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Current and Future Therapeutic Options in the Management of Invasive Aspergillosis

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Abstract

The past decade has witnessed significant progress in the management of invasive aspergillosis. Potent, relatively non-toxic antifungal drugs, data on early chest CT scanning and the availability of a non-invasive diagnostic test (serum galactomannan) are the key advances; among these, the contribution of the recently available drugs is the most significant. Safer and earlier intervention resulting in reduced mortality and improved outcome is being demonstrated. Newer strategies enable clinicians to provide drug therapy in a highly targeted manner, such that empirical use of antifungal drugs may decline. Voriconazole has become the drug of choice for primary therapy, while posaconazole shows

promise as a prophylactic drug. Echinocandins are effective for salvage therapy and are under evaluation for primary therapy. Preliminary data for efficacy of combination therapy with a mould-active azole plus an echinocandin are of promise and clinical trials are under way. Reports of emergence of less-susceptible *Aspergillus* spp. during azole therapy are of concern and close monitoring is needed. Remarkably, the era of polyenes appears to be nearing the end in the therapy of invasive aspergillosis. The promise of newer classes of drugs, immune-modulating therapies and vaccines are exciting future additions to the arsenal against invasive aspergillosis.

Aspergillosis comprises a wide variety of manifestations, the principal entities being acute invasive forms, notably pulmonary disease with or without dissemination, and chronic forms, namely chronic necrotizing aspergillosis, pulmonary/sinus aspergilloma (fungal balls) and allergic bronchopulmonary aspergillosis. Although several species of *Aspergillus* have been reported as human pathogens, *A. fumigatus* is the most common aetiological agent of aspergillosis followed by *A. flavus* (table I);[1] the organs primarily affected being the lungs and the sinuses. The most devastating infection in immunocompromised (cancer/transplant) patients is the invasive form with a 357% increase in mortality rates reported in the US between 1980 and 1997.[2] This trend is probably related to the increased number of individuals at risk of the infection as a result of advances in modern medicine, such as bone marrow and solid organ transplantation, and prolonged survival of critically ill and susceptible patients. In addition, the aging of the population has increased the number of susceptible individuals.

Table I. Characteristics of various pathogenic *Aspergillus* spp.

Species	Frequency in clinical infection (%)	Clinical significance
A. fumigatus	65	Most common cause of IPA. Resistance to triazoles reported
A. flavus	14	Frequent cause of sinusitis and skin infections. Resistance to triazoles reported
A. niger	5	Role in invasive infections less well established. Less pathogenic probably because larger conidia do not reach the alveoli
A. terreus	5	Usually susceptible to triazoles. Increasing reports of resistance to amphotericin B. Blood cultures may be positive
A. ustus	1	Intrinsically higher MIC values (resistance) to triazoles and polyenes

IPA = invasive pulmonary aspergillosis; **MIC** = minimum inhibitory concentration.

The number of patients undergoing transplantation has vastly increased in recent years. Transplant recipients are among the most significant subgroups of immunosuppressed hosts at risk for invasive aspergillosis.[2-4] Transplantation practices, immunosuppressive regimens and the characteristics of patients undergoing transplantation have continued to evolve. Current data show that invasive aspergillosis in stem cell recipients now predominantly occurs late after engraftment in non-neutropenic patients in whom graft-versus-host disease (GVHD) and its management with increasingly intense immunosuppression have emerged as major risk factors.[5] Despite the heightened awareness of the profiles of patients at risk for invasive aspergillosis and a number of therapeutic options today (table II),[6] the mortality rate remains high. High mortality from invasive aspergillosis has been due to compromised host immunity, delayed diagnosis and the limited availability of safe and effective antifungal drugs.[7]

1. Diagnosis of Invasive Pulmonary Aspergillosis

This section is a brief overview of the recent advances in the diagnostic techniques of invasive aspergillosis. For a detailed discussion, the reader is referred to a review by Hope et al.[8]

Table II. An overview of antifungal drugs approved for invasive aspergillosis[6]

Drug	Absorption/protein binding	Metabolism	Mechanism of action
Triazoles			
Voriconazole	Bioavailability 96%; food ↓ absorption; not pH dependent; ≈60% protein binding Good CNS concentrations	CYP3A4, CYP2C9/19 Excreted in bile and stools	Inhibit lanosterol demethylase
Posaconazole	No IV formulation; food with fat ↑ absorption; not pH dependent; bioavailability 96%; ≈98% protein binding Good CNS concentrations	CYP3A4 only Excreted in bile and stools	
Itraconazole	IV not pH dependent Oral pH dependent; food ↑ absorption; bioavailability 55%; ≈99.8% protein binding Minimal CNS concentrations	CYP3A4 only Excreted in bile and stools	
Echinocandins			
Caspofungin	Significant protein binding Minimal to undetectable CNS concentrations Tissue concentrations are unknown	No CYP metabolism N-acetylation in liver Eliminated in bile and stools (35%), urine (41%)	Inhibit 1,3-β-D-glucan
Micafungin		Hepatic-aryl sulfatase, COMT and hydroxylation Eliminated in stools	
Anidulafungin		Chemical degradation to a ring-opened peptide that lacks antifungal activity	
Polyenes			
AMB deoxycholate AMB colloidal dispersion Liposomal AMB AMB lipid complex	AMB deoxycholate and liposomal AMB have significant protein binding CNS concentrations of liposomal AMB are more than deoxycholate, lipid complex and colloidal dispersion AMB Peak concentrations in liver, spleen and lung Excreted in urine and stools	No CYP metabolism Metabolism pathway unclear	Bind to ergosterol resulting in membrane pores

AMB = amphotericin B; **COMT** = catechol-O-methyl transferase; **CYP** = cytochrome P450 enzyme; **IV** = intravenous; ↑ indicates increase; ↓ indicates decrease.

The lack of reliable and non-invasive diagnostic procedures remains a major obstacle in the successful early intervention of invasive pulmonary aspergillosis. Clinical signs and symptoms are non-specific, culture and microscopy of lower respiratory tract specimens have a low sensitivity, and tissue for histopathological examination is not easy to obtain because of the frequent presence of thrombocytopenia and coagulation abnormalities. Thus, most clinical cases of invasive pulmonary aspergillosis are classified as possible or probable infections in view of the diagnostic difficulty. In recent years, efforts have been directed towards identifying non-invasive markers for rapid and reliable diagnosis of invasive aspergillosis. In particular, tests based on identifying fungal antigens or metabolites released into the circulation have become available.

Galactomannan is a polysaccharide cell wall component of *Aspergillus* spp. that is released into the circulation during fungal growth in the tissues.[9] The double-sandwich, enzyme-linked immunosorbent assay, which can detect galactomannan, is a useful tool for the early diagnosis of *Aspergillus* infection. Studies evaluating the role of galactomannan assay in the diagnosis of invasive aspergillosis have largely been conducted with patients undergoing cancer chemotherapy or haematopoietic stem cell transplantation (HSCT) recipients. In these patients, a sensitivity of 67–100% and a specificity of 86–98.8% has been documented. When serially monitored, the galactomannan test preceded the diagnosis of invasive aspergillosis by an average of 6–14 days. False positives with this assay are not

uncommon; cross-reactivity of Platelia™ [1] *Aspergillus* galactomannan enzyme immunoassay (EIA) with *Penicillium* spp. and bacteria, such as *Bifidobacterium* spp., has been noted but is deemed to be of little clinical relevance as these are rarely pathogens in humans.[5,10] The use of antifungal agents may lower the sensitivity of the galactomannan assay by decreasing the fungal load. Use of piperacillin/tazobactam and amoxicillin/clavulanic acid may result in a false-positive test for galactomannan.[11] The timing of collection of the sample may influence the test results, with false-positive results being less likely in samples collected at trough concentrations or prior to the administration of the dose. Despite the shortcomings of the test, galactomannan antigen testing is under active scrutiny for the diagnosis of invasive aspergillosis, especially in the transplant population.

1,3-β-D-Glucan is an integral component of the cell walls of a number of pathogenic yeasts and filamentous fungi. In addition to aspergillosis and candidiasis, it may be detected in infections caused by less common fungi (e.g. *Fusarium*, *Trichosporon*, *Acremonium* and *Saccharomyces* spp.). The sensitivity and specificity of the test has ranged from 67% to 100% and 84% to 100%, respectively.[10]

Polymerase chain reaction (PCR)-based molecular diagnostic tests for *Aspergillus* are not commercially available and remain largely nonstandardized. Such assays, when performed on blood or bronchoalveolar lavage samples, have shown a negative predictive value for invasive aspergillosis ranging from 92% to 99%. PCR results are usually positive when the galactomannan assay is highly positive; 12 of 20 PCR assays that yielded a positive result were observed in association with high galactomannan values.[12]

A prospective comparison of real-time PCR, galactomannan and 1,3-β-D-glucan assays as weekly screening for invasive aspergillosis in patients with haematological disorders showed that the galactomannan test was relatively more sensitive in predicting the diagnosis.[12]

Chest radiographic findings are not sensitive and a high-resolution chest CT scan is the preferred method to detect early changes of invasive pulmonary aspergillosis; radiographic contrast is not required. However, the radiological signs (halo sign and air crescent sign) are not specific and other entities may mimic invasive pulmonary aspergillosis.[13]

2. Management

2.1 Definitive Therapy

The drugs currently approved for the primary management of invasive aspergillosis include amphotericin B deoxycholate (conventional formulation) and voriconazole;[14-16] echinocandins are also under investigation[17] (table II). Amphotericin B has been the gold standard for the treatment of invasive aspergillosis for many decades; however, its toxicities, particularly renal toxicity, are well known. The therapeutic advantages of amphotericin B include excellent fungicidal activity, rapid clearance of organisms from tissue and the paucity of emergence of resistant organisms.[6]

The landmark study that led to the approval of voriconazole for the primary treatment of invasive aspergillosis was published in 2002 by Herbrecht et al.[18] The study was performed in patients with haematological malignancy, including those who underwent HSCT. This randomized, open-label trial evaluated 144 patients in the voriconazole group and 133 patients in the amphotericin B deoxycholate group. Patients were followed for a period of 12 weeks; at the end of the study period, the clinical response rate was 52.8% among voriconazole recipients (complete response in 20.8% and partial response in 31.9%) and 31.6% among amphotericin B recipients (complete response in 16.5% and partial response in 15%). There was a significant difference in drug adherence between the two groups; 62 of 144 patients in the voriconazole arm continued to take the drug, compared with only 2 of 133 patients in the amphotericin B arm, reflecting poor tolerance

1 The use of trade names is for identification purposes only and does not imply endorsement.

to the latter drug. The difference in survival rate at the end of 12 weeks was statistically significantly between the two groups, with 71% and 58% surviving in the voriconazole and amphotericin B arms, respectively. Similar results were observed in the intent-to-treat population that included all randomized patients. Since the publication of this study, voriconazole is widely accepted as the drug of first choice for the treatment of invasive aspergillosis.

Whether initial therapy with higher than standard doses (3–5 mg/kg) of a lipid form of amphotericin B would have a better outcome has been debated. A recent, randomized, double-blind, prospective trial (AmBiLoad Trial) compared the safety and efficacy of a high-dose regimen of liposomal amphotericin B versus the standard dose as initial therapy for invasive aspergillosis. The standard-dose group received 3 mg/kg/day and the high-dose group received 10 mg/kg/day of liposomal amphotericin B for 14 days, followed by 3 mg/kg/day in both groups. The favourable response to treatment at 12 weeks was similar in both study groups: 50% with the standard dose and 46% with the high dose. Survival rate at 12 weeks was similar as well, with 72% and 59% with the standard-dose and high dose-regimens, respectively. Nephrotoxicity occurred in 14% of the standard-dose group versus 31% of the high-dose group ($p < 0.02$). The authors concluded that there was no beneficial effect with administration of a high loading dose of liposomal amphotericin B and, in fact, the high dose was associated with an increased risk of nephrotoxicity.[19]

In an analysis of 85 allogeneic HSCT recipients (78% with invasive aspergillosis) treated with amphotericin B lipid complex, the overall response rate was 41%, with a 44% response in patients with GVHD.[20] These results are comparable to those achieved with other available drugs.

With its better tolerability, voriconazole is preferred to amphotericin B or its lipid formulations.[21] It is to be noted that the efficacy of voriconazole has not been compared with that of a lipid formulation of amphotericin B and such a study is unlikely to be performed. Amphotericin B, either conventional or a lipid formulation, may be preferred to voriconazole in certain situations as follows: (i) previous use of a mould-active drug for prophylaxis or empiric therapy; (ii) concomitant use of drugs with major interactions with voriconazole, such as sirolimus, rifampin or warfarin; (iii) presence of significant hepatic impairment; (iv) high suspicion for zygomycosis; or (v) the presence of cardiac risk factors such as prolonged QT interval and cardiomyopathy.

Echinocandins have undergone very limited evaluation as first-line therapy for invasive aspergillosis. The study by Candoni et al.[17] reported 32 patients with haematological malignancies who received caspofungin as primary or first-line therapy for probable or proven aspergillosis. Most patients (97%) were neutropenic and had pulmonary localization. All patients with neutropenia (97%) received granulocyte colony-stimulating factor (G-CSF) in addition to caspofungin. The overall response rate was 56%, with neutrophil recovery and stable haematological disease (remission) associated with a favourable response rate. A small proportion of patients who had partial response were rescued with voriconazole. The second study by Denning et al.[14] evaluated the efficacy of micafungin alone or in combination with other antifungal agents. Although there were 331 patients enrolled in the study, the group that received micafungin alone was small (23 patients) and the response rate was 50% in that group. Although echinocandins appear promising, monotherapy with echinocandins for invasive aspergillosis is not recommended pending data from definitive studies.

With the established diagnosis of invasive aspergillosis, primary therapy must be initiated with voriconazole. With clinical and radiological improvement, therapy is continued for a period of at least 3 months. Lack of response or progression of infection could be secondary to host factors or drug failure. If drug failure is suspected, switching to a different class of antifungal agents or addition of a second agent (i.e. echinocandin) has become a common clinical practice. In situations where there is a concern with the pharmacokinetics (specifically, drug absorption), the drug concentration should be

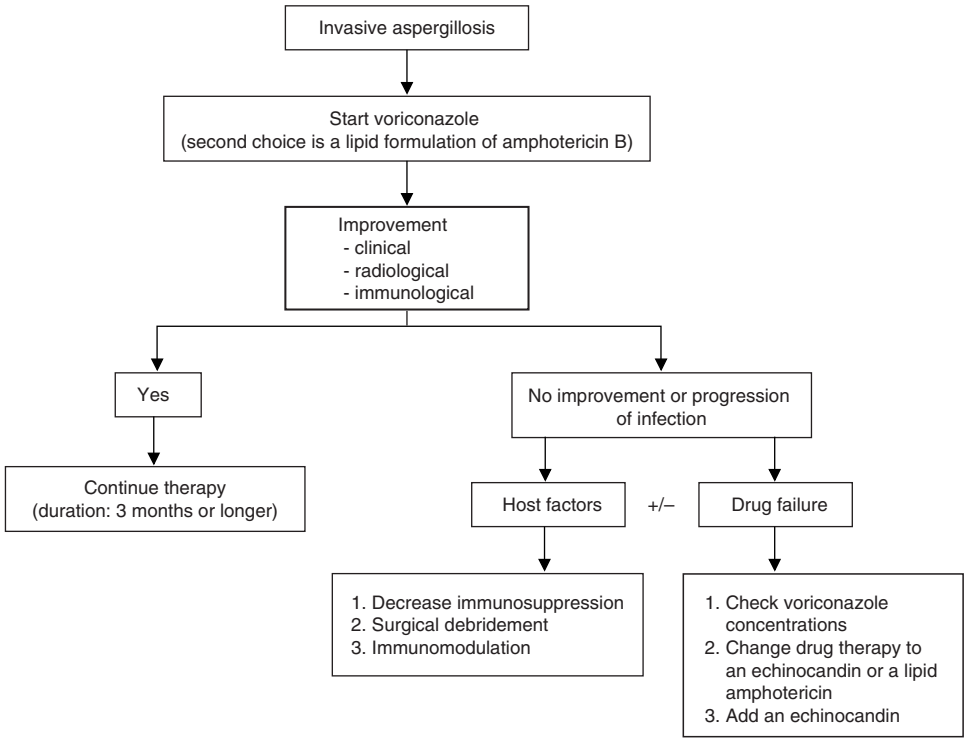

Fig. 1. An algorithm for the clinical management of invasive aspergillosis.

obtained and the dose of voriconazole adjusted accordingly (figure 1).

2.2 Prophylactic Therapy

Institution of therapy for aspergillosis in a high-risk patient without any clinical and/or radiological evidence or history of aspergillosis is considered primary prophylaxis. Several factors should be considered prior to initiating prophylactic therapy, including the frequency and outcome of infection, difficulty with early diagnosis, drug efficacy, drug interactions, safety and cost. The antifungal drugs that may be effective as prophylaxis against invasive aspergillosis include itraconazole, voriconazole, posaconazole, conventional or lipid formulations of amphotericin B, caspofungin, micafungin and anidulafungin (table II).[6]

Mattiuzzi et al.[22] performed a randomized, open-label trial to compare the efficacy and safety of liposomal amphotericin B versus an unusual combination (fluconazole plus itraconazole) as prophylaxis against invasive fungal infections in patients undergoing induction chemotherapy for newly diagnosed acute myelogenous leukaemia or myelodysplastic syndrome. A total of 49% of the liposomal amphotericin B group and 48% of the fluconazole plus itraconazole group completed prophylactic therapy and the azole combination, as expected, had a better tolerability profile. There was no difference observed in the incidence of infection or the mortality rates between the two groups. The concept of using two azoles simultaneously for prophylaxis may not be appealing to most clinicians.

A meta-analysis of 13 randomized controlled trials compared the efficacy of itraconazole versus fluconazole for the primary prophylaxis of invasive fungal infections in neutropenic patients with haematological malignancies. The study assessed 3597 patients and concluded that itraconazole significantly decreased the incidence of and mortality from invasive fungal infections. A statistically sig-

nificant reduction (48 ± 21%) in the incidence of invasive aspergillosis was observed in the itraconazole (solution) group. Importantly, the authors noted that the effect of prophylaxis was dependent on the pharmacokinetics and oral bioavailability of itraconazole.[23] The solution is preferred to the capsule formulation and the frequent drug-drug interactions with itraconazole make it a not-so-desirable drug.[6]

Two clinical trials have compared the prophylactic efficacy of fluconazole versus itraconazole against invasive fungal infections in patients undergoing allogeneic HSCT. The earlier study by Winston and colleagues[24] involved 138 patients who received either itraconazole or fluconazole prophylaxis and were followed for a period of 180 days post-HSCT. In this relatively small study, itraconazole prevented more invasive fungal infections than fluconazole, probably because of the efficacy of the former drug against invasive moulds. The second trial by Marr et al.[25] found no difference between the efficacies of fluconazole and itraconazole during the study period; however, in a subset of patients who tolerated itraconazole, fewer patients developed invasive mould infection while receiving prophylaxis. The trial had to be terminated because of the high incidence of adverse effects (renal and hepatic toxicities) reported in the itraconazole group, which were perhaps due to a larger than standard dose of itraconazole (200 mg three times daily) that was administered.

Recently, Cornely and colleagues[26] compared the prophylactic efficacy of posaconazole and either fluconazole or itraconazole in patients with prolonged neutropenia in the setting of acute myelogenous leukaemia or myelodysplastic syndrome. In this randomized multicentre study, a total of 304 patients received posaconazole and 298 patients received fluconazole (240 patients) or itraconazole (58 patients). Proven or probable invasive fungal infections were reported in 7 patients (2%) in the posaconazole group and in 25 patients (8%) in the fluconazole or itraconazole group; the difference was statistically significant. Posaconazole reduced the number of invasive fungal infections and improved survival, with an all-cause mortality rate of 16% in the posaconazole group versus 22% in the standard azole group ($p = 0.048$). Posaconazole was superior to fluconazole and itraconazole as prophylaxis treatment against invasive aspergillosis and was as well tolerated as the other two drugs.

As GVHD is a significant risk factor for invasive aspergillosis in HSCT recipients, Ullmann et al.,[27] in an randomized, double-blind international trial, compared the prophylactic efficacy of posaconazole (301 patients) versus fluconazole (299 patients) in this population. At the end of the fixed 112-day treatment period, posaconazole was found to be as effective as fluconazole in preventing all invasive fungal infections (5.3% vs 9%; $p = 0.07$) but was superior to fluconazole in the prevention of invasive aspergillosis (2.3% vs 7%; $p = 0.006$) and was associated with a lower incidence of breakthrough fungal infections (2.4% vs 7.6%; $p = 0.004$), particularly invasive aspergillosis (1% vs 5.9%; $p = 0.001$).

In summary, while itraconazole has lost its appeal in view of its unfavourable adverse effect profile, posaconazole appears to be an effective prophylactic drug in high-risk patients and data for voriconazole are awaited. Lack of an intravenous formulation of posaconazole at the present time is a drawback, particularly in cancer and transplant patients with gastrointestinal abnormalities. Additionally, drug interactions involving azoles remain a significant concern.

Echinocandins are attractive agents for prophylaxis, as they have good anti-*Aspergillus* activity and have an excellent adverse effect profile with minimal drug interactions. Caspofungin has been evaluated as a prophylactic agent in comparison with intravenous itraconazole in patients undergoing induction chemotherapy for high-grade myelodysplastic syndrome or acute myelogenous leukaemia. There was no significant difference in the incidence of invasive fungal infections in the two groups (5 of 86 in the itraconazole group and 7 of 106 in the caspofungin group).[28] Van Burik et al.[29] compared the prophylactic efficacy of micafungin with fluconazole against invasive fungal infections during pre-engraftment neutropenia in patients undergoing

HSCT. Both drugs had comparable efficacy in the prevention of candida infection. However, there were few patients with invasive aspergillosis because the study was conducted during the relatively short pre-engraftment neutropenic period. The disadvantages of echinocandins include the lack of an oral formulation and their relatively narrow spectrum of activity.

There are no data addressing the issue of how long the prophylaxis should continue and so clinicians frequently opt to provide chemoprophylaxis during long periods of intense immunosuppression (e.g. neutropenia or GVHD treated with high-dose corticosteroids).

The term 'secondary prophylaxis' is used when the drug is administered to patients previously treated for invasive aspergillosis but who are still at risk because of continued immunosuppression. A common example is a patient who has a history of aspergillosis and is receiving reinduction chemotherapy for relapsed leukaemia. Voriconazole has largely replaced the use of itraconazole or a polyene for secondary prophylaxis. A retrospective review by Martino et al.[30] analysed the outcome of 129 patients with a history of invasive aspergillosis who underwent allogeneic HSCT, of whom 57 (44%) received a reduced-intensity conditioning. Overall, 27 patients with invasive aspergillosis relapsed or recurred after the allogeneic HSCT (cumulative incidence at 2 years of 22%). Antifungal drugs used for prior therapy included liposomal amphotericin B, amphotericin B lipid complex, caspofungin and voriconazole. Since several different strategies of drug therapy were used for secondary antifungal prophylaxis during conditioning and post-transplantation follow-up, it was difficult to identify differences among specific strategies. However, a subset analysis showed that the use of voriconazole for secondary prophylaxis reduced the risk of progression of invasive aspergillosis (12% in 31 voriconazole recipients compared with 22% in the remaining 98 patients; $p < 0.15$).

2.3 Empirical Antifungal Therapy

Empirical treatment is antifungal therapy provided to high-risk patients with signs and symptoms suggestive of invasive fungal infection, such as patients with neutropenia and fever unresponsive to broad-spectrum antibacterials. The difficulties associated with the early diagnosis of invasive fungal infections led to the introduction of empirical antifungal therapy in the early 1980s. The scientific rationale for such treatment was based on two prospective randomized trials. The first trial, with a modest number of patients, concluded that empirical antifungal therapy resulted in a lower incidence of invasive fungal infections, particularly invasive candidiasis.[31] The second larger EORTC (European Organisation for Research and Treatment of Cancer) trial assessed the role of adding empirical antifungal therapy to antibacterial therapy after 4 days of persistent febrile neutropenia in 132 patients. The clinical response rate in the amphotericin B arm was 69% versus 53% in the control group. There were six (9%) documented fungal infections in the control group and one (1%) in the amphotericin B group. No fungal-related deaths occurred in the amphotericin B group compared with four (6%) in the control group ($p = 0.05$).[32]

Although the emphasis in these trials was on invasive candidiasis, they paved the way for further studies in empirical therapy for invasive aspergillosis.[33] Subsequently, there have been several trials that have evaluated empirical therapy with different antifungal drugs. Either liposomal or conventional amphotericin B was compared with different antifungal drugs: fluconazole,[34-37] itraconazole,[38] voriconazole[39-41] or caspofungin.[42,43] Most trials have used composite endpoints. Excluding the toxicities associated with the conventional amphotericin B preparation, similar outcomes were observed with liposomal or conventional amphotericin B with respect to clinical response, survival and the number of cases of breakthrough aspergillosis. Itraconazole was found to be as effective as amphotericin B when used for empirical therapy in patients with cancer and persistent febrile neutropenia, although significant nephrotoxicity was seen in the

latter group.[38] More recently, voriconazole failed to meet the pre-specified non-inferiority criteria in a prospective, randomized, open-label, multicentre, international trial, when compared with liposomal amphotericin B. When defervescence, a less sensitive endpoint, was removed from the composite endpoint, results with voriconazole were improved.[39] Walsh et al.[42] have showed caspofungin to be as effective and better tolerated than liposomal amphotericin B for empirical therapy of patients with persistent febrile neutropenia. Importantly, a meta-analysis of 24 randomized trials published in 1997 has questioned the role of empirical antifungal therapy in the management of patients with persistent febrile neutropenia.[44]

It is clear that antifungal drugs, when given empirically, are frequently administered to a large number of patients with no fungal infection. With the availability of non-invasive diagnostic markers (e.g. chest CT scan and serum galactomannan antigen test), it is anticipated that the high-risk patients can be better identified and hence empirical therapy may decline in clinical practice.

2.4 Pre-Emptive Therapy

Pre-emptive therapy for invasive aspergillosis is gaining momentum with the availability of the galactomannan antigen test for invasive aspergillosis. This strategy is a risk-based intervention for high-risk patients with persistent febrile neutropenia plus the presence of other evidence of invasive fungal infection, such as positive surveillance cultures, radiological features or a positive galactomannan antigen test.[45] The concept of pre-emptive therapy for invasive aspergillosis is akin to the utilization of antigen/PCR measurement for cytomegalovirus (CMV) infection in transplant patients. On the basis of the currently available diagnostic tests, pre-emptive therapy is considered appropriate in the setting of compatible radiological findings and antigen tests in high-risk patients. However, the false positives/negatives associated with the galactomannan antigen test and the non-standardized PCR techniques make it difficult for a reliably uniform pre-emptive approach.[8]

A recent study by Maertens and colleagues[46] assessed the value of pre-emptive therapy in patients with acute leukaemia or allogeneic HSCT and persistent febrile neutropenia, based on serum galactomannan antigen test results. A total of 88 patients received prophylactic therapy with fluconazole and were followed with daily serum galactomannan testing and thorax CT scans if needed. Liposomal amphotericin B was instituted as pre-emptive therapy in patients with positive galactomannan tests or compatible radiological findings with culture or histopathological confirmation. A total of 19 patients had proven or probable invasive aspergillosis. Overall, 35% of patients met criteria for empirical therapy (i.e. persistent febrile neutropenia), whereas only 7% of patients received therapy based on the pre-emptive strategy; there was a 78% reduction in the use of antifungal agents between the two strategies. It is noteworthy that the galactomannan antigen test could be falsely negative in the presence of circulating *Aspergillus* antibodies or in patients receiving anti-*Aspergillus* drugs for prophylactic or empirical therapy; the test could be falsely positive in patients receiving β-lactam drugs (e.g. piperacillin/tazobactam).[11] Lin et al.[47] used a PCR screening assay with panfungal primers for the early detection of invasive fungal infections and therapy with amphotericin B was instituted only in patients with two positive PCR results. The authors showed that a PCR-based therapeutic approach reduced mortality in cancer patients with febrile neutropenia and fungal infections. Although employing PCR tests for pre-emptive strategies appears promising, the tests are not standardized and are not yet commercially available.

Chest CT scans play a critical role in the early diagnosis of invasive pulmonary aspergillosis. Greene et al.[48] evaluated baseline chest CT scan findings in 235 patients with invasive pulmonary aspergillosis. The authors compared the response to treatment and survival after 12 weeks of treatment in 143 patients with a halo sign (a feature of early infection) to 79 patients with other CT findings. The former group had a significantly better response to treatment (52% vs 29%; $p < 0.001$) and improved

survival (71% vs 53%; p = 0.01). Thus, early initiation of antifungal treatment on the basis of identification of a halo sign on the chest CT scan was associated with a better outcome. Also, a prospective review of the baseline imaging and treatment response of 343 immunocompromised patients reported that patients at risk for developing invasive pulmonary aspergillosis and presenting with compatible radiological findings benefit from early antifungal therapy.[49] One study noted that detection of serum galactomannan antigen by EIA did not precede detection of compatible lesions on a thoracic CT scan.[50]

Since daily galactomannan testing may not be a practical approach, Stephanie et al.[45] have proposed determining serum galactomannan levels at the time of onset of fever, repeated after 72 hours with a chest CT scan if fever persists and then twice weekly thereafter.

On the basis of data from these studies, chest CT scans and serum galactomannan antigen tests are being increasingly utilized in pre-emptive strategies for the management of invasive aspergillosis. There is considerable overlap between the empirical and pre-emptive strategies. As non-invasive diagnostic tools become refined and more reliable for early diagnosis, the empirical approach may be replaced by pre-emptive therapy.

2.5 Combination Therapy

In recent years, compared with amphotericin B, voriconazole has contributed to improved outcomes in invasive aspergillosis. Nevertheless, therapeutic success rates continue to be suboptimal, particularly in stem cell recipients. In a further attempt to improve outcome, based on data from *in vitro* and animal studies, several antifungal drug combinations have been tried with variable success.[51,52] In the past, the concurrent use of amphotericin B and an azole elicited controversy, given the potential antifungal antagonism. The introduction of the echinocandin class of drugs with a different target site has invigorated the prospects of combination therapy. There are *in vitro* and animal data to show that echinocandins may provide additive or synergistic activity in combination with triazoles.[53,54]

The combination of voriconazole and caspofungin (n = 40) as primary therapy for invasive aspergillosis in solid organ transplant recipients was investigated by Singh et al.[52] in a prospective multicentre study. The authors reported that combination therapy was independently associated with an improved 90-day survival, particularly in patients with renal failure or *A. fumigatus* infection.

Marr et al.[55] conducted a retrospective study of 47 patients with invasive pulmonary aspergillosis after HSCT and chemotherapy. Most patients in this study received amphotericin B as primary therapy and were switched to either voriconazole alone or a combination of voriconazole plus caspofungin as salvage therapy. The mortality rate in patients who received the combination was lower than that in the monotherapy group. At present, the sequential addition of an echinocandin is being increasingly used as a salvage strategy.

The pilot Combistrat trial (n = 30 patients) studied the efficacy of the combination of caspofungin plus liposomal amphotericin B (3 mg/kg/day) versus monotherapy with high-dose liposomal amphotericin B (10 mg/kg/day) as primary therapy for invasive aspergillosis. At the end of treatment period, the favourable overall response rate was 67% for the combination versus a relatively low 27% for the high-dose liposomal amphotericin B group (p = 0.028). The study concluded that the combination of caspofungin and liposomal amphotericin B could be more efficacious in high-risk patients with haematological malignancies. As this was a phase IV clinical trial, carefully controlled and well designed randomized clinical trials are needed before any firm recommendations are made.[56]

Another echinocandin, micafungin, was evaluated in two- and three-drug combinations with amphotericin B and azoles as salvage therapy. The combination was found to be well tolerated and effective for refractory aspergillosis in bone marrow transplant patients.[57]

At present, for patients with refractory/progressive aspergillosis, the addition of a second agent (an

echinocandin) may be reasonable (figure 1). In practice, an azole plus an echinocandin as initial therapy in seriously ill patients is becoming common place in the absence of evidence based data. As the two drug classes target entirely different sites, potential synergism is a reasonable expectation. However, as drug-related toxicities and cost may negate the potential clinical benefits, a randomized trial for primary therapy comparing a single drug (triazole) to a combination (triazole plus echinocandin) of drugs needs to be performed.

2.6 Salvage Therapy

Lipid amphotericin B formulations and caspofungin have been approved by the US FDA for salvage therapy of invasive aspergillosis. The lipid amphotericin formulations maintain a broad spectrum of antifungal activity with fewer infusion related toxicities. However, the EORTC group[16] demonstrated that the initial choice of antifungal therapy is critical to a successful outcome. In their study of 139 patients, patients received initial therapy with either voriconazole or amphotericin B deoxycholate and were switched to other licensed therapies as needed. The overall response rate was 30% in the amphotericin B group and 55% in the voriconazole group, independent of the salvage therapy that was administered.

The efficacy of caspofungin as salvage therapy was evaluated in an open-label, non-comparative, multicentre trial that demonstrated a 45% favourable response to caspofungin therapy.[58] This led to the use of caspofungin as salvage therapy for invasive aspergillosis in North America and Europe. Echinocandin as initial monotherapy for invasive aspergillosis is under evaluation. In addition, it is unclear whether higher than 'standard doses' of echinocandin may have improved efficacy against invasive aspergillosis.

In an open-label, multicentre study, Walsh et al.[59] investigated the efficacy and safety of posaconazole in patients with invasive aspergillosis and other mycoses who were refractory to or intolerant of conventional antifungal therapy. The study included 107 posaconazole recipients and 86 control subjects. The overall success rate was 42% for the posaconazole group and 26% for the control subjects ($p = 0.006$). This benefit was observed as early as 30 days into salvage therapy and continued to the end of the study; survival advantage was also noted with posaconazole. Perfect et al.[60,61] studied the use of voriconazole as salvage therapy for refractory fungal infections, including invasive aspergillosis. The efficacy rate of voriconazole for invasive aspergillosis was 43.7%; the drug was well tolerated and treatment-related discontinuation occurred in <10% of patients.

Salvage therapy is generally instituted at the time of clinical failure of initial therapy. Studies of salvage therapy show an ≈40% response rate regardless of the second drug type (40% rule). As physicians become desperate because of clinical failure, the addition of one, two or more drugs to existing therapy commonly occurs. Besides drug failure, other possible causes for therapeutic failure need to be evaluated and managed accordingly (figure 1).

2.7 Role of Surgery

The indications for surgery in invasive aspergillosis include infected catheters, infection of skin and soft tissue, chest wall or pericardial invasion, haemoptysis from a single pulmonary lesion, selected cases of invasive sinusitis, amenable solitary CNS lesions, endocarditis and osteomyelitis. Besides lobectomy, open-wedge resection and video-assisted thoracoscopic surgical interventions are also feasible.[62] In a retrospective analysis comparing medical and surgical treatment, lung resection was associated with a better survival rate and reduced mortality, especially for solitary pulmonary lesions.[63] Lung resection should be considered in patients with clinical and radiological signs of solitary/localized lesions with severe haemoptysis.[64]

3. Future Approaches

3.1 Antifungal Agents in the Pipeline

Several new classes of antifungal agents with anti-*Aspergillus* activity are in development or have been tested in animals.[65-67]

Ravuconazole, a derivative of fluconazole, has demonstrated good activity when compared with that of amphotericin B and itraconazole in a neutropenic rabbit model of disseminated aspergillosis and a murine model of invasive pulmonary aspergillosis.[68]

The polyene liposomal nystatin has shown good *in vitro* activity against *Aspergillus* spp. It was also effective against disseminated aspergillosis in neutropenic rabbits where it prolonged survival and reduced residual tissue fungal burden. The EORTC group conducted a study of 26 patients with probable or definite aspergillosis and concluded that liposomal nystatin could be effective for salvage therapy of invasive aspergillosis, although infusion-related toxicity has limited its further development.[69]

The pradimicins and the structurally similar benanomicins constitute a unique class of fungicidal antibiotics derived from the culture filtrates of *Actinomycetes*. Both have activity against *Aspergillus* spp. The pradimicin BMS-181184 was associated with severe hepatotoxicity in early clinical trials and had to be withdrawn from clinical investigation.[70] Nikkomycins are potent inhibitors of chitin-synthase and recent *in vitro* studies have demonstrated synergistic activity against *A. fumigatus* when used in combination with an azole.[71]

Sordarins are natural fungal products that exert their antifungal effects by inhibition of the protein synthesis elongation cycle in yeasts without affecting the protein synthesis machinery in mammalian systems. Sordarin derivatives, GM222712 and GM237354, have shown good activity against a wide range of pathogenic fungi, including certain filamentous fungi. Pharmacokinetic, safety and *in vivo* data are pending and will delineate if this class of drugs can be further developed.[72] Novel β-D-glucan synthase inhibitors, such as the glycolipid papulacandins and the acidic terpenoids, are under investigation. Terbinafine is an allylamine that has demonstrated good fungicidal activity *in vitro* and *in vivo* against various *Aspergillus* spp., including *A. terreus*.[66,67]

3.2 Immunomodulatory Therapies

The increasing incidence of invasive aspergillosis, the limited efficacy of the available drugs and the high mortality underscore the need for additional or alternative treatment strategies. It is well known that host defence (neutrophils, monocytes/macrophages) is paramount in the management of these infections and, therefore, immunomodulatory therapy is a rational approach. Phagocytosis and killing of conidia requires optimal monocyte/macrophage function, while neutrophils are essential for the phagocytosis of the hyphal elements. Corticosteroid use is a well known risk factor for invasive aspergillosis through its inhibition of the phagocytic activity of neutrophils and macrophages, chemotaxis, oxidative bursts and activity against hyphae. Since neutrophil count and function are both essential for the primary defence against *Aspergillus* spp., most studies on immunomodulatory therapy[65-67] revolve around augmentation of the quantity as well as the quality of neutrophils and T cells. Also, withdrawal or reduction in the dosages of immunosuppressive drugs is a key principle for the successful restoration of the immune function.

3.2.1 Recombinant T Helper Cell Type 1 Cytokines

Although neutrophils play an important role in the defence against invasive aspergillosis, HSCT recipients remain susceptible to this infection long after neutrophil recovery because of a prolonged and defective T cell immune response that is seen in these patients. The most important cytokines that are pertinent to host defence against invasive aspergillosis include interleukins (ILs) and interferon (IFN)-γ.[65-67]

Interleukins

Host invasion by *A. fumigatus* results in the stimulation of two subsets of CD4+ (T helper [T_h]) cells. Generally, T_h1 cells confer protection against fungal diseases and T_h2 cells are associated with disease progression. Activated CD4+ T_h1 cells that secrete various cytokines, including IFNγ, IL-2 and IL-15, and macrophages that produce IL-12 have been shown to confer protection against invasive aspergillosis.[73,74] Animal studies have demonstrated re-

sistance to disease progression associated with elevated production of these cytokines. However, increased levels of IL-12 in animals has also been shown to be deleterious because of the induction of a profound immune-inflammatory response that has been a major concern for use in humans.[73-75]

Interferon-γ

A. fumigatus stimulates IFNγ release by T_h cells, which in turn promote tumour necrosis factor (TNF)-α production by the monocyte-macrophage system. IFNγ has been shown to increase the oxidative burst and fungicidal activity of polymorphonuclear cells against *A. fumigatus* hyphae *in vitro*; it also restores the corticosteroid-induced suppressed activity of monocytes and leucocytes. The use of IFNγ in humans has not been extensively evaluated, with the exception of a study that showed its prophylactic efficacy in patients with chronic granulomatous disease. There are some case reports of its use in combination with other cytokines and antifungal drugs in immunocompromised patients with variable success.[76]

Tumour Necrosis Factor-α

TNFα is a pro-inflammatory cytokine secreted by activated macrophages in response to respiratory pathogens, including *A. fumigatus*. *In vitro*, it has demonstrated crucial activity at the time of invasion, with increases in oxygen radical production, activation of alveolar macrophages and phagocytosis. It also potentiates late defence with increased recruitment of polymorphonuclear lymphocytes. Administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNFα has been shown to counteract the immune deficiency associated with corticosteroid therapy both *in vitro* and *in vivo*. Treatment with neutralizing antibodies to TNFα reduced neutrophil migration to the lungs and is associated with a delay in fungal clearance.[67,77] Murine studies have also revealed that intratracheal administration of TNFα 3 days prior to inoculation with *A. fumigatus* conidia (results in macrophage priming) showed survival benefits but not with simultaneous administration.[76] However, the toxicity associated with the effective doses has been a major impediment for further use.

3.2.2 Donor Granulocyte Transfusions

The major predictors of invasive aspergillosis during chemotherapy or in the early post-transplant period are the severity and the duration of neutropenia. Donor granulocyte infusions have been in use since the 1960s.[65,67] Transfusions have been used for primary prophylaxis during the engraftment period or as secondary prophylaxis in patients with a recent history of invasive aspergillosis with some encouraging results. The prognostic factors associated with a successful outcome include an adequate dose of infusion used, granulocyte compatibility between donor and recipient, and a short duration of neutropenia. However, randomized prospective trials are needed to investigate their clinical efficacy before any strong recommendation can be made.

3.2.3 Recombinant Growth Factors

Large prospective data with colony stimulating factors to treat invasive aspergillosis are not available, although there is anecdotal evidence that they may favourably alter the course of the disease process when used in conjunction with antifungal therapy. G-CSF not only increases the number of circulating neutrophils, but also enhances the phagocytic activity and oxidative burst metabolism of circulating polymorphonuclear leukocytes. Prophylaxis with human G-CSF and amphotericin B or itraconazole has shown some additive effect in neutropenic animal models of invasive aspergillosis. In a neutropenic murine model, human G-CSF, by itself, was ineffective but in combination with amphotericin B demonstrated synergistic fungicidal activity.[67]

Unlike G-CSF, GM-CSF promotes the differentiation and proliferation of mononuclear cells in addition to neutrophils.[78] It also prevents the defective *in vitro* antifungal activity of corticosteroid-treated monocytes and enhances the phagocytic activity of polymorphonuclear leukocytes. An *in vivo* murine model demonstrated that GM-CSF administered prior to dexamethasone prevented the deleterious effects but if given after dexamethasone could not undo the damage on macrophages.[79] GM-CSF has also been shown to reduce the fungal infection associated mortality from 19% to 2% in a clinical trial of patients with acute myelogenous leukae-

mia.[80] A note of caution is the rapid return of neutrophils in the presence of CSF, resulting in an exuberant inflammatory response and subsequent clinical deterioration. Fatal haemoptysis has been reported in the presence of such an exaggerated inflammatory response.[67] Adjuvant treatment with G-CSF or GM-CSF could be considered in select groups of HSCT recipients with serious infections and in those patients receiving systemic corticosteroid therapy, especially in the setting of GVHD.

Treatment with IFNγ and growth factors may be attempted as salvage therapy for patients with refractory invasive aspergillosis. However, use of these immunemodulators must be considered early in the course of therapy, especially in the severely immunocompromised hosts, since introducing them late in the course of fungal infection may not have a significant impact on the outcome.[65]

3.2.4 Pentraxin

Pentraxin is a highly conserved super family of proteins secreted by diverse cell types, including mononuclear phagocytes, dendritic cells and endothelial cells, in response to pro-inflammatory signals such as TNF, IL-1 and selected microbial moieties. Laboratory studies have been encouraging and show a potential therapeutic role in preventing fungal infections and a possible role as an adjuvant immune-enhancing agent in combination with antifungal drug therapy. Additional studies are needed to elucidate the role of this novel agent and its potential applicability in clinical practice.[65]

3.2.5 Pathogen-Specific Immune Therapy

In addition to the non-specific augmentation of the host immunity, there are immunotherapies directed at the specific pathogen. Potential targeted therapies include the use of transfer factors to potentiate the immune response to fungal antigens, administration of a specific inactivated fungal antigen to induce active immunity, pathogen-directed intravenous immunoglobulin and the use of specific monoclonal antibodies by genetic engineering.[77] Most of the research in this field has been conducted in patients with candidaemia or disseminated candidiasis, and data pertaining to invasive aspergillosis are lacking.

Improved understanding of the pathogenesis of invasive pulmonary aspergillosis at the molecular level, and of the complex interaction of the host immunity and pathogen at the cellular level will provide critical information, essential to further evaluate the role of immunomodulating agents in the management of invasive aspergillosis.

3.3 Vaccines

The development of a vaccine against aspergillosis in immunocompromised hosts is a new and challenging paradigm in vaccinology.[81-84] The difficulty of designing a vaccine lies with the diversity of the immunocompromised population and the heterogeneity of immune defects that predisposes patients to invasive aspergillosis. To date, several studies have demonstrated protection using crude *A. fumigatus* vaccines.[85,86] Most murine models have a single immune-deficiency state.[87] Therefore, it is unclear if vaccine-induced protection covers a wide range of immune deficits, such as those resulting from T-cell dysfunction, CMV disease, corticosteroid therapy, neutropenia and GVHD. Current efforts have focused on active immunisation protocols using crude antigen preparations or individual antigens. Novel adjuvants, cytokines and other immunomodulators may be used to increase the efficacy of these vaccines.[86-89] Cenci et al.[90,91] used a killer anti-idiotype monoclonal antibody to protect mice from a lethal *A. fumigatus* challenge during experimental bone marrow transplantation. An intriguing approach is the use of adoptive transfer of previously immunised cells providing a combination of active and passive immunisation. Adoptive transfer of *Aspergillus*-specific donor T cells is under investigation for the treatment of invasive aspergillosis.[65] Although the initial results from preliminary studies appear encouraging, one of the major concerns is the susceptibility of patients to other unrelated fungal pathogens such as *Fusarium* and *Zygomycetes* spp.[92]

4. Antifungal Drug Resistance

Despite 50 years of amphotericin B use, resistance to the drug has been rarely documented in

most *Aspergillus* spp. In fact, resistance is rare even as a secondary event either during or after therapy with amphotericin B. In contrast, although voriconazole has only been in use for the past 3 years, several transplant centres have already reported breakthrough infections with less susceptible *Aspergillus* and *Candida* spp., and the innately resistant *Zygomycetes* spp.[93,94] With the increasing utilization of voriconazole in various transplant centres, multiple reports of clinical failures during therapy of aspergillosis have emerged, the cause for which may be multifactorial (host factors, serum drug concentrations and drug resistance).[95-100] Although less susceptible *Aspergillus* spp. have emerged as pathogens (e.g. *A. ustus*), it is unclear whether azole-resistance may develop in common *Aspergillus* spp. (e.g. *A. fumigatus, A. flavus*) during mould-active azole exposure. Echinocandin resistance during treatment of aspergillosis is not reported as yet.

5. Conclusion

Despite considerable progress in the diagnosis and management of invasive aspergillosis over the last 2 decades, frequency of infection and mortality remain high. Early diagnosis with antigen-based testing and high-resolution chest CT scans in high-risk patients has greatly facilitated the administration of antifungal drugs in a targeted fashion. Voriconazole has become the drug of choice for primary therapy, while posaconazole shows promise as a prophylactic drug. Echinocandins are effective for salvage therapy and are under evaluation for primary therapy. Preliminary data for efficacy of combination therapy with mould-active azole plus an echinocandin are intriguing; clinical trials are under way. Clearly, better therapeutic options have become available, but there is an unmet need for non-invasive and more reliable, rapid and simple diagnostic tools. The promise of newer classes of drugs and immunomodulating therapies are exciting future additions to the arsenal against invasive aspergillosis.

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