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REVIEW

Strategies to enhance immune function in hematopoietic transplantation recipients who have fungal infections

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The challenges in the treatment of systemic fungal infections after HSCT include: (1) changing epidemiology as less drug-susceptible saprophytic fungi are increasingly associated with human disease; (2) the difficulty of early and correct diagnosis, even with the new generation of enzymatic immunoassays; (3) the inability to reduce or eliminate predisposing factors, especially severe immune suppression in most transplant patients with these infections and (4) the uncertain role of antifungal drug combinations and risk of drug antagonism complicating effective empiric-pre-emptive therapy. Current, developing and future immune enhancement strategies including recombinant granulocyte- and granulocyte macrophage-colony stimulating factor (GM-CSF), interferon-gamma (IFN-γ), adjuvant pro-inflammatory cytokine therapy during mobilized donor granulocyte transfusions, therapeutic potential of pentraxin, adaptive immune transfer and dendritic cell fungal vaccines. Improved understanding of the molecular pathogenesis of fungal infections and of the complexity of host antifungal immune responses has provided the critical information to readdress existing treatment paradigms and further evaluate the role of GM-CSF and IFN-γ early in the course of therapy against life-threatening fungal infections in high-risk patients following stem cell transplantation.
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Introduction

In patients with hematologic malignancies who undergo HSCT, invasive fungal infections (IFI) are a prominent cause of morbidity and death.[1] Even in the present era of highly active anti-*Aspergillus* drugs such as voriconazole and caspofungin, the response to treatment in patients with severe neutropenia after HSCT remains mostly unfavorable.[2,3] Amelioration of the underlying predisposing conditions is important in achieving a favorable response to antifungal drug therapy.[4] For example, persistent granulocytopenia due to myeloablative preparatory therapy occurs before engraftment, or results from graft failure, or relapsed cancer, severely compromising the outcome of drug therapy.[5] Late post-transplant fungal infections are commonly noted in patients receiving treatment for GVHD; in these non-neutropenic patients, a favorable response to antifungal treatment requires reduction or, when possible, discontinuation of drug-induced immune suppression.[6] During the post-transplant period, immunosuppressive viral infections (e.g. CMV infection) may also increase the risk of secondary opportunistic bacterial and fungal infections.[7]

In this article, I will review the changing spectrum of IFI after HSCT, the limitations of the new generation of diagnostic tests, and the limited response of IFI to systemic antifungal drug therapy alone and then explore emerging and re-emerging immune enhancement strategies to improve the response to antifungal therapy.

Changing epidemiology

The decline in systemic yeast infections in the past decade has been attributed mostly to the common use of fluconazole or itraconazole prophylaxis in patients undergoing HSCT.[8–10] Such prophylaxis benefits granulocytopenic patients during the early post-transplantation period, and even non-neutropenic patients with GVHD have benefited from anti-*Candida* prophylaxis during the late post-transplantation phase.[11] Despite the increase in difficult-to-treat *Candida glabrata* and *Candida krusei* breakthrough infections, triazoles prophylaxis during the high-risk periods has been viewed favorably, as the overall incidence of systemic candidiasis has diminished considerably in the past decade.[8,11]

Infections due to filamentous moulds, on the other hand, have increased, and furthermore, this rise in part reflects the emergence of non-*Aspergillus* infections (Table 1), which are less susceptible to conventional antifungal drugs.[12] For example, infections due to the black moulds,

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Table 1 Amphotericin B non-susceptible filamentous fungi associated with invasive disease

Hyalohyphomycoses (hyaline or non-pigmented septate fungi)	*Phaeohyphomycoses (dematiaceous or pigmented septate fungi)*
Common[a]	Increasing in frequency
Fusarium	*Altenaria*
F. solani complex	*Curvularia*
Increasing in frequency	*Bipolaris*
Aspergillus terreus	*Scedosporium*
Paecilomyces	*S. prolificians*
P. lilacinus	*S. apiospermum*
P. variotti	*Pseudallescheria*
Less common	*P. boydii*
Aureobasidium	Less common
Acremonium	*Cladophialophora*
Aureobasidium	*Cladosporium*
Chrysosporium	*Fonsecaea*
Fusarium oxysporum	*Exophiala*
	Exserohilum

[a]*Fusarium solani* is a frequent cause of disseminated mycosis in severely immunocompromised patients undergoing HSCT in high-risk regions, such as south central US. This common plant pathogens is probably ingested along with contaminated uncooked or partially cooked vegetables and this unusual route of entry may probably explain the distinctly different clinical disease noted with fusariosis compared with other molds infections that arise following inhalation of the infectious microconidia.

especially *Scedosporium prolificans*, are resistant to most available antifungal agents, and disseminated infections due to *Fusarium* species, *Pseudallescheria* species, *Scedosporium apiospermum* and other dematiaceous molds have variable drug susceptibility profiles; owing to the high rates of failure with amphotericin B-based therapy, treatment of infections due to these non-*Aspergillus* molds is approached as salvage therapy, often with combinations of antifungal drugs.

The unanticipated rise in zygomycoses, especially in patients receiving voriconazole prophylaxis, in the past few years[13] has been complicated by the fact that these infections are more serious leading to greater than 80% mortality in transplant recipients compared to other IFI.

Diagnosing IFI

Definite diagnosis of IFI requires histologic demonstration of tissue invasion by fungi and systemic signs of disease along with cultures and fungal identification. As obtaining tissue samples from most HSCT recipients is not feasible, the emphasis in these high-risk patients is placed on minimally invasive or non-invasive laboratory tests with better diagnostic yields than conventional culture-based methods. Furthermore, in severely immunosuppressed HSCT recipients, the isolation of saprophytic environmental molds from blood culture samples[14] or non-*Scedosporium* species including *S. apiospermum*, and *S. prolificans*, from bronchoalveolar lavage specimens has been noted to have a low association with IFI.[15]

The findings on non-contrast enhanced computed tomography chest scan are important in determining the empiric course of therapy for patients with a pulmonary

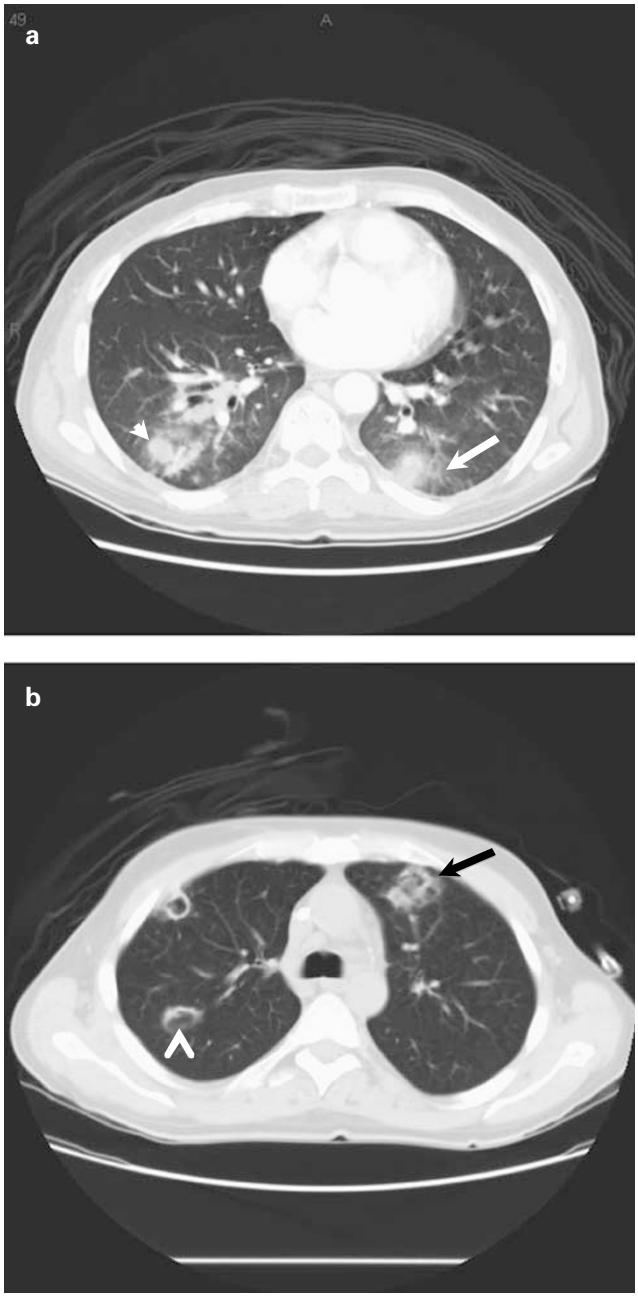

Figure 1 Chest computed tomography scans showing typical findings of IFI in HSCT recipients. (**a**) Posterior, pleural-based nodular lesion in the left lower lung lobe showing characteristic ground glass attenuation, the 'hypodense sign' or 'halo sign' (arrow). In right lower lobe a large, dense, peripheral, pleomorphic nodules are highly suggestive of invasive fungal infection (arrow head). (**b**) Bilateral thick-walled cavities (arrow head) and a multiloculated cavity (arrow) in the left upper lung lobe in an allogeneic HSCT recipient following donor lymphocyte infusion for relapsed CLL with *Aspergillus* and *Rhizopus* pulmonary mycosis.

processes. In Figure 1a, characteristic ground glass attenuation, the 'hypodense sign' or 'halo sign' in a patient with invasive aspergillosis is present in $>80\%$ of IFI within first 7 days of infection and is highly indicative of invasive fungal disease.[16,17] Pleomorphic, irregular, multicentric and often peripheral, plural-based pulmonary nodules, espe-

cially those presenting with thick-walled cavities are highly indicative pulmonary mycoses (Figure 1b). Whereas, small lung nodules, <10 mm in diameters are more common in patients with viral infections and not likely to represent IFI.[18] The 'crescent sign' is a late finding and may be noted in patients following recovery of neutropenia.[19,20]

The current approach to the diagnosis of IFI after HACT includes: (1) an overall assessment of predisposing factors and determination of a patient's susceptibility to invasive mycosis, (2) fungal cultures from sterile body sites, (3) radiographic findings consistent with or suggestive of IFI, especially in the lungs and (4) the recently available enzyme immunoassays that detects fungal antigens (galactomannan (GM EIA) and D-glucan) or an investigational polymerase chain reaction (PCR) assay, which provides a measure of fungal tissue burden but is presently not available for clinical use.[21,22] The overall diagnostic sensitivity of galactomannan assay in 27 studies between 1966 and 2005 was 71% and specificity was 89%; in patients with hematologic malignancies and HSCT recipients the results were more useful compared to patients with solid-organ cancer or solid-organ transplantation. The sensitivity and specificity of *Aspergillus* antigen immunoassays in these high-risk population are both nearly 90%; albeit in patients receiving mold-active drugs for prophylaxis or therapy, the sensitivity is markedly reduced (Table 2).[23] Furthermore, due to cross reactivity with piperacillin-tazobactam false positive GM EIA may complicate interpretation of these results and definite diagnostic tests such as PCR are sought. In recent reports, proven or probable invasive aspergillosis among patients with hematologic malignancy PCR analysis of whole blood (92% sensitivity, 95% vs specificity, 60% positive predictive value and 99% negative predictive value)[24] or serum samples (67% sensitivity, 88% specificity, 67% positive predictive value and 90% negative predictive value)[25] was promising; there was also 77% diagnostic concordance with GM EIA and high-resolution computed tomography scan.[24]

Responses to antifungal therapy

In allogeneic HSCT recipients with IFI, the rate of response to amphotericin B-based therapy is low, and despite treatment, the infection-associated mortality rate remains high.[26] Overall, the response to voriconazole as primary therapy for invasive aspergillosis is significantly better than that to amphotericin B; however, this therapeutic advantage is markedly reduced in patients with severe neutropenia after HSCT.[2] Salvage therapy with caspofungin has also led to modest improvement in IFI treatment responses, especially among the sub-group of patients who receive caspofungin owing to intolerance to the primary antifungal therapy rather than true treatment-refractory IFI.[3]

Antifungal drug combinations appear to have additive and/or synergistic activity in experimental animal studies. In early clinical studies, caspofungin plus amphotericin B lipid preparation[27–29] and caspofungin plus voriconazole[27,30] combinations were tolerated without serious adverse events and resulted in modest improvements in IFI response compared to single drug therapy in response, especially in severely immunosuppressed HSCT recipients. Increasing the caspofungin dose resulted in favorable survival 12 weeks after therapy compared with patients who were treated with standard dose caspofungin combination therapy.[31,32] It is important to recognize that some drug combinations are antagonistic and thus may reduce therapeutic efficacy if used.

Abelcet®, a less nephrotoxic amphotericin B lipid preparation, is suggested as the drug of choice for patients with invasive zygomycosis. Recent studies of posaconazole were encouraging, as nearly 50% of patients with zygomycoses responded to posaconazole alone.[33,34] Posaconazole response in patients with other refractory non-*Aspergillus* species infections such as disseminated fusariosis also appears promising.[35] The roles of newer echinocandins, micafungin, and anidulafungin in the treatment of invasive mold infections have not been determined and need further study.

Table 2 Diagnostic variability of galactomannan enzyme immunoassays (GM EIA) and impact of systemic antifungal drug exposure

Reference (no.)	*Samples*	*Antifungals*	*Test – index cutoff*	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPP (%)*
Maertens *et al.*[78]	Serum	Yes	GM EIA 1.0	89.7	98.1	87.5	98.4
Becker *et al.*[79]	CT-based BAL	No	GM EIA 1.0	100	100	100	100
	Serum	No	GM EIA 1.0	47	93	73	82
	BAL	Yes (>3 days)	GM EIA 1.0	0	0	0	0
Musher *et al.*[22]	BAL	No	GM EIA 1.0	61	98	NA	NA
		No	GM EIA 0.5	76	94	NA	NA
Maertens *et al.*[80]	Serum	Yes	GM EIA 1.0	92.6	95.4	93	95
Marr *et al.*[23]	Serum	No	GM EIA 1.0	72	98	NA	NA
		No	GM EIA 0.5	89	92	NA	NA
		Yes	GM EIA 1.0	30	97	NA	NA
		Yes	GM EIA 0.5	52	91	NA	NA
Marr *et al.*[81]	Serum	No	GM EIA 1.0	87.50	NC	NA	NA
		Yes	GM EIA 1.0	20	100	NA	NA
		Yes	GM EIA 0.5	81.8	77.1	NA	NA
Machetti *et al.*[82]	Serum	NA	GM EIA>1.0	60	82	NA	NA

Abbreviations: PPV = positive predictive value; NPV = negative predictive value; BAL = bronchoalveolar lavage; NA = not available; NC = non-calculable.

Antifungal immune responses

The host's innate immune response plays an important role in protection against invading fungal pathogens (Figure 2). The first line of defense includes acellular microbicidal components such as products of complement activation cascade, cationic proteins, and proteolytic enzyme secreted along the mucosal lining of the respiratory tract. The principle cellular component during this early non-antigen specific innate immune response is mediated via phagocytic activity of alveolar macrophages and recruited neutrophils; Toll-like receptor (TLR) 2 and TLR 4, were recently recognized as critical in the early recognition of infective *Aspergillus* conidia.[36,37] Via a complex signaling cascade, these receptor leads to translocation of nuclear-factor-κB and induction of various proinflammatory cytokine genes and thus to improved effector cell immune function. Interestingly, TLR 4-mediated immune activation is more focused against the fungal microconidia; whereas, TLR 2 is important for eliciting immune response against microconidia and the developed fungal hyphae.[38] Tumor necrosis factor (TNF) has also been shown to be an essential mediator of early immune activation following ingestion of infectious fungal spores or microconidia.[39] TNF is also important in promoting selective early recruitment of leukocytes at the site of fungal invasion/infection.[40] Other important chemoattractants for neutrophil recruitment are the ELR-positive subset of CXC chemokines and corresponding CXCR2 receptors.[41] For the entry of mononuclear cells at the infection site, chemokine ligand 3 (CCL3)/macrophage inflammatory protein-1 alpha and CCL2/monocyte chemoattractant protein-1 are critical in

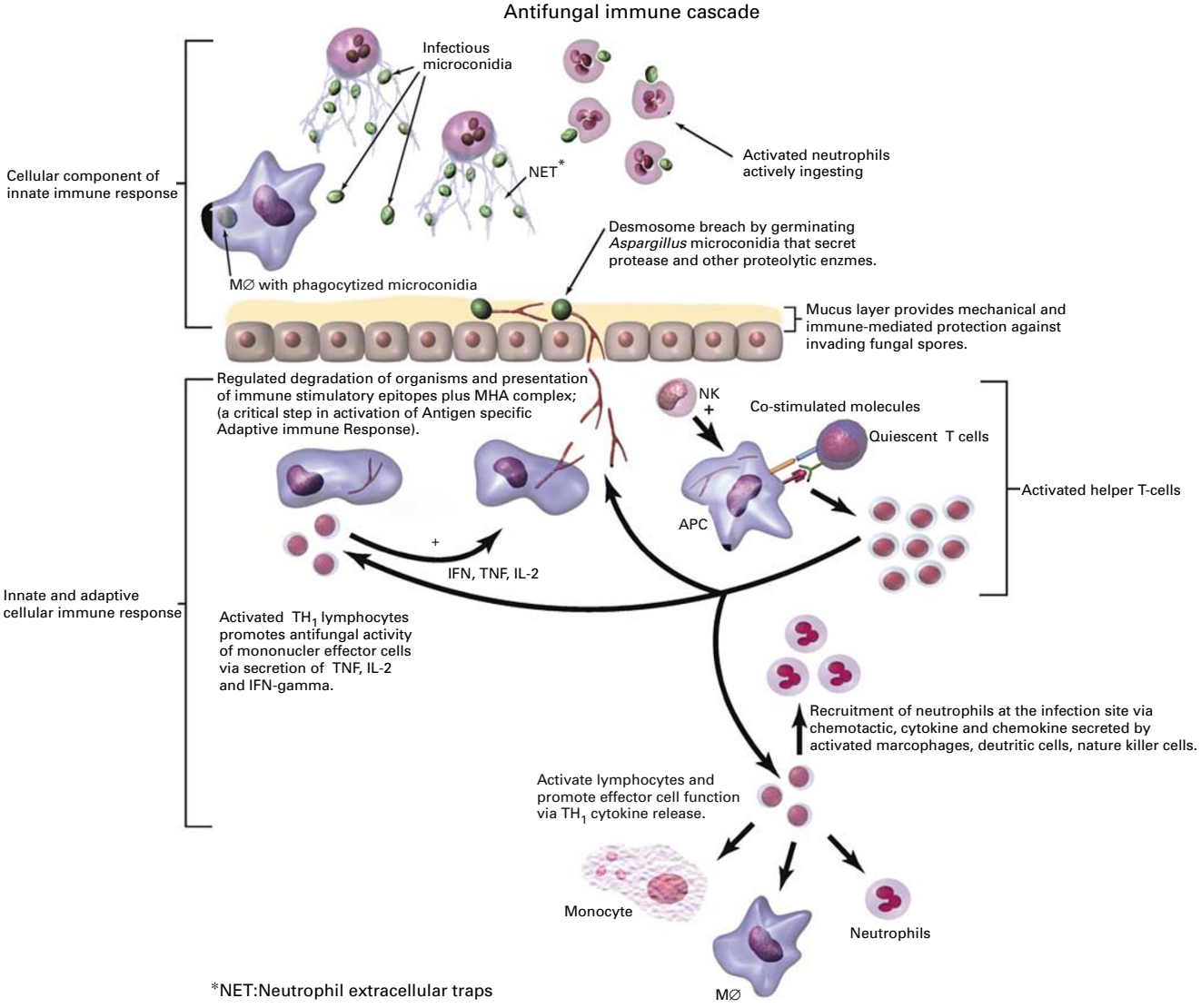

Figure 2 The pathogen–host interaction following inhalation of infectious, metabolically active germinating fungal microconidia. The various components of acellular and cellular innate immune response and activation of adaptive antigen-specific immune response. The germinating *Aspergillus* microconidia secrete various enzymes and toxins. The proteases disorganize the actin cytoskeleton and destroy the epithelial cell attachment (cell-to-cell adhesions), which near-exponentially increases capability of infectious microconidia to invade alveolar epithelial barrier.[95]

the recruitment of monocytes and natural killer cells.[42] Other major chemokines that modulate early innate immunes response against filamentous mold infections include the macrophage-derived chemokines (CCL22), which enhance immune response among monocytes; DC; and natural killer cells.

In contrast, the T_H2 driven thymus and activation-regulated chemokines (CCL17) have immunosuppressive effects and impair the pulmonary antifungal innate immune response.[43] The T_H1 adaptive cellular immune response mediated via IL-12/IL-23, IL-18, IL-2 and IFN-γ, the protagonist in this group, promotes the fungicidal activity of the host's mononuclear cells (Figure 2). Moreover, dominance of T_H2 cytokines such as IL-4 and IL-10 abrogates the antifungal protection rendered by the T_H1-dependent antimycotic response.[44,45] This defective T_H1 cytokine-mediated antifungal activity can be restored in experimental animals that have invasive aspergillosis by treatment with T_H2 cytokine inhibitors.[46]

Interestingly, the host's cellular and humoral immune responses appear to be restricted to only the viable and germinating infectious fungal spores that carry a risk for invasive diseases; the mechanism by which the host's adaptive immune system differentiates between metabolically active and non-disease-causing inactive spores remains unknown.[47] Various pathways have been explored in selective recognition of germinating, infectious fungal microconidia, such as Dectin-1, and myeloid differentiation factor 88, these important regulatory molecules may be central in preventing indiscriminate activation of innate immune-inflammatory cytokine response against inhaled non-infectious environmental fungal spores.[48,49]

A number of factors adversely influence the host's antifungal immunity, including hematologic malignancy, antineoplastic chemotherapy, radiation therapy, HSCT, immunosuppressive agents given for the treatment of GVHD, including systemic corticosteroids.[1,9,50] Severe and prolonged chemotherapy-induced or cancer-related granulocytopenia is a well-known risk factor for IFI.[51]

Among pathogen-associated factors that may adversely influence IFI include, gliotoxin, the most abundant mycotoxin produced by *Aspergillus fumigatus*, was recognized as an important virulence factor that suppresses the host's antifungal innate and cellular immune responses. A toxin-induced apoptosis of antigen-presenting cells is considered central for disease-causing fungi to evade host immune surveillance.[52]

Immune enhancement strategies

The infectious complications and immune dysfunction after HSCT varies in time of onset, duration, type and severity among patients undergoing myeloablative versus non-myeloablative HSCT (Table 3). It was interesting to note that frequency of infection and infection-associated death among recipients of non-myeloablative and myeloablative HSCT were comparable (16–77 and 7–88%, respectively). Furthermore, in patients undergoing either type of transplantation, higher infection rates occurred even in patients who had low rates of GVHD, which is considered as the most important predictor of IFI during late HSCT period.

The restoration of immune function is critical to effective treatment of IFI in these patients. Immune enhancement therapies include (1) mobilized donor granulocyte transfusion (GTX), (2) recombinant growth factors, (3) recombinant T_H1 cytokine therapy and (4) experimental modalities such as target-specific adaptive immune transfer.

Donor granulocyte transfusions

The severity and duration of granulocytopenia in cancer patients are two important predictors of the development of serious systemic bacterial and fungal infections.[53] Since the early 1960s, donor GTX have been used for infection prevention and/or treatment in patients with profound granulocytopenia.[54] The success of GTX depends on the dose of granulocytes transfused; however, large doses require sophisticated cell separation and leukocyte collection techniques and enhancement of the number of circulating granulocytes by pretreating the donor with recombinant growth factors and corticosteroids.[55–57] Donor priming with GM-CSF is desirable, although its use in healthy donors is limited owing to drug intolerance; in most institutions, G-CSF and dexamethasone are commonly used for this purpose.[58]

In patients with a recent history of IFI, secondary GTX prophylaxis has had encouraging results.[58] However, whereas, GTX may help to prevent serious bacterial and fungal infections in patients with chemotherapy-induced severe neutropenia, the results in immunosuppressed cancer patients, have been variable. A recent meta-analysis of GTX for primary prevention of infection, the following three factors have been associated with significantly improved GTX efficacy: (a) an adequate granulocyte dose ($>5.0\times10^{10}$ cells per transfusion), (b) donor/recipient leukocyte compatibility and (c) most important, a short duration (<2 weeks) of neutropenia, which was shown to reduce the risk of serious bacterial or fungal infection by nearly 80%.[59]

The efficacy for adjuvant GTX in neutropenic patients with severe systemic bacterial or fungal infections treated between 1997 and 2005 was 50–82%.[49] Even in severely neutropenic patients with *Candida* species fungemia, despite the presence of multiple predictors of poor outcome including prolonged neutropenia, GTX were shown to improve short-term survival.[60]

Immune enhancement with GTX

Neutrophils, after migrating to the site of infection, engulf and kill fungi through the actions of proteolytic enzymes, antimicrobial proteins, and toxic oxygen radicals. Similar to recently described neutrophil extracellular traps (NET) composed of granules and nuclear structures that are effective in killing extracellular bacteria, these activated phagocytes may inundate and kill extracellular fungal pathogens without eliciting the severe inflammatory response that often occurs following non-NET indiscriminate release of oxygen radicals and other proteolytic enzymes.[61] In *ex vivo* models, granulocytes' intracellular and perhaps extracellular antimicrobial activity may be augmented via

Table 3 Infectious complication following myeloablative and non-myeloablative hematopoietic transplantation

Reference/year (No.)	*No.*	*Age*[a]	*Diagnosis*	*Conditioning Regimen*	*Engraftment (range) days*	*Primary/ secondary failure*	*aGVHD*	*cGVHD*	*Relapse*	*Infections*[b]	*Infection related mortality*
Non-myeloablative HSCT											
Parker J *et al.* (1993–2000)[83]	23	48	MDS	Flu/Bu/Campath-1H	16 (14–24)	3 (18%)	4/23 (17%)	3/20 (15%)	4 (17%)	8 (35%)	7 (31%)
Couriel D *et al.* (1996–2000)[84]	63	59	AML, CML, CLL, MDS, NHD	Flu, Ida, Cyta/Flu, Cyta, Cy/Flu, Cyta, Ritu	No reported	5 (8%)	6 (11%)	55 (7%)	No reported	No reported	2 (1%)
Diaconescu R *et al.* (1997–2000)[85]	73	54	AML, ALL, CML, CLL, MDS, MM, NHL, HL, WG	Flu, rTBI/rTBI	9 (1–39)	5 (7%)	19%	56%	No reported	42 (58%)	12 (16%)[c]
Canals C *et al.* (1996–2001)[86]	27	53	AL, CML, CLL, MDS, MM, HL, Myelofibrosis	Flu, Me-Bu	14 (11–23)	No reported	33%	38%	8 (19%)	No reported	1 (4%)
Petersen SL *et al.* (1997–2001)[87]	15	51	CLL, MDS, MM, NHL	Flu, rTBI	No reported	No reported	55%	80%	No reported	No reported	No reported
Valcárcel D *et al.* (1995–2002)[88]	57	51	AML, ALL, CLL, CML, MDS, MM, NHL, HL	Flu, Me-Bu	15 (11–26)	No reported	35%	68%	14 (24%)	No reported	3 (5%)
Alyea EP *et al.* (1997–2002)[89]	71	58	AML, ALL, CML, CLL, MDS, NHL, CMML	Flu, Bu	No reported	No reported	20 (29%)	No reported	33 (46%)	No reported	16 (22%)
Kojima R *et al.* (1998–2002)[90]	70	57	AML, ALL, CML, MDS	Cla/Flu/rTBI	12 (9–30)	1 (1%)	38 (56%)	37/57 (65%)	23	11 (16%)	1 (1%)[c]
Massenkeil G *et al.* (1998–2002)[91]	25	44	AML, ALL	Flu, Bu, ATG	12 (9–31)	1 (2%)	13 (52%)	12 (63%)	15 (60%)	4 (16%)[c]	1 (1%)
Sorror ML *et al.* (2000–2002)[92]	60	54	AL, CML, CLL, MDS, MM, NHL, HL	Flu, rTBI	15	No reported	77%	No reported	No reported	46 (77%)	5 (8%)
Scott BL *et al.* (1998–2003)[93]	38	62	AML, MDS	Flu, rTBI	No reported	4 (11%)	54%	55%	11 (29%)	No reported	9 (24%)
Meijer E *et al.* (2000–2003)[94]	40	56	AML, CLL, MDS, MM, NHD	Flu/rTBI	7	No reported	22 (54%)	24 (61%)	No reported	10 (25%)[c]	No reported
Myeloablative HSCT											
Parker J *et al.* (1993–2000)[83]	29	37	MDS	BuCy/-TBI/Campath	21 (13–30)	2 (7%)	16/28 (55%)	12/21 (57%)	2 (7%)	5 (17%)	No reported
Couriel D *et al.* (1996–2000)[84]	74	45	AML, CML, MDS, NHL	BuCy/Flu, Me	No reported	2 (3%)	23 (34%)	70 (40%)	No reported	No reported	1 (1%)
Diaconescu R *et al.* (1997–2000)[85]	73	48	AML, ALL, CML, CLL, MDS, MM, NHL, HD	Cy, TBI/Bu, Cy-TMI-TBI	16 (7–37)	0	16%	43%	No reported	64 (88%)	22 (30%)[c]
Canals C *et al.* (1996–2001)[86]	23	50	AL, CML, CLL, MDS, MM, HL	Cy, TBI/BuCy/Thio, Cy, TBI/Thio, Bu, Cy	13 (9–30)	1 (4%)	35%	17%	17%	No reported	6 (26%)
Petersen SL *et al.* (1997–2001)[87]	50	40	AML, ALL, CML, CLL, NHL	Cy, TBI-Bu	No reported	No reported	40%	40%	No reported	No reported	No reported
Valcárcel D *et al.* (1995–2002)[88]	100	39	AML, ALL, CML, CLL, MDS, MM, NHL	Cy, TBI/Cy, Bu	14 (9–33)	1 (1%)	46%	59%	24%	No reported	13 (13%)
Alyea EP *et al.* (1997–2002)[89]	81	54	AML, ALL, CML, CLL, MDS, NHL	Cy, TBI-Bu	No reported	No reported	22 (27%)	No reported	30%	No reported	7 (9%)
Kojima R *et al.* (1998–2002)[90]	137	52	AML, ALL, CML, MDS	Cy, Bu/TBI	15 (5–27)	2 (15%)	56 (43%)	60/104 (58%)	38	9 (7%)	13 (9%)[c]
Massenkeil G *et al.* (1998–2002)[91]	50	38	AML, ALL	Cy, TBI/Cy, Eto	17 (9–35)	1 (2%)	32 (64%)	21 (50%)	20 (40%)	9 (18%)[c]	11 (22%)
Sorror ML *et al.* (2000–2002)[92]	74	41	AL, CML, CLL, MDS, MM, NHL, HL	Cy, TBI-Bu	18	No reported	91%	No reported	NA	65 (88%)	13 (18%)
Scott BL *et al.* (1998–2003)[93]	112	53	AML, MDS	Cy, Bu	17 (10–35)	1 (<1%)	78%	64%	26 (23%)	No reported	19 (17%)
Meijer E *et al.* (2000–2003)[94]	38	44	AML, ALL, CML, CLL, MDS, MM, NHD	Cy/TBI	13	No reported	22 (58%)	19 (50%)	No reported	32 (84%)[c]	No reported

[a]Median age.
[b]Represent infections that occur during the treatment course.
[c]Fungal infections[3] 12 out of 33 were fungal not specified by group.[8]
One non-myeloablative and four myeloablative deaths due to fungal[9] represent only the fungal[12] one non-myeloablative and five myeloablative.
Abbreviations: AML = acute myelogenous leukemia; ALL = acute lymphocytic leukemia; CLL = chronic lymphocytic leukemia; CML = chronic myelogenous leukemia; MDS = myelodysplactic syndrome; MM = myeloma multiple; NHL = non-Hodgkin lymphoma; HL = Hodgkin lymphoma; Flu = fludarabine; Bu = busulfan; rTBI = reduce total body irradiation (2Gy); Cla = cladribine; ATG = antithymocyte globulin; TBI = total body irradiation; Ida = idarubicin; Cyta = cytarabin; Cy = cyclophosphamide; Ritu = rituxan; Me = melphalan; Thio = thiothepa; Eto = etoposide; TMI = total marrow irradiation.

recombinant growth factors and pro-inflammatory cytokines.[62] For this purpose, recombinant GM-CSF and recombinant IFN-γ-1b (rIFN-γ-1b) had been used in HSCT recipients with systemic infections; these cytokines were associated with no serious sequelae such as GVHD or relapsed cancer.[63,64] We recently showed that adjuvant therapy with recombinant cytokines including GM-CSF and rIFN-γ-1b in patients receiving donor GTX was well tolerated and possibly had a therapeutic benefit in patients with difficult-to-treat IFI.[65] Randomized, prospective trials are needed to further investigate strategies to improve the clinical efficacy of donor GTX.

Recombinant growth factors

Following allogeneic HSCT, the use of G-CSF has improved engraftment, graft survival, and overall survival among patients with poor graft function.[66] The use of G-CSF has also been successfully explored in the treatment of various systemic infections.[58] The therapeutic efficacy of G-CSF probably represents (1) increase in circulating neutrophils and (2) enhancement of the antimicrobial ability of neutrophils, especially by promoting killing of phagocytosed fungal microconidia via reactive oxygen species. It remains to be seen whether G-CSF and/or GM-CSF favorably influence the extracellular microbicidal activity by releasing antimicrobial factors into the extracellular medium or whether these immune modulators may promote and generate extracellular fibers or NET,[61] which may play an important role in killing non-phagocytosed invading filamentous molds. Another interesting mechanism by which GM-CSF may promote effective fungicidal activity is the induction of chitotriosidase, an important and highly regulated enzyme active against chitin (an integral component of fungal cell walls)-containing pathogens.[67,68] GM-CSF also reverses steroid-induced dysfunction of alveolar and tissue macrophages' handing of the *Aspergillus* microconidia.[69,70] Treatment with GM-CSF may enhance antifungal immune resistance in patients receiving systemic corticosteroids, especially in the setting of GVHD. Adjuvant treatment with G-CSF plus GM-CSF should be considered in select group of HSCT recipients with serious IFI, and even in non-neutropenic patients, especially those receiving treatment with systemic corticosteroids, GM-CSF 250–500 μg daily may be added to the antifungal regimen for the initial 2–3 weeks of therapy.

Recombinant T_H1 cytokines

IFN-γ-1b enhances the host's antimicrobial defense by promoting the intracellular microbicidal activity of effector mononuclear cells and polymorphonuclear phagocytes, which are critical in the defense against filamentous mold infections.[64,65] Recombinant human IFN-γ-1b (rhIFN-γ-1b) had been given safely in patients with IFI following allogeneic HSCT, with no serious adverse events, including no cytokine-related GVHD exacerbation; in fact, a number of patients with acute and chronic GVHD had measurable improvements following rhIFN-γ-1b therapy.[64] Treatment with rhIFN-γ-1b is currently regarded as salvage therapy for patients with refractory IFI that fails to respond to conventional therapy. However, I believe that the use of adjuvant immune modulators such as GM-CSF, rhIFN-γ-1b should be considered early in the course of IFI therapy, especially in the severely immunosuppressed recipients of allogeneic HSCT. Introducing these immune modulators late in the course of advanced fungal disease may not have a significant impact on infection outcome. However, recombinant IL-2 and IL-12 are associated with high rates of systemic toxicity and therefore, are not recommended for patients with IFI. Other T_H1 promoters such as IL-18 and IL-25 need further evaluation.

The recommended dose of rhIFN-γ-1b in adults is 50 μg/m^2 of body surface area given every other day until complete clinical and radiographic resolution of the infection occurs. In patients with severe, multicentric or disseminated IFI, GM-CSF may be initially added with rhIFN-γ-1b; however, because capillary leak syndrome may occur with GM-CSF, my preference is not to use GM-CSF for longer than 4–6 weeks.

Pentraxin. Pentraxin (PTX) is a highly conserved superfamily of proteins secreted by diverse cell types including mononuclear phagocytes, DC, and endothelial cells in response to pro-inflammatory signals such as TNF-α, IL-1 and selected microbial moieties.[71] PTX3 is an important component of the innate antimycotic immune response and acts by promoting complex effector pathways. Even in the unfavorable antifungal T_H2-predominant cytokine milieu, in animal experiments PTX3 facilitated macrophage anti-*Aspergillus* response and activated and improved DC-mediated antifungal activity by acting as a non-redundant soluble target-specific 'pattern-recognition' receptor against pathogenic fungi.[71]

Laboratory studies have been encouraging and show a potential therapeutic role of PTX3 in preventing fungal infections and a possible role as an adjuvant immune-enhancing agent in combination with antifungal drug therapy.[72] Additional evaluation is needed to elucidate the role of this novel agent and its potential applicability in clinical practice.

Adoptive cell transfer and DC vaccines. After HSCT and during treatment for GVHD, patients have a severely reduced ability to develop antigen-specific T cells. This deficit is often seen during the early post-transplant period owing to dysfunctional DC; in the later period, in patients with chronic GVHD, an inadequate T-cell response reflects a more complex immune defect involving disruption of several facets of immune activation. Adoptive transfer of *Aspergillus* (fungal species)-specific T cells is currently being explored as a treatment for IFI. *Ex vivo*-generation *Aspergillus*-specific donor T-cell clones have been successfully given to patients following HSCT; these target-specific T cells ($CD3^+/CD4^+$) are long-lasting T_H1 (high IFN-γ/low IL-10) cells and in early trials did not lead to GVHD or compromise the stem cell graft.[73] Although the early results appear encouraging, the transfer of *Aspergillus*-specific T cells[74] may render patients susceptible to severe invasive disease due to other unrelated fungal species like *Fusarium*, or the dematiaceous (black) molds.

Following T-cell depleted HSCT, patients also have marked defects in the function of DC, cells critical in the

initial stages of the adaptive cellular immune response against the inhaled infectious fungal microconidia. DC activated via pattern-recognition receptors such as Toll-like receptor 2, PTX3, are highly antigen specific and finely differentiate between conidia and *Aspergillus* hyphae, which in turn results in a specific adaptive immune response. The plasticity of these valuable cells in triggering a sustained antifungal response has been explored in the development of DC vaccines. In experimental models, DC stimulated *ex vivo* using live fungi or fungal nucleic acid resulted in the generation of local and systemic antigen-specific IFN-γ-producing T cells. This desirable antifungal T_H1 response was also seen in animals following allogeneic SCT.[75]

Various other methods of DC activation have been explored including RNA transfection, which may improve antifungal immune responses by enhancing protective T_H1 responses mediated by endogenous IL-12 and IL-18 and by enhancing IFN-γ-mediated effector cell responses. The adoptive transfer of DC may also improve regulatory T-cell functions by promoting antigen-specific T-cell responses, which are severely inadequate in patients following T-cell depleted SCT.[76] Further clinical trials are needed to assess the clinical viability of this technology in HSCT patients at risk for IFI.

Conclusions

IFI are associated with significantly increased risk of infection-associated death especially in patients between ages 12–35 years, concurrent pleural effusion, low monocyte count (<120 cells/mm^3), systemic corticosteroids of >2 weeks within 2 months of IFI diagnosis, and uncontrolled GVHD.[77] Effective IFI therapy in this high-risk population poses a serious challenge on many fronts: (1) a rise in infections due to saprophytic molds with reduced drug susceptibility; (2) significantly reduced diagnostic efficacy of the new generation of enzymatic immunoassays in patients receiving systemic antifungal drugs; (3) it is difficult to reduce or eliminate predisposing factors in most patients with IFI; and (4) modest or no measurable increase in response to antifungal drug combinations compared with single drug therapy; furthermore potential drug–drug antagonism make selection of empiric-pre-emptive antimicrobial therapy for severely immunosuppressed HSCT recipients a daunting task. Improved understanding of the molecular pathogenesis of fungal infections and of the complexity of host antifungal immune responses has provided the critical information to readdress existing treatment paradigms and further evaluate the role of GM-CSF and IFN-γ early in the course of therapy against life-threatening fungal infections in high-risk patients following stem cell transplantation.

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References

1 Safdar A, Armstrong D. Infectious morbidity in critically ill patients with cancer. *Crit Care Clin* 2001; **17**: 531–570.
2 Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW *et al*. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; **347**: 408–415.
3 Maertens J, Raad I, Petrikkos G, Boogaerts M, Selleslag D, Petersen FB *et al*. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. *Clin Infect Dis* 2004; **39**: 1563–1571.
4 Jantunen E, Ruutu P, Niskanen L, Volin L, Parkkali T, Koukila-Kohkola P *et al*. Incidence and risk factors for invasive fungal infections in allogeneic BMT recipients. *Bone Marrow Transplant* 1997; **19**: 801–808.
5 Junghanss C, Marr KA, Carter RA, Sandmaier BM, Maris MB, Maloney DG *et al*. Incidence and outcome of bacterial and fungal infections following nonmyeloablative compared with myeloablative allogeneic hematopoietic stem cell transplantation: a matched control study. *Biol Blood Marrow Transplant* 2002; **8**: 512–520.
6 Grow WB, Moreb JS, Roque D, Manion K, Leather H, Reddy V *et al*. Late onset invasive *Aspergillus* infection in bone marrow transplant patients at a university hospital. *Bone Marrow Transplant* 2002; **29**: 15–19.
7 Safdar A, Papadopoulous EB, Armstrong D. Listeriosis in recipients of allogeneic blood and marrow transplantation: thirteen year review of disease characteristics, treatment outcomes and a new association with human cytomegalovirus infection. *Bone Marrow Transplant* 2002; **29**: 913–916.
8 Safdar A, van Rhee F, Henslee-Downey JP, Singhal S, Mehta J. *Candida glabrata* and *Candida krusei* fungemia after high-risk allogeneic marrow transplantation: no adverse effect of low-dose fluconazole prophylaxis on incidence and outcome. *Bone Marrow Transplant* 2001; **28**: 873–878.
9 MacMillan ML, Goodman JL, DeFor TE, Weisdorf DJ. Fluconazole to prevent yeast infections in bone marrow transplnatation patients: a randomized trial of high versus reduced dose, and determination of the value of maintenance therapy. *Am J Med* 2002; **112**: 369–379.
10 Winston DJ, Maziarz RT, Chandrasekar PH, Lazarus HM, Goldman M, Blumer JL *et al*. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. *Ann Intern Med* 2003; **138**: 705–713.
11 Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changing in epidemiology and risk factors. *Blood* 2002; **100**: 4358–4366.
12 Groll AH, Walsh TJ. Uncommon opportunistic fungi: new nosocomial threats. *Clin Microbiol Infect* 2001; **7** (Suppl 2): 8–24.
13 Kontoyiannis DP, Lionakis MS, Lewis RE, Chamilos G, Healy M, Perego C *et al*. Zygomycosis in a tertiary-care cancer center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis* 2005; **191**: 1350–1360.
14 Safdar A, Singhal S, Mehta J. clinical significance of non-*Candida* fungal blood isolation in patients undergoing high-risk allogeneic hematopoietic stem cell transplantation (1993–2001). *Cancer* 2004; **100**: 2456–2461.
15 Lionakis MS, Kontoyiannis DP. The significance of isolation of saprophytic moulds from the lower respiratory tract in patients with cancer. How do the European Organization for

Research and Treatment of Cancer/Mycosis Study Group criteria apply? *Cancer* 2004; **100**: 165–172.
16 Horger M, Hebart H, Einsele H, Lengerke C, Claussen CD, Vonthein R *et al*. Initial CT manifestation of invasive pulmonary aspergillosis in 45 non-HIV immunocomprmised patients: association with patient outcome. *Eur J Radiol* 2005; **55**: 437–444.
17 Hoger M, Einselle H, Schumacher U, Wehrman M, Hebart H, Lengerke C *et al*. Invasive pulmonary aspergillosis: frequency and meaning of the 'hypodense sign' on unenhanced CT. *Br J Radiol* 2005; **78**: 697–703.
18 Franquet T, Muller NL, Gimenez A, Martinez S, Madrid M, Domingo P. Infectious pulmonary nodules in immunocompromised patients: usefulness of computed tomography in predicting their etiology. *J Comput Assist Tomogr* 2003; **27**: 461–468.
19 Kim MJ, Lee KS, Kim J, Jung KJ, Lee HG, Kim TS. Crescent sign in invasive pulmonary aspergillosis: frequency and related CT and clinical factors. *J Comput Assist Tomogr* 2001; **25**: 305–310.
20 Caillot D, Couaillier JF, Bernard A, Casasnovas O, Denning DW, Mannone L *et al*. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* 2001; **19**: 253–259.
21 Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F *et al*. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002; **34**: 7–14.
22 Musher B, Fredricks D, Leisening W, Balajee SA, Smith C, Marr KA. Aspergillus galactomanna enzyme immunoassays and quantitative PCR for diagnosis of invasive aspergillosis with bronchoalveolar lavage fluid. *J Clin Microbiol* 2004; **42**: 5517–5522.
23 Marr KA, Laverdiere M, Gugel A, Leisenring W. Antifungal therapy decreases sensitivity of the *Aspergillus* galactomannan enzyme immunoassays. *Clin Infect Dis* 2005; **40**: 1762–1769.
24 White PL, Linton CJ, Perry MD, Johnson EM, Barnes RA. The evolution and evaluation of a whole blood polymerase chain reaction assay for the detection of invasive aspergillosis in hematology patients in a routine clinical setting. *Clin Infect Dis* 2006; **42**: 479–486.
25 Florent M, Katsahian S, Vekhoff A, Levy V, Rio B, Marie JP *et al*. Prospective evaluation of a polymerase chain reaction-ELISA targeted to *Aspergillus fumigatus* and *Aspergillus flavus* for the early diagnosis of invasive aspergillosis in patients with hematologic malignancies. *J Infect Dis* 2006; **193**: 741–747.
26 Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systemic review of the literature. *Clin Infect Dis* 2001; **32**: 358–366.
27 Marr KA, Boeckh M, Carter RA, Kim HW, Corey L. Combination antifungal therapy for invasive aspergillosis. *Clin Infect Dis* 2004; **39**: 797–802.
28 Aliff TB, Maslak PG, Jurcic JG, Heaney ML, Cathcart KN, Sepkowitz KA *et al*. Refractory Aspergillus pneumonia in patients with acute leukemia: successful therapy with combination caspofungin and liposomal amphotericin. *Cancer* 2003; **97**: 1025–1032.
29 Kontoyiannis DP, Hachem R, Lewis RE, Rivero GA, Torres HA, Thornby J *et al*. Efficacy and toxicity of caspofungin in combination with liposomal amphotericin B as primary or salvage treatment of invasive aspergillosis in patients with hematologic malignancies. *Cancer* 2003; **98**: 292–299.
30 Marr KA, Boeckh M, Carter RA, Kim HW, Corey L. Combination antifungal therapy or invasive aspergillosis. *Clin Infect Dis* 2004; **39**: 797–802.
31 Safdar A, Rodriguez G, Rolston KV, Kantarjian H, Bodey G, Champlin R *et al*. Efficacy of high-dose (100 mg daily) caspofungin (HD-CASP) combination antifungal therapy in cancer patients with invasive fungal infections (IFIs): case-control observational analysis during 2002–2004. *The 45th Interscience Conference on Antimicrobial Agents and Chemotherapy* 2005, December 16–19, 2005, Washington, DC (abstr. M-955).
32 Maertens J, Glasmacher A, Herbrecht R, Aoun M, Thiebaut A, Cordonnier C *et al*. Multicenter, noncomparative study of caspofungin (CAS) combination with other antifungals in adults with invasive Aspergillus (IA) refractory® or intolerant (I) to prior therapy (Rx): final data. *The 45th Interscience Conference on Antimicrobial Agents and Chemotherapy*. December 16–19, 2005, Washington, DC (abstr. M-954).
33 Greenberg RN, Mullane K, van Burik JA, Raad I, Abzug MJ, Anstead G *et al*. Posaconazole as salvage therapy for zygomycosis. *Antimicrob Agents Chemother* 2006; **50**: 126–133.
34 van Burik JA, Hare RS, Solomon HF, Corrado ML, Kontoyiannis DP. Posaconazole is effective as salvage therapy in zygomycosis: a retrospective summery of 91 cases. *Clin Infect Dis* 2006; **42**: e61–e65.
35 Raad II, Hachem RY, Herbrecht R, Graybill JR, Hare R, Corcoran G *et al*. Posaconazole as salvage treatment for invasive fusariosis in patients with underlying hematologic malignancy and other conditions. *Clin Infect Dis* 2006; **42**: 1398–1403.
36 Balloy V, Si-Tahar M, Takeuchi O, Philippe B, Nahori MA, Tanguy M *et al*. Involvement of Toll-like receptor 2 in experimental invasive pulmonary aspergillosis. *Infect Immun* 2005; **73**: 5420–5425.
37 Wang JE, Warris A, Ellingsen EA, Jorgensen PF, Flo TH, Espevi KT *et al*. Involvement of CD14 and toll-like receptors in activation of human monocytes by *Aspergillus fumigatus* hyphae. *Infect Immun* 2001; **69**: 2402–2406.
38 Netea M, Warris A, Van der Meer JWM, Fenton MJ, Verver-Janssen TJ, Jacobs LE *et al*. *Aspergillus fumigatus* evades immune recognition during germination through loss of toll-like receptor-4-mediated signal transduction. *J Infect Dis* 2003; **188**: 320–326.
39 Rolides E, Dimitriadou-Georgiadou A, Sein T, Kadiltsoglou I, Walsh TJ. Tumor necrosis factor alpha enhances antifungal activities of polymorphonuclear and mononuclear phagocytes against *Aspergillus fumigatus*. *Infect Immunol* 1998; **66**: 5999–6003.
40 Mehrad B, Strieter RM, Standiford TJ. Role of TNF-α in pulmonary host defense in murine invasive aspergillosis. *J Immunol* 1999; **162**: 1633–1640.
41 Mehrad B, Strieter RM, Moore TA, Tsai WC, Lira SA, Standiford TJ. CXC chemokine receptor-2 ligands are necessary components of neutrophil-mediated host defense in invasive pulmonary aspergillosis. *J Immunol* 1999; **163**: 6086–6094.
42 Traynor TR, Herring AC, Dorf ME, Kuziel WA, Toews GB, Huffnagle GB. Differential roles of CC chemokine ligand 2/monocyte chemotactic protein-1 and CCR2 in the development of T1 immunity. *J Immunol* 2002; **168**: 4659–4666.
43 Carpenter KJ, Hogaboam CM. Immunosuppressive effects of CCL17 on pulmonary antifungal responses during pulmonary invasive aspergillosis. *Infect Immun* 2005; **73**: 7198–7207.
44 Cenci E, Mencacci A, Del Sero G, Bacci A, Montagnoli C, d'Ostiani CF *et al*. Interleukin-4 causes susceptibility to invasive pulmonary aspergillosis through suppression of protective type I response. *J Infect Dis* 1999; **180**: 1957–1968.
45 Hebart H, Bollinger C, Fisch P, Sarfati J, Meisner C, Baur M *et al*. Analysis of T-cell responses to *Aspergillus fumigatus*

antigens in healthy individuals and patients with hematologic malignancies. *Blood* 2002; **100**: 4521–4528.
46 Mencacci A, Perruccio K, Bacci A, Cenci E, Benedetti R, Martelli MF *et al*. Defective antifungal T-helper 1 (TH1) immunity in a murine model of allogeneic T-cell depleted bone marrow transplantation and its restoration by treatment with TH2 cytokine antagonists. *Blood* 2001; **97**: 1483–1490.
47 Rivera A, Van Epps HL, Hohl TM, Rizzuto G, Pamer EG. Distinct CD4+-T-cell responses to live and heat-inactivated *Aspergillus fumigatus* conidia. *Infect Immun* 2005; **73**: 7170–7179.
48 Gersuk GM, Underhill DM, Zhu L, Marr KA. Dectin-1 and TLR permit macrophages to distinguish between different *Aspergillus fumigatus* cellular states. *J Immunol* 2006; **176**: 3717–3724.
49 Hohl TM, Van Epps HL, Rivera A, Morgan LA, Chen PL, Feldmesser M *et al*. *Aspergillus fumigatus* triggers inflammatory response by stage-specific b-glucan display. *PLoS Pathog* 2005; **1**: e30.
50 Lionakis MS, Kontoyiannis DP. Glucocorticoids and invasive fungal infections. *Lancet* 2003; **362**: 1828–1838.
51 Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitaive relationship between circulating leukocytes and infection in patients with acute leukemia. *Ann Intern Med* 1966; **64**: 328–340.
52 Stanzani M, Orciuolo E, Lewis R, Kontoyiannis DP, Martins SL, St John LS *et al*. *Aspergillus fumigatus* suppresses the human cellular immune response via gliotoxin-mediated apoptosis of monocytes. *Blood* 2005; **105**: 2258–2265.
53 Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationship between circulating leukocytes and infection in patients with acute leukemia. *Ann Intern Med* 1966; **64**: 328–340.
54 Freireich EJ, Morse EE, Bronson W, Carbone PP. Transfusion of granulocytes from donors with chronic myelocytic leukemia to leukopenic recipients. *Proc IXth Congr Int Soc Hematol* 1962; **1**: 549–557.
55 Freireich EJ, Judson G, Levin RH. Separation and collection of leukocytes. *Cancer Res* 1965; **25**: 1516–1520.
56 Freireich EJ. White cell transfusions born again. *Leuk Lymph* 1993; **11** (Suppl 2): 161–165.
57 Hubel K, Carter RA, Liles WC, Dale DC, Price TH, Bowden RA *et al*. Granulocyte transfusion therapy for infections in candidates and recipients of HPC transplantation: a comparative analysis of feasibility and outcome for community donors versus related donors. *Transfusion* 2002; **42**: 1414–1421.
58 Mousset S, Hermann S, Klein SA, Bialleck H, Duchscherer M, Bomke B *et al*. Prophylactic and interventional granulocyte transfusions in patients with haematological malignancies and life-threatening infections during neutropenia. *Ann Hematol* 2005; **84**: 734–741.
59 Vamvakas EC, Pineda AA. Determinants of the efficacy of prophylactic granulocyte transfusions: a meta-analysis. *J Clin Apher* 1997; **12**: 74–81.
60 Safdar A, Hanna HA, Boktour M, Kontoyiannis DP, Hachem R, Lichtiger B *et al*. Impact of high-dose granulocyte transfusions in patients with cancer with candidemia. Retrospective case-control analysis of 491 episodes of *Candida* species bloodstream infections. *Cancer* 2004; **101**: 2859–2865.
61 Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS *et al*. Neutrophil extracellular traps kill bacteria. *Science* 2004; **303**: 1532–1535.
62 Roilides E, Uhlig K, Venzon D, Pizzo PA, Walsh TJ. Enhancement of oxidative response and damage caused by human neutrophils to *Aspergillus fumigatus* hyphae by granulocyte colony-stimulating factor and gamma interferon. *Infect Immunol* 1993; **61**: 1185–1193.
63 Schuening FG, Nemunaitis J, Appelbaum FR, Storb R. Hematopoietic growth factors after allogeneic marrow transplantation in animal studies and clinical trials. *Bone Marrow Transplant* 1994; **14** (Suppl 4): 74–77.
64 Safdar A, Rodriguez G, Ohmagari N, Kontoyiannis DP, Rolston KV, Raad II *et al*. The safety of interferon-γ-1b therapy for invasive fungal infections after hematopoietic stem cell transplantation. *Cancer* 2005; **103**: 731–739.
65 Safdar A, Rodriguez GH, Lichtiger B, Dickey BF, Kontoyiannis DP, Freireich EJ *et al*. Recombinant interferon γ1b immune enhancement in 20 patients with hematologic malignancies and systemic opportunistic infections treated with donor granulocyte transfusions. *Cancer* 2006; **106**: 2664–2671.
66 Bittencourt H, Rocha V, Filion A, Ionescu I, Herr AL, Garnier F *et al*. Granulocyte colony-stimulating factor for poor graft function after allogeneic stem cell transplantation: 3 days of G-CSF identifies long-term responders. *Bone Marrow Transplant* 2005; **36**: 431–435.
67 Hubel K, Dale DC, Liles WC. Therapeutic use of cytokines to modulate phagocyte function for the treatment of infectious diseases: current status of granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, and interferon gamma. *J Infect Dis* 2002; **185**: 1490–1501.
68 van Eijk M, van Roomen CPAA, Renkema GH, Bussink AP, Andrews L, Blommaart EF *et al*. Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. *Intern Immunol* 2005; **17**: 1505–1512.
69 Choi JH, Brummer E, Kang YK, Jones PP, Stevens DA. Inhibitor kappaB and nuclear factor kappaB in granulocyte-macrophage colony-stimulating factor antagonism of dexamethasone suppression of the macrophage response to *Aspergillus fumigatus* conidia. *J Infect Dis* 2006; **193**: 1023–1028.
70 Brummer E, Kamberi M, Stevens DA. Regulation by granulocyte-macrophage colony-stimulating factor and/or steroids given *in vitro* of proinflammatory cytokine and chemokine production by bronchoalveolar macrophages in response to *Aspergillus* conidia. *J Infect Dis* 2003; **187**: 705–709.
71 Garlanda C, Hirsch E, Bozza S, Salustri A, De Acetis M, Nota R *et al*. Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. *Nature* 2002; **420**: 182–186.
72 Gaziano R, Bozza S, Bellocchio S, Perruccio K, Montagnoli C, Pitzurra L *et al*. Anti-*Aspergillus* efficacy of pentraxin 3 alone and in combination with antifungals. *Antimicrob Agents Chemother* 2004; **48**: 4414–4421.
73 Perruccio K, Tosti A, Burchielli E, Topini F, Ruggeri L, Carotti A *et al*. Transferring functional immune responses to pathogens after haploidentical hematopoietic transplantation. *Blood* 2005; **106**: 4397–4406.
74 Beck O, Topp MS, Koehl U, Poilides E, Simitsopoulou M, Hanisch M *et al*. Generation of highly purified and functionally active human TH1 cells against *Aspergillus fumigatus*. *Blood* 2006; **107**: 2562–2569.
75 Bozza S, Perruccio K, Montagnoli C, Gaziano R, Bellocchio S, Burchielli E *et al*. A dendritic cell vaccine against invasive aspergillosis in allogeneic hematopoietic transplantation. *Blood* 2003; **102**: 3807–3814.
76 Bozza S, Gaziano R, Spreca A, Bacci A, Montagnoli C, di Francesso P *et al*. Dendritic cells transport conidia and hyphae of *Aspergillus fumigatus* from the airways to the draining lymph nodes and initiate disparate Th responses to the fungus. *J immunol* 2002; **168**: 1362–1371.
77 Cordonnier C, Ribaud P, Herbrecht R, Milpied N, Valteau-Couanet D, Morgan C *et al*. Prognostic factors for death due

to invasive aspergillosis after hematopoietic stem cell transplantation: a 1-year retrospective study of consecutive patients at Freach transplantation centers. *Clin Infect Dis* 2006; **42**: 955–963.
78 Maertens J, Verhaegen J, Lagrou K, Eldere JV, Boogaerts M. Screening for circulating galactomannan as a noninvasive diagnositic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001; **97**: 1604–1610.
79 Becker MJ, Lugtenburg EJ, Cornelissen JJ, Van Der Schee C, Hoogstenden HC, De Marie S. Galactomannan detection in computerized tomography-based broncho-alveolar lavage fluid and serum in haematological patients at risk fro invasive pulmonary aspergillosis. *Br J Haematol* 2003; **121**: 448–457.
80 Maertens J, Verhaegen J, Demuynck H, Brock P, Verhoef G, Vandenberghe P *et al*. Autopsy-controlled prospective evalaution of serial screening for circulating galactomnannan by sandwich enzyme-linked immunosorbant asay for hematological patients at risk for invasive aspergillosis. *J Clin Microbiol* 1999; **37**: 3223–3228.
81 Marr KA, Balajee SA, McLaughlin L, Tabouret M, Bentsen C, Walsh TJ. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. *J Infect Dis* 2004; **190**: 641–649.
82 Machetti M, Feasi M, Mordini N, Van Lint MT, Bacigalupo A, Latge JP *et al*. Comparison of an enzyme immunoassay and a latex agglutination system for the diagnosis of invasive aspergillosis in bone marrow transplant recipients. *Bone Marrow Transplant* 1998; **21**: 917–921.
83 Parker JE, Shafi T, Pagliuca A, Mijovic A, Devereux S, Potter M *et al*. Allogeneic stem cell transplantation in the myelodysplastic syndromes: interim results of outcome following reduced-intensity conditioning compared with standard preparative regimens. *Br J Haematol* 2002; **119**: 144–154.
84 Couriel DR, Saliba RM, Giralt S, Khouri I, Andersson B, de Lima M *et al*. Acute and chronic graft-versus-host disease after ablative and nonmyeloablative conditioning for allogeneic hematopoietic transplantation. *Biol Blood Marrow Transplant* 2004; **10**: 178–185.
85 Diaconescu R, Flowers CR, Storer B, Sorror ML, Maris MB, Maloney DG *et al*. Morbidity and mortality with nonmyeloablative compared with myeloablative conditioning before hematopoietic cell transplantation from HLA-matched related donors. *Blood* 2004; **104**: 1550–1558.
86 Canals C, Martino R, Sureda A, Altes A, Briones J, Subira M *et al*. Strategies to reduce transplant-related mortality after allogeneic stem cell transplantation in elderly patients: Comparison of reduced-intensity conditioning and unmanipulated peripheral blood stem cells vs a myeloablative regimen and CD34+ cell selection. *Exp Hematol* 2003; **31**: 1039–1043.
87 Petersen SL, Madsen HO, Ryder LP, Svejgaard A, Jakobsen BK, Sengelov H *et al*. Haematopoietic stem cell transplantation with non-myeloablative conditioning in the outpatient setting: results, complications and admission requirements in a single institution. *Br J Haematol* 2004; **125**: 225–231.
88 Valcarcel D, Martino R, Sureda A, Canals C, Altes A, Briones J *et al*. Conventional versus reduced-intensity conditioning regimen for allogeneic stem cell transplantation in patients with hematological malignancies. *Eur J Haematol* 2005; **74**: 144–151.
89 Alyea EP, Kim HT, Ho V, Cutler C, Gribben J, DeAngelo DJ *et al*. Comparative outcome of nonmyeloablative and myeloablative allogeneic hematopoietic cell transplantation for patients older than 50 years of age. *Blood* 2005; **105**: 1810–1814.
90 Kojima R, Kami M, Kanda Y, Kusumi E, Kishi Y, Tanaka Y *et al*. Comparison between reduced intensity and conventional myeloablative allogeneic stem-cell transplantation in patients with hematologic malignancies aged between 50 and 59 years. *Bone Marrow Transplant* 2005; **36**: 667–674.
91 Massenkeil G, Nagy M, Neuburger S, Tamm I, Lutz C, le Coutre P *et al*. Survival after reduced-intensity conditioning is not inferior to standard high-dose conditioning before allogeneic haematopoietic cell transplantation in acute leukaemias. *Bone Marrow Transplant* 2005; **36**: 683–689.
92 Sorror ML, Maris MB, Storer B, Sandmaier BM, Diaconescu R, Flowers C *et al*. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplantation comorbidities. *Blood* 2004; **104**: 961–968.
93 Scott BL, Sandmaier BM, Storer B, Maris MB, Sorror ML, Maloney DG *et al*. Myeloablative vs nonmyeloablative allogeneic transplantation for patients with myelodysplastic syndrome or acute myelogenous leukemia with multilineage dysplasia: a retrospective analysis. *Leukemia* 2006; **20**: 128–135.
94 Meijer E, Dekker AW, Lokhorst HM, Petersen EJ, Nieuwenhuis HK, Verdonck LF. Low incidence of infectious complications after nonmyeloablative compared with myeloablative allogeneic stem cell transplantation. *Transplant Infect Dis* 2004; **6**: 171–178.
95 Kogan TV, Jadoun J, Mittelman L, Hirschberg K, Osherov N. Involvement of secreted Aspergillus fumigatus proteases in disruption of the actin fiber cytoskeleton and loss of focal adhesion sites in infected A549 lung pneumocytes. *J Infect Dis* 2004; **189**: 1965–1973.