Direct effects of non-antifungal agents used in cancer chemotherapy and organ transplantation on the development and virulence of *Candida* and *Aspergillus* species

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**Key words:** Candida, Aspergillus, chemotherapy, calcineurin inhibitors, TOR, HSP90, tyrosine kinase, autophagy, DNA topoisomerase, corticosteroids

**Abbreviations:** 5AC, 5-azacytidine; 17-AAG, 17-allylamino-17-demethoxygeldanamycin; ara-C, cytosine arabinoside; AMB, amphotericin B; ATO, arsenic trioxide; CDR, Candida drug resistance; Crz1p, calcineurin-regulated zinc finger-containing 1 protein; CsA, cyclosporine; 17,-DMAG, 17-desmethoxy-17-N,N-dimethylaminoethylaminogeldanamycin; FK-506, tacrolimus; FKBP12, tacrolimus binding protein 12; 5FU, 5-fluorouracil; Hsp90, heat shock protein 90; HDAC, histone deacytelase; HDACi, histone deacetylation inhibitor; IFI, invasive fungal infection; MDR, multidrug resistance; MG-CD0103, moccetinostat; MIC, minimal inhibitory concentration; PG-paclitaxel, polyglutamate bound paclitaxel; Pgp, P-glycoprotein; SRE, steroid responsive element; TSA, trichostatin A; SAHA, suberoylanilide hydroxamic acid; TK, tyrosine kinase; TKI, tyrosine kinase inhibitor; TOR, target of rapamycin; TORC, target of rapamycin complex

**Introduction**

Patients receiving chemotherapy for cancer and individuals who are immunosuppressed after hematopoietic stem cell or organ transplantation are at increased risk for opportunistic fungal infections with high attendant morbidity and mortality.1,2 Although previously uncommon pathogens, including non-*fumigatus* *Aspergillus* species, Zygomycetes and Fusarium, have emerged, most invasive fungal infections (IFIs) are caused by *Candida* species and *Aspergillus fumigatus*.1,3

Predisposing factors for IFIs are well established. Specifically, modern chemotherapy for neoplastic diseases, with its immunosuppressive effects such as profound neutropenia, is a major risk factor.1,2,4 The dysregulatory effects of antineoplastic agents on the inhibition of host leukocyte cell lineages and on other host immune responses that result in increased susceptibility to IFIs are well studied. Detailed descriptions of these immune modulatory effects are beyond the scope of this article but are comprehensively discussed elsewhere.5-7 Interestingly, various anticancer agents, such as glucocorticoids, cytotoxic agents and calcineurin inhibitors, also have direct inhibitory effects on and/or have altered the biology of fungal cells.8-11 Yet relatively little is known about these effects on the pathogenesis of IFIs and consequent clinical significance.

The advent of novel antineoplastic agents, such as the taxanes and inhibitors of histone deacytelase (HDAC), tyrosine kinase (TK) and heat shock protein 90 (Hsp90) complexes, has shifted cancer treatment paradigms from time-limited cytotoxic chemotherapy cycles to continuous low-dose treatment...
Many targets of new anticancer agents have homologs in fungi and are components of critical cell-signaling pathways. Thus, studying new molecular targets and their inhibitors in the context of drug-pathogen interactions will provide insight into the pathogenesis of IFIs and may assist with antifungal drug development. This is pertinent given the current limited antifungal drug armamentarium.

In this article, we review current knowledge of the direct effects of conventional and investigational antineoplastic and/or immunosuppressive agents (Tables 1 and 2) on fungi. For each class of agent, we focus on the effects on fungal growth, morphological development and virulence, of the two most important pathogen genera, Candida and Aspergillus. Data on these fungi from direct in vitro and in vivo animal model-based studies of anticancer drugs, as well as from experiments using genetically engineered strains, are reviewed. We caution that these data are largely derived from "artificial" experimental systems, raising uncertainty about their application in the clinical setting. Nonetheless, understanding the fundamental in vitro and in vivo findings is an essential first step in informing the design of future studies that explore the clinical relevance of these observations where appropriate; such studies are currently lacking.

### Conventional Cytotoxic Agents

Cytotoxic agents frequently used in cancer therapy include alkylating agents, antimetabolites, vinca alkaloids, anthracyclines (e.g., daunorubicin) and the antibiotic bleomycin (Table 1) (also see section on DNA topoisomerase inhibitors). Alkylating agents exert their cytotoxic effects by adding alkyl groups to cancer-cell DNA; e.g., 5'-azacytidine (5-AC), a cytidine analog that specifically causes DNA hypomethylation. Antimetabolites such as cytosine arabinoside (ara-C) are structural analogs of naturally occurring competitive inhibitors of enzymes and molecules that inhibit progression to the S phase in DNA replication and, in some instances, inhibit DNA and RNA polymerases. Vinca alkaloids inhibit microtubule aggregation.

**Effects on Candida. Antifungal activity.** Cytotoxic agents have variable in vitro activity against Candida spp. (Table 1). In one report, the minimum inhibitory concentrations (MICs) of methotrexate, cyclophosphamide, vincristine, bleomycin and mitomycin C for three yeast strains (Candida albicans, Candida tropicalis and Candida kefyr) were high (500–1,500 μg/ml), whereas those of asparaginase and 5-fluorouracil (5-FU) were less than 100 μg/ml. Both asparaginase and 5-FU were the most active against C. tropicalis (MIC, <1 μg/ml) suggesting Candida species-specific antifungal activity. In contrast, Graybill and colleagues noted that bleomycin was toxic in several Candida spp. in vitro, with minimum lethal concentrations ranging from 12.5 to 50.0 μg/ml (MICs, 0.39–12.5 μg/ml), but had no in vivo antifungal activity in mice with candidiasis. It is unlikely that bleomycin results in clinically meaningful activity in vivo against Candida, as bleomycin is given once or twice weekly and in various doses with differing resultant plasma levels. Indeed, the literature contains few data correlating the in vitro activity of cytotoxic agents with their in vivo efficacy. The anti-Candida activity of 5-FU is anticipated because 5-flucytosine, the less toxic precursor of 5-FU, is a well-established antifungal agent. Flucytosine is converted by cytosine deaminase to 5-FU in fungi, leading to blockage of protein synthesis. Unlike griseofulvin, which also inhibits fungal tubulin formation, the vinca alkaloids vincristine and vinblastine, even at clinically relevant concentrations, have little direct impact on fungal growth.

Studies of combinations of antifungal and antineoplastic agents have demonstrated inhibitory drug combinations whose MICs were lower than those for the respective agents alone. In general, the combination of a polyene and an antineoplastic agent was the most potent inhibitor of growth of multiple Candida spp. Specifically, 5-FU had the most synergistic interaction with amphotericin B (AMB), but investigators observed similar interactions for AMB with methotrexate, mitomycin C and doxorubicin. However, cyclophosphamide and bleomycin antagonized AMB. Again, the clinical relevance of these observations is not known. Large-scale testing of Candida strains across species using clinically relevant doses of various antineoplastic agents in clinically meaningful host models of infection is required to answer this question.

**Morphology and virulence.** Various effects of antineoplastic agents on Candida morphogenesis and virulence have been reported in vitro (Table 1). For example, both yeast-phase growth and germ-tube formation by C. albicans were inhibited by treatment with actinomycin D, bleomycin, 5-FU and hydroxyurea at varying concentrations (100–1,000 μg/ml). These effects were enhanced when the drugs were combined with AMB; in particular, actinomycin similar to the peak plasma concentrations of these agents that result from typical dosing; also, treatment with doxorubicin, vincristine and vinblastine inhibited Candida filamentation. These effects were potentiated when these drugs were combined with AMB; actinomycin D-AMB combinations resulted in 90% loss in cell viability. In another study, at drug concentrations typically used during therapy, C. albicans isolates exposed to methotrexate but not cyclophosphamide, cytarabine or dacarbazine produced significantly higher amounts of catalase (a marker of oxitativ stress) than did unexposed isolates. As catalase activity in Candida spp. is suggested to reduce the effects of AMB by protecting the species against oxidative damage, methotrexate may contribute to AMB resistance.

In vitro exposure of Candida glabrata to high-dose methotrexate (500–1,500 μg/ml) also has inhibited the growth of and enhanced pseudomycelial formation by this species while restricting the uptake of RNA and DNA precursors into fungal into fungal cells. Morphological alterations of Candida spp. following treatment with antineoplastic agents may explain the frequent isolation of morphologically-impaired isolates from patients with cancer.

**Effects on Aspergillus.** The effects of ara-C and 5-AC on the nonpathogenic species Aspergillus nidulans are shown in Table 1. The “fluffy” phenotypes of A. nidulans and Aspergillus niger are characterized by severely impaired asexual sporulation, abundant vegetative hyphae and uncontrolled growth. Similarly, exposure of an A. fumigatus strain to...
Table 1. Direct effects of antineoplastic agents and antibiotics on the growth and virulence of Candida and Aspergillus

| Chemotherapeutic agent | Antifungal activity | Growth and morphogenesis | Virulence trait | Antifungal activity | Growth and morphogenesis | Virulence trait |
|------------------------|---------------------|--------------------------|----------------|---------------------|--------------------------|----------------|
| Conventional cytotoxic agents (nonmetals) | | | | | | |
| Alkylation agents (e.g., cyclophosphamide, 5-AC) | Inhibition of Candida spp.; MICs <100-1500 μg/ml, MICs of 5-FU and asparaginase against C. tropicalis, < 1 μg/ml | Inhibition of filamentation, especially in combination with AMB | Increase in catalase activity in C. albicans (MTX) | No data | Conversion of A. nihilo and A. niger to fluffy phenotype (5-AC) | Increase in elastase activity and angiogenesis of A. fumigatus (5-AC) |
| Aminonucleotides (e.g., MTX, 5-FU, ara-C) | Inhibition of growth in combination with AMB | Enhanced formation of pseudomycelia and limitation of macromolecule uptake in C. glabrata (MTX) | | | | |
| Vinca alkaloids (e.g., vinristine, vinblastine) | | | | | | |
| Antibiotics (e.g., bleomycin) | | | | | | |
| Heavy metals | | | | | | |
| Cioplatin (cis-diammine-dichloroplatinum [II], see also Hsp90 inhibitors) | Inhibition of C. albicans at 40–180 μg/ml | Inhibition of C. albicans hyphae and budding | Increase in growth, adhesion to HeLa cells, and endolysate and proteinase activity in C. albicans | | Induced mitotic recombination with A. nihilo | |
| Arsenic trioxide | No data | No data | No data | No data | No direct effects observed | No data |
| Zinc | No data | No data | No data | No data | No direct effects observed | No data |
| Taxanes | Doctaxel | No effect on actin skeleton in C. albicans (paclitaxel) | No observed effect on actin skeleton in C. albicans (paclitaxel) | No data | No direct effects observed | No data |
| Paclitaxel | No data | No data | No data | No data | No direct effects observed | No data |
| Cabazitaxel | No data | No data | No data | No data | No direct effects observed | No data |
| Paclitaxel | No data | No data | No data | No data | No direct effects observed | No data |
| PG-paclitaxel | No data | No data | No data | No data | No direct effects observed | No data |
| Hsp90 inhibitors | Geldanamycin analogs (17-AC, 17-DMAG, radicicol, oxime derivatives) | Increased efficacy of fluconazole in vitro (17-AC, 17-DMAG) | Improved clinical outcomes in invasive candidiasis (recombinant anti-Hsp90 antibody against C. albicans) | | Enhanced activity of caspofungin and voriconazole in A. fumigatus and A. nihilo (geldanamycin) | No data |
| Purine small-molecule inhibitors | Blocked de novo azole resistance and abrogation of resistance already evolved | Blockage of azole activity in C. albicans | Combined 17-AC/17-DMAG and an azole rescued larvae from C. albicans infection | | | |
| Coumarins (novebucin analogs) | Blocked resistance to micafungin | Blockage of azole activity in C. albicans | Combined 17-AC/17-DMAG and an azole rescued larvae from C. albicans infection | | | |
| Mycofast (antifungal Hsp90) | Reduced paradoxical effect of micafungin and abrogated micafungin-voriconazole antagonism | Blockage of azole activity in C. albicans | Combined 17-AC/17-DMAG and an azole rescued larvae from C. albicans infection | | | |
| CisPlatin (see above) | In vitro activity against Candida (MICs 0.15-16 μg/ml; MGC290) | Increase in white-to-opaque cell switching (TSA) | Reduced adherence to pneumocytes (TSA, voronostat) | In vitro synergy with voriconazole and posaconazole (MGC290) | Delay in growth and sporulation of A. nihilo | No data |
| HDACI (see below) | Reduction of trilling and fluconazole MICs (TSA) | Inconsistent inhibition of germ tube formation (TSA) | Inconsistent inhibition of germ tube formation (TSA) | Increase in small-molecule production in A. nihilo | Increase in small-molecule production in A. nihilo | No data |
| Histone deacetylase inhibitors | Decrease in upregulation of CDR1/2 and ERG11 genes | In vitro synergy with fluconazole (voriconostat) | In vitro synergy with fluconazole (voriconostat) | In vitro synergy with fluconazole (voriconostat) | In vitro synergy with fluconazole (voriconostat) | No data |

5-AC, 5-azacytidine; 17-AC, 17-allylaminio-17-demethoxygeldanamycin; ara-C, cytosine arabinoside; AMB, amphotericin B; CDR, Candida drug resistant; 17-,DMAG; 17-desmethoxy-17-N,N-dimethylaminoethylaminogeldanamycin; 5FU, 5-fluorouracil; Hsp90, heat shock protein 90; HDACI, histone deacetylase inhibitor; 3-MA, 3-methyladenine; MTX, methotrexate; MIC, minimal inhibitory concentration; PG-paclitaxel, polyglutamate bound paclitaxel; TSA, trichostatin A. CDR1/CDR2 are genes coding for the multidrug efflux transporters of the ATP-binding cassette transporter family. ERG11 is the gene encoding for the enzyme sterol 14α-demethylase.

High concentrations (250–500 mM) of 5-AC induced high-frequency conversion of the strain to a developmental mutant with impaired light-dependent conidiation accompanied by increased elastase and proteolytic activity, greater angioinvasion in vitro than that in the wild-type strain, and overexpression of genes encoding for cell wall-associated proteins. Mutants of this type remained pathogenic in fruit fly and murine model systems. However, the concentrations of 5-AC required to induce the fluffy phenotype exceeded those achievable in humans during treatment.
**Table 1 (continued). Direct effects of antineoplastic agents and antibiotics on the growth and virulence of Candida and Aspergillus**

| Chemotherapeutic agent | Major effects on *Candida* spp. | Major effects on *Aspergillus* spp. |
|------------------------|---------------------------------|------------------------------------|
| **DNA topoisomerase II inhibitors** | **Antifungal activity** | **Growth and morphogenesis** | **Virulence trait** | **Antifungal activity** | **Growth and morphogenesis** | **Virulence trait** |
| Anthracyclines (mitomycin C) | In vitro MICs against *Candida* (50-100 μg/ml; <2 μg/ml for aclarubicin) | Inhibition of hyphal formation in *C. albicans* | Deformation of plasma membrane and mitochondria in *Candida utilis* (doxorubicin) | No data | Induction of parameiosis in *A. nidulans* (doxorubicin) | No data |
| Doxorubicin | Inhibition of *C. glabrata*, and *C. albicans* (doxorubicin, idarubicin) | Increases tolerance to AMB at 24 h in *C. albicans* (doxorubicin) | Disruption of respiration in *C. albicans* (doxorubicin) | No data | No data | No data |

| Bleomycin | In vivo activity against *C. albicans* | Inhibition of self-splicing | No data | Inhibition of growth in *A. fumigatus* | No data | No data | No data |

| Cisplatin | Effects on Candida. Researchers have studied the effects of cisplatin on a number of yeasts, including *Saccharomyces cerevisiae* and *C. albicans* with variable results. One study showed that cisplatin inhibited *C. albicans* growth only at concentrations much higher (100–800 μg/ml) than those achievable in vivo, whilst another using the strain *C. albicans* ATCC 10261 noted persistent (up to 40 h) inhibition at concentrations as low as 20 μg/ml. Serum concentration exposures of cisplatin vary according to the underlying malignancy. In testicular cancer cases, plasma levels of cisplatin equivalent to a Cmax of 1.95 μg/ml are typical targets. 34 In addition to causing aberrant cross-linking of DNA, cisplatin causes DNA strand breaks and DNA-protein cross-links, leading to cell death. The mechanism of action of cisplatin involves the formation of interstrand and intrastrand DNA cross-links, which disrupt DNA replication and transcription. Cisplatin also induces apoptosis in cancer cells by activating the caspase pathway. Cisplatin binds to DNA through the formation of a dihydropyrimidine adduct, which is further modified to a tetrahydropyrimidine adduct. The latter is a potent DNA cross-linking agent, which ultimately leads to cell death.

**Metal Complexes**

The platinum compounds cisplatin, carboplatin and transplatin; arsenic trioxide (ATO); and zinc-containing agents have emerged as clinically useful metal complexes in the treatment of cancer (Table 1). Data on their effects on the growth and/or development of fungi, however, are relatively lacking.

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5-AC, 5-azacytidine; 17-AAG, 17-allylamino-17-demethoxygeldanamycin; ara-C, cytosine arabinoside; AMB, amphotericin B; CDR1/CDR2, Candida drug resistant; 17,-DMAG, 17-desmethoxy-17- N,N-dimethylaminooethylaminogeldanamycin; 5FU, 5-fluorouracil; Hsp90, heat shock protein 90; HDACi, histone deacetylase inhibitor; 3-Methyladenine; MTX, methotrexate; MIC, minimal inhibitory concentration; PG-paclitaxel, polyglutamate bound paclitaxel; TSA, trichostatin A. CDR1/CDR2 are genes coding for the multidrug efflux transporters of the ATP-binding cassette transporter family; ERG11 is the gene encoding for the enzyme sterol 14α demethylase.
Table 2. Direct effects of corticosteroids and other immunosuppressive agents used in transplantation on the growth and virulence of Candida and Aspergillus spp.

| Drug class          | Antifungal activity | Growth and morphogenesis | Virulence                      | Antifungal activity | Growth and morphogenesis | Virulence |
|---------------------|---------------------|--------------------------|--------------------------------|---------------------|----------------------------|-----------|
| **Corticosteroids** |                     |                          |                                |                     |                            |           |
| Prednisone          | Decrease in antifungal effects of nystatin in skin (topical methylprednisolone) | Enhanced germ-tube formation in serum | Increase in colonization of oral mucosa and gastrointestinal tract of mice by *C. albicans* and bloodstream translocation | Increase in growth rates of *A. fumigatus* and *A. flavus* (hydrocortisone) | - | - |
| Methyprednisolone   |                     |                          |                                |                     |                            |           |
| Hydrocortisone      |                     |                          |                                |                     |                            |           |
| Dexamethasone       |                     |                          |                                |                     |                            |           |
| **TOR inhibitors**  |                     |                          |                                |                     |                            |           |
| Rapamycin and analogs 2, 21, 18, and 9 | Inhibition of growth in vitro (fungicidal) of *C. albicans* | Inhibition of filamentation in *C. albicans* | Protection of mice against *C. albicans* | Inhibition of growth of *A. fumigatus*, *A. flavus*, and *A. niger* | Enhancement of azole, caspofungin, and amphotericin B activity in vitro | No data |
| **Calcineurin inhibitors** |                     |                          |                                |                     |                            |           |
| Cyclosporine and analogs (CsA:211-810) | Demonstration of in vitro and in vivo synergy with AMB and 5-flucytosine in vitro against *C. albicans* | Inhibition of germ-tube formation in *C. albicans* | Gene deletion studies indicate decreased virulence in systemic and ocular mouse models | Inhibition of growth of *A. fumigatus* | Demonstration of synergistic activity in vitro with azoles and caspofungin | No survival advantage in an intravenous mouse model of aspergillosis |
| Tacrolimus (FK-506) and analogs | Demonstration of in vitro and in vivo synergy with azoles and terbinafine against Candida spp., including in biofilm-associated models | No data | Gene deletion studies indicate decreased virulence in systemic and ocular mouse models | Inhibition of growth of *A. fumigatus* | Synergistic activity in vitro with azoles and caspofungin | Conferring survival advantage in an intravenous mouse model of aspergillosis |

Host DNA strands, cisplatin inhibits rRNA synthesis as well as the self-splicing reaction of the group-I intron ribozyme of pre-rRNA in mammalian cells. Although *C. albicans* possesses this group I self-splicing intron, whether cisplatin exerts its inhibitory effect via this mechanism is not known. Pretreatment of *C. albicans* with AMB (and miconazole) has increased its sensitivity to cisplatin in vitro, suggesting a degree of synergistic antifungal activity of these agents (Table 1), but these observations require confirmation. In studying changes in the morphology and growth of *C. albicans* using fluorescence microscopy, Chandrasekar et al. showed that cisplatin exposure (6 μg/ml) markedly inhibited hyphal and bud development. Also, *C. albicans* cells exhibited increased uptake of ethidium bromide and were unusually large before dying. These changes were concomitant with suppression of growth of cisplatin-exposed *C. albicans* compared with untreated cells. In addition, cisplatin may potentiate the virulence...
of Candida cells. In one study, cells from two Candida strains cultured in the presence of 10–100 μg/ml cisplatin proliferated more rapidly than did untreated cells, demonstrated increased adhesion to HeLa cells via lectin binding, had increased enolase and acid proteinase activity, and were more resistant to neutrophil killing than were unexposed to cisplatinum *C. albicans* control cells. The researchers also observed similar results for 5-FU (250 μg/ml). In contrast with previous findings, however, cisplatin decreased the sensitivity of Candida cells to AMB when the half-maximal inhibitory concentration of this agent was increased 1.5- to 2.0-fold.9 The clinical implications of these effects have not yet been investigated.

**Effects on Aspergillus.** At the time of this writing, whereas investigators have shown that cisplatin promotes mitotic recombination in *A. nidulans*, no reported data have indicated that this occurs in *A. fumigatus* (Table 1).

**Arsenic (ATO).** Despite the fact that arsenic is considered a poison, ATO is a valuable treatment option for cancer, especially acute promyelocytic leukemia. Although its predominant antineoplastic activity occurs via induction of apoptosis, ATO also induces intracellular activity of reactive oxygen species, activates enzyme kinases, and is involved in inhibition of P-glycoprotein and potentiation of tubulin polymerization.38

**Effects on fungi.** Genome-wide analyses of transcription patterns in *S. cerevisiae* in response to treatment with arsenic have revealed a complex response that influences signaling pathways including protein kinases such as the mitogen-activated protein kinase and target of rapamycin (TOR) complex 1 (TORC1) systems.39,40 Arsenic toxicity results from inhibition of TORC1 kinase and modulation of the activity of downstream factors, leading to activation of the transcription factors that are targets of TORC1 and protein kinase A.60 Whether arsenic has similar effects in *Candida* and *Aspergillus* spp. is unknown. In other studies, *A. niger* isolates recovered from arsenic-rich environments demonstrated reduced radial growth (~50%), formed tiny black compact colonies with dense sporulation and exhibited greater catalase activity than did laboratory control strains.41,42

The relevance of these in vitro findings is unknown.

**Taxanes**

The diterpene taxanes are also integral parts of modern cancer treatment regimens.12,43 They reorganize and stabilize cell microtubules, interfering with their normal breakdown during cell division.43 Licensed agents include paclitaxel, docetaxel and carbazitaxel and less toxic nanoparticle albumin-bound formulations (e.g., nab-paclitaxel) and paclitaxel derivatives (e.g., bound to a polyglutamate polymer, PG-paclitaxel).44 Taxanes are produced by several fungi, yet in vitro (as well as in vivo) data regarding their direct effects on the biology of pathogenic fungi are scarce. Possible effects may be assessed in the context of studies of *S. cerevi*ae. In wild-type *S. cerevisiae*, paclitaxel does not bind to fungal tubulin. However, *S. cerevisiae* strains with mutated β-tubulin genes leading to diminished activity of tubulin exhibit evidence of mitotic blockage with cell apoptosis after exposure to paclitaxel.46 The microfilament physiology in of *C. albicans* is known to play a critical role in germ-tube formation during pH-regulated morphological yeast to hyphal transition.47 However, whether taxanes administered in pharmacological doses (e.g., typical paclitaxel doses of 135–175 mg/m² result in plasma C<sub>max</sub> values of 0.195–0.355 μg/ml) affect microtubule formation in vitro or in vivo is unknown. Although the cytoskeletal inhibitors cytochalasin A and nocodazole disrupted microfilament formation in endothelial cells and subsequently impaired phagocytosis of *C. albicans* in one study, the pattern of actin polymerization-driven phagocytosis of *C. albicans* by endothelial cell was unaffected by treatment with 1 μM paclitaxel.48

**Corticosteroids**

The vulnerability to IFIs of patients with cancer receiving long-term glucocorticoid-based therapy (e.g., dexamethasone, hydrocortisone, methylprednisolone) at supraphysiological doses is attributed to dysregulation of macrophage and polymorphonuclear function by excessive plasma cortisol.3 Treatment with steroids reduces the ability of innate effectors to kill microorganisms via multiple mechanisms, including production of reactive oxygen species. Data on the direct effects of corticosteroids on fungi are summarized in Table 2.

**Effects on Candida.** Early observations that treatment with glucocorticoids increased the *C. albicans* burden in the gastrointestinal tract and frequency of fungal translocation from the gastrointestinal tract to the bloodstream in mice and rats,49,50 raised the possibility that steroids can augment the growth rate of *Candida* spp.51 Their direct effects on Candida growth per se however, remain undetermined.

Nonetheless, investigators have shown that glucocorticoids enhance adhesion of *Candida* spp. to vaginal epithelial cells.52 Also, treatment with dexamethasone (0.01% w/v) increased adhesion (by 17–44%) of these species to buccal epithelial cells, although that with cortisone acetate and hydrocortisone (both at 0.01% w/v) decreased the adhesion by 16–32%.53 In another study, exposure to glucocorticoids stimulated secretion of Candida phospholipase and aspartyl proteinase, whereas Gyervai et al. found that high-dose treatment (4 mM) with methylprednisolone stimulated *C. albicans* growth, the germination rate in serum and phospholipase release from *C. albicans*. Surprisingly, these effects were not accompanied by increased pathogenicity in intraperitoneal and intravenous mouse models of infection. Methylprednisolone-based treatment also increased the sensitivity of *C. albicans* cells to menadione via inhibition by inhibiting antioxidant enzymes and enhanced the deleterious effects of AMB.55 Adhesion to human epithelial cells and secretion of extracellular proteinases and phospholipases are virulence traits of *C. albicans*.56

The mechanisms by which corticosteroids affect Candida biology and growth are not well understood. Researchers identified a corticosteroid-binding protein (CBP) on the surface of *C. albicans* cells.57 Investigation is warranted to determine whether steroid-CBP binding enhances Candida virulence. Of interest, treatment with ketoconazole, at concentrations readily achievable in therapeutic settings, has displaced corticosterone...
from the CBP site in *C. albicans*.\(^58\) Genome-wide analyses of *C. albicans* cells treated with the human steroid progesterone have identified enhanced expression of multidrug resistance genes of the ATP-binding cassette transporter [Candida drug resistance 1 (CDR1) and CDR2] superfamily, suggesting the presence of a relationship between steroid-induced stress and azole resistance in *C. albicans*.\(^59\) The expression of several genes associated with induction of hyphal growth and establishment of pathogenesis in *C. albicans* was upregulated with specific progesterone-responsive sites in the promoter regions of these genes.\(^59\) In a detailed study of the *C. albicans* CDR1 promoter, researchers identified two cis-regulatory regions responsive to progesterone and β-estradiol, respectively: steroid responsive element (SRE)1 and SRE2.\(^60\) Whether glucocorticoids in clinical use have similar roles in regulation of the expression of CDR1 and other genes in *C. albicans* remains to be elucidated.

**Effects on Aspergillus.** Treatment with glucocorticoids may also alter the biological fitness of *Aspergillus* spp. (Table 1). Ng et al. first showed that in vitro exposure to pharmacological doses of hydrocortisone increased the growth (by 30–40%) of *A. fumigatus* and *Aspergillus flavus*.\(^8\) This direct growth effect may poten-
tiate the immunosuppressive action of hydrocortisone, facilitating dissemination of Aspergillus in vivo.\(^5\) Interestingly, treatment with progesterone and estradiol had no effects on *A. fumigatus* growth.\(^8\) Since many patients with cancer simultaneously receive glucocorticoids and antifungal drugs, the combined effects of these agents on Aspergillus growth would be a relevant topic of study. In another study, treatment with a therapeutic concentra-
tion (1 μM) of hydrocortisone induced a significant increase in susceptibility to itraconazole but not to AMB in *A. fumigatus* (Table 2).\(^61\) The mechanisms of these interactions, and whether the interactions are clinically meaningful, are not known.

**Inhibitors of Calcineurin and TOR**

Agents that inhibit the Ca\(^{2+}\)/calmodulin-activated protein phosphatases calcineurin and TOR are the cornerstones of therapy in patients who undergo organ or bone marrow transplantation as well as chemotherapy for cancer. Despite having intrinsic anti-
fungal activity, the development of currently-available agents as anti-infective drugs has been limited by their potent immuno-
suppressive effects. Indeed, the relative high rates of IFIs in patients receiving these agents emphasise the complex interplay of host and pathogen-specific factors that influence the composite host response to any one drug.

The relevance of calcineurin and TOR to fungal biology relates to the central roles of (1) TOR kinase and (2) calcineu-
rin and its modulator Hsp90 in regulating cell growth and stress responses. Calcineurin is essential for fungal morphogenesis and virulence and positively regulates cell-wall synthesis through in *FKS2* gene expression via the calcineurin-regulated zinc finger-containing protein (Crz1p) or its homologs.\(^10,62,63\)

Inhibitors of these conserved targets—cyclosporin A (CsA), tacrolimus (FK-506; calcineurin), rapamycin (sirolimus; TOR), Hsp90 inhibitors, and their less immunosuppressive analogs have become foci of study for their effects on fungal development (Table 2). Key findings have revealed interplay among TOR, calcineurin and Hsp90 inhibitors in regulating polarized growth and stress responses of fungi, which have opened up the possibility of exploiting these pathways to design novel synergistic combination therapies between conventional antifungals and inhibitors of these targets (Fig. 1). Expert reviews have detailed these signaling cascades.\(^10,62,64\)

**Lessons from studies in *S. cerevisiae*.** Researchers first identified the TOR kinases as in *S. cerevisiae* and showed that rapamycin forms a complex with the tacrolimus-binding protein FKBP12 prolyl isomerase, which then binds to TOR kinase, blocking its function. Although *S. cerevisiae* contains two TOR complexes (TORC1 and TORC2), other fungi have single TOR homologs.\(^10\) Rapamycin inhibits ribosome synthesis, mating and filamentation and induces autophagy in *S. cerevisiae*.\(^65\) In vitro, rapamycin MICs range from 0.1 to 5.0 μg/ml, whereas CsA and tacrolimus MICs are 10- to 1,000-fold higher.\(^66\)

**Effects on Candida.** The advent of less immunosuppress-
ive analogs of the calcineurin pathway (CsA, tacrolimus) and the lipid-formulated rapamycin, and the finding that the afore-
mentioned compounds are synergistic in vitro with AMB and 5-flucytosine against *Candida* spp. renewed interest in their potential antifungal activity in the clinic.\(^67\) Prior studies established that rapamycin and its analogs (particularly analogs 23 and 2) were fungicidal against *C. albicans* because of FKB12-
dependent inhibition of TOR; the rapamycin MICs in these studies ranged from less than 0.02 to 0.20 μg/ml with an MFC of 0.39 μg/ml.\(^66,68\) At sublethal concentrations, rapamycin blocked *C. albicans* filamentation via expression of genes associated with nitrogen use, such as *mep2* and *gln3*.\(^69,65,69\) Mutants defective in these genes were avirulent in mice with disseminated candidia-
sis.\(^69\) In another study, oral administration of 10 and 12.5 mg/kg rapamycin protected 50% and 90%, respectively, of mice against death from *C. albicans* infection.\(^70\)

Despite having high MICs (>10–100 μg/ml) in Candida spp., both tacrolimus and CsA (which complex with FKBP12 and cyclophilin, respectively) may influence the efficacy of anti-
fungal drugs and reduce *C. albicans* virulence (Table 2).\(^62,71,72\) These in vitro results are supported by gene deletion studies showing that calcineurin-defective *C. albicans* mutants were more sensitive to stress conditions than isogenic wild-type con-
trols, were markedly hypersensitive to azoles, and had attenuated virulence in mice with systemic candidiasis.\(^73,75\) Although that hypovirulence is attributed to sensitivity of *C. albicans* to killing by serum mediated by Ca\(^{2+}\), no reported data have indicated that pharmacological inhibition of calcineurin also protects against calcium stress. Interestingly, loss of function crz1 *C. albicans* mutants, defective in *crz1* which encoded a downstream tran-
scription effector of the calcineurin, Crz1, were not sensitive to serum and exhibited no decrease in virulence.\(^76\)

Synergistic interaction among CsA, tacrolimus and fluconazole in vitro (Table 2) is attributed to conversion of the static effect of fluconazole to fungicidal activity in *Candida* species. This inter-
action extends to the other azoles as well as terbinafine and was observed in both susceptible *C. albicans* and azole-resistant spe-
cies, including *C. glabrata*.\(^71,73\) Checkerboard microdilution and
Figure 1. Mammalian targets of cancer chemotherapy agents reported to affect the biology of pathogenic fungi. Arrows represent activation; blocked lines represent inhibition. NFAT, nuclear factor of activated T cells; HSP90, heat shock protein 90; HSP70, heat shock protein 70; P, Phosphate group.

time-kill experiments found that at a concentration of 0.625 μg/ml (i.e., at a concentration achievable in vivo), CsA was synergistic with fluconazole, reducing viable Candida counts by 2 logs. Importantly, testing of Candida isolates obtained from liver transplant recipients receiving tacrolimus showed that treatment with this agent did not select for resistance toward calcineurin inhibitors.

In general, these data are supported by the results of in vivo animal studies. Treatment with a CsA-fluconazole combination (at therapeutic drug levels) was more effective than that with either drug alone against experimental candidal endocarditis and in mice with Candida keratitis. In a rat study, C. albicans embedded in biofilm and was resistant to fluconazole, CsA and tacrolimus alone but highly susceptible to CsA-fluconazole and tacrolimus-fluconazole combinations. However, the influence of calcineurin on C. albicans virulence appears to be host- and niche-specific. Investigators observed that calcineurin-defective C. albicans strains were hypovirulent in mice with systemic or ocular infections but fully virulent in mice with lung, vaginal or oropharyngeal infections. Such C. albicans mutants may
be unable to survive serum-induced calcium stress, accounting for their reduced virulence in systemic infections. Recently, a *C. glabrata* calcineurin mutant, defective in the calcineurin regulatory B subunit, exhibited increased susceptibility to both azoles and micafungin and was hypovirulent in mice. Unlike with *C. albicans*, loss of the *C. glabrata* homolog Crz1 did not result in increasedazole susceptibility.62 The in vitro and in vivo (in animals) synergy of CSA/tacrolimus and azoles is intriguing; both classes of drug may be immuno suppressive again emphasizing the need to study host as well as and pathogen-specific factors in predicting likely response of a pathogen to any therapeutic agent.82,83

Reported data on the effects of CsA and tacrolimus on Candida morphogenesis are few. In one study, CsA administered at concentrations of 50–1,000 μg/ml inhibited germ-tube induction (50% inhibition at 100–500 μg/ml) and mycelial transformation of blastospores in *C. albicans* (Table 2).84

**Effects on Aspergillus.** Extensive studies of *A. fumigatus* indicated that calcineurin has a critical role in regulating polarized hyphal growth of and tissue invasion by this species.62 In *A. nidulans* for instance, cnaA, the gene that encodes calcineurin, is essential for conidial germination. CnaA mutants in *A. fumigatus* animal models exhibited defective conidial and hyphal extension and significant attenuation of pathogenicity (mortality rate, 10% vs. 90%); hyphae were almost completely absent from lung tissue.82,84 cnaA *A. fumigatus* mutants defective in the calcineurin A unit also demonstrated aberrant hyphal growth and reduced pathogenicity.85,86

Direct evidence of the effects of calcineurin and TOR inhibitors on the phenotype of *Aspergillus* spp. (Table 2) include reports that rapamycin inhibited the growth of *A. fumigatus, A. flavus* and *A. niger* in vitro (MICs, ~50 μg/ml) but that this inhibition was not sustained.87,88 In other studies, CsA and tacrolimus had intrinsic activity against *A. fumigatus* (CsA: MICs, 3–10 μg/ml; geometric mean MIC, 6.25 μg/ml; tacrolimus: MICs, 0.2–0.4 μg/ml; geometric mean MIC, 1.56 μg/ml).66,89 Calcineurin and TOR inhibitors also have converted azoles (e.g., iraconazole) and the echinocandin caspofungin into fungicidal compounds against *A. fumigatus, A. flavus* and *Aspergillus terreus*.87,89 Inhibition of the calcineurin and TOR pathways may decrease the threshold of deleterious effects of echinocandins. Alternatively, combinations of caspofungin and calcineurin inhibitors may inhibit expression of the *A. fumigatus* FKS1 homolog.89 As for Candida spp., long-term CsA and tacrolimus exposure in vivo did not make these species resistant to these agents.88

Animal data have indirectly confirmed the CsA- and tacrolimus-induced anti-Aspergillus effects described above in vivo. For example, a study showed a 100% mortality rate in granulocytopenic rabbits given ara-C but a 100% survival rate in those given CsA and methylprednisolone.80 In another, *A. fumigatus* model, survival rates were significantly higher and survival durations were significantly longer in mice receiving low-dose (1 mg/kg/day) intravenous tacrolimus but not CsA. Also, researchers observed numerous Aspergillus hyphae in the brains and kidneys of CsA-treated mice but no hyphae in the brains of tacrolimus-treated animals; it is unclear if these observations are directly related to an in vitro drug effect and/or from effects on the host immune system11 and again, their relevance (or not) in the clinical setting remains to be established.

Tacrolimus and CsA exhibit differences in their effects on the morphology of *Aspergillus* spp. (Table 2). For example, authors reported gross stunting of the growth with blunting of hyphal tips after tacrolimus but not CsA exposure.88,90 However, administered alone, both agents delayed filamentation of *A. fumigatus*, which was enhanced when either agent was given with caspofungin. Quantification of cell wall 1,3-β-glucan synthase activity revealed decreased levels of activity after calcineurin inhibition, supporting the hypothesis that calcineurin inhibition operates via cell-wall biosynthesis.91 Identification of fungus-specific downstream effectors of calcineurin in fungi may lessen any broad cross-reactivity of current calcineurin inhibitors with human targets. A study showed that the CrzA homolog in *A. fumigatus* (not found in mammals) was essential for the growth, germination, sexual development and pathogenicity of *A. fumigatus*.94 Inhibition of CrzA is worthy of consideration as one of the next generation of calcineurin inhibitors.

**Effects on other fungi.** Although beyond the scope of this review, rapamycin and the calcineurin inhibitors have inhibited the growth of other fungi, most notably *Cryptococcus neoformans*.62 The calcineurin pathway is integral to cryptococcal pathogenesis and parallels regarding other pathogenic fungi may be drawn from reported data on this pathogen.82,95

**Hsp90 Inhibitors**

The molecular chaperone Hsp90 is part of a multichaperone complex essential for the conformation and function of a diverse set of client proteins, including key oncogenic proteins, calcineurin, TKs and serine/threonine kinases, many of which regulate cell signaling and stress response.13,96,97 ATPase activity combined with ADP/ATP exchange in this complex is essential for the control of client-protein binding and fate and involves the association and/or dissociation of co-chaperones (e.g., Hsp40, Hsp90/70 organizing protein). Pharmacological inhibitors of Hsp90 typically dock in the N-terminal nucleotide binding site with high avidity, interrupting ATPase activity and resulting in downstream degradation of client proteins.13

The first-in-class Hsp90 inhibitor tested in clinical trials for cancer treatment was the ansamycin antibiotic geldanamycin analog 17-allylamino-17-demethoxygedanamycin (17-AAG; geldanamycin itself is too toxic for clinical use) administered either as a single agent or in combination with cisplatin and paclitaxel. Other Hsp90 inhibitors include another geldanamycin analog, 17-desmethoxy-17-N,N-dimethylaminoethylaminogeldanamycin (17-DMAG); the oxime derivatives of radicicol; pyrazoles; purines; novobiocin analogs; and inhibitors of Hsp90 isoforms, including a recombinant antibody against fungal Hsp90 (described below). Cisplatin, which binds to the Hsp90 C-terminal domain; paclitaxel; and the HDAC inhibitors (HDACs), which induce acetylation of Hsp90 (described below), also decreases Hsp90 function.13,96,97 Hsp90 inhibitors are already known to inhibit viral replication.98 Because Hsp90 is highly conserved in eukaryotes, their pharmacological inhibition may affect fungal development.
Effects on Candida. Researchers first obtained data linking Hsp90 with the emergence of antifungal drug resistance (Fig. 1) in *S. cerevisiae*. Similarly, investigators have shown that the Hsp90-calcineurin signaling network plays a key role in the rapid development and maintenance of *C. albicans* resistance to azoles as well as the echinocandins (Table 1). At concentrations well tolerated in humans, treatment with radicicol prevented the emergence of resistance to azoles and abrogated the resistance of laboratory mutants of *C. albicans* as well as *C. albicans* strains in which resistance to azoles developed in a human host.99,100

More recently, 17-AAG and 17-DMAC administered at concentrations with minimal direct activity against *C. albicans* were synergistic in vitro with fluconazole.101 The efficacy of fluconazole with these two Hsp90 inhibitors in an invertebrate model (larvae of the wax moth *Galleria mellonella*) was greater than that when it was administered alone as the combination therapy rescued larvae from lethal infection with *C. albicans*. That study also showed fungicidal activity of azoles when administered along with geldanamycin in viability assays and in mice with candidiasis.102 Singh and colleagues further showed that pharmacological (geldanamycin) and genetic impairment of Hsp90 function reduced the micafungin tolerance of laboratory *C. albicans* strains and micafungin resistance of clinical *C. albicans* isolates, resulting in a fungicidal combination of micafungin and geldanamycin.103 Investigators have expressed concerns with the potential for reduced activity of *echinocandins* at high concentrations, known as the “paradoxical” effect. In one study, treatment with radicicol reduced the paradoxical effect of micafungin in vitro, reduced tolerance to voriconazole and abrogated micafungin-voriconazole antagonism in *C. albicans*.105 Intriguingly, Hsp90 inhibition-induced filamentation of *C. albicans* via regulation of cyclic adenosine monophosphate-dependent protein kinase A signaling, and may be relevant to the virulence of *C. albicans*.104

Further, studies have shown that *C. albicans* Hsp90 is an immunodominant antigen in mice and humans. For example, researchers tested a recombinant *C. albicans*-specific anti-Hsp90 antibody (efungumab) to determine its antifungal activity.105,106 Although Candida isolates display high efungumab MICs of 128–256 μg/ml, efungumab was synergistic with both AMB and caspofungin against Candida against these isolates. Furthermore, a clinical trial of invasive candidiasis demonstrated a therapeutic benefit of efungumab when administered with AMB.107 Efungumab likely affects the host immune responses to the pathogen because the antibody is unable to cross the fungal cell wall and enter the cytosol, where Hsp90 inhibitors exert their activity.108 Overall, given the promising in vitro results obtained with the HSP90 inhibitors, further studies systematically exploring the clinical efficacy, particularly of efungumab formulations, are warranted to determine their position in the anti-Candida drug armamentarium.

Effects on Aspergillus. Unlike *C. albicans*, little is known about the direct effects of Hsp90 inhibitors on the development of drug resistance in *Aspergillus* spp., although a recent study confirmed that Hsp90 is required for basal resistance of these species to the echinocandins.109 Treatment with geldanamycin has enhanced the activity of caspofungin against single strains of *A. fumigatus* as well as against *A. terreus*.99,101 Inhibition of Hsp90 enhanced the activity of voriconazole against *A. fumigatus*, although this effect depended on environmental conditions.101 Finally, combination treatment with geldanamycin and caspofungin rescued *G. mellonella* larvae from lethal infection with *A. fumigatus*.102

HDACs

HDACs are chromatin-modeling enzymes that deacetylate lysine residues on core histones and other cell proteins. Central to gene regulation, HDACs control a range of cellular functions, including proliferation and death. HDACs thus modulate the acetylation status of chromatin and a range of cellular proteins, including Hsp90, leading to transcriptional cell-signaling events that induce growth arrest, apoptosis and autophagy (described below). HDACs exhibit promise in the treatment of cancer and certain parasitic and viral infections.14,109

The HDACs include trichostatin A (TSA), romidepsin/dep-sipeptide, suberoylanilide hydroxamic acid (SAHA; vorinostat), panobinostat and MG-CD0103 (mocetinostat). However, only SAHA and romidepsin are licensed for clinical use (for cutaneous T cell lymphoma).14,108,111 As HDACs potentiate the cytotoxicity of DNA methylation inhibitors (e.g., cisplatin, the anthracyclines, dexamethasone), they may be used together with these inhibitors.109-111 Mammalian and fungal HDACs are substantially homologous and investigators have identified several classes and subclasses of HDACs in fungi (e.g., the class I enzymes RpdA and HosA, the class II enzymes HdaA and HosB).112 Amongst proteins within the HDAC complex, H3K56ac has a key role in the DNA damage response; this protein may be fungal-specific.113

Effects on Candida. *C. albicans* have two distinct cell types—white and opaque—with phenotypic differences in cell shape, mating properties and virulence. White cells are typically found in the bloodstream, whereas opaque cells are mainly located on mucosal surfaces and skin. HDACs regulate these phenotypes.14 Treatment with TSA can increase the occurrence of white-to-opaque cell transition by inhibiting the expression of HDAC genes *HDAI* and *RPD3* (Table 1).14,114 TSA may also impactazole susceptibility in *Candida* spp. In one study, treatment with TSA at concentrations ranging from 2 to 3 μg/ml markedly reduced the azole trailing effect and enhanced azole susceptibility by reducing the upregulation of expression of the *CDR* and *ERG* genes in a small number of strains of *C. albicans*, *C. tropicalis* and *Candida parapsilosis* but not *C. glabrata*.115 Conversely, treatment with TSA at 1–32 μg/ml had a minimal effect on in vitro growth of, germ-tube formation by (in 20% serum) and heat sensitivity of these strains,115 although another study found that both TSA and SAHA inhibited serum-induced germ-tube formation in *C. albicans*.116 In combination with fluconazole, treatment with several investigational uracil-based hydroxamates inhibited induction of resistance to fluconazole in *Candida* spp.117 TSA and SAHA also caused a 90% reduction in *C. albicans* adherence to human cultured pneumocytes.118 Increasing experience in using HDACis to treat cancer will aid testing of the efficacy of combinations of HDACis and azoles.
against Candida spp. at pharmacologically relevant concentrations and in in vivo models of infection.

Novel HDACs with activity against Candida HDACs, particularly those selective for HDAC6 and Hda1p, are worthy of investigation as next-generation HDACs because of their potential antifungal activity. The protein Hda1p appears to play a key role in development of drug resistance in Candida spp. as such resistance cannot develop in HDA1-deficient strains. In addition, data have shown that HDAC6 regulates Hsp90 acetylation and that hyperacetylation of the Hsp90 complex leads to inhibition of it. Finally, reduced levels of H3K56ac activity (regulated by the C. albicans RTT109 gene) have sensitized C. albicans to hydroxyurea, the azoles, and echinocandins and are associated with attenuated C. albicans virulence in mice. Inhibition of Hst3p in C. albicans results in loss of cell viability with abnormal filamentous growth. Researchers have tested the activity of at least one new HDACi, MG-CD290, against a range of Candida spp. and molds (described below; Table 1).

**Effects on Aspergillus.** The effects of HDACis on *A. nidulans* are summarized in Table 1. Inhibition of HDACs targets primarily the class II enzyme HdaA, which accounts for the majority of HDAC activity in Aspergillus. Deletion of the RPD3-type HDAC RpdA in *A. nidulans* leads to a pronounced reduction in growth and sporulation of this species.

Pfaller et al. examined the antifungal activity of MGCD290, a novel Hos2 fungus-specific HDACi, in combination with fluconazole, voriconazole and posaconazole against a range of yeasts (including Candida; *n* = 30) and molds (including Aspergillus; *n* = 10). MGCD290 MICs ranged from 0.5 to 16.0 μg/ml for Candida spp., including azole-resistant strains, whereas the MICs ranged from 8 to >32 μg/ml for *Aspergillus* spp. Notably, MGCD290 (8–32 μg/ml) was synergistic with fluconazole against 87% of Candida isolates and unexpectedly enhanced fluconazole activity against six Aspergillus isolates. Importantly, in *Aspergillus* spp., MGCD290 was synergistic with voriconazole (six isolates) and posaconazole (four isolates). Current studies are examining optimal dosing of MGCD290 with phase I first-in-human clinical studies demonstrating safety and favorable pharmacokinetic properties of this agent when administered alone or with fluconazole (www.methylgene.com).

**DNA Topoisomerase Inhibitors**

That DNA topoisomerase inhibitors directly affect the growth and biology of fungi is not a new concept. These compounds include the fluoroquinolones, anthracyclines and bleomycin.

**Fluoroquinolones.** Fluoroquinolones inhibit DNA gyrase (topoisomerase II) and topoisomerase IV, resulting in cell death. Topoisomerases are universally present in eukaryotes, with high levels of topoisomerase I and II in fungi. Data have indicated the potential of fluoroquinolones, especially ciprofloxacin, ofloxacin and moxifloxacin, to enhance the efficacy of antifungal drugs despite lacking intrinsic antifungal activity.

**Effects on Candida.** Assessment of in vitro pharmacodynamic interactions between antifungal and nonantifungal agents is complicated by the absence of a common antifungal end point using conventional fractional inhibitory concentration index criteria. However, recent in vitro isobolographic analyses showed that significant pharmacodynamic interactions between ciprofloxacin and AMB and between voriconazole and caspofungin do occur in *C. albicans*. Specifically, AMB and ciprofloxacin were synergistic at ciprofloxacin concentrations less than 10.64 μg/ml and AMB concentrations less than 0.33 μg/ml but may be antagonistic at higher ciprofloxacin concentrations. Other fluoroquinolone-AMB and fluoroquinolone-azole interactions vary according to the fluoroquinolone (Table 1). Variable interactions between fluoroquinolones and caspofungin also have occurred, with indifferent to synergistic results. Fluoroquinolones may inhibit fungal DNA replication, but this effect is apparent only when combined with antifungal drugs. Also, increased cell-membrane permeability resulting from treatment with antifungal drugs may increase intracellular fluoroquinoloxin levels, leading to synergy of the antifungal drugs and this fluoroquinolone. Alternatively, ciprofloxacin may interact with cholesterol-containing liposomes and participate in pore formation in fungal cell membranes, thus enhancing AMB activity.

The above in vitro observations are supported by limited animal data—synergistic interactions of ciprofloxacin plus fluconazole, ciprofloxacin plus AMB and fluconazole plus ofloxacin in vivo in mice with *C. albicans* infection are described. However, liposomal AMB and moxifloxacin were not synergistic in a rabbit model of *C. albicans* endophthalmitis for this species. In another study, moxifloxacin protected cyclophosphamide-injected mice against *C. albicans* bronchopneumonia and reduced mortality rates. Yet, long term quinolone use is known to be a risk factor for Candida infection emphasizing the complexity of drug-pathogen interaction in a specific host environment and the need to study fungal pathogenesis in appropriate clinical contexts.

**Effects on Aspergillus.** Likewise, researchers have observed synergistic interactions of AMB with ciprofloxacin and levofloxacin (but not moxifloxacin) in *A. fumigatus*. Combinations of ciprofloxacin with voriconazole and caspofungin were also synergistic in these studies (Table 1). For ciprofloxacin-AMB combinations, investigators detected synergy at low concentrations of both ciprofloxacin (0.78–1.85 μg/ml) and AMB (0.24–0.39 μg/ml) but noted antagonism at higher concentrations. Animal models of invasive aspergillosis have supported the enhanced efficacy of antifungals when combined with fluoroquinolones. Genus-specific differences between Candida and Aspergillus in the interactions of fluoroquinolones with individual antifungals may be related to different affinities of each quinolone for fungal topoisomerases. Determining the clinical relevance (or lack thereof) of these experimental interactions is indicated.

**Anthracyclines.** The anthracycline antibiotics induce faulty retyping of DNA strands by impairing topoisomerase II activity. Doxorubicin (Adriamycin) also affects mitochondrial function by selectively binding to cardiolipin in the mitochondrial cell membrane. Other anthracyclines are daunorubicin, aclacinomycin and idarubicin.

**Effects on Candida and Aspergillus.** In studies of simulated blood cultures, doxorubicin inhibited the growth of *C. albicans*,
whereas at physiologically achievable concentrations, idarubicin inhibited the growth of *C. glabrata* but not *C. albicans* or *Candida krusei*. In another study, aclarubicin displayed fungistatic activity against *C. albicans* (MIC range, 0.8–7.3 μg/ml), but doxorubicin, idarubicin and daunorubicin had much higher MICs (>26 μg/ml); furthermore, idarubicin inhibited hyphal formation by *C. albicans*. Exposure of *C. albicans* to doxorubicin resulted in mitochondrial dysfunction, decreased in ergosterol content in fungal cell membrane (ergosterol is the target binding site of AMB), all culminating in increased in the tolerance of this species to AMB. Most recently, Kofla et al. reported that doxorubicin induced *CDR1/CDR2* expression in *C. albicans* at both the mRNA and protein level and caused a 4–8-fold increase in MICs to fluconazole. Expression of the multidrug resistant 1 (MDR1) gene was not affected. Data on the effects of antracyclines on *Aspergillus* spp. are limited (Table 1).

**Bleomycin.** The glycopeptide bleomycin induces oxidative-mediated DNA damage manifested by double- and single-strand DNA breaks and mediates RNA degradation at sites adjacent to guanosine residues.

Early studies of mice with candidiasis demonstrated activity of bleomycin against *C. albicans* (Table 1). Recently, a study showed that bleomycin inhibited self-splicing of the 25S rRNA Group I intron of *C. albicans* at a half-maximal inhibitory concentration of 1.2 μM, resulting in accumulation of RNA precursors and inhibition of *C. albicans* growth by about 44%. The intron-containing *C. albicans* strain had a bleomycin MIC of 1.56 μg/ml, whereas the intronless strain had a MIC of 6.25 μg/ml. This difference in MICs is thought to be related to selective interaction of bleomycin with folded RNA structures.

Bleomycin also can impact the phenotype of *A. nidulans* (Table 1). Researchers have observed direct activity of bleomycin against *A. fumigatus* (MIC, 3.2 μg/ml) with arrest of conidial germination, prevention of hyphal development and completion of cell-wall septation. A possible mechanism of cell-wall damage caused by bleomycin is increased cell-wall permeability via disruption of glycosidic cross-linking structures.

**Other antibiotics.** The sulphonamides, rifampicin and metronidazole have also been studied alone or in combination with polyenes or azoles in vitro and in animal models for their antifungal effects. Sulphamethoxazole drugs were found to be active against most *Aspergillus* species and may help to prevent invasive aspergillosis in AIDS patients (reviewed in ref. 143). Ketoconazole-sulphamethoxazole combinations were synergistic against *C. albicans*. Rifampicin, a macrocyclic antibiotic in combination with AMB has synergistic interactions against certain *Candida* and *Aspergillus* spp. Likewise, additive to synergistic effects have occurred between AMB and metronidazole.

The clinical implications of these observations have yet to be determined.

**TK Inhibitors**

TK inhibitors (TKIs) target enzymes whose dysregulated expression and activity are associated with various cancers. As such, small-molecule TKIs that prevent autophosphorylation and propagation of downstream intracellular signals and monoclonal antibodies against TKs that block ligand binding have revolutionized the treatment of certain cancers. Small-molecule TKIs compete with ATP binding to the TK domain and abrogate the catalytic activity of the TKI receptors but may also be "non-receptor-targeted" (non-receptor TKIs, e.g., the src TK family). These include imatinib mesylate (a nonreceptor TKI) and its derivatives, nilotinib and dasatinib, and the receptor-targeted TKIs gefitinib and erlotinib. TKIs are most often prescribed for prolonged periods, often in combination with other drugs, including antifungal agents, thus, an understanding of their effects on fungal development is essential, as several protein kinase classes regulate key intracellular signals that impact fungal cell differentiation and death.

**Effects on Candida and Aspergillus.** The only reported direct effects of TKIs on *Candida* and *Aspergillus* spp. are derived from in vitro observations of TKIs not in clinical use. Work involving the TKIs genistein, herbimycin, tyrphostin-47 and calphostin C provide insight into what may be expected for the modulation of key aspects of Candida biology by TKIs in general (Table 1).

Genistein appears to positively impact the growth of, rate of germination of and Hsp90 expression in vitro in *C. albicans*. In view of its activity as a phytoestrogen, these effects may result from interaction of it with, for example, the Candida estrogen-binding protein. Both genistein and tyrphostin-47 have blocked Candida-induced tyrosine phosphorylation and subsequent endocytosis by endothelial cells. *C. albicans* is known to induce its own uptake by endothelial cells, a process that requires tyrosine phosphorylation. Using immunofluorescence microscopy, researchers observed that phosphotyrosine-containing endothelial cell proteins associated with the cell cytoskeleton surrounding *C. albicans* germ tubes while undergoing endocytosis. Blocking of adhesion of endothelial cells to vitronectin molecules could result in inhibition of the critical first step in the escape of *C. albicans* from the intravascular compartment.

In *Aspergillus* spp., phagocytosis of conidia is an actin-dependent process that requires TK activity. In one study, two broad-range TKIs (genistein and AG18) and inhibitors that specifically blocked src kinases strongly reduced *A. fumigatus* conidia uptake by human and murine macrophages. This could have implications for *Aspergillus* virulence as phagocytosis of conidia (e.g., in the lung) is required for their elimination.

**Interactions with antifungal drugs.** In addition to directly affecting fungi, TKIs are extensively metabolized by cytochrome P450 (CYP) enzymes. Certain TKIs are also substrates or inhibitors of the drug transporters P-glycoprotein (Pgp), breast cancer resistance protein and organic cation carrier 1. Because of their potential drug-drug interactions withazole antifungals, understanding the pharmacokinetics of TKIs is essential. Imatinib is metabolized primarily by the CYP isoenzyme 3A4 but is also a substrate of organic cation carrier 1, Pgp and BCRP. Fluconazole and itraconazole are dual CYP3A4 and Pgp inhibitors; thus, administration of them with imatinib may lead to increased plasma and intracellular concentrations of imatinib and increased toxicity of this agent. Drug-drug interactions should be considered when administering TKIs with other inhibitors of
the CYP3A family, most notably voriconazole. Similar interactions can be expected for dasatinib and nilotinib.

**Autophagy Inhibitors**

Autophagy is a conserved process by which eukaryotes recycle cytoplasm and organelles for survival during stress and starvation. Implicated in cancer development, autophagy is also harnessed by host cells to eliminate microorganisms. It may be induced by rapamycin exposure via inhibition of TOR kinase and subsequent activation of the autophagy pathway. Active autophagy is likely to be incompatible with rapid fungal growth. Inhibition of the process of autophagy may occur by pharmacological means, such as class I and III inhibitors of phosphatidylinositol-3-kinase—3-methyladenine and wortmannin, respectively—and their derivatives or by disruption of genes (i.e., ATG) encoding for autophagy proteins (e.g., Atg1 and Atg8 in *S. cerevisiae*). Because the functional significance of autophagy in pathogenic fungi has yet to be established, the effects of autophagy inhibitors on fungi can only be surmised.

**Effects on Candida.** Autophagy appears to have a minor role in *C. albicans* yeast-hyphal transition, chlamydospore differentiation and virulence in mice with systemic candidiasis as demonstrated in experiments using autophagy-defective mutants without the ATG9 gene homolog. However, autophagy-defective *C. glabrata* mutants lacking expression of the CgATG11 and CgATG17 genes were associated with decreased survival in *C. glabrata*, with the double mutant being highly sensitive to engulfment by macrophages. Current data suggest that autophagy inhibitors (as well as other agents that indirectly induce autophagy) reduce survival of *C. glabrata* in vitro but have little direct effect on *C. albicans* survival and morphogenesis. Yet since autophagy is profoundly important to eukaryote cell biology, it is unlikely to be completely superfluous in *C. albicans*.

**Effects on Aspergillus.** Reported data on the direct effects of autophagy inhibitors on Aspergillus development are also scarce, although *Aspergillus* spp. are reported to be resistant to wortmanin. In *Aspergillus oryzae* autophagy appears to be involved in nutrient recycling during starvation; mutants of this species with deletions of the ATG1 or ATG8 gene were unable to undergo asexual development and acquire nutrients. In a study of *A. fumigatus*, autophagy was required for aerial hyphae formation, conidial germination, hyphal foraging and growth under nutrient-deficient conditions; recent data have also suggested that autophagy contributes to metal ion homeostasis but the mechanism by which this occurs requires further investigation. As for *C. albicans*, autophagy does not appear to be essential for virulence in mice with aspergillosis, the reason for which remains to be defined. One possible explanation is that for *A. fumigatus*, the fungus is able to survive on dead/decaying matter and is relatively resistant to nutrient stress.

**Perspectives**

Collectively, the scientific literature indicates that antineoplastic and other immunosuppressive therapies can directly affect fungal growth, development and virulence. Certain classes of antineoplastic agents, such as the calcineurin inhibitors and HDACis may significantly influence antifungal drug susceptibility. The specific effects and magnitude thereof of these agents on each of these aspects of fungal biology vary both with the agent class and within each class. Of note, these effects are observed not only with new antineoplastic agents but also with established cytotoxic drugs.

Studies of novel and investigational anticaner drugs show that signal transduction networks that modulate crucial events during fungal invasion can be targeted in the development of drug-pathogen interaction-based cancer-management strategies. Pertinent observations in these studies include findings that inhibitors of calcineurin and/or TOR perturb morphogenesis, membrane stress responses, survival of fungi in serum and fungal virulence in experimental models of infection. These inhibitors can also reduce azole tolerance and may be considered for novel therapies for recalcitrant azole-resistant candidiasis and biofilm-associated infections. Results in Aspergillus suggest that these inhibitors inhibit growth and filamentation, but in vivo animal data are conflicting. Inhibition of candidal Hsp90 activity and modulation of fungal gene expression by Candida-specific HDAC inhibition indicate that they are feasible approaches to the treatment of invasive candidiasis. TKIs have good potential for inhibiting fungal entry into epithelial cells and subsequent phagocytosis by macrophages but may interact significantly with antifungal agents.

These data however, should be considered in the context in which they were obtained. Specifically, researchers largely observed the direct effects of all classes of anticaner agents on *Candida* and *Aspergillus* spp. under in vitro conditions. Uncertainty regarding the wider applicability of these results and their clinical relevance stems from the use of these “artificial” laboratory conditions, incorporating technical variables such as the effects of different media, inocula and growth conditions. Furthermore, investigators conducted many of the experiments using only single strains or small numbers of strains. Testing of larger numbers of strains of given species is required to determine drug effects in a population-based context and confirm prior observations. It is intriguing that the use of anticaner/immunosuppressive agents with the potential to affect fungal biology or growth largely occurs is in a clinical context where there is a relatively high rate of IFIs (i.e., in transplant and cancer patients). These “paradoxical” observations emphasize the complexity of the host immune response and host-pathogen interactions and that laboratory observations, even if robust, should not be used alone to predict clinical response to a drug.

Another limitation of the current data set is evaluation of antineoplastic drugs as single agents. Because these drugs are typically given in combinations, assessment of their net combined effects on fungal biology is required. To date, these combined effects remain unknown. Anticaner agents that may have no discernible effects when given alone may well have significant effects when given with other agents. Further clinical studies, in particular those testing the in vivo synergy of antineoplastic agents with currently used antifungals, are warranted.
In summary, the direct effects of antineoplastic agents on the development of Candida and Aspergillus infections and the virulence of their species as well as on antifungal drug resistance are varied. Many questions remain unanswered. In particular, results obtained using both in vitro and in vivo experimental systems may not reflect the clinical context in which a host is exposed to a defined inoculum of a fungus in a complex, pleiotropic environment (Fig. 1). The resultant ability of various antineoplastic and immunosuppressive agents to create permissive conditions for the initiation and progression of the fungal are multifaceted and difficult to predict. The occurrence of such infection is dependent on not only the intrinsic virulence properties of the fungal strain, but also local and systemic metabolic factors and the effects of antifungal and other chemotherapeutic agents in the pathogen's environmental milieu. Because fungal infections frequently begin on mucosal surfaces in multi-organism communities, chemotherapy drugs may also affect the quorum-sensing ability of the infecting organisms. Evaluation of the effects on fungi of antineoplastic drugs administered with and without antifungal agents in more clinically plausible systems that reflect “real-life” scenarios is awaited with interest.

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