Recent advances in antileukemia therapies and supportive care have resulted in steadily improving survival rates among pediatric leukemia patients [1,2]. Infection-related mortality, which is as high as 6–16% in children receiving remission-induction chemotherapy for high-risk leukemia, has also declined as a result of advances in diagnostic modalities and antimicrobial therapies for infectious diseases [3–6]. Bacterial pathogens remain the most common cause of microbiologically documented infections and of infectious causes of death in pediatric leukemia patients [4,5]. Infections with multidrug-resistant Pseudomonas aeruginosa and enteric pathogens, vancomycin-resistant Enterococcus and methicillin-resistant Staphylococcus aureus have been increasingly reported since the widespread use of empiric and prophylactic antibiotics in high-risk cancer patients [7–9]. However, a review of the management of bacterial infections in pediatric leukemia patients is beyond the scope of this article. This review aims to summarize the management of fungal and viral infections in pediatric leukemia patients, which is less standardized and therefore more challenging for the clinician. When the existing knowledge about viral and fungal infections in children with leukemia is limited, management approaches have been derived from studies involving adult patients or in some cases, based on experience in hematopoietic stem cell transplant (HSCT) patients.

In general, life-threatening viral infections tend to occur infrequently and episodically in pediatric leukemia patients. By contrast, fungal infections have been a continuing threat, although the pattern of life-threatening fungal infections have shifted over the past three decades from predominantly Candida spp. infections, to predominantly Aspergillus spp. infections, to an emerging trend towards preponderance of nonaspergillus mold infections.

Invasive fungal infections in leukemia

Invasive fungal infections (IFIs) remain a major cause of morbidity and mortality in children with leukemia, ranging in incidence from 5 to 28% [10–14]. In a recent single-center prospective study, 13 (2%) out of 614 febrile neutropenic episodes in children with cancer or after HSCT were attributed to IFIs [15]. In a multicenter study of healthcare-associated infections in pediatric cancer patients in Germany, fungal infection was uncommon. However, 10% of the infections were due to Aspergillus spp., which accounted for six out of the eight deaths attributed to infection [16]. A recent review from Australia reported that the incidence of IFIs was 21% in acute lymphoblastic leukemia (ALL), but only 15% in acute myeloid leukemia (AML) [17]. This unexpected finding may be related to the relatively less intense chemotherapy and lack of corticosteroids in the treatment regimens used in this particular AML population. Profound (absolute neutrophil count [ANC] <100 cells/mm³) and prolonged (>2 weeks) neutropenia are the major risk factors for IFI. Other risk factors include broad-spectrum antibiotic use, parenteral nutrition, corticosteroid use and...
central venous catheters. In the recent study from Australia, Hale and colleagues identified risk factors for IFI in children with leukemia to include high-risk ALL, relapsed disease and intensive care admission [17].

Candida and Aspergillus spp. are the most common etiologies of fungal infection in children with leukemia. One third of infection-related deaths in leukemia patients have been associated with infection with these fungi [4]. However, the risk of these fungal infections varies according to both the underlying type of leukemia and the degree of treatment-induced immunosuppression. In general, children with AML are at higher risk for IFI than children with ALL [10-12]. In a recent study, children with AML were randomized to receive either standard versus intensive chemotherapy followed by either stem cell transplant or chemotherapy [18]. Intensive therapy was associated with more infections in general, including fungal infections, compared with standard therapy [18]. Major changes in the epidemiology of IFIs have occurred, coinciding with changes in antifungal use. Since the introduction of fluconazole prophylaxis in leukemia patients in the 1990’s, nonalbicans, triazole-resistant candida, such as Candida glabrata and Candida krusei, are increasingly identified [19-22]. In addition, the incidence of nonaspergillus mold infections, such as zygomycetes and dematiaceous molds, has increased [23-26]. Potential factors accounting for this shift in IFI epidemiology include the increasing use of broad-spectrum antifungal agents, such as triazoles, improved survival of patients, prolonged duration of neutropenia and improved diagnostic methods [27]. Lamaris and colleagues noted that voriconazole, but not amphotericin or caspofungin, enhanced infection with Mucor and Rhizopus in animal models [28]. This unexpected observation that voriconazole can actually enhance growth of zygomycetes may help explain some of the recent shift in patterns of IFI in settings where voriconazole use is widespread.

Figure 1 summarizes the classification of clinically significant fungi commonly observed in the immunocompromised host.

Candidiasis
Manifestations of infections due to Candida spp. span a broad spectrum ranging from mild, such as oropharyngeal thrush, vaginitis, and dermatitis, to severe, such as esophagitis and disseminated infection. Systemic candidiasis is a serious disease that predominately involves the lungs, liver, spleen, kidneys and occasionally the brain. Risk factors associated with candidemia in pediatric cancer patients include prolonged neutropenia, severe mucositis, younger age, corticosteroid therapy, parenteral nutrition, and use of prophylactic antibiotics [29-31]. Disseminated candidiasis that involves multiple organs has become less frequent over the past decades since the introduction of antifungal prophylaxis. Candidemia is more likely to occur in immunosuppressed patients with central venous catheters [32]. Although native valve endocardial wall infective endocarditis, due to candida, is rare, it was more frequently reported in patients with indwelling central venous catheters [33].

Chronic disseminated candidiasis, also called hepatosplenic candidiasis, presents with persistent fever not responding to broad-spectrum antibiotics, abdominal pain, hepatosplenomegaly and elevation of alkaline phosphatase. Characteristic radioimaging findings include the 'bull's eye' sign, which is a hypoechoic lesion with hyperechoic center on ultrasonography, or multiple hypodense lesions in the liver and spleen visualized on CT scan only after recovery of neutropenia [34]. Blood cultures are often sterile, despite systemic candidiasis. Candidemia is documented in less than 20% of the patients with chronic disseminated candidiasis [35-37]. In the absence of a positive blood culture, definitive diagnosis of disseminated candidiasis requires histopathologic examination and staining, and culture of tissue biopsy specimens. Among 423 patients with acute leukemia, young age, prolonged neutropenia for 15 days or longer, and prophylactic fluoroquinolone use were shown to be independent risk factors for chronic disseminated candidiasis [31].

Other yeast infections
Cryptococcal infection is uncommon in children with leukemia. Cryptococcus may manifest as skin or lung lesions, meningitis or prolonged fever. Recognition of this infection is of particular importance since infections with these organisms are not covered by echinocandins.

Trichosporon infections may manifest with persistent fever in the setting of prolonged neutropenia and papular or necrotic skin lesions indicating disseminated infection, sometimes diagnosed by positive blood culture [38]. Localized infections involving only the lungs or bloodstream have also been reported. Trichosporon infections are resistant to echinocandins.

Pneumocystis jirovecii was recently reclassified from a protozoan to a fungus (Figure 1A) [39,40]. This organism classically causes interstitial pneumonia. Rarely, pneumocystis infection may present as a consolidated pneumonia or may occur at other sites. These organisms are generally not susceptible to conventional antifungal agents, although echinocandins have some in vitro activity against pneumocystis spores.

Aspergillosis
Aspergillosis is uncommon in children with ALL, but approximately 4% of pediatric AML patients develop invasive aspergillosis [41]. Although several hundred species of Aspergillus have been identified, only approximately 12 species are associated with human infection. The most common species in human infections are Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus and Aspergillus niger. Infection occurs when spores are inhaled or contaminate a wound. Construction can increase exposure to Aspergillus spores. Despite the improving diagnostic and therapeutic modalities, mortality rates due to invasive aspergillosis are still reported to be as high as 60% [41,42]. Zaoutis et al. recently reviewed a national database in the USA and found that the incidence of invasive aspergillosis in hospitalized, immunocompromised pediatric patients was 0.4%, with an 18% mortality rate [41]. The clinical features of Aspergillus infection include sinusitis, pneumonitis, hemoptysis, tracheobronchitis, brain abscess or infiltrate, cutaneous necrosis, and ulceration. Pneumonitis, sinusitis and skin infections are the most common manifestations.
Managing fungal & viral infections in pediatric leukemia

Fusariosis

Fusariosis has been increasingly recognized in immunocompromised patients, particularly those with hematologic malignancies. *Fusarium solani* is the most frequently isolated species. Fusariosis in the immunocompromised patients is characterized by disseminated invasive disease with poor prognosis. Fusariosis-associated mortality has been reported to range from 79 to 100% [43,44]. Risk factors for invasive fusariosis include persistent neutropenia and corticosteroid therapy [43]. A periungual infection, usually on a toe, may be observed. The appearance of multiple purpuric nodular lesions with central necrosis should raise suspicion for disseminated fusarium infection. Biopsy of these nodules reveals branching angioinvasive septate hyphae. Fusarium is characterized by relative resistance to most antifungal agents, with susceptibility profiles varying according to the species and strain [45].

Zygomycoses

Zygomycetes are an increasingly recognized cause of invasive mold infection in immunocompromised patients. The epidemiology, clinical presentation and course of zygomycetes infections have been derived from a review of case reports [23,46]. Dehority and colleagues recently reported that the majority of children with hematologic malignancies complicated by zygomycosis had ALL [23]. The overall mortality rate was 42.5%. However, mortality rates decreased from 100% in the decade 1950–1959 to 25.8% in the period between 2000 and 2007. Zygomycoses may present as sinusitis; pulmonary infiltrate or infarct; a localized erythematous papule or eczema or eschar-like cutaneous lesion; rhinocerebral infiltration; and disseminated infection. Children with an isolated skin infection had a good prognosis, while disseminated infection was associated with significantly increased

Figure 1. Classification of clinically significant (A) yeasts and (B) molds commonly observed in the immunocompromised host.

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its anamorph, *prolificans* are the only available antifungal agents with activity against zygo-
(biopsy/autopsy) or otherwise sterile body fluids remains the mycetes. Optimal therapy requires aggressive antifungal therapy coupled with the cessation of immunosuppression (if feasible) and surgical resection of all infected tissue where possible [47,48].

**Other mold & fungal infections**

Infections with molds that were once rarely isolated are becoming increasingly common (Figure 1B) [49]. In particular, these include the dematiaceous fungi; *Pseudallescheria boydii* and its anamorph, *Scedosporium apiospermum*; and *Scedosporium prolificans* [50,51]. The presentation and course of these infections are not readily distinguishable from other mold infections; however, these organisms should be suspected in particular when skin lesions on the lower extremities are encountered. Histoplasmosis, blastomycosis and coccidioidomycosis are occasionally encountered in leukemia patients, depending on the geographic location [52,53].

**Diagnosis of invasive fungal infection**

The specific diagnosis of IFI is often challenging. Autopsy studies have shown that fungal infections at the time of death are frequently not diagnosed antemortem [54]. In general, yeast infections are most often definitively diagnosed by blood culture or by blood antigen test. By contrast, aspergillosis, zygomycosis and dematiaceous infections are rarely diagnosed by blood culture, although some molds are occasionally found in the blood, especially *Fusarium* spp. Biopsy of skin lesions, pulmonary lesions and sinus membranes is especially helpful in the definitive diagnosis of mold infection. Histologic examination with silver stain is usually positive if the specimen yields a positive culture, and is often positive even in the absence of a culture isolate. Upon histologic examination, it is generally not possible to distinguish *Aspergillus from Fusarium* or *Pseudallescheria*, a limitation that has significant clinical implications given the varying antifungal susceptibilities of these molds. The increasing frequency of previously rare mold infections and the ability to perform antifungal susceptibility testing in specialized laboratories argues strongly for obtaining a biopsy to attempt to isolate the infecting organism. The major risks of biopsy are hemorrhage and poor wound healing with secondary infection, especially in neutropenic patients.

Culture of the mouth, urine or stool often yields *Candida* spp., but such isolation merely establishes colonization. However, febrile neutropenic children who are not receiving antifungal prophylaxis and who are colonized with *Candida tropicalis* are approximately tenfold more likely to develop invasive candidiasis than those colonized with *Candida albicans* [55]. Culture and histology of bronchoalveolar lavage (BAL) fluid is typically of low yield [56], except in pneumocystosis. Cancer patients with pneumocystosis typically have few organisms visible in BAL fluid, in contrast to HIV-infected patients with pneumocystosis. Interpretation of candida isolated from BAL fluid is often challenging. However, isolation of a mold in conjunction with a typical pulmonary lesion can be useful to guide therapy. Owing to the low yield of BAL we generally favor needle or open biopsy as the diagnostic procedure of choice for pulmonary lesions when mold infection is suspected.

Computerized tomography (CT) is very helpful in screening for pulmonary mold infections and disseminated candidiasis, with the caveat that candidal lesions are often not apparent until neutrophil recovery in the neutropenic patient. Radiologic findings include pulmonary infiltrates and wedge-shaped lesions, and less commonly, the classic ‘halo signs’ and ‘air crescent signs’ indicative of aspergillosis. ‘Reversed halo sign’, which is defined as a rounded area of ground-glass appearance surrounded by a complete ring of consolidation, can be indicative of IFI, especially infections with zygomycetes [57]. In a recent study, less than 25% of children with invasive aspergillosis had a classic radiographic finding of IFI, such as a cavitary lesion, halo sign or air crescent sign [58]. Neutropenic children may lack any focal findings until neutrophil recovery. CT scans of sinuses showing a soft tissue mass within the sinus, mucosal thickening, fluid level, and bony invasion strongly suggest mold infection of the sinuses, usually aspergillosis [59]. In the febrile neutropenic child with leukemia and regardless of the use of empiric antifungal therapy, we generally advocate CT of the chest to screen for mold infections if there is fever beyond 4 or 5 days of treatment with broad-spectrum antibiotics or if the patient is not clinically well. The finding of lesions indicative of mold infection is an indication for biopsy and may alter the recommended empiric antifungal regimen. By contrast, CT examination of the abdomen can often be delayed until neutrophil recovery because candidal lesions are often not visible until recovery and most empiric antifungal therapy regimens are effective for disseminated candidiasis.

Recently, there have been significant advances in the diagnosis of IFIs, especially aspergillosis, using noninvasive methods based on the detection of the fungal antigen or DNA elements in the serum [60]. *Aspergillus galactomannan* (GM) is a polysaccharide cell wall component that can be detected by ELISA as a marker of invasive aspergillosis [61–64]. The detection of GM in serum has been reported to precede the development of clinical signs and symptoms or radioimaging findings by several days [65,66]. However, a study of 56 pediatric oncology patients found that a positive test result is predictive of invasive aspergillosis, but has a low sensitivity [67]. A more recent pediatric study evaluated the performance of the GM assay in 195 periods at risk for invasive aspergillosis following chemotherapy and HSCT [63]. Similar to the previous pediatric study, GM assay was found to have a poor sensitivity of 32%, but a good specificity and negative predictive value of 98% and 98%, respectively [63]. A recent meta-analysis evaluated the performance of GM assay for the diagnosis of proven invasive aspergillosis and reported a sensitivity of 71%, a specificity of 89%, a negative predictive value of 92–98% and a positive predictive value of 25–62% [68]. This meta-analysis included studies that varied with regard to the age of the evaluated patient population (adults vs pediatrics), underlying cancer
diagnosis, definition of proven or probable invasive aspergillosis and the cutoff level of positivity of the GM assay. Table 1 summarizes the GM assay performance at various cutoff levels of positivity [68]. False-positive results have been associated with the use of certain β-lactam antibiotics (piperacillin, piperacillin–tazobactam, amoxicillin and amoxicillin–clavulanic acid), ingestion of protein-rich food items, such as milk and soybeans, the use of cyclophosphamide, presence of mucositis and receipt of hematopoietic stem cell transplant. False-positive results were more likely to occur in children than adult patients [66,69]. GM has also been detected in clinical specimens other than serum including BAL, urine and CSF. The sensitivity and specificity of GM detection in BAL fluid was estimated to be 88 and 87%, respectively [70]. False-negative results are more likely to occur in patients receiving antifungal agents that are active against aspergillus [61].

Another noninvasive assay for the diagnosis of IFI detects (1 → 3)-β-D-glucan, which is a universal component of the fungal cell wall [71–73]. This assay was highly sensitive for the detection of IFI in patients with AML or myelodysplastic syndrome, but as expected, it was not specific for any type of fungus [71]. False-positive results have been associated with the use of cellulose membranes in dialysis machines, certain antimicrobial use (e.g., some cephalosporins, carbapenems, and β-lactam antibiotics, such as amoxicillin–clavulanic acid and ampicillin–sulbactam), and with *Pseudomonas aeruginosa* infection [74,75]. The antigen-based tests have been mainly used as an adjunctive diagnostic test in adult patients with a clinical picture suggestive of IFI and screening of high-risk patients, such as those undergoing allogeneic stem cell transplantation. The use of (1 → 3)-β-D-glucan assay in pediatric patients has been limited because of a lack of established baseline reference ranges of glucan levels in uninfected children. Recently, Smith and colleagues measured (1 → 3)-β-D-glucan levels in serum samples collected from 120 immunocompetent healthy children [76]. The median (1 → 3)-β-D-glucan level was 32 pg/ml, and the mean value was 68 (±128) pg/ml, which was higher than the values previously reported in healthy adults. The only pediatric report in immunocompromised patients is a small series of two pediatric recipients of HSCT and two low-birthweight neonates with proven IFI who had a (1 → 3)-β-D-glucan level higher than 523 pg/ml [77]. More pediatric studies are needed to evaluate the role of (1 → 3)-β-D-glucan assay in diagnosing IFIs in immunocompromised children.

Other recently developed diagnostic tools include molecular techniques to detect fungal DNA, such as PCR, which is characterized by high sensitivity and rapid results. However, the clinical role of PCR-based techniques still needs to be further investigated before it can be recommended for clinical practice.

The recent advances in the noninvasive fungal diagnostic techniques, including high-resolution CT scans, GM and (1 → 3)-β-D-glucan assays, and detection of fungal DNA by PCR have prompted the revision of definitions of ‘proven’, ‘probable’, and ‘possible’ IFI, which have been published recently [78]. Although the revised definitions are derived from studies involving adults, they are widely adopted in the pediatric patient population.

### Treatment approach for IFI

Early diagnosis of IFI and prompt initiation of antifungal therapy have been associated with an improved outcome [79]. Traditionally, amphotericin B deoxycholate has been the drug of choice for most severe fungal infections in neutropenic patients, however, its use has been limited over the years because of its toxicity profile and the development of lipid formulations of amphotericin B in the mid-1990s. Currently, three lipid formulations are available: amphotericin B colloidal dispersion, amphotericin B lipid complex and liposomal amphotericin B. Lipid formulation amphotericin B is associated with fewer infusion-related adverse events and less nephrotoxicity than amphotericin B deoxycholate. Liposomal amphotericin is preferred for the treatment of meningitis [80]. Amphotericin lipid complex may be advantageous in treating pulmonary infection, although its nephrotoxicity may be higher than liposomal amphotericin [81]. The recommended dosage of liposomal amphotericin B varies in children from 3 to 5 mg/kg/day depending on the suspected resistance of the infecting organism, and the formulation is generally well tolerated by pediatric cancer patients. Several new antifungal agents have been developed for clinical use over the past decade. It should be noted that although commonly used in children, pediatric use of most of the antifungal agents, including amphotericin B, voriconazole, echinocandins and posaconazole have not been approved by the US FDA. The newer generation triazoles (voriconazole and posaconazole) are characterized by their broad spectrum of antifungal coverage that includes molds. There is no established dosing of voriconazole in children younger than 12 years of age. In adults and children aged 12 years or more, an intravenous loading dose of 6 mg/kg for two doses followed by a maintenance

### Table 1. Performance of the galactomannan assay for the diagnosis of invasive aspergillosis.

<table>
<thead>
<tr>
<th>Cutoff value for GM assay positivity</th>
<th>Proven invasive aspergillosis</th>
<th>Proven or probable invasive aspergillosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pooled sensitivity (95% CI)</td>
<td>Pooled specificity (95% CI)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.27 (0.06–0.61)</td>
<td>0.79 (0.74–0.83)</td>
</tr>
<tr>
<td>1.0</td>
<td>0.79 (0.71–0.87)</td>
<td>0.87 (0.85–0.88)</td>
</tr>
<tr>
<td>1.5</td>
<td>0.68 (0.58–0.76)</td>
<td>0.92 (0.91–0.93)</td>
</tr>
</tbody>
</table>

GM: Galactomannan. Reproduced with permission from [68].
dose of 4 mg/kg every 12 h has been recommended. Blood levels of voriconazole should be monitored to ensure therapeutic levels are achieved, particularly when using an oral formulation. Other than amphotericin B, posaconazole is the only currently available drug that is active against zygomycetes. The factors that limit the use of triazoles include hepatotoxicity and interaction with drugs, chemotherapy in particular, metabolized by cytochrome P450 or P3A4 enzymes [82]. Posaconazole is only available as an oral formulation and must be administered with a high-fat food for optimal absorption (administration with nonlight ice cream is an effective strategy in children). Posaconazole serum levels should be monitored in patients with mucositis, poor oral intake, severe diarrhea, or in those who are receiving proton-pump inhibitors. Posaconazole has been approved by FDA only for prophylaxis against invasive Aspergillus and Candida infections in patients 13 years of age and older. Posaconazole dosing in children younger than 13 years of age has not been established. Echinocandins’ antifungal coverage is limited to Candida spp. and Aspergillus spp. Echinocandins (including caspofungin and micafungin) are characterized by the safest toxicity profiles among antifungal drug classes and limited drug interactions because these agents have no effect on the cytochrome P450 enzyme. Anidulafungin is a new echinocandin that is effective in treating esophageal candidiasis, including azole-resistant Candida spp. In a recent randomized clinical trial, anidulafungin was shown to be noninferior to fluconazole in the treatment of candidemia and invasive candidiasis [83]. However, existing knowledge regarding anidulafungin is based on the adult population. More clinical studies are needed to evaluate its role in pediatric patients.

Empiric versus pre-emptive antifungal therapy

By definition, empiric antifungal therapy is initiated in a symptomatic patient prior to the determination of proven IIFI, usually in the setting of fever with neutropenia. The Infectious Diseases Society of America (IDSA) guidelines for the management of febrile and neutropenic patients recommend the use of empiric antifungal therapy in neutropenic patients with persistent fever on day 3–5 of adequate broad-spectrum antibacterial coverage [84]. Liposomal amphotericin B has been considered the antifungal agent of choice for empiric therapy because of its broad coverage. However, the use of newer antifungal agents for empiric therapy has been assessed in recent randomized clinical trials. In a double-blind, multinational, noninferiority clinical trial, 1095 neutropenic patients were randomized to receive either caspofungin or liposomal amphotericin B [85]. Caspofungin was shown to be as efficacious as liposomal amphotericin B, as determined by a five-component end point that consisted of successful treatment of any baseline fungal infection, absence of any breakthrough fungal infection during therapy or within 7 days after the completion of therapy, survival for 7 days after the completion of therapy, no premature discontinuation of study therapy because of drug-related toxicity or lack of efficacy, and resolution of fever during neutropenia [85]. A recent randomized, double-blind, multicenter trial was designed to assess the safety of caspofungin versus liposomal amphotericin B as empiric antifungal therapy in neutropenic children with persistent fever [86]. Efficacy of caspofungin compared with liposomal amphotericin B was also evaluated as a secondary end point and was defined as the accomplishment of the following five criteria: successful treatment of any baseline IIFI, absence of any breakthrough fungal infection during therapy or within 7 days after the completion of therapy, survival for 7 days after the completion of therapy, no premature discontinuation of study therapy because of drug-related toxicity or lack of efficacy, and resolution of fever during neutropenia [86]. Caspofungin was shown to be as safe and effective as liposomal amphotericin B as empiric antifungal therapy in persistently febrile neutropenic children when administered on a body surface area basis, rather than according to weight [86]. Another open-label, randomized, multinational noninferiority trial indicated that voriconazole is an adequate alternative to liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever [87]. Moreover, voriconazole was associated with significantly reduced rate of breakthrough IIFI [87]. The IDSA is in the process of updating its guidelines for the management of febrile neutropenic patients.

In contrast to empiric therapy, pre-emptive antifungal therapy is initiated at the earliest sign of IIFI, such as CT scan findings or positive GM assay and before the appearance of clinical signs and symptoms. A recent multicenter, open-label, randomized, noninferiority trial compared these two strategies of antifungal therapy in febrile neutropenic patients mostly receiving chemotherapy for AML – empiric versus pre-emptive approaches [88]. Empiric treatment was initiated in patients with persistent or recurrent fever. Pre-emptive treatment was defined as initiation of antifungal therapy in patients who had clinical, imaging, or galactomannan findings suggesting IIFI. Intention-to-treat analysis showed no difference in the survival rates between the two study arms [88]. A subgroup analysis in AML patients undergoing induction chemotherapy showed better survival rates in those who received empirical antifungal coverage when compared with pre-emptive therapy [88]. In addition, pre-emptive treatment failed to reduce nephrotoxicity. It is worth noting that the success of any pre-emptive approach is largely dependent on the performance of the tests and the choice of the surrogate marker. For example, the sensitivity and specificity of the GM detection assays vary according to the cutoff level used [89]. More trials are needed to evaluate the role of pre-emptive antifungal therapy in clinical care, but at this time the use of empiric therapy for children with suspected fungal infection is widely practiced.

Definitive therapy of invasive fungal infections

Table 2 summarizes the IDSA guidelines for the treatment of several fungal infections. Table 3 provides suggestions for initial management of uncommon fungal infections pending antifungal susceptibility testing. Table 4 summarizes key gaps in coverage of the major antifungal agents.

Candidiasis

The primary antifungal therapy for candidiasis depends on the patient’s neutrophil count, his/her previous exposure to azoles and the condition or organ involvement in the infection.
Fluconazole is the primary antifungal agent for the definitive treatment of candidemia in non-neutropenic patients, symptomatic cystitis, pyelonephritis, chronic disseminated candidiasis, candida osteoarticular infections and esophageal candidiasis [90]. Echinocandins are the drugs of choice for definitive treatment of candidemia in neutropenic patients or empirical treatment of suspected invasive candidiasis in both neutropenic and non-neutropenic patients [90]. Patients with recent exposure to azoles should receive an echinocandin or lipid formulation amphotericin B as the initial therapy [90]. Esophageal candidiasis is treated with fluconazole, an echinocandin or amphotericin B [90]. Disseminated candidiasis is treated with fluconazole or amphotericin B. Echinocandins are an alternative regimen for this indication [90]. In general, transition to oral fluconazole or voriconazole after initial therapy with an echinocandin is appropriate in many cases, especially if the *Candida* isolate is azole-susceptible and the patient’s neutropenia has resolved.

**Trichosporon infection**

Trichosporon species are resistant to echinocandins [91,92]. At our institution, combination therapy with high-dose voriconazole (patients should be monitored for hepatic and other toxicity carefully) and terbinafine have been effective in treating a small number of patients with trichosporon infection, which has a high mortality historically. Use of antifungal susceptibility testing may be helpful.

### Table 2. Infectious Diseases Society of America treatment guidelines for various fungal pathogens.

<table>
<thead>
<tr>
<th>Fungal pathogen</th>
<th>Primary therapy</th>
<th>Alternative therapy</th>
<th>Comments</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Candidiasis</strong></td>
<td>Caspofungin, micafungin, fluconazole</td>
<td>Azoles, lipid formulation amphotericin B</td>
<td>• Primary combination therapy is not routinely recommended based on lack of clinical data</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Indications for surgical intervention are lesions that are contiguous with the great vessels or pericardium, lesions causing hemoptysis from a single focus, and lesions causing erosion into the pleural space or ribs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Micafungin has been used as salvage therapy for invasive aspergillosis, but remains investigational for this indication</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Posaconazole is US FDA-approved only for prophylaxis against invasive aspergillosis and <em>Candida</em> infections in patients 13 years of age and older</td>
<td></td>
</tr>
<tr>
<td><strong>Aspergillosis</strong></td>
<td>Voriconazole</td>
<td>Lipid formulation amphotericin B, caspofungin, posaconazole</td>
<td></td>
<td>[95]</td>
</tr>
<tr>
<td><strong>Cryptococcosis</strong></td>
<td>Fluconazole Induction with amphotericin B plus flucytosine followed by consolidation with fluconazole</td>
<td>Amphotericin B plus flucytosine Amphotericin B plus fluconazole Flucytosine Itraconazole</td>
<td>• Lumbar puncture is recommended to rule out CNS infection</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Monitor renal function because of flucytosine toxicity</td>
<td></td>
</tr>
<tr>
<td><strong>Coccidioidomycosis</strong></td>
<td>Amphotericin B Lipid formulation amphotericin B Fluconazole</td>
<td>Itraconazole Ketoconazole</td>
<td></td>
<td>[214]</td>
</tr>
<tr>
<td><strong>Blastomycosis</strong></td>
<td>Itraconazole Lipid formulation amphotericin B followed by itraconazole</td>
<td></td>
<td>• Long-term suppressive treatment may be required during immunosuppression</td>
<td>[215]</td>
</tr>
<tr>
<td><strong>Histoplasmosis</strong></td>
<td>Itraconazole Amphotericin B followed by itraconazole</td>
<td></td>
<td>• Itraconazole solution is preferred for better absorption than the capsule</td>
<td>[216]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Methylprednisolone during the first 1–2 weeks of antifungal therapy is recommended for patients with moderately severe to severe acute pulmonary histoplasmosis, mediastinal lymphadenitis or pericarditis</td>
<td></td>
</tr>
<tr>
<td><strong>Sporotrichosis</strong></td>
<td>Lipid formulation amphotericin B followed by itraconazole Itraconazole</td>
<td></td>
<td>• Surgery combined with antifungal therapy is recommended for localized disease</td>
<td>[217]</td>
</tr>
</tbody>
</table>
Table 3. Suggested treatment of uncommon fungal infections pending susceptibility testing.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Treatment recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichosporon</td>
<td>Voriconazole, high dose plus terbinafine</td>
</tr>
<tr>
<td>Zygomycetes</td>
<td>Liposomal amphoterin B or posaconazole as investigational</td>
</tr>
<tr>
<td>(e.g., Mucor)</td>
<td>salvage therapy</td>
</tr>
<tr>
<td>Dematiaceous</td>
<td>Posaconazole or liposomal amphoterin B</td>
</tr>
<tr>
<td>Fusarium</td>
<td>Posaconazole or voriconazole, high dose</td>
</tr>
<tr>
<td>Scedosporium</td>
<td>Posaconazole or voriconazole, high dose; addition of</td>
</tr>
<tr>
<td></td>
<td>terbinafine may be considered for <em>Scedosporium prolificans</em></td>
</tr>
</tbody>
</table>

Pneumocystosis

Conventional antifungals are generally ineffective. Intravenous trimethoprim–sulfamethoxazole with steroids is the preferred treatment unless there is an allergy to sulfa drugs or pneumocystis broke through trimethoprim–sulfamethoxazole prophylaxis (very rare in compliant leukemia patients in our extensive experience). Typically, response to treatment is not evident for 2–3 days, and a favorable outcome is dependent on early diagnosis and treatment. Atovaquone, pentamidine, clindamycin plus primaquine, and dapsone plus trimethoprim are alternative therapies. Second to trimethoprim–sulfamethoxazole, intravenous pentamidine is the most extensively evaluated drug and is considered the alternative treatment option in patients intolerant to trimethoprim–sulfamethoxazole [93]. However, intravenous pentamidine use is limited by its toxicities, including renal dysfunction, prolonged QT interval, electrolyte abnormalities and pancreatitis. A randomized, double blind clinical trial demonstrated that the three treatment regimens, trimethoprim–sulfamethoxazole, trimethoprim–dapsone and clindamycin–primaquine, had similar efficacy in the treatment of mild to moderate pneumocystis pneumonia in AIDS patients [94]. No clinical trials have been performed to compare the various treatment regimens of pneumocystis pneumonia in immunocompromised non-AIDS patients. Systemic steroids have been used to prevent further deterioration of the respiratory status following the initiation of antipneumocystis therapy.

Aspergillosis

Voriconazole is the primary drug for the treatment of invasive aspergillosis [95–98]. A large, randomized clinical trial for the primary treatment of invasive aspergillosis has shown that outcomes of patients treated with voriconazole are as good as or better than outcomes in patients treated with amphotericin B [95]. Surgical intervention with excision of localized lesions is indicated in some cases [95].

Zygomycosis & other molds

Data regarding the antifungal therapy of zygomycoses are derived from case series. There are no comparative clinical trials available. Amphotericin B is considered the standard of care for zygomycosis, although treatment failure is common.

Combination antifungal therapy

Combination antifungal therapy for mold infections including aspergillosis has been used in individualized reported cases, some of which suggest a possible benefit. However, there is concern that amphotericin B and voriconazole has been shown to be antagonistic *in vitro* [103–105]. Furthermore, the combination of caspofungin and amphotericin B lipid complex has been shown to improve the survival rates of diabetic ketoacidotic mice with disseminated zygomycosis when compared with amphotericin B lipid complex monotherapy [106]. Steinbach et al. recently summarized the evidence regarding the combination antifungal therapy and showed inconsistent results from the different preclinical studies [107]. The use of more than one agent from the same class should be avoided because of added toxicity without any known benefit. In addition, there is the aforementioned concern regarding voriconazole stimulation of zygomycete infection found in animal models [28]. Clinical studies are needed to evaluate the role of combination antifungal therapy in clinical practice.

Adjuvant immune therapy

Several immune-modulating approaches have been investigated as adjuvant therapy, directed at the risk factors predisposing patients to infections [108].

Table 4. Deficiencies in coverage by antifungal agents.

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Lack of coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomal amphoterin</td>
<td>Trichosporon, some Candida, Fusarium, Pseudallescheria,</td>
</tr>
<tr>
<td></td>
<td>Scedosporium</td>
</tr>
<tr>
<td>Micafungin/caspofungin</td>
<td>Cryptococcus, Histoplasma, Blastomyces, Coccidioides,</td>
</tr>
<tr>
<td></td>
<td>Trichosporon, some Candida, Zygomycetes (e.g., Mucor),</td>
</tr>
<tr>
<td></td>
<td>Fusarium, Scedosporium</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Some Candida, Zygomycetes, some dematiaceous fungi</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>Some Candida</td>
</tr>
</tbody>
</table>
Colony-stimulating factors
Granulocyte–macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) stimulate the proliferation, maturation and mobilization of myeloid progenitor cells and hence reduce the duration and severity of neutropenia [109]. Most of the growing evidence supporting the potential role of colony stimulating factors in improving treatment outcomes has been derived from studies involving adults with cancer. The recent guidelines issued by the American Society of Clinical Oncology (ASCO) recommends the prophylactic use of CSF after completion of the consolidation chemotherapy for adults with AML because its use decreases the duration of severe neutropenia, the incidence of infection and the likelihood of hospitalization [109]. A recent meta-analysis of 16 multicenter randomized clinical trials of prophyactic CSF use in leukemic children reported significant reductions in the incidence of fever and neutropenia, documented infections, and length of hospitalization [110]. However, the major limitation to its prophylactic use in children is the concern regarding the potential risk for secondary AML or myelodysplastic syndrome or relapse of AML associated with CSF use [109,111,112]. The therapeutic use of a CSF may be considered in children with febrile neutropenia and poor prognostic factors and who are at high-risk for infection-associated complications, including prolonged (>10 days) and profound (<100/µl) neutropenia, uncontrolled primary disease, pneumonia, hypotension, multiorgan dysfunction or invasive fungal infection [109,113].

Recombinant human IFN-γ
Recombinant human IFN-γ (rhIFN-γ1b) reverses the phagocyte dysfunction induced by steroids and enhances the ability of the immune system to control fungal infection [114–116]. However, the potential risk of drug-induced toxicity, generalized systemic inflammatory reaction, exacerbation of graft-versus-host disease, relapse of hematologic malignancy and lack of data regarding its therapeutic efficacy remain the limiting factors for its use [117].

Granulocyte transfusion
The potential benefit of dexamethasone-primed or G-CSF-mobilized granulocyte transfusion as adjunctive therapy of invasive fungal infections in neutropenic children has been reported in small studies [116,118,119]. However, controlled trials are needed to further evaluate its efficacy and safety as an adjuvant therapy.

Antifungal chemoprophylaxis
Despite the recent advances in diagnostic modalities, the diagnosis of IFI remains difficult because of nonspecific symptoms, lack of inflammatory signs due to neutropenia, and the invasiveness of tissue biopsy procedures for histologic examination. Therefore, preventive measures, including antifungal prophylaxis, have been investigated as strategies to reduce fungal-related mortality. The randomized clinical trials of antifungal prophylaxis differed in their outcome measures, antifungal agents used, patient populations studied and the type of prophylaxis – whether primary or secondary.

Primary antifungal prophylaxis
Early evidence of the efficacy of fluconazole or itraconazole in primary IFI prophylaxis was derived from clinical trials conducted in adult recipients of HSCT [120–124]. However, efficacy of antifungal prophylaxis is not as well established in patients with hematologic malignancies. Cornely and colleagues have reviewed 50 clinical trials of primary antifungal prophylaxis in more than 9000 patients [124]. The authors concluded that evidence to support routine primary antifungal prophylaxis in patients with hematologic malignancies not undergoing allogeneic stem cell transplantation is poor; however, there was no evidence of worse outcome with antifungal prophylaxis [124]. On the other hand, a meta-analysis that evaluated 38 trials involving 7014 patients concluded that antifungal prophylaxis in patients with chemotherapy-induced prolonged neutropenia was effective in reducing the incidence of invasive fungal infections and of fungal infection-related mortality, even though invasive aspergillosis and overall mortality rate were not reduced [125]. A recent meta-analysis evaluated the role of antifungal prophylaxis in cancer patients after chemotherapy or HSCT [126]. Most of the evaluated studies involved adult patients with acute leukemia. Overall, antifungal prophylaxis significantly decreased all-cause mortality in cancer patients compared with placebo, no treatment or nonsystemic antifungals. However, only the reduction in fungal-related mortality and documented IFI was significant in acute leukemia patients. It is important to note that the antifungal agents evaluated in these clinical trials included fluconazole, itraconazole, ketoconazole, miconazole, and amphotericin B.

Newer generation triazoles that have activity against aspergillus, voriconazole and posaconazole, have been evaluated in later studies. In a recent open-label study that randomized 127 patients with AML or high-risk myelodysplastic syndrome to receive either voriconazole or itraconazole prophylaxis, voriconazole was shown to be as effective and safe as itraconazole in preventing IFIs [127]. Although larger, randomized clinical trials to evaluate the use of voriconazole prophylaxis in high-risk leukemia patients are lacking, the results of one recent trial demonstrated its beneficial effect as prophylaxis in HSCT patients [128,129]. In two large, randomized, blinded clinical trials, posaconazole prophylaxis has been shown to be superior to fluconazole or itraconazole in preventing IFI [130,131]. One trial evaluated patients who were 13 years of age or older and had neutropenia for 7 days or more that resulted from remission-induction chemotherapy given for newly diagnosed, or the first relapse of, AML or MDS [130]. Posaconazole prophylaxis was administered with each cycle of chemotherapy and continued until recovery from neutropenia and complete remission of the underlying malignancy, occurrence of an IFI, or until 12 weeks completed, whichever event occurred first. Proven or probable IFIs, invasive aspergillosis, and all-cause mortality were significantly reduced in patients who received posaconazole prophylaxis when compared with those who received fluconazole or itraconazole [130]. In another trial, 600 HSCT recipients with GVHD were randomized to receive either posaconazole or fluconazole prophylaxis [131]. At the end of the follow-up period from randomization to day 112
of treatment, posaconazole was shown to be as effective as fluconaclone in preventing all IFIs, and superior to fluconazole in preventing proven or probable invasive aspergillosis [131]. Patients who received posaconazole had a similar overall mortality rate but significantly lower fungal-related mortality [131]. The recent IDSA guidelines recommended the use of posaconazole for antifungal prophylaxis against aspergillosis in neutropenic patients [95]. Posaconazole is only available as oral formulation, which is a limitation to its use in patients with poor oral intake or absorption.

The concern for the potential of emergence of triazole-resistant pathogens, such as Aspergillus fumigatus, due to selection pressure has recently been raised [132,133]. However, because mold infection typically results from infection from the environment, in contrast to Candida derived from mucosal flora, emergence of resistant molds through patient to patient spread or hospital environmental contamination would not be anticipated.

Secondary antifungal prophylaxis

The role of secondary antifungal prophylaxis after a recent proven or probable pulmonary IFI was evaluated in a multicenter study that involved 166 adult patients with AML [134]. Recurrent IFI occurred in 26 patients (15.7%). Aspergillus spp. were the most frequently identified cause of breakthrough IFI. Risk factors for recurrence or breakthrough invasive fungal infection while on secondary prophylaxis included duration of neutropenia, receipt of high-dose cytarabine, number of prior antibiotics used as therapy, partial response as outcome of prior IFI, and newly diagnosed AML [134]. The rates of recurrent IFI did not differ among patients receiving or not receiving secondary antifungal prophylaxis [134]. This unexpected outcome may reflect a high degree of susceptibility in AML patients who have already had one documented IFI. In a small study involving pediatric patients, 11 adolescents with acute leukemia and a history of possible or probable invasive pulmonary aspergillosis received secondary prophylaxis with liposomal amphotericin B followed by oral voriconazole [135]. Three patients died, two due to secondary possible or probable invasive pulmonary aspergillosis [135].

Viral infections in leukemia

Viral infections are common in children with hematological malignancies and are characterized by a wide spectrum of manifestations that range from mild illness similar to that which occurs in immunocompetent patients to a complicated course with significant morbidity and mortality [136]. Viral infections were identified as the source of fever in 10–25% of the febrile neutropenic episodes in children with cancer [137,138]. The most common viral infections in children with leukemia include respiratory syncytial virus (RSV), herpes simplex virus (HSV), varicella zoster virus (VZV), influenza virus, and parainfluenza virus.

Figure 2 provides an overview of the clinically important viral pathogens encountered in children with leukemia. Table 5 summarizes antiviral therapy for various viral infections.

Respiratory viruses

Respiratory viral infections are frequent in children with hematologic malignancies and include rhinovirus, parainfluenza, influenza A and B, RSV, adenovirus, and the recently identified human metapneumovirus and bocavirus. In a prospective multicenter study, a respiratory viral etiology was identified in 44% of 138 febrile episodes in children with leukemia using culture, antigen detection and PCR tests [139]. These viruses present a spectrum of manifestations that range from the common cold to sinusitis, pharyngitis, tracheobronchitis, bronchiolitis and pneumonia. Respiratory viral infections in patients with leukemia are associated with prolonged viral shedding, prolonged duration of illness, increased rates of complications, progression to lower respiratory tract infections and, infrequently, death. The risk of developing pneumonia complicating viral infection has been estimated to range from 3 to 19% with mortality rates as high as 7% [139,140].

Respiratory syncytial virus infection is a particular concern in young children with AML. Progression to lower respiratory infection after upper respiratory infection with RSV is more common during periods of profound lymphopenia and in patients under 2 years of age, and is associated with increased morbidity and mortality [144]. There are no clinical trials conducted to evaluate the optimal treatment regimen of RSV infection in immunocompromised children regarding the timing of initiation of treatment (pre-emptive vs after onset of symptoms), single or combination of agents (intravenous or aerosolized ribavirin, IVIG, palivizumab), and the patient population to treat according to risk stratification. However, previous studies involving both adults and children have
Managing fungal & viral infections in pediatric leukemia

Review

Table 5. Antiviral therapy for various viral infections in leukemia.

<table>
<thead>
<tr>
<th>Viral infection</th>
<th>Primary therapy</th>
<th>Alternative therapy</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A and B virus</td>
<td>Oseltamivir, zanamivir, amantadine (A only), rimantadine (A only)</td>
<td></td>
<td>Choice of antiviral agent is based on the susceptibility pattern of the circulating influenza virus for the season</td>
</tr>
<tr>
<td>Parainfluenza virus</td>
<td>Supportive care</td>
<td>IVIG</td>
<td>IVIG may be considered</td>
</tr>
<tr>
<td>RSV</td>
<td>Ribavirin</td>
<td>Palivizumab, IVIG</td>
<td></td>
</tr>
<tr>
<td>Metapneumovirus</td>
<td>Supportive care</td>
<td>Ribavirin is active in vitro</td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Cidofovir</td>
<td>IVIG</td>
<td></td>
</tr>
<tr>
<td>HSV</td>
<td>Acyclovir, farniclovir, valacyclovir</td>
<td>Foscarnet, cidofovir</td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td>Acyclovir</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>Ganciclovir</td>
<td>Foscarnet, cidofovir</td>
<td></td>
</tr>
<tr>
<td>HHV-6</td>
<td>Foscarnet, ganciclovir</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parvovirus</td>
<td>IVIG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bocavirus</td>
<td>Supportive care</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BK virus</td>
<td>Cidofovir</td>
<td>Leflunamide, ciprofloxacin, IVIG</td>
<td></td>
</tr>
</tbody>
</table>

CMV: Cytomegalovirus; HHV-6: Human herpes virus-6; HSV: Herpes simplex virus; IVIG: Intravenous immunoglobulin; RSV: Respiratory syncytial virus; VZV: Varicella zoster virus.

suggested the safety and efficacy of aerosolized ribavirin with or without palivizumab or IVIG in preventing the progression of RSV upper respiratory infection to lower respiratory infection, treating pneumonia and preventing death in patients with hematologic malignancies and HSCT recipients [142–144].

Similarly, it has been shown that influenza infection in children with leukemia is characterized by prolonged symptoms and viral shedding, increased hospitalization and significant delays in chemotherapy [145–149]. The emergence of the pandemic 2009 H1N1 influenza A virus has added to the complexity of the interaction between influenza virus and immunocompromised host because of lack of immunity to this novel strain. Vaccination with the 2009 seasonal influenza vaccine induced no cross-reactive antibody response to 2009 H1N1 influenza virus [150]. The clinical course of 2009 H1N1 influenza infection in immunocompromised patients has ranged from mild illness in the majority of patients to severe illness requiring mechanical ventilation and admission to an intensive care unit [151–153]. Oseltamivir and zanamivir are the antiviral agents of choice for the treatment of 2009 H1N1 influenza A infection. Prolonged viral shedding associated with the development of oseltamivir resistance has been reported in immunosuppressed patients [154–156].

Human metapneumovirus (hMPV) is a paramyxovirus discovered earlier in this century [157]. It is ubiquitous and most children are seropositive by 5 years of age. Like RSV, hMPV can manifest as both upper and lower respiratory tract disease and is associated with more severe disease in immunosuppressed patients [158]. Diagnosis is based on PCR assays. Culture of this virus is difficult and antigen detection assays are not commercially available. There is no established treatment for hMPV.

However, a combination of intravenous ribavirin and IVIG has been used in the successful management of hMPV pneumonia in immunocompromised patients in two case reports [159,160].

Human bocavirus is a newly discovered virus classified in the Paroviriridae family [161]. It has been isolated from respiratory specimens collected from patients with respiratory symptoms where no other pathogens have been detected. Reports suggest that it plays a pathogenic role in respiratory tract disease [162–164]. Furthermore, human bocavirus has been isolated from blood, stool and respiratory specimens collected from a pediatric recipient of a HSCT who was thought to have disseminated viral infection [165]. However, more evidence is needed to confirm bocavirus’ role as a respiratory pathogen because of the high rates of copathogens detected in respiratory samples [161], and its detection in patients without any respiratory symptoms [166].

Viral culture of respiratory specimens has always been the gold standard for definitive diagnosis. Over the past decade, major advances have been achieved in the molecular diagnostic techniques for respiratory viruses. Rapid tests that detect influenza viral antigen are commercially available for clinical use. However, they are limited by their poor sensitivity. PCR assays that detect nucleic acid elements from RSV, influenza A and B, and parainfluenza viruses in nasopharyngeal wash or swab specimens have been characterized by their high sensitivity and specificity.

A yearly influenza vaccination is recommended for all children with hematologic malignancies who are 6 months of age or older. Data regarding the effectiveness of palivizumab immunoprophylaxis against RSV in immunocompromised children are limited [139,141]. In a study designed to develop a decision-analysis model.
Herpes viruses

Infections with herpes viruses are a common complication of the antileukemia myelosuppressive therapy because of their propensity to reactivate from a latent infection following immunosuppression. Children with leukemia are also at increased risk for acquiring a primary infection. Clinical manifestations include HSV stomatitis and esophagitis, localized and disseminated VZV infection, cytomegalovirus (CMV) pneumonitis, retinitis and enteritis, Epstein–Barr virus (EBV) mononucleosis and lymphoproliferative disorder, and human herpes virus-6 (HHV-6) hepatitis and encephalitis [136].

Varicella zoster virus infections manifest as either Varicella (chickenpox) or Herpes zoster (shingles). Chickenpox results from a primary VZV infection, while herpes zoster results from VZV reactivation. In addition to the cutaneous involvement, VZV may cause more severe clinical syndromes including trigeminal zoster with keratitis and retinal necrosis, encephalitis and Ramsay–Hunt syndrome. Complications include secondary bacterial and yeast skin infections and post-herpetic neuralgia. It has been estimated that 15% of children with ALL developed primary varicella in the prevaccine era [170]. Primary varicella infection in ALL patients have been associated with a particularly severe and complicated course that can be life-threatening [170]. However, the implementation of the population-based varicella vaccination program in 1995 has decreased the incidence of varicella infections in the general population and thus the exposure of immunocompromised children.

The safety and efficacy of VZV vaccines in children with ALL have been evaluated in prelicensure clinical trials at a time when the herd immunity was poor and the risk of exposure was higher. In one study, 96 leukemic children who were in remission but still receiving maintenance chemotherapy at the time of varicella vaccination were matched according to chemotherapeutic protocol with 96 children who had experienced natural varicella [171]. The incidence of herpes zoster was significantly (threefold) lower in vaccine recipients (0.80 per 100 person-years) than in the matched leukemic children who had experienced natural varicella (2.46 per 100 person-years) [171]. Other similar studies have also supported the efficacy of VZV vaccine in preventing severe natural varicella disease in immunocompromised children [172,173]. However, because of vaccine Oka strain-induced rashes, although rare, the use of this live attenuated virus vaccine is not recommended in immunocompromised children, particularly in the era following the population-based varicella vaccination program that markedly reduced the potential exposure of immunocompromised children.

Treatment of VZV infections requires the early initiation of intravenous acyclovir at higher doses for presumed clinical findings suggestive of VZV, while awaiting the results of antigen detection testing or viral cultures. Routine antiviral prophylaxis for zoster in leukemia patients is not recommended. In a recent double-blind controlled trial, 77 hematopoietic cell transplant recipients at risk of VZV reactivation were randomized to receive either oral acyclovir prophylaxis or placebo for 1 year after transplantation [174]. Acyclovir significantly reduced VZV infections at 1 year after transplantation. However, there was no significant difference in VZV infections after discontinuation of the acyclovir prophylaxis [174]. Post-exposure immunoprophylaxis with IVIG is indicated within 96 h of exposure of patients with hematologic malignancies (Varicella zoster immunoglobulin, is no longer available in the USA). If more than 96 h have elapsed since exposure to VZV, chemoprophylaxis with acyclovir beginning 7–10 days after exposure could be considered in immunocompromised patients without evidence of immunity.

Cytomegalovirus is a significant cause of morbidity and mortality in patients with impaired T-cell immunity. The existing knowledge about CMV infection in immunocompromised patients has been largely derived from HSCT recipients. Manifestations of CMV disease include pneumonitis, enterocolitis, hepatitis, encephalitis, cystitis, nephritis, bone marrow suppression and multiorgan system failure. In contrast to patients with AIDS, CMV retinitis is very rare in immunocompromised patients with cancer. Diagnosis of CMV disease can be confirmed by the use of rapid viral culture, CMV antigen-detection assays and PCR-based molecular tests. Antiviral agents directed against CMV include ganciclovir, foscarnet, cidofovir and CMV-specific IVIG. CMV-negative or leukocyte-depleted blood products should be used in CMV-seronegative patients to prevent CMV infection.

HHV-6 is the cause of exanthem subitum or roseola in healthy children. HHV-6 reactivation is most frequently reported in stem cell transplant recipients. In a prospective study, Chemaly et al. evaluated 37 adult patients with leukemia using molecular diagnostic methods for aspergillosis, CMV, and HHV-6 [175]. HHV-6 DNA was detected in 30% of patients using whole blood specimens, most of whom had fever, respiratory symptoms, diarrhea and rash [175]. The life-threatening clinical manifestations, most commonly seen in HSCT patients, include pneumonitis, hepatitis, encephalitis, myelosuppression and failure of engraftment. Diagnosis can be made by PCR assays using blood and cerebrospinal fluid. Finding markedly elevated levels of HHV-6 suggests active infection. Chromosomal integration of HHV-6 (present in almost 1% of the population) can be excluded by comparing HHV-6 levels in whole blood and serum. Foscarnet and ganciclovir have been used as treatment for HHV-6 infections.
Epstein–Barr virus is a ubiquitous human herpesvirus that infects the vast majority of the population early in childhood. After primary infection, EBV predominantly persists in memory B-lymphocytes that are kept under control by the T-lymphocytes in healthy individuals. Hence, T-cell immunosuppressive conditions predispose to EBV replication. Furthermore, EBV has been implicated in the etiology of several malignancies, including Burkitt’s lymphoma, nasopharyngeal carcinoma, post-transplant lymphoproliferative disorders, Hodgkin’s disease, non-Hodgkin’s lymphoma, and leiomysarcomas. In addition, herpes viruses, particularly EBV, are the most common infectious etiology of hemophagocytic lymphohistiocytosis, a potentially life-threatening disease characterized by uncontrolled T-cell and macrophage activation and infiltration of the liver, spleen, bone marrow, and the CNS [176]. Patients with EBV-related hemophagocytic lymphohistiocytosis present with fever, two or three lineage cytopenia, splenomegaly, hypertriglyceridemia and/or hypofibrinogemia, and hemophagocytosis.

**Adenovirus**

Adenovirus is a common cause of mild upper respiratory or gastrointestinal infection in immunocompetent patients. However, in immunocompromised patients, particularly HSCT recipients, adenovirus may cause pneumonia, hepatitis, hemorrhagic cystitis, nephritis, keratoconjunctivitis, pancreatitis, encephalitis and disseminated disease with viremia. Profound lymphopenia is a major risk factor for disseminated disease and mortality secondary to adenovirus infection [177]. Detection of adenovirus DNA from blood is considered predictive of disseminated disease [178]. Adenovirus DNA can be detected in the blood 2–3 weeks before the development of clinical disease in HSCT recipients [179]. Therefore, HSCT recipients are regularly monitored using PCR assays for the early detection of adenovirus DNA in the blood to initiate pre-emptive therapy with cidofovir before development of disseminated disease [180]. Routine monitoring is generally not warranted in leukemic patients who have not received HSCT. There are no specific antiviral agents of proven efficacy in the treatment of adenovirus infections. Several agents, including cidofovir, ribavirin, ganciclovir, vidarabine and IVIG, have been reported as successful and/or hypofibrinogemia, and hemophagocytosis.

**BK virus**

BK virus is a ubiquitous polyoma virus with an estimated seroprevalence of up to 90% of the adult population. BK virus has been reported to cause graft nephropathy and failure in renal transplant recipients. It has also been implicated as the cause of hemorrhagic cystitis and nephropathy in both pediatric and adult patients with hematological malignancy with and without HSCT [187–190]. Most of the clinical experience with BK virus has been derived from transplant patients. Definitive diagnosis of BK virus-associated nephropathy requires the confirmation of polyomavirus cytopathic changes on a renal biopsy tissue and the presence of polyomavirus antigen by immunohistochemical staining. However, owing to the invasiveness of the procedure, alternative surrogate markers have been investigated, mainly detection of DNA elements in blood and urine by PCR. The cutoff values of viral loads associated with development of BK virus-associated nephropathy haven’t been identified. In a case–control study of HSCT patients with hemorrhagic cystitis, Erard and colleagues reported that detection of more than 10,000 copies of BK virus per ml of plasma was independently associated with the development of late-onset hemorrhagic cystitis after engraftment [191,192]. Treatment of hemorrhagic cystitis is supportive using intravenous hydration and platelet transfusions. Treatment of BK virus nephropathy requires reduction of immunosuppression, which might put patients at risk for relapse. Cidofovir, at a weekly dose of 5 mg/kg, has been reported to have some clinical activity against BK virus in hematopoietic and renal transplant recipients [193]. However, no clinical trials have been carried out. The major limitation of cidofovir use is its nephrotoxicity. For this reason, intravenous hydration and probenecid should be administered with cidofovir to reduce its renal toxicity. Another approach to prevent the cidofovir-induced nephrotoxicity is to use a low-dose regimen of cidofovir. A weekly dose of 1 mg/kg without probenecid has been suggested to be safe and successful in achieving BK viremia clearance in the blood and urine and in symptom resolution [194–196]. Other treatment regimens reported to be of potential efficacy in case reports include lufenamid, fluoroquinolones, IVIG and local bladder instillation of cidofovir [197–199].

**Parvovirus**

Infection with parvovirus B19, which is responsible for erythema infectiosum, typically presents with fever and viral exanthem. However, it is also an important cause of prolonged cytopenia, especially anemia, in leukemia patients, resulting in increased need for red blood cell transfusions and significant delays in chemotherapy [184–186]. Lindblom and colleagues reported that 15% of consecutive bone marrow samples collected from patients with ALL without any clinical manifestations suggestive of viral infection were positive for parvovirus B19 by PCR [184]. No specific antiviral therapy for parvovirus B19 infection is available.

**Molecular diagnosis of viral infections**

Diagnosis of viral infections has markedly improved over the past years owing to advances in the molecular-based techniques such as PCR. However, the cutoff levels of viral copies that are considered diagnostic of active disease have not yet been established [136]. This may be attributed to the evaluation of different patient populations, molecular techniques and outcome measures, whether clinical disease or response to pre-emptive therapy. Therefore, the interpretation of the specific value of viral copies and comparison of results from different molecular-based assays should be practised with caution.
Immunization against childhood infections
As antiviral therapy is of limited availability and efficacy, immunization would be the best preventive measure of viral infections in children with leukemia. The Advisory Committee on Immunization Practices (ACIP) recommends against the administration of live-virus vaccines (live-attenuated influenza vaccine, measles–mumps–rubella, the oral polio vaccine, the VZV vaccine, yellow fever and rotavirus vaccine) in immunocompromised children due to concerns about their safety \[200,201\]. An alternate, possibly effective, approach is to vaccinate the household contacts of the immunosuppressed children to prevent exposure. Timitilli and colleagues have tried this approach by vaccinating the seronegative household contacts of the seronegative immunocompromised children with VZV vaccine \[202\]. However, their data was not evaluable because of the small number of patients and physicians compliant with this strategy. Thus, this approach, which has theoretical appeal, has practical challenges.

The efficacy of inactivated vaccines in children with leukemia has been investigated owing to concerns about different responses depending on the degree of immune suppression. Several studies have demonstrated that only a small proportion of children with ALL maintained protective antibody levels against vaccine-preventable pathogens when measured immediately after completion of chemotherapy \[203\]. However, the majority of these patients achieved protective levels when revaccinated 6 months or more following completion of treatment \[203,204\]. On the other hand, other studies suggested that children with ALL might benefit from vaccination during maintenance therapy \[205–208\]. Awaiting larger trials to clarify the role, timing and schedule of immunization in children with leukemia, the ACIP recommends that patients who have been vaccinated within 2 weeks of starting immunosuppressive therapy or while receiving immunosuppressive therapy should be considered unvaccinated and should be revaccinated at least 3 months after therapy is discontinued if immune competence has been restored \[200\].

For leukemic children with viral infections and low immunoglobulin levels, passive immunization with IVIG may be therapeutic.

Infection control & prevention
Prevention of infections in children with leukemia is essential to improve the overall survival rate in this high-risk patient population. Effective measures that can protect patients against infections include patients’ and parents’ education about pathogen transmission, hand hygiene, and avoidance of contact with sick individuals. Strict compliance with infection control and isolation measures reduces the risk of nosocomial transmission of bacterial, fungal and viral pathogens. These measures include: avoidance of construction work close to the wards where neutropenic patients are admitted because of increased risk of invasive aspergillosis associated with such exposure \[209\]; use of high-efficiency particulate air filtration and laminar airflow which directs the air current from the patient to the exhaust systems \[210–212\]; and a higher air pressure in the patient room than in the hallways \[213\].

Expert commentary
Despite the recent advances in diagnostic and therapeutic modalities for fungal and viral infections, these pathogens remain a major cause of morbidity and mortality in immunocompromised patients. *Candida* and *Aspergillus* spp. are the most common etiologies of fungal infection in children with leukemia. However, the past decade has witnessed an epidemiologic shift to an increased incidence of nonaspergillus mold infections, such as zygomycetes and dematiaceous molds. Early diagnosis of invasive fungal infections and prompt initiation of antifungal therapy have been associated with an improved outcome. More trials are needed to decide on the best treatment approach, whether empiric or pre-emptive. The definitive treatment guidelines for various fungal infections have been updated by the IDSA.

Viral infections in immunocompromised patients can lead to a complicated, life-threatening course with significant morbidity and mortality. Diagnosis of viral infections has advanced using molecular-based techniques such as PCR. However, more research is still needed in order to evaluate the role of various PCR techniques and viral copy levels in clinical decision making.

Five-year view
Despite the significant improvements in the cure rates of pediatric leukemia, infections remain a major cause of morbidity and mortality. Major breakthroughs in antifungal or antiviral therapy

### Key issues
- Despite improvements in antileukemia chemotherapeutic regimens and supportive care in pediatric leukemia, infections remain a major cause of morbidity and mortality.
- The etiology of life-threatening fungal infections has evolved from *Candida* spp. to *Aspergillus* spp., to a more recent increase in nonaspergillus molds.
- New molecular diagnostic techniques have the potential for earlier, noninvasive diagnosis of aspergillosis and the development of new broad-spectrum antifungal agents has a significant role in improving the outcome of invasive fungal infections in children with leukemia.
- Voriconazole is the treatment of choice for *Aspergillus* infections, but could enhance infections with zygomycetes.
- Viral infections may account for 10–40% of febrile, neutropenic episodes, varying with the seasons.
- Infection control measures that prevent person-to-person spread of respiratory viral infections may be the most promising approach to decreasing the incidence of viral infections in children receiving treatment for leukemia.
- Intravenous immunoglobulin may be therapeutic for viral infections in children with low immunoglobulin levels.
are not anticipated in the short term. Future research needs to focus on targeted antileukemic cell therapy in order to reduce the associated infectious complications. In addition, further work is needed to enable the identification of subgroups of patients who are at high risk of infection and who may benefit from risk-stratified management, such as antimicrobial prophylaxis or a pre-emptive therapy approach.

References
Papers of special note have been highlighted as:
• of interest
•• of considerable interest

Financial & competing interests disclosure
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Managing fungal & viral infections in pediatric leukemia
Review

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- Reports on several factors affecting the performance of galactomannan antigen detection assay.


- Evaluates the diagnostic performance of galactomannan detection assay in invasive aspergillosis.

- A randomized, international, multicenter clinical trial has shown that voriconazole is as effective as, but with less infusion-related side effects and nephrotoxicity than, amphotericin B, when used as empirical antifungal therapy in febrile neutropenic patients.


- The only clinical trial that directly compares empiric with pre-emptive antifungal therapy approaches in febrile neutropenic patients.


- A recent systematic review of the performance of galactomannan detection assays for the diagnosis of invasive aspergillosis.


- IDSA guidelines for the treatment of candidiasis.


• Summarizes the role of primary antifungal prophylaxis in preventing breakthrough fungal infections.


• Evaluates the role of antifungal prophylaxis in neutropenic patients.


• Evaluates the role of antifungal prophylaxis in preventing fungal infections in patients with cancer.


• An important randomized clinical trial that has demonstrated that posaconazole prophylaxis was more effective than either fluconazole or itraconazole in preventing invasive fungal infections in neutropenic patients with acute myeloid leukemia or myelodysplastic syndrome.


• An important randomized double-blind clinical trial that has demonstrated that posaconazole prophylaxis was more effective than fluconazole in preventing invasive aspergillosis in patients with graft-versus-host disease.


** A systematic review of the role of antiviral prophylaxis in preventing viral infections in patients with hematologic malignancies.


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• The Advisory Committee on Immunization Practices guidelines for the immunization of high-risk patients including those with malignancies.


• Recent updates on the recommended immunization schedule.


203 van Tilburg CM, Sanders EA, Rovers MM, Wolfs TF, Bierings MB. Loss of antibodies and response to (re-)vaccination in children...
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•• IDSA guidelines for the treatment of coccidioidomycosis.


•• IDSA guidelines for the treatment of blastomycosis.


•• IDSA guidelines for the treatment of histoplasmosis.


•• IDSA guidelines for the treatment of sporotrichosis.