; Andes, D., et al. Clinical breakpoints for the Echinocandins and *Candida*, revisited. Presented at Interscience Conference on Antimicrobial Agents and Chemotherapy. Poster M-369; 12–15 September 2010; MA, USA.
132. Bennett JE. Echinocandins for candidemia in adults without neutropenia. *N. Engl. J. Med.* 2006; 355(11):1154–1159. [PubMed: 16971721]
133. Hajdu R, Thompson R, Sundelof JG, et al. Preliminary animal pharmacokinetics of the parenteral antifungal agent MK-0991 (L-743,872). *Antimicrob. Agents Chemother.* 1997; 41(11):2339–2344. [PubMed: 9371330]
134. Hoang A. Caspofungin acetate: an antifungal agent. *Am. J. Health Syst. Pharm.* 2001; 58(13):1206–1214. [PubMed: 11449878]
135. Paderu P, Garcia-Effron G, Balashov S, Delmas G, Park S, Perlin DS. Serum differentially alters the antifungal properties of echinocandin drugs. *Antimicrob. Agents Chemother.* 2007; 51(6):2253–2256. [PubMed: 17420211]

136. Odabasi Z, Paetznick V, Rex JH, Ostrosky-Zeichner L. Effects of serum on *in vitro* susceptibility testing of echinocandins. *Antimicrob. Agents Chemother.* 2007; 51(11):4214–4216. [PubMed: 17785512]
137. Wiederhold NP, Najvar LK, Bocanegra R, Molina D, Olivo M, Graybill JR. *In vivo* efficacy of anidulafungin and caspofungin against *Candida glabrata* and association with *in vitro* potency in the presence of sera. *Antimicrob. Agents Chemother.* 2007; 51(5):1616–1620. [PubMed: 17307976]
138. Garcia-Effron G, Park S, Perlin DS. Improved detection of *Candida* spp. fks hot spot mutants using the CLSI M27-A3 document by the addition of bovine serum albumin (BSA). *Antimicrob. Agents Chemother.* 2011 (Epub ahead of print).
139. Arendrup MC, Rodriguez-Tudela JL, Park S, et al. Echinocandin susceptibility testing of *Candida* spp. using the EUCAST 1 EDef 7.1 and CLSI M27-A3 standard procedures: analysis of the influence of bovine serum albumin supplementation, storage time and drug lots. *Antimicrob. Agents Chemother.* 2011; 55(4):1580–1587. [PubMed: 21245440]
140. Ben-Ami R, Garcia-Effron G, Lewis RE, et al. The fitness and virulence cost of fks1 mutations causing echinocand in-resistance in *Candida albicans*. *Proc. Natl Acad. Sci. USA.* 2010
141. Wiederhold NP, Lewis JS 2nd. The echinocandin micafungin: a review of the pharmacology, spectrum of activity, clinical efficacy and safety. *Expert Opin. Pharmacother.* 2007; 8(8):1155–1166. [PubMed: 17516879]
142. Cappelletty D, Eiselstein-McKittrick K. The echinocandins. *Pharmacotherapy.* 2007; 27(3):369–388. [PubMed: 17316149]
143. Vazquez JA. Anidulafungin: a new echinocandin with a novel profile. *Clin. Ther.* 2005; 27(6):657–673. [PubMed: 16117974]
144. 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(4):1108–1111. [PubMed: 10722525]
145. Chiller T, Farrokhsad K, Brummer E, Stevens DA. The interaction of human monocytes, monocyte-derived macrophages, and polymorphonuclear neutrophils with caspofungin (MK-0991), an echinocandin, for antifungal activity against *Aspergillus fumigatus*. *Diagn. Microbiol. Infect. Dis.* 2001; 39(2):99–103. [PubMed: 11248522]
146. Brown GD, Gordon S. Immune recognition. A new receptor for β -glucans. *Nature.* 2001; 413(6851):36–37. [PubMed: 11544516]
147. Brown GD, Taylor PR, Reid DM, et al. Dectin-1 is a major β -glucan receptor on macrophages. *J. Exp. Med.* 2002; 196(3):407–412. [PubMed: 12163569]
148. Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. Dectin-1 mediates the biological effects of β -glucans. *J. Exp. Med.* 2003; 197(9):1119–1124. [PubMed: 12719478]
149. Herre J, Willment JA, Gordon S, Brown GD. The role of Dectin-1 in antifungal immunity. *Crit. Rev. Immunol.* 2004; 24(3):193–203. [PubMed: 15482254]
150. Steele C, Rapaka RR, Metz A, et al. The β -glucan receptor dectin-1 recognizes specific morphologies of *Aspergillus fumigatus*. *PLoS Pathog.* 2005; 1(4):E42. [PubMed: 16344862]
151. Wheeler RT, Fink GR. A drug-sensitive genetic network masks fungi from the immune system. *PLoS Pathog.* 2006; 2(4):E35. [PubMed: 16652171]
152. Aimanianda V, Bayry J, Bozza S, et al. Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature.* 2009; 460(7259):1117–1121. [PubMed: 19713928]
153. Hohl TM. Stage-specific innate immune recognition of *Aspergillus fumigatus* and modulation by echinocandin drugs. *Med. Mycol.* 2008; 12:1–7. [PubMed: 18608931]
154. Hohl TM, Rivera A, Lipuma L, et al. Inflammatory monocytes facilitate adaptive CD4 T cell responses during respiratory fungal infection. *Cell Host Microbe.* 2009; 6(5):470–481. [PubMed: 19917501]
155. Hohl TM, Van Epps HL, Rivera A, et al. *Aspergillus fumigatus* triggers inflammatory responses by stage-specific β -glucan display. *PLoS Pathog.* 2005; 1(3):E30. [PubMed: 16304610]
156. Hohl TM, Feldmesser M, Perlin DS, Pamer EG. Caspofungin modulates inflammatory responses to *Aspergillus fumigatus* through stage-specific effects on fungal β -glucan exposure. *J. Infect. Dis.* 2008; 198(2):176–185. [PubMed: 18500928]

157. Lamarin GA, Lewis RE, Chamilos G, et al. Caspofungin-mediated β -glucan unmasking and enhancement of human polymorphonuclear neutrophil activity against *Aspergillus* and non-*Aspergillus* hyphae. *J. Infect. Dis.* 2008; 198(2):186–192. [PubMed: 18500936]
158. Katragkou A, Kruhlak MJ, Simitsopoulou M, et al. Interactions between human phagocytes and *Candida albicans* biofilms alone and in combination with antifungal agents. *J. Infect. Dis.* 2010; 201(12):1941–1949. [PubMed: 20415537]
159. Schwartz RE, Smith SK, Onishi JC, et al. Isolation and structural determination of enfumafungin, a triterpene glycoside antifungal agent that is a specific inhibitor of glucan synthesis. *J. Am. Chem. Soc.* 2000; 122(20):4882–4886.
160. Pelaez F, Cabello A, Platas G, et al. The discovery of enfumafungin, a novel antifungal compound produced by an endophytic *Hormonema* species biological activity and taxonomy of the producing organisms. *Syst. Appl. Microbiol.* 2000; 23(3):333–343. [PubMed: 11108011]
161. Wilkening, R.; Apgar, J.; Meng, D., et al. Enfumafungin derivatives: orally active glucan synthase inhibitors. Presented at: 2010 Interscience Conference on Antimicrobial Agents and Chemotherapy. F1–846; 12–15 September 2010; MA, USA.
162. Peel, M.; Fan, W.; Mamai, A., et al. Enfumafungin derivatives: orally active glucan synthase inhibitors. Presented at: Interscience Conference on Antimicrobial Agents and Chemotherapy. F1–845; 12–15 September 2010; MA, USA.
163. Motyl, MR.; Tan, C.; Liberator, P., et al. MK-3118, an oral enfumafungin with potent *in vitro* activity against *Candida* and *Aspergillus* spp. Interscience Conference on Antimicrobial Agents and Chemotherapy F1–847; 12–15 September 2010; MA, USA.
164. Flattery, AM.; Abruzzo, GK.; Gill, C., et al. Evaluation of orally active enfumafungin derivative mk-3118 in mouse models of disseminated candidiasis. Presented at: Interscience Conference on Antimicrobial Agents and Chemotherapy; 12–15 September 2010; Boston, MA, USA.
165. Flattery, AM.; Abruzzo, GK.; Gill, C., et al. evaluation of enfumafungin derivative MK-3118 in two mouse models of disseminated aspergillosis. Presented at: Interscience Conference on Antimicrobial Agents and Chemotherapy. F1–F849; 12–15 September 2010; MA, USA.

Executive summary

Echinocandin antifungal drugs

- The echinocandin drugs anidulafungin, CSF and MCF are the first members of the lipopeptide class of antifungal drugs that target the fungal cell wall by inhibition of β -1,3-D-glucan synthase.
- These drugs show concentration-dependent antifungal activity against susceptible *Candida* spp. and *Aspergillus* spp. and are effective against azole-resistant yeasts and moulds; they are also active on most biofilms.

Glucan synthase complex

- The enzyme complex in *Candida* spp. consists of a catalytic subunit that is the target of echinocandin drugs and is encoded by three related genes, *FKS1*, *FKS2* and *FKS3*; it also contains a regulatory component Rho1.

FKS resistance mechanism

- Echinocandin resistance resulting in clinical failure from susceptible *Candida* species is largely uncommon, although population-based surveillance studies suggest the level of *in vitro* resistance in some species is approximately 3%.
- Echinocandin resistance resulting in clinical failure is associated with mutations in two highly conserved 'hot spot' regions of *FKS1* and/or *FKS2*. These mutations confer cross-resistance among the class of drugs and result in several log order decreased sensitivity at the level of glucan synthase.
- In *Candida albicans* and most other *Candida* spp., mutations occur in hot spot regions of *FKS1*, which is the most prominently expressed *FKS* gene. In *C. glabrata*, *FKS1* and *FKS2* are major sources of resistance.

Clinically important nonsusceptible fungi

- These fungi typically have glucan synthase enzymes that are sensitive to drug but fail to show significant antifungal susceptibility.
- The resistance mechanism is largely unknown but may involve compensatory changes in cell-wall composition or presence of excess glucan that acts as a drug sink.

MIC & breakpoints

- An initial clinical break point for susceptible isolates was established for all three echinocandin drugs and all species of *Candida*, which followed from an analysis of existing clinical, pharmacokinetics/pharmacodynamics (PK/PD), epidemiological and microbiologic data.

FKS-mediated resistance helps redefine breakpoints

- Clinical isolates containing characteristic *FKS* hot spot mutations resulting in clinical failures were not captured by the initial susceptibility cut-off value. A new echinocandin breakpoint was proposed based on studies of PK/PD, enzyme inhibition kinetics and epidemiologic MIC data to better account for these isolates.

FKS mutations & virulence

- *FKS* mutations conferring elevated MICs are associated with reduced virulence due to alterations in the catalytic potential of mutant glucan synthase and the net result on cellular physiology and morphology.

PK/PD considerations

- Echinocandin drugs show concentration-dependent killing and a prolonged post-antifungal effect.
- Pharmacodynamic studies demonstrate that the 24-h area under the concentration-time curve (AUC)/MIC best describes the echinocandin exposure-response relationship.

Drug-bug-immune responses

- Exposure of *Candida* and *Aspergillus* to echinocandins alters the cell wall composition resulting in surface exposure of glucans that stimulate host immune responses.

Oral glucan synthase inhibitors

- Enfumafungins are potent inhibitors of glucan synthase that are chemically distinct from the echinocandins and are being developed as a next generation oral antifungal class.

Figure 1. The three approved echinocandin drugs
Cyclic hexapeptides are best differentiated by their aliphatic tails (circled).

Figure 2. Morphological change in *Aspergillus fumigatus* hyphae following exposure to caspofungin
Cells were grown for 18 h in Roswell Park Memorial Institute (RPMI) medium in the (A) absence or (B) presence of caspofungin at 0.5 mg/ml and visualized by light microscopy (100 $\times$).

Figure 3. Membrane topology model for glucan synthase showing putative resistance mutations and action of echinocandin drugs

Transmembrane segments deduced from analysis of amino acid relative hydrophobicity.

Figure 4. Effect of *FKS* mutations on sensitivity of glucan synthase to echinocandin drugs
Glucan synthases with characteristic hot spot one amino acid substitutions were isolated and inhibition of activity (IC_{50}) by each of the echinocandin drugs was determined, as described by Park *et al.* [85].

Figure 5. Distribution and frequency of FKS mutants in *Candida albicans* and *Candida glabrata* Specific mutations in referred clinical isolates (n = 88) are shown for hot spot one mutations in *FKS1* for *C. albicans* and both *FKS1* and *FKS2* for *C. glabrata*.

Figure 6. IC₅₀ as a function of MIC for echinocandin drugs

A composite of *Candida* spp. in reference library ($n = 55$) containing WT and *FKS* mutations were plotted as a function of IC₅₀ and MIC for each of the echinocandin drugs, as shown. The original CLSI breakpoint of $2 \mu\text{g/ml}$ (solid line) fails to capture a significant number of *FKS* mutants resulting in treatment failure, especially for micafungin and anidulafungin. The new proposed breakpoint (broken line) of $0.25\text{--}0.5 \mu\text{g/ml}$ is more inclusive of all *FKS* mutants. The WT ECR represents *Candida* spp. (e.g., *Candida parapsilosis*) with inherent elevated MIC levels.

CLSI: Clinical Laboratory Standards Institute; ECR: Echinocandin resistance; WT: Wild-type.

Partially adapted with permission from [42,43].

Table 1

Epidemiological cut-off values for anidulafungin, caspofungin and micafungin and eight species of *Candida*.

Species	Antifungal agent	No. of isolates tested	MIC (µg/ml)			Isolates with MIC of 2 µg/ml (%)
			Range	Mode	Epidemiological cut-off values (%) [†]	
<i>Candida albicans</i>	Anidulafungin	4283	0.007–1	0.03	0.12 (99.7)	100.0
	Caspofungin	4283	0.007–0.5	0.03	0.12 (99.8)	100.0
	Micafungin	4283	0.007–0.5	0.015	0.03 (97.7)	100.0
<i>Candida glabrata</i>	Anidulafungin	1236	0.015–4	0.06	0.25 (99.4)	99.9
	Caspofungin	1236	0.015–8	0.03	0.12 (98.5)	99.8
	Micafungin	1236	0.007–2	0.015	0.03 (98.2)	100.0
<i>Candida tropicalis</i>	Anidulafungin	996	0.007–2	0.03	0.12 (98.9)	100.0
	Caspofungin	996	0.007–>8	0.03	0.12 (99.4)	99.9
	Micafungin	996	0.007–1	0.015	0.12 (99.1)	100.0
<i>Candida kefyr</i>	Anidulafungin	61	0.015–0.12	0.06	0.25 (100.0)	100.0
	Caspofungin	61	0.007–0.03	0.015	0.03 (100.0)	100.0
	Micafungin	61	0.015–0.06	0.06	0.12 (100.0)	100.0
<i>Candida krusei</i>	Anidulafungin	270	0.015–0.5	0.03	0.12 (99.3)	100.0
	Caspofungin	270	0.015–1	0.06	0.25 (96.3)	100.0
	Micafungin	270	0.015–0.25	0.06	0.12 (97.8)	100.0
<i>Candida lusitanae</i>	Anidulafungin	99	0.06–1	0.5	2 (100)	100.0
	Caspofungin	99	0.03–1	0.25	0.5 (98.0)	100.0
	Micafungin	99	0.007–1	0.12	0.5 (99.0)	100.0
<i>Candida parapsilosis</i>	Anidulafungin	1238	0.015–4	2	4 (100.0)	93.1
	Caspofungin	1238	0.015–4	0.25	1 (98.6)	99.9
	Micafungin	1238	0.015–2	1	4 (100)	100.0
<i>Candida guilliermondii</i>	Anidulafungin	88	0.06–4	2	16 (100.0)	92.0
	Caspofungin	88	0.03–>8	0.5	4 (95.5)	95.5
	Micafungin	88	0.015–>8	0.5	4 (98.9)	98.9

[†] Percentage of isolates for which MIC is less than or equal to the epidemiological cut-off values.

Adapted from [123].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript