

Treatment Options in Invasive Aspergillosis

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Opinion statement

Aspergillus species are septated molds that cause a wide spectrum of clinical syndromes. Among these, invasive aspergillosis (IA) causes very high morbidity and mortality among the most severely immunosuppressed, especially those with profound qualitative or quantitative neutropenia. Empirical, pre-emptive, and targeted approaches have been attempted to blunt establishment of infection with variable success. The preferred treatment of primary IA is voriconazole, which has been found to be superior to amphotericin B. Azoles interfere with the synthesis of ergosterol found in the fungal cell membrane, whereas polyenes—such as amphotericin B—interfere with ergosterol function. An echinocandin that disrupts fungal cell wall synthesis—caspofungin—and itraconazole have been approved for salvage therapy of IA. Lipid amphotericin B formulations are used in those intolerant to amphotericin B deoxycholate. Posaconazole is used for prophylaxis in high-risk groups such as those with acute myelogenous leukemia, myelodysplastic syndrome, and stem cell transplant recipients with graft versus host disease to reduce the incidence of invasive fungal infections (IFI) such as IA. Combining mold-active azoles or a lipid amphotericin B formulation with an echinocandin may have a role in refractory IA. Immunomodulatory properties of antifungals, growth factors, cytokines, and immune cell infusions may enhance host ability to facilitate adjunctive control of infection, but an uncontrolled, exuberant inflammatory response can also cause significant pathology. Surgical resection may be a last resort when angioinvasion of critical structures places a patient at high risk for bleeding, thrombosis, or embolic phenomena, despite medical

therapy. Nevertheless, immune reconstitution with myeloid lineage recovery is the key to successful outcomes.

Introduction

Aspergillus species are ubiquitous Ascomycetes that may cause a variety of syndromes depending on the degree of immunosuppression in the host. In ascending order of immunocompromise, these include allergic bronchopulmonary aspergillosis (ABPA), saprophytic aspergillomas, chronic necrotizing aspergillosis, and invasive aspergillosis (IA) (Fig. 1). Angioinvasion is the hallmark of IA, with sinopulmonary involvement being the most common manifestation, while dissemination to the central nervous system, gastrointestinal tract, skin, or contiguously may occur amongst the most severely immune impaired. Definitions of proven and probable IA and complete or partial response to therapy have been previously published [1•, 2•]. The most important species include *A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans*, which may have varying resistance patterns [3••].

IA causes tremendous morbidity and mortality, particularly among those with prolonged and severe functional or quantitative [absolute neutrophil count (ANC) <500 cells/ μ L] neutropenia. For instance, data from the Transplant Associated Infections Surveillance Network (TRANSNET) from 2001 to 2006 revealed a 25.4 % and 59 % 1-year survival from IA among hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients, respectively. While the IA 1-year cumulative incidence may be 1–8 % in such populations, the 1-year overall cumulative mortality incidence is 18–42 % [4•, 5•]. More recently, the Prospective Antifungal Therapy (PATH) alliance registry reported a 12-week survival of 66.3 % for IA among a variety of risk groups [6•].

The timing of interventions—empiric, pre-emptive, or targeted—affects outcomes with early interventions, generally ameliorating success endpoints and potentially lowering drug-related toxicities, costs, and resistance. For instance, two recent multicenter, randomized clinical trials compared pre-emptive approaches based on biomarkers such as galactomannan or PCR, radiographic signs (e.g., “halo”), and clinical symptoms in high-risk patients to (1) empirical approaches based on persistent febrile neutropenia despite broad-spectrum antibacterials and (2) targeted approaches based on culture and/or histology. In the first study, the pre-emptive model was found to be non-inferior to empirical therapy with respect to survival assessed 14 days after neutrophil recovery and was not statistically different 4 months after study inclusion among those with hematologic malignancies or autologous HSCT (the lower 95 % CI margin for mortality difference was –5.9 %, which was within the non-inferiority margin of –8 %). However, it was also associated with a 2.5-fold increased IA incidence, particularly during induction chemotherapy ($p < 0.01$), yet it decreased antifungal costs by 35 % [7]. In the second study comparing pre-emptive to directed modalities among allogeneic HSCT and those receiving chemotherapy for acute leukemia, use of biomarkers reduced empirical antifungal usage by 17 % ($p < 0.002$) at 26 weeks following randomization, but drug adverse events and all-cause or IA-related mortality did not differ between the groups; the latter perhaps due to the higher-risk IA population included in this study [8]. Thus, empiric therapy may be advisable in high-risk patients,

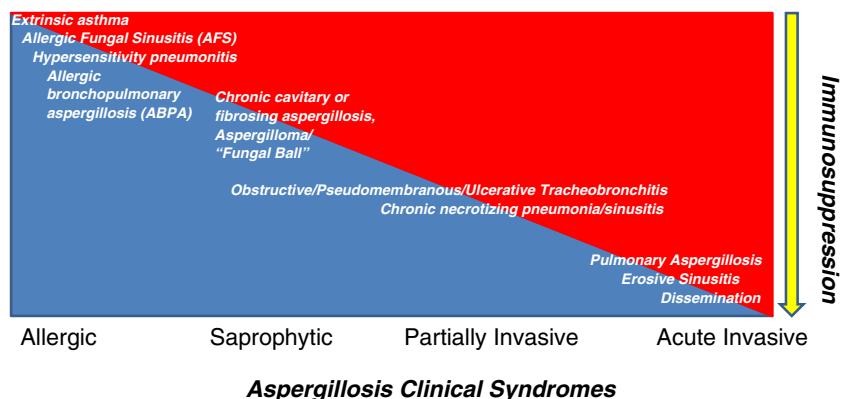


Fig. 1. Spectrum of aspergillosis disease as a function of immunosuppression. With decreasing cell-mediated immunity, the likelihood of invasive disease increases.

whereas a pre-emptive approach may be supported in low-risk patients.

Treatment options in IA are evolving, with novel antifungals introduced within the past decade. The use of

combination antifungals, immunotherapy, and surgery for refractory disease has become particularly appealing in certain situations. This review gives an overview of current practices in the management of IA.

Treatment

Pharmacologic treatment

Antifungals (Table 1)

Mold-active triazoles

Triazoles inhibit a step in fungal cell membrane ergosterol biosynthesis by blocking 14 α -demethylase, a fungal cytochrome P450 (CYP) enzyme.

Table 1. Antifungal use for invasive aspergillosis. Duration may be dependent on the level of immunosuppression and clinical/radiographic response

Antifungal	Dose	Approved indication
<i>Azoles</i>		
Itraconazole*	200 mg IV/PO BID-TID \times 3–4 days then 200 mg IV/PO QD-BID	Salvage (refractory or intolerant to OLAT****)#
Voriconazole**	6 mg/kg IV/PO q12h \times 1 then 4 mg/kg IV/PO q12h	Primary therapy#
Posaconazole***	200–400 mg PO QID-BID	Prolonged neutropenia prophylaxis (e.g., AML, MDS, GVHD, HSCT)
<i>Polyenes</i>		
Amphotericin B deoxycholate (AmB-d)	0.5–1.0 mg/kg IV QD to 1.5 mg/kg IV QOD	Primary Therapy#
Liposomal amphotericin B (L-AmB)	3–5 mg IV QD	Primary Therapy; empiric febrile neutropenia therapy#
Amphotericin B lipid complex (ABLC)	5 mg/kg IV QD	Salvage (refractory or intolerant to AmB-d)#
Amphotericin B colloid dispersion (ABCD)	3–6 mg/kg IV QD	Salvage (refractory or intolerant to AmB-d)
<i>Echinocandins</i>		
Caspofungin	70 mg IV \times 1 then 50 mg IV QD	Salvage (refractory or intolerant to OLAT);# empiric febrile neutropeniatherapy
Micafungin	100–150 mg IV QD	N/A
Anidulafungin	200 mg IV \times 1 then 100 mg IV QD	N/A

BID twice daily, *IV* intravenous, *N/A* not applicable, *OLAT* other licensed antifungal therapy, *PO* oral, *q12h* every 12 h, *QD* once daily, *TID* three times daily

* Oral solution with greater bioavailability than capsule (target trough >250 ng/ml)

** Target trough 1 μ g/mL and 5.5 μ g/mL

*** Target trough >0.5 μ g/mL, a delayed-release tablet that has improved oral absorption with food is now available

****Other licensed antifungal therapy

Refers to the Infectious Diseases Society of America (IDSA) guidelines [3••]

Fluconazole does not have mold activity. The intravenous formulation of itraconazole contains hydroxypropyl cyclodextrin while intravenous voriconazole contains sulfobutyl cyclodextrin, both of which may cause renal toxicity. Intravenous itraconazole is now no longer routinely available in the USA, but may be available in other countries. In a study by Caillot et al., a complete or partial response was seen at the end of therapy (EOT) in 15 (48 %) of 31 patients receiving 12 days of intravenous itraconazole followed by 12 weeks of oral itraconazole capsule. Itraconazole has erratic oral bioavailability, though the oral solution has improved upon the capsule form in this regard. In general, a trough plasma concentration of itraconazole >250 ng/mL is considered to be the minimum efficacy level [9]. Hydroxy-itraconazole is also antifungal and present in nearly equal concentrations to native drug. In a non-comparative study by Denning et al., oral itraconazole capsules (600 mg/day for 4 days followed by 400 mg/day) afforded 39 % complete and partial response at the EOT, with failure rates varying according to site of infection (pulmonary and tracheobronchial disease: 14 %; sinus disease: 50 %; central nervous system disease: 63 %) and degree of immunosuppression (SOT: 7 %; allogeneic HSCT: 29 %; neutropenia >2 weeks: 14 %; AIDS: 44 %) [10]. In addition, it has been used in the prevention of fungal infections, including IA, among those with neutropenia and chronic granulomatous disease (CGD), an entity that predisposes to infections with organisms such as *Staphylococcus*, *Serratia*, *Burkholderia*, *Nocardia*, and *Aspergillus* [11, 12]. In CGD, *A. nidulans* may be more virulent than *A. fumigatus*, causing tissue plane invasion rather than true angioinvasion, but paradoxically are not killed by reactive oxygen species [13, 14]. Based on these studies, itraconazole received indication for IA among those intolerant of or who are refractory to amphotericin B therapy.

Voriconazole received approval for primary therapy of IA in 2002 based on the pivotal randomized controlled trial (RCT) by Herbrecht et al. [15••], which found it to be superior over the deoxycholate version of amphotericin B (AmB-d). A loading dose of 6 mg/kg every 12 h for the first day followed by 4 mg/kg twice daily was given in order to achieve steady state more rapidly (i.e., 24 h), which may otherwise take 5–7 days. Voriconazole achieved 52.8 % 12-week global response rate versus 31.6 % in the AmB-d arm and a 22 % reduction in overall mortality [12-week survival: 70.8 vs. 57.9 %; hazard ratio=0.59 (95 % CI 0.44–0.88), $p=0.02$] [15••]. In the subsequent analysis, Patterson et al. found that fewer patients receiving voriconazole switched to other antifungals than in the AmB-d arm due to disease progression or intolerance (24 vs. 70 %, $p<0.001$) and, despite the switch, success at 12 weeks was less common in the latter than the former group (32 vs. 55 %, $p<0.001$) [16]. Ravuconazole, isavuconazole, and albaconazole have a structure similar to voriconazole with a fluorinated pyrimidine ring and may offer extended half-lives, but are still in the research stages of clinical development [17]. Clinical trials of isavuconazole in Europe are nearing completion.

Posaconazole is currently only available as an oral formulation and is structurally similar to itraconazole but has a broader spectrum of activity

(e.g., certain Mucorales). It is indicated for prophylaxis during neutropenia, particularly in acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS), and graft versus host disease (GVHD). Cornely et al. demonstrated a significant reduction in IA [2 (1 %) vs. 20 (7 %), $p < 0.001$] and prolonged survival (100-day survival, $p = 0.04$) in those receiving posaconazole during neutropenia with AML or MDS compared with itraconazole or fluconazole [18]. Ullmann et al. showed that those allogeneic HSCT who received posaconazole during GVHD had an 83 % reduction in breakthrough IA infections compared with those receiving fluconazole (1.0 vs. 5.9 %, $p = 0.001$) and decreased fungal-related mortality (1 vs. 4 %, $p = 0.046$) [19]. Like itraconazole but unlike voriconazole, the oral absorption of posaconazole suspension is dependent upon gastric acidity and gut integrity. Based on these data, posaconazole received indication for prophylaxis against IA among such patients at high risk in 2006. A new delayed-release tablet is now available with improved oral absorption with food (300 mg twice-daily loading dose on the first day followed by 300 mg daily).

Unfortunately, azole drug interactions via the CYP enzymes (CYP3A4 and 2C19) may lead to significant perturbations in levels of co-administered medications such as cyclosporine, proton pump inhibitors, HIV protease inhibitors, HMG-CoA reductase inhibitors, warfarin, and corticosteroids. Use with quinolones may lead to prolongation of the corrected QT interval. Concomitant use of voriconazole with sirolimus and efavirenz is contraindicated. The dose of calcineurin inhibitors such as tacrolimus and cyclosporine should be reduced by 25 to 75 % when used with fluconazole and voriconazole or posaconazole, respectively. Liver function should be monitored, as a transaminitis five times the upper limit of normal in an asymptomatic individual would preclude its continued use. Voriconazole can cause a severe photosensitivity reaction; it can cause reversible visual changes, such as blurring, light sensitivity, or abnormal color perception. Moreover, azole-resistant isolates of *Aspergillus*, via alterations of the target enzyme CYP51A, have been reported [20]. In The Netherlands, the TR/L98H mutation in the CYP51A gene was harbored by 64 % of azole-naïve patients' *A. fumigatus* isolates, which correlated with an 88 % overall mortality and significant cross-azole resistance was observed [21••]. This may have been due to the agriculture use of azoles in this particular region and its spread has been limited thus far [22]. The epidemiologic cut-off value (ECV) in this context may be valuable, as it can permit the tracing of where resistance changes have occurred. The ECV is the minimum inhibitory concentration (MIC) value identifying the upper limit of the wild-type (WT) population, as defined as the predominant species strain without acquired resistance mutations to an antifungal, which enables discrimination of WT from acquired resistance phenotypes in population-based studies [23].

Azoles may also have immunomodulatory effects, but less so than lipid amphotericin formulations and echinocandins. For instance, transcriptional profiles of Toll-like receptor (TLR)-2 and tumor necrosis factor (TNF)- α in human monocytes were upregulated in the presence of voriconazole—even at sub-inhibitory concentrations (0.1 $\mu\text{g}/\text{mL}$)—compared with hyphae alone and this correlated with hyphal

damage. In addition, nuclear translocation of nuclear factor (NF)- κ B—a key common event in the inflammatory response to cytokine via signal transduction and activator of transcription (STAT) pathways—was more pronounced in the presence of voriconazole [24]. However, other investigators have found these effects to be abrogated by conidia [25].

Polyenes

Amphotericin B formulations that disrupt the fungal ergosterol membrane by forming pores have been the “gold standard” antifungal for IA for more than 50 years [26, 27••]. The deoxycholate version (AmB-d) can be prohibitively toxic (infusion- and kidney-related) and has consequently been replaced largely by lipid formulations: liposomal amphotericin B (L-AmB) 3–5 mg/kg/day and amphotericin B lipid complex (ABLC) 5 mg/kg/day primarily, while amphotericin B colloid dispersion (ABCD) 3–5 mg/kg/day is no longer used much [28–30]. In a double-blind RCT comparing ABCD 6 mg/kg/day to AmB-d 1.0–1.5 mg/kg/day for the treatment of IA, those receiving ABCD had lower renal toxicity (25 vs. 49 %; $p=0.002$), with a longer median time to onset of nephrotoxicity (301 vs. 22 days; $p=0.001$), but efficacy was equivalent (therapeutic response: 52 vs. 51 %; $p=1.0$, and death due to IA: 32 vs. 26 %; $p=0.7$) [30]. Infusion-related toxicities due to ABCD were much higher (30 %), however, which has led to its disuse—favoring L-AmB and ABLC. In the AmBiLoad trial, Cornely et al. compared 3 and 10 mg/kg/day of L-AmB and found a comparable overall favorable response for IA (50 vs. 46 %; $p=0.65$) but greater nephrotoxicity and hypokalemia in the high-dose arm [31].

The different lipid formulations vary in their tissue distribution and toxicity, with L-AmB having a better therapeutic index while ABLC achieves higher lung and kidney tissue concentrations [32]. However, all three reach high concentrations in the liver, spleen, and other reticuloendothelial system organs including the bone marrow. Nonetheless, tissue penetration may be modified by site-specific inflammation. For instance, in a murine model of cerebral aspergillosis, investigators found intravenous L-AmB 3 mg/kg daily to be most efficacious in reducing brain colony forming unit burden with high localization to the capillary endothelium [33]. The efficacy of these lipid formulations in IA treatment is comparable, with approximately 40–50 % response rates, though head-to-head comparisons have been only done for febrile neutropenia. Nevertheless, certain species such as *A. terreus*, *A. nidulans*, and *A. ustus* may have intrinsic resistance to the polyenes [34–36].

The liposomal carrier may produce immunomodulatory activity that attenuates a dysregulated inflammatory response to IA by promoting non-oxidative leukocyte killing mechanisms [37]. In addition, L-AmB may facilitate transition from TLR-2 to TLR-4 pattern recognition by neutrophils with consequent balance away from pro-inflammatory cytokines such as TNF- α towards anti-inflammatory interleukin (IL)-10, but preserved polymorphonuclear (PMN) leukocyte phagocytosis ability and degranulation rather than radical oxidative induction [38].

Echinocandins

Echinocandins (caspofungin, micafungin, and anidulafungin) work by inhibiting primarily fungal β -1,3-glucan synthase, which makes a key component in the cell wall, but are fungistatic for *Aspergillus*—blunting hyphal growth with distended balloon-like tips [39]. In a non-comparative study by Maertens et al., caspofungin was found to have a 45 % favorable response in salvage IA therapy—the majority with refractory pulmonary disease [40]. Based on data such as this, caspofungin received approval for salvage but not primary IA therapy. Echinocandins may differ in their fungicidal and post-antifungal effects, with micafungin and anidulafungin having a two- to ten-fold lower minimum effective concentration (MEC)—“the lowest drug concentration at which small, rounded, and compact hyphal forms are observed” at 24–48 h incubation [41]—than caspofungin [42]. Among the TRANSNET cohort isolates, more than 95 % of isolates from proven or probable IA cases had echinocandin MECs less than the ECV, but there was inter-species variability; 17 % of *A. terreus* isolates had an MEC greater than the ECV [41].

All three echinocandins may have a role in empirical febrile neutropenia therapy among those with hematologic malignancy and recipients of stem cell and solid organ transplantation, though only caspofungin is licensed for this indication with comparable 34 % overall success to L-AmB 3 mg/kg/day [43, 44]. Micafungin has been shown to be comparable to caspofungin among an adult febrile neutropenic cohort in terms of deaths, breakthrough fungal infections, and adverse events, but the dose may need to be higher in the pediatric population [45, 46]. However, breakthrough IA in patients receiving caspofungin is increasingly being reported: 13 % in one cohort compared with none among those receiving amphotericin B [47]. A fourth echinocandin—aminocandin—is in development and has potent fungicidal *Aspergillus* activity in a murine model of disseminated disease, reducing organ burden and improving survival over other echinocandins and itraconazole [48].

Echinocandins are metabolized in the liver with a half-life of 9–26 h that permits once-daily dosing. However, they are highly protein bound (>95 %). Only intravenous formulations are made, permitting distribution to a wide variety of organs, including the brain, but low eye and cerebrospinal fluid (CSF) concentrations that preclude use in infections affecting these compartments [49]. Minor dose adjustments in hepatic insufficiency are only needed with caspofungin and micafungin but not anidulafungin, and only micafungin does not require a loading dose. Caspofungin may interact with calcineurin inhibitors reducing the tacrolimus area under the plasma concentration–time curve (AUC) levels by 20 %, but having 35 % increased AUC levels in the presence of cyclosporine.

Micafungin was found to have a dose-dependent anti-inflammatory effect on human polymorphonuclear leukocytes in the presence of *A. fumigatus* conidia in vivo through decreasing TNF- α and increasing IL-10 via the TLR-2/dectin-1 and TLR-3/TRIF (TIR-domain-containing adapter-inducing interferon- β) pathway signaling [50]. Echinocandins may unmask β -glucans to facilitate immune cell recognition by monocytes via pattern recognition receptors such as dectin-1 and modulate subsequent

inflammatory response due to TNF and chemokine ligand (CXCL)2 in a fungal growth stage-specific manner over time [51]. In addition, fungal wall re-modeling through compensatory cytoskeletal synthesis mechanisms such as Rho-1p—the regulatory subunit of the β -glucan synthase complex—that are triggered during fungal stress may lead to epitope modification and subsequent fungal clearance through undetermined mechanisms [52].

Combination antifungal therapy

Based upon in vitro and animal study data, several investigators noted the potential for improved outcomes for IA through synergistic or additive effects of drugs with complimentary mechanisms of action such as combining mold active triazoles or an amphotericin B product with an echinocandin [53, 54]. More recently, Elefanti et al. [55] performed serum inhibitory and fungicidal interaction studies against *Aspergillus* species by combining echinocandins with amphotericin B or voriconazole. The greatest additive inhibitory interactions were found with micafungin (3.6-fold) > anidulafungin (2.9-fold) > caspofungin (2-fold) and with *A. flavus* > *A. fumigatus* > *A. terreus*, but the lowest serum interaction indices were found with amphotericin B–caspofungin and *A. terreus* [55].

A few human observational studies and small-scale clinical trials have been published to support the use of such combination therapy for IA. In a retrospective cohort study, Kontoyiannis et al. demonstrated that L-AmB combined with caspofungin resulted in no significant difference in composite response among those who received it for primary versus refractory or intolerant-to-treatment IA (53 vs. 35 %; $p=0.36$) [56]. In a recently completed larger, randomized clinical trial comparing voriconazole and anidulafungin combination therapy to voriconazole alone for primary IA therapy, Marr et al. noted a marginal 12-week survival effect of combination versus monotherapy (95 % CI -21.4 to 1.09; $p=0.08$). Notably, global success favored monotherapy in this trial (95 % CI -21.6 to 1.15; $p=0.08$) [57••]. Given the difficulties (e.g., patient accrual) in completing this trial of primary IA treatment comparing modalities, the probability of a similar appropriately powered and comparative, double-blinded, multicenter trial for salvage therapy is low.

As a consequence, Panackal et al. [58••] performed a recent comprehensive systematic review and meta-analysis looking at a combination of mold-active triazoles or lipid amphotericin B with echinocandins versus monotherapy with such a triazole or a lipid amphotericin B. The rationale was that the three drug classes have comparable efficacies in the salvage IA setting (i.e., primarily refractory disease). We demonstrated that the conglomerate evidence suggests improved 12-week survival [Peto odds ratio (OR)=1.80 (95 % CI 1.08–3.01)] and success [Peto OR=2.17 (95 % CI 1.21–3.91)] with combination therapy by our fixed-effects model. This remained significant after applying a random-effects approach as a sensitivity analysis [12-week survival (Peto OR=1.90 [95 % CI 1.04–3.46] and unchanged value for success]. Restriction to high-quality studies and including echinocandins as the comparator for refractory IA revealed an adjusted OR of 1.72 (95 % CI 0.96–3.09; $p=0.07$) for global success while the survival endpoint remained unaltered. However, these findings should be interpreted with caution given the

inherent limitations of meta-analyses and applied only in certain clinical settings. Moreover, time-varying parameters, such as changes in conditioning regimens for HSCT resulting in improved patient care, may make our conclusions based on past studies yield uncertain applicability with future studies (i.e., a cohort effect) [58••].

Adjuvant immunotherapy/immunoprophylaxis

Growth factors and cytokines

Apart from the immunomodulatory properties of antifungals (especially L-AmB and echinocandins > triazoles), specific immunotherapy/immunoprophylaxis for IA has only been studied on a limited basis (Table 2). For example, myeloid growth factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) are mostly used to hasten cellular immune recovery in the susceptible host. Recombinant cytokines such as interferon (IFN)- γ may facilitate cell-mediated *Aspergillus* killing. G-CSF and IFN- γ have been shown to augment neutrophil oxidative burst by 24–75 and 52–96 %, respectively, regardless of serum opsonization of *Aspergillus* hyphae in vitro, and the effects may be additive; these cytokines caused 35–40 % hyphal damage alone, but nearly 50 % when combined [59].

Some animal studies combining amphotericin B, a mold-active triazole, or an echinocandin with G-CSF in murine models receiving immunosuppressive medications have shown differences in survival. For example, mice given intraperitoneal cyclophosphamide followed by intravenous *Aspergillus* conidia, which were subsequently given caspofungin and G-CSF with or without lipid amphotericin, had a 78.9 % improved survival rate—prolonging life by 25 days [60]. In contrast, as corticosteroids can lead to demargination of neutrophils and impaired chemotaxis, G-CSF has been reported to worsen lung abscesses by increasing fungal tissue burden and PMN leukocyte infiltrates and shorten lives of mice treated with posaconazole with corticosteroid-associated IA [61]. Therefore, differences between cytotoxic and anti-inflammatory chemotherapies may lead to a

Table 2. Adjunctive immunotherapy/immunoprophylaxis for invasive aspergillosis

Agent	Dose	Setting
G-CSF	5–6 $\mu\text{g}/\text{kg}/\text{day}$ SQ	High-risk febrile neutropenia, especially cyclical/congenital vs. cancer-related neutropenia; may prolong polymorphonuclear leukocyte survival
GM-CSF	4–5 $\mu\text{g}/\text{kg}/\text{day}$ SQ	High-risk febrile neutropenia, stimulating myeloid lineages; may improve IFI survival; capillary leak syndrome may occur
IFN- γ	50 $\mu\text{g}/\text{m}^2$ BSA SQ every other day	Chronic granulomatous disease prophylaxis; possibly adjunctive refractory mold therapy in cancer chemotherapy and stem cell transplant recipients (graft versus host disease caution)
Granulocyte transfusion	N/A	Profound neutropenia as a bridge to engraftment in HSCT or natural neutrophil recovery in other conditions (CMV donor/recipient discordance and possible acute injury caution)
Dendritic cell infusions	N/A	Experiment prophylaxis model

BSA body surface area, CMV cytomegalovirus, G-CSF granulocyte colony-stimulating factor, GM-CSF granulocyte macrophage colony-stimulating factor, HSCT hematopoietic stem cell transplant recipients, IFI invasive fungal infection, IFN- γ recombinant interferon-gamma-1b, N/A not applicable, SQ subcutaneous

differential response to G-CSF in murine models of IA.

However, in humans, two double-blinded RCTs of G-CSF demonstrated decreased duration of febrile neutropenia by approximately 15 % but did not decrease the risk of infections, including invasive fungal infections (IFI) in patients with AML [62, 63]. Based on such data, the American Society of Clinical Oncology and European Organization for Research and Treatment of Cancer recommend myeloid growth factors when the risk of febrile neutropenia is greater than 20 % [64]. Limited data exist favoring the use of G-CSF in IA. For instance, G-CSF was used in five neutropenic children with hematologic and solid tumor malignancies and IA along with L-AmB, with three surviving with or without surgery [65]. In an open, randomized clinical trial of amphotericin B deoxycholate (1 mg/kg/day) with G-CSF (3–5 µg/kg/day) compared with the polyene alone, among 29 neutropenic hematologic/bone marrow transplant patients with IFI, 62 % (95 % CI 43.3–80.9) receiving both had a favorable response compared with 32 % (95 % CI 15.4–51.2) with monotherapy ($p=0.03$), and the combination was found to be cost effective [66].

Corticosteroids disrupt nuclear translocation and activation of NF-κB, which interferes with macrophage functionality—another defense in the clearance of pulmonary *Aspergillus* conidia. GM-CSF degrades inhibitors of NF-κB, thereby restoring macrophage ability to lead to a pro-inflammatory response via the JAK2–STAT5–NF-κB pathway [67]. Although pulmonary alveolar macrophages can phagocytose and kill *Aspergillus* conidia, GM-CSF (and IFN-γ) may also augment superoxide hyphal killing by monocytes [68]. Nevertheless, in a retrospective human study of 66 neutropenic patients who received GM-CSF, Safdar et al. noted a 24 times higher odds of antifungal treatment failure in the 15 % receiving high-dose corticosteroids, but IFI-related deaths were significantly lower among GM-CSF responders (9 %) than in non-responders (94 %) ($p=0.0001$), based on clinical and/or radiographic progression. No serious toxicities were reported, though increased capillary permeability issues had been suggested previously [69].

IFN-γ augments neutrophil oxidative hyphal killing—preventing corticosteroid suppression of such activity—and improves monocyte conidiacidal ability [59, 68]. In 1999, recombinant IFN-γ received approval by the US FDA for prophylaxis against infections (not specifically IA) in CGD based on a randomized, double-blind, placebo-controlled study of 128 patients with CGD that showed a 67 % relative risk reduction in infection rate among recombinant IFN-γ recipients, but phagocyte function was not significantly altered [70]. The data for IFN-γ use in treating rather than preventing IFI, such as refractory IA, are scant. To illustrate, in a retrospective study of 32 HSCT patients, 26 with refractory IA received adjuvant recombinant IFN-γ-1b and antifungals (ABLC, voriconazole, posaconazole, and/or caspofungin)—the majority of whom had an ANC >100 cells/µL. Of these, the attributable IA mortality was 54 %, but four (36.3 %) of 11 with proven/probable IA, five (50 %) of ten with possible IA, and three (60 %) of five with disseminated IA responded. Although there have been concerns in using such pro-inflammatory cytokines in the setting of GVHD, only two (9.5 %) of the 21 with acute or chronic GVHD in this cohort had persistent GVHD [71]. Similarly, sporadic case reports of the successful adjuvant use of IFN-γ for IA in the CGD and HIV population have been mentioned [72–74].

Based on such reports and expert opinion, the Infectious Diseases Society of America (IDSA) has given a category B-III recommendation for G-CSF and GM-

CSF in high-risk neutropenic patients based on severity and duration, and IFN- γ in “non-neutropenic” patients with CGD as adjunctive therapy in IA [3••]. Larger multicenter RCTs taking such a multi-pronged approach to IA with G-CSF, GM-CSF, and IFN- γ are needed to determine their true therapeutic index among select patient populations with known immune deficits.

Granulocyte transfusions

Human donors primed with G-CSF have had subsequent granulocytes harvested and infused in the adjunctive management of a variety of refractory IFI, including fusariosis and IA. This has been investigated most often in profoundly neutropenic hosts, with favorable responses that extended 3–12 weeks after therapy [75, 76]. Such studies suggest that granulocyte transfusions should be reserved for persistent IFI in the setting of severe neutropenia, as a bridge to neutrophil recovery and not as a long-term solution. Moreover, caution is advisable as cytomegalovirus transmission and transfusion-related acute lung injury (TRALI) has been noted to occur, particularly if the product is not strictly screened [77, 78].

Dendritic cell infusions

Adoptive transfer of dendritic cells (DCs) pulsed ex vivo with *Aspergillus* conidia and RNA have shown promise as a vaccine in a murine allogeneic HSCT model at risk for IA. Bozza et al. [79] demonstrated that such DCs infused into the model produced antigen-specific T cells that produced IFN- γ [T helper-1 (Th1) skewed]. The efficacy of such pulsed DCs infused subcutaneously among mice subsequently administered intravenous *A. fumigatus* was significantly greater in terms of survival >60 days (95–100 vs. 0 %) and reduced organ burden compared with mice that did not receive the infusion, received unpulsed DCs, or received hyphal pulsed DCs. However, adoptive transfer of *Aspergillus*-specific Th1 cells did not produce the same survival effect. Interestingly, the immune response of similarly pulsed human DCs mirrored that of their murine counterpart [79]. Intriguing animal studies such as this suggest the need to study such therapy in primates prior to clinical investigation in humans [80].

Adjunctive surgery

In certain cases refractory to medical management, surgery may be necessary. For example, angioinvasion may lead to life-threatening hemoptysis that endoscopic embolization procedures may solve [81, 82]. In other situations, the extent of disease may be such that open surgical resection becomes necessary. To illustrate, in a retrospective study of pediatric oncology patients with IA, 24 surgical interventions ranging from pulmonary wedge resection to lobectomy or neurosurgical and abdominal surgeries for extrapulmonary cases were performed. The 3-month overall survival was 94.4 % (95 % CI 66.6–99.2), with minimal complications and half of these were still alive years later [83]. Similar results have been found in the hematologic malignancy adult population [84]. Based on such data, the IDSA has given a category B-II recommendation for adjunctive surgery in cases of IA where lesions are contiguous with the great vessels or pericardium, infiltrating the chest wall or pleural space, causing hemoptysis (e.g., single cavity), or in-

vading skin and soft tissues, bone, sinuses, or brain. In addition, surgery may be advised in single pulmonary lesion IA cases prior to HSCT or intensive chemotherapy, but clinical decision making should be individualized [3••].

Conclusion

To conclude, IA causes high mortality among mostly neutropenic hosts once infection is established. Early interventions such as empiric and pre-emptive strategies may assist in ameliorating outcomes when applied in a risk-stratified manner. Voriconazole is the drug of choice for primary IA, but other triazoles, lipid amphotericin B formulations, and echinocandins may have a role in empiric and salvage therapy for IA in certain situations. Combination therapy based on synergism between antifungals that work on the fungal cell membrane (i.e., mold active triazoles and amphotericin B preparations) and those that disrupt the fungal cell wall (i.e., echinocandins) may have a role in refractory IA, but improvements in practice over time—such as less toxic but equally efficacious conditioning regimens in HSCT—may impact this approach in the future. The role of immunotherapy for adjunctive management is less well-defined and larger clinical trials are needed to determine its utility. Surgery is a last resort when disease progresses despite medical therapy, or if life-threatening invasive manifestations occur. Finally, recovery of neutrophil numbers and functioning ultimately dictates the likelihood for successful outcomes.

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Compliance with Ethics Guidelines

Conflict of Interest

Peter Williamson declares no conflicts of interest.

Anil Panackal is employed at the NIH and has stock in AMCAP.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:

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