J Antimicrob Chemother 2011; **66** Suppl 1: i25–35
doi:10.1093/jac/dkq439

Journal of Antimicrobial Chemotherapy

Systematic review and mixed treatment comparison of randomized evidence for empirical, pre-emptive and directed treatment strategies for invasive mould disease

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Randomized controlled trials (RCTs) provide the most reliable estimates of the effects of treatments. However, not all treatments are compared in available RCTs, making comparison of treatments problematic. Mixed treatment comparisons (MTCs) can provide estimates of the comparative effects of treatments across a range of available therapeutic options. MTCs use networks of available direct comparisons to estimate differences in treatments that have not been estimated in trials via a common comparator. We conducted a systematic review and MTCs of comparative RCTs in haematological patients of anti-mould active agents used for the empirical treatment of febrile neutropenia (Analysis 1), and pre-emptive therapy (Analysis 2) of invasive mould diseases. In addition, we summarized the evidence available associated with the use of directed treatment strategies (Analysis 3). For empirical therapy, caspofungin proved superior to amphotericin B, liposomal amphotericin B, amphotericin B lipid complex and voriconazole in the outcome of survival, but no agents showed superiority for treatment response. There was no evidence of a difference between pre-emptive and empirical strategies on mortality outcomes. For directed therapy, voriconazole was superior to amphotericin B for overall survival, and both voriconazole and liposomal amphotericin B were superior to amphotericin B and amphotericin B colloidal dispersion on the outcome of response. While limited to some degree by the availability of RCTs, the MTCs reported here provide the best available evidence of relative therapeutic success for different available treatment strategies.

Keywords: invasive fungal disease, randomized controlled trials, empirical therapy, pre-emptive therapy

Introduction

Invasive mould diseases (IMDs) are still a major cause of morbidity and mortality in patients with haematological malignancies. Two decades ago, amphotericin B (AmB) and itraconazole were the only agents available for treating IMDs. New antifungal agents, such as alternative lipid formulations of AmB, the echinocandins, e.g. caspofungin, and extended-spectrum azoles, e.g. voriconazole and posaconazole, have now been developed. These newer drugs provide increased efficacy (voriconazole) for directed therapy of *Aspergillus* infections, as well as in other rarer mould infections such as mucormycosis, fusariosis or scedosporiosis. Others, such as caspofungin, afford better tolerance for empirical therapy. The European Conference on Infections in Leukemia (ECIL) has provided guidelines for the prophylaxis of invasive aspergillosis, for empirical therapy of persistent febrile neutropenia and for directed therapy of invasive aspergillosis (Table 1).[1]

Significant improvement in diagnostic tests allows earlier diagnosis of IMD, also resulting in better outcome. Early high-resolution CT scans help identify typical pulmonary lesions suggestive of invasive aspergillosis, such as nodules with or without a halo sign.[2] New biomarker detection tests (e.g. *Aspergillus* galactomannan detection, β-glucan detection test, PCR) allow IMDs to be suspected at very early stages, sometimes before typical clinical or radiological signs are present.[3,4]

More treatment options and the availability of new diagnostic tools have led to changes in treatment strategies. Although empirical antifungal therapy of persistent febrile neutropenia is still largely used, prophylaxis with newer azoles has now been shown to be effective against invasive aspergillosis, and directed therapy applies to all mycologically documented infections. Beside these three therapeutic approaches in haematological patients, there is an unmet need to develop pre-emptive approaches. Pre-emptive antifungal treatment in haematological patients aims to treat patients with minimal disease early, at a stage where antifungal treatment may have optimal efficacy (Table 2).[5] Identifying these patients by way of the new diagnostic tools allows the targeting of costly antifungal agents to patients most in need of treatment, as opposed to prophylaxis,

Table 1. ECIL-3 recommendations[a] on antifungal prophylaxis in leukaemic and haematopoietic stem cell transplant patients, for empirical therapy of febrile neutropenia and for first-line and salvage-directed treatment of invasive aspergillosis[1,20,21]

	Prophylaxis				Directed treatment of invasive aspergillosis	
Drug	allogeneic HSCT: neutropenic phase	allogeneic HSCT: GVHD phase	induction chemotherapy of acute leukaemia	Empirical therapy	first line	salvage
Amphotericin B deoxycholate	CI	CI	CI	efficacy BI, safety DI	DI	—
Liposomal amphotericin B	BII (aerosolized plus fluconazole)	—	BI (aerosolized plus fluconazole)	efficacy AI, safety AI	BI	BIII
ABCD	—	—	—	efficacy BI, safety BI	DI	—
ABLC	—	—	—	efficacy BI, safety BI	BII	BIII
Itraconazole	BI	BI	CI	efficacy BI, safety BI	CIII	CIII
Posaconazole	—	AI	AI	—	—	BII
Voriconazole	Provisional AI	Provisional AI	—	efficacy BI, safety BI	AI	BII
Caspofungin	—	—	—	efficacy AI, safety AI	CII	BII
Micafungin	CI	—	—	efficacy BII, safety BII	—	—

HSCT, haematopoietic stem cell transplantation; GVHD, graft versus host disease; ABLC, amphotericin B lipid complex; ABCD, amphotericin B lipid dispersion.
[a]Evidence was graded using the following criteria: I, evidence from at least one well-executed randomized trial; II, evidence from at least one well-designed clinical trial without randomization, cohort or case–controlled analytical studies (preferably from more than one centre), multiple time series studies, or dramatic results from uncontrolled experiments; III, evidence from opinions of respected authorities based on clinical experience, descriptive studies or reports from expert committees. The following recommendation levels were used: A, strong evidence for efficacy and substantial clinical benefit; B, strong or moderate evidence for efficacy, but only limited clinical benefit; C, Insufficient evidence for efficacy, or efficacy does not outweigh possible adverse consequences (e.g. drug toxicity or interactions) or cost of chemoprophylaxis or alternative approaches; D, moderate evidence against efficacy or for adverse outcome—generally not recommended; E, strong evidence against efficacy or of adverse outcome—never recommended.

where antifungal agents are given to all patients at high risk to prevent a disease that will occur in only a limited proportion of them. Similarly, empirical therapy of persistent febrile neutropenia leads to overtreatment of many patients who experience fever not related to an IMD.

In this setting, with the development of new agents, new diagnostic approaches and various therapeutic strategies, we aimed to undertake a systematic review of randomized comparative trials in haematological patients of anti-mould active agents used for the empirical treatment of febrile neutropenia (Analysis 1), and pre-emptive therapy (Analysis 2) of IMD. In addition, we summarized the available evidence associated with the use of directed treatment strategies (Analysis 3; Table 2).

Randomized controlled trials (RCTs) provide reliable and unbiased estimates of treatment effect. Where a single question is addressed across several trials, it is straightforward to utilize standard methods for meta-analysis, and these have become very well established in health technology assessment in order to provide an overall synthesis of treatment effects. Where there are multiple potential therapies available, and there is an interest in comparing them but not all treatments have been compared adequately with each other, mixed treatment comparisons (MTCs) provide the opportunity to derive conditional estimates of the treatment effect across a network, and supplement data from direct comparisons with those from indirect comparisons where treatments are linked through a common comparator or comparators.[6] Network diagrams provide a graphical description of the directly randomized data that are available, and summarize the data upon which a mixed treatment comparison is based.

Methods

Citation searching and assessment for inclusion

Patient population

This review sought to summarize the evidence involving patients at high risk of invasive aspergillosis (IA) and other IMDs, including:

- Patients with haematological malignancies—mostly acute leukaemia or myelodysplastic syndromes requiring induction or consolidation chemotherapy.
- Patients receiving immunosuppressive therapy for allogeneic haematopoietic stem cell transplantation (HSCT), particularly those with graft versus host disease (GVHD).
- Patients who are at lower risk from autologous HSCT and, for directed therapy of IMD, other groups at risk of these infections, such as solid organ transplant recipients, HIV-positive patients or patients treated with steroids.

Definition of interventions

For the purposes of Analyses 1 and 2 of this review, any randomized controlled study involving the following antifungal agents for empirical or pre-emptive therapy were included: AmB (all formulations), itraconazole, voriconazole, caspofungin and micafungin.

For the purposes of Analysis 3, an intervention was considered to be any strategy for directed therapy compared with empirical therapy (utilizing the following pharmacological agents: any formulation of AmB,

Table 2. Therapeutic strategies in haematological patients at high risk of invasive mould diseases and objectives of the systematic review (adapted from Herbrecht and Berceanu)[5]

	Clinical signs and symptoms				
Parameter	no symptoms	persistent febrile neutropenia	no symptoms or persistent febrile neutropenia	clinical or radiological signs consistent with fungal infection	clinical or radiological signs consistent with fungal infection
Mycological results	negative	negative	positive biomarker[a]	negative	positive biomarkers or positive microscopy, culture or histopathology[b]
Category in EORTC/MSG definitions	not categorized	not categorized	not categorized	possible IMD	probable or proven IMD
Type of treatment	prophylactic	empirical	pre-emptive	pre-emptive	directed
Objective in this systematic review	not considered	Analysis 1	Analysis 2	Analysis 2	Analysis 3

EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group.
[a]Biomarkers include *Aspergillus* galactomannan, β-D-glucan and PCR.
[b]Positive microscopy, culture or histopathology from a clinically relevant sample.

itraconazole, voriconazole, posaconazole, caspofungin, micafungin or anidulafungin) for the patient groups defined above.

In all included cases, the anti-mould agents were administered systemically and early when there was either only a persistent febrile neutropenia (empirical therapy), or when there was clinical or radiological evidence for infection (possible IMD diagnosis), or indirect mycological evidence using *Aspergillus* galactomannan detection or PCR (which justified following a pre-emptive strategy).

Types of outcome

Studies that specify mortality/survival (all-cause or disease-specific), complete treatment response and/or associated morbidity as outcomes were included.

Study design

Only RCTs that met all inclusion criteria above have been considered in this review.

Exclusion criteria

Studies that include participants who had received previous antifungal treatment, were on combination therapy or were on prophylactic therapy were excluded. Additionally, studies examining patients with candidiasis or other yeast infections were excluded, as well as studies comparing two different dosages of the same antifungal agent. Studies dedicated exclusively to paediatric patients were also not included in this review.

Although the toxicity of these agents may be relevant to clinical outcome, this factor will not be included in the analysis.

Search strategy

Search strategies appropriate for the purposes of the review were developed and adapted for use with the following electronic bibliographic databases: MEDLINE, EMBASE, CINAHL, the Cochrane Library, Web of Science and the Health Management Information Consortium (HMIC) for economic comparisons. The strategy was limited to citations published in the English language and, additionally, to those published in the period between 2000 and 2010 for Analysis 2 (as assessments of pre-emptive strategies would not have been undertaken prior to this period).

The search terms used in the strategy for each analysis of the review are listed below.

For Analyses 1 and 2 (MTC of RCTs involving anti-mould active agents in haematological patients) the following terms were used:

- Immunosuppressed patients/mortality or survival, tolerability, adverse events, cost.
- Leukaemia/linked with invasive fungal infections, therapy, dose regimen, time and duration of therapy.
- Leukaemia/linked to acute, myeloid or stem-cell transplant, organ transplant, mortality or survival.

These were combined with the following substance names (generic and trade names): conventional AmB (Fungizone®), AmB lipid complex [ABLC (Abelcet®)], liposomal AmB [L-AmB (Ambisome®)], AmB colloidal dispersion [ABCD (Amphocil®, Amphotec®)], itraconazole (Sporanox®), voriconazole (Vfend®), posaconazole (Noxafil®), caspofungin (Cancidas®), micafungin (Mycamine®), anidulafungin (Eraxis®, Ecalta®).

For Analysis 3 (directed versus empirical therapy), the following MeSH headings with associated terms were used:

- Leukaemia/linked with acute, myeloid or stem-cell transplant, organ transplant, complications, invasive fungal infections, antifungal drug therapy.
- Mycosis and invasive fungal disease (IFD)/IMD linked with diagnosis or diagnostics, non-culture/proven, probable or possible.
- Fungal infections or mycosis and treatment/linked with empirical, early, endpoint, diagnosis, outcome, risk, mortality or survival, morbidity, duration.

The following single term/keyword searches were also undertaken as part of the strategy: aspergillosis or *Aspergillus*, moulds, *Fusarium* or zygomycosis, invasive, opportunistic, nosocomial, 'non-culture based fungal diagnostics', galactomannan antigen immunoassay (GM), Bio-Rad Platelia® aspergillus EIA, CT scan, PCR-based diagnosis, pre-emptive, early targeted, empirical, human, clinical, age, adult, children, adolescents, economics, cost-outcomes, cost-effectiveness/cost utility and risk ratio.

Additionally, hand-searches were conducted on the following: websites of relevant agencies (European Medicines Agency, Evidence for Policy and Practice Information and Co-ordinating Centre, the Food and Drug Administration, the National Institute for Health and Clinical Excellence, the National Guideline Clearinghouse and the Scottish Intercollegiate Guidelines Network), company databases, meeting abstracts, reference lists of relevant guidelines (ECIL-3) and reference lists of citations included in this review.

Citations retrieved from each bibliographic database listed were imported and combined in a single Endnote® library (Thomson Reuters, New York, NY, USA) with the program set to remove duplicates. Relevant hand-searched citations were then added to the library.

The titles and abstracts for these citations were checked in order to identify full texts that needed to be retrieved, in order to assess suitability for inclusion in the review. The assessment for inclusion was conducted by one member of the reviewing team and checked by another.

Data extraction for general characteristics, quality assessment criteria and the main findings from included studies was done by one member of the reviewing team and checked by another.

Statistical analysis

We conducted mixed treatment analyses using the procedures developed by Lu and Ades[6] to estimate conditional effects for all treatments of interest, compared with a single common therapy. An advantage of this approach is that it can provide a best-estimate ranking of treatment estimates for different therapies, even when all the interventions have not been compared directly in randomized trials. The principal analyses used a fixed effects approach, and we conducted further supportive analyses using random effects approaches, assessing model fit in both cases using the Bayesian deviance information criterion (DIC).[7] The DIC can help select between competing statistical models, as long as the models describe the data appropriately. Analyses were conducted separately for studies in patients receiving directed and empirical therapy. The outcomes of response and survival were examined in separate statistical models. We used standard methods for exact meta-analysis for the comparison of pre-emptive versus empirical therapy.

Results

General characteristics and quality assessment of included studies

There were 10 RCTs that met the inclusion criteria for Analyses 1 and 3 of the review.[8–17] The general characteristics of these trials are presented in Tables 3–5. These trials randomized in excess of

Table 3. General characteristics of studies included in the comparison of antifungal agents used in the empirical treatment of invasive mould diseases (Analysis 1)

Study	Comparison/treatment strategy	Study design	Patient population	Primary endpoint	Definition of response	Mortality included as an outcome?
Boogaerts 2001	itraconazole (200 mg iv over 48 h, days 3–14, 200 mg iv daily; day 15 onwards, 400 mg/day oral) vs. C-AmB (0.7–1.0 mg/kg/day iv, for up to 28 days/empirical	multisite, open-label, equivalence	haematological malignancy, with intensive myelosuppressive cytotoxic therapy, with or without autologous haematopoietic stem-cell support (≥18 years)	favourable response at end of treatment	patient afebrile (daily oral peak temperature <38°C) and recovered from neutropenia (neutrophils >500/μL on ≥2 successive days)	yes
Prentice 1997	L-AmB (1–3 mg/kg/day iv) vs. C-AmB (1 mg/kg/day iv)/empirical	multisite, open-label, superiority	neutropenic patients (<500/μL) who present following 96 h of fever, defined as temperature ≥38°C, and not responding to broad-spectrum antibacterial therapy (uncertain)	incidence of serious toxicity	minimum of 3 consecutive days without fever (<38°C) that continued until study end, indicated by recovery of neutrophils to 500/μL	
Walsh 1999	L-AmB (3 mg/kg/day iv) vs. C-AmB (0.6 mg/kg/day iv)/empirical	multisite, double-blind, superiority	patients receiving chemotherapy for leukaemia, lymphoma or other cancers or had undergone bone marrow or peripheral HSCT, and had received empirical antibacterial therapy for at least 5 days while continuing to have neutropenia (<500/μL) and fever (2–80 years of age)	composite of five treatment response-related criteria.	composite of 5 criteria: survival for 5 day after initiation of therapy; resolution of fever during the period of neutropenia; successful treatment of any baseline fungal infection if present; absence of breakthrough fungal infections during administration of study drug or within 7 days after completion of treatment; and absence of premature discontinuation of study drug because of toxicity or lack of efficacy	yes
Walsh 2002	voriconazole (day 1, 6 mg/kg iv every 12 h, followed by maintenance dose of 3 mg/kg iv every 12 h or 200 mg every 12 h oral after ≥3 days of iv) vs. L-AmB (3 mg/kg/day iv)/empirical	multisite, open-label, non-inferiority	patients who had received chemotherapy for leukaemia, lymphoma or other cancers or had undergone HSCT, and had received >96 h of systemic antibacterial therapy while continuing to have fever (oral temperature >38°C within 24 h before randomization) and neutropenia (<500/μL for 96 h and <250/μL within 24 h before randomization) (≥12 years)	treatment success based on composite of treatment response-related criteria	composite of 5 criteria: no breakthrough fungal infection, survived 7 days beyond end of therapy, did not discontinue therapy prematurely, had resolution of fever during the period of neutropenia, and was successfully treated for any baseline fungal infection	yes
Walsh 2004	caspofungin (70 mg on day 1 and 50 mg once daily thereafter) vs. L-AmB (3 mg/kg/day iv)/empirical	multisite, double-blind, superiority	patients who had received chemotherapy for cancer or had undergone HSCT and if they had had a neutrophil count <500/μL for at least 96 h, had fever (temperature >38.0°C), and had received parenteral antibacterial therapy for at least 96 h (≥16 years)	favourable overall response, as determined by 5 treatment response-related criteria	composite of 5 criteria, similar to Walsh 2002 above	yes
White 1998	ABCD (4 mg/kg/day iv) vs. C-AmB (0.8 mg/kg/day iv)/empirical	multisite, double-blind, superiority	patients who had received chemotherapy for haematological malignancy or had undergone HSCT in the previous 3 months, and were neutropenic, i.e. neutrophils <500/μL or <1000/μL and expected to decline to <500/μL within 2 days) for >7 days (uncertain)	Treatment success based on a composite of treatment response-related criteria	all of the following: survival for ≥7 days after last dose of study drug, lack of suspected or documented fungal infection during the study and within 7 days of last dose of study drug, lack of study drug discontinuation because of adverse events, and lack of fever on day of discontinuation of therapy.	yes
Wingard 2000	ABLC (5 mg/kg/day iv) vs. L-AmB (5 mg/kg/day or 3 mg/kg/day)/empirical	multisite, double-blind, superiority	neutropenic patients (neutrophils <500/μL) who were aged 2 to 84 years were enrolled in this study if they had a suspected fungal infection, as demonstrated by fever after at least 72 h of broad-spectrum antibacterial therapy (>2 years)	frequency of infusion-related chills/rigors during infusion or for up to 1 h after infusion on day of first dose (day 1)	fever resolution during neutropenic period; improvement/cure for patients with proven baseline fungal infection; absence of treatment-emergent probable or proven fungal infections; non-occurrence of death with fungal infection as primary or contributing factor, either during the study or within 7 days of last administration of study drug; no discontinuation of study drug due to toxicity; and no administration of alternative systematic antifungal agent for a probable or proven fungal infection	yes

ABCD, amphotericin B colloidal dispersion; ABLC, amphotericin B lipid complex; C-AmB, conventional amphotericin B; L-AmB, liposomal amphotericin B; HSCT, haematopoietic stem cell transplantation; iv, intravenous.

Table 4. General characteristics of studies included in the comparison of pre-emptive and empirical treatment of invasive mould diseases (Analysis 2)

Study	Location	Study design	Patient population	Pre-emptive strategy	Initiation of empirical therapy	Antifungal regimen
Cordonnier 2009	France	multicentre; open-label, non-inferiority, RCT	febrile neutropenic patients treated for haematological malignancies (patients aged ≥18 years were eligible if they had haematological malignancies and were scheduled for chemotherapy or autologous HSCT that was expected to cause neutropenia (≤500/μL) for at least 10 days	based on clinical, imaging or galactomannan antigen assay evidence	based on persistent or recurrent fever	all patients received first-line antifungal treatment with C-AmB (1 mg/kg/day) or L-AmB (3 mg/kg/day)
Hebart 2009	Germany	multicentre, RCT	allogeneic HSCT patients (bone marrow or peripheral blood progenitor cell transplantation from related and unrelated donors between July 1998 and June 2001 in 5 different centres)	based on 1 positive PCR	based on febrile neutropenia for >120 h	recommendation was to give L-AmB at 3 mg/kg body weight for 3 days followed by 1 mg/kg body weight in clinically stable patients; dose was titrated based on physician's discretion and lasted a minimum of 3 days

ABCD, amphotericin B colloidal dispersion; ABLC, amphotericin B lipid complex; C-AmB, conventional amphotericin B; L-AmB, liposomal amphotericin B; HSCT, haematopoietic stem cell transplantation; RCT, randomized controlled trial.

4000 participants (4094), with a range of 66 to 1095 in individual sample size. Seven studies were multinational.[8–17]

Five were open-label trials[8,10–12,14] and the remaining five employed a double-blind study design.[9,13,15–17] Additionally, White *et al.*[16] was a pilot study, and three trials used a non-inferiority or equivalence design.[8,10,14]

In five of these cases, the aim was to compare AmB with other preparations of the agent.[9,11–13,16] Wingard *et al.*[17] compared the safety of ABLC and L-AmB, while Boogaerts *et al.*[8] and Walsh *et al.*[15] compared itraconazole and caspofungin, respectively, with preparations of AmB. Herbrecht *et al.*[10] and Walsh *et al.*[14] compared AmB formulations with voriconazole. The drug comparison involved empirical therapy in seven cases,[8,12–17] while three studies used directed strategies.[9–11]

The age group of participants enrolled was not specified in two cases, but both these studies involved adult and paediatric patients.[12,18] In all other cases, the minimum age for enrolment was set at 2,[9,13,17] 12,[10,14] 16[11,15] or 18 years.[8]

The primary endpoint was response to treatment in eight cases,[8–11,13–16] while Prentice *et al.*[12] and Wingard *et al.*[17] specified safety criteria as the primary endpoint. The definitions of response used in each study are detailed in Tables 3–5.

Independent or blinded confirmation of diagnosis at study entry was conducted in five studies,[9,10,13–15] and blinded assessment of outcome was reported in three studies.[9,10,15] In two of these, this role was performed by an independent committee.[10,15] The method of allocation concealment was not discussed in any of the study reports. The main analysis was conducted on an intention-to-treat basis in seven cases (with five of these reporting the use of a modified intention-to-treat population, which included only patients that received at least one dose of the study drug). The population included in the analysis was unclear in three cases.[8,9,16]

There were two RCTs that examined the impact of a pre-emptive strategy on outcomes in patients with invasive fungal infections (IFIs; Analysis 2), and these are discussed below.[18,19]

Cordonnier *et al.*[19] attempted to demonstrate the non-inferiority (margin of 8%) of the use of a pre-emptive as compared with an empirical strategy in an open-label trial involving 293 febrile neutropenic patients treated for haematological malignancies. Antifungal treatment was AmB or L-AmB, and the initiation of pre-emptive treatment was based on clinical, imaging or galactomannan antigen assay evidence. This was a well-conducted, open-label RCT with rigorous methods, including a computer-generated randomization sequence and blinded adjudication of outcomes. There was an overall drop-out rate of about 10% due to protocol violations. Intention-to-treat analysis demonstrated the non-inferiority of pre-emptive treatment, compared with empirical treatment, with regard to mortality 2 weeks after recovery from neutropenia (the primary outcome).

Hebart *et al.*[18] investigated the impact of a PCR-based pre-emptive treatment strategy using L-AmB on the incidence of IFIs, and the overall and IFI-related mortality in patients. This

Table 5. General characteristics of studies included in the comparison of antifungal agents used in the directed treatment of invasive mould diseases (Analysis 3)

Study	Comparison/treatment strategy	Study design	Patient population	Primary endpoint	Definition of response	Mortality included as an outcome?
Bowden 2002	ABCD (6.0 mg/kg/day iv) vs. C-AmB (1.0 or 1.5 mg/kg/day iv)/directed (for invasive aspergillosis)	multisite, double-blind, superiority	haematological malignancy, HSCT, disease requiring immunosuppressive therapy (solid organ transplant or solid tumours), chronic obstructive pulmonary disease or other immune-compromising conditions (≥2 years)	therapeutic response, (composed of complete response, partial response or stable disease)	complete response, partial response, stable disease, failure[a]	√
Herbrecht 2002	voriconazole (day 1: 6 mg/kg iv every 12 h, day 2 onwards: 4 mg/kg iv every 8 h for ≥7 days, followed by switch to oral) vs. C-AmB (1–1.5 mg/kg/day iv)/directed (for invasive aspergillosis)	multisite, open-label, non-inferiority	haematological malignancy, HSCT, aplastic anaemia, myelodysplastic syndrome; or other immunocompromising conditions; including AIDS, receipt of corticosteroid therapy, and solid organ transplantation (≥12 years)	global response at week 12 (to demonstrate non-inferiority)	complete response, partial response, stable disease, failure[a]	√
Leenders 1998	L-AmB (5 mg/kg/day iv) vs. C-AmB (1 mg/kg/day iv)/directed (for invasive fungal infections)	uncertain, open-label, superiority	severely neutropenic (<500/μL) or those who presented within 14 days of recovery from severe neutropenia (≥18 years)	response after 14 days	complete response, partial response, failure, relapse[a]	√

ABCD, amphotericin B colloidal dispersion; ABLC, amphotericin B lipid complex; C-AmB, conventional amphotericin B; L-AmB, liposomal amphotericin B; HSCT, haematopoietic stem cell transplantation.

[a]Complete response: normalization of all pre-treatment signs and symptoms together with, if applicable, progressive improvement of chest X-rays. Partial response: decrease of pre-treatment signs and symptoms and a stable or improved chest X-ray (≥50%). Stable disease: absence of change from baseline <50% improvement in radiological findings. Failure: unchanged or progressive pre-treatment. Relapse: recurrence of any sign or symptom of fungal infection during follow-up after an initial response.

Table 6. Quality assessment of included trials

Study	Blinding	Allocation concealment	Loss to follow-up
Studies assessing the impact of empirical therapy (Analysis 1)			
Boogaerts 2001[8]	no	yes (centralized computer)	9% (394 enrolled, 360 analysed)
Prentice 1997[12]	no	yes (each centre provided with a set of blinded, numbered envelopes which required sequential opening)	3% (338 enrolled, 335 analysed)
Walsh 1999[13]	yes (double)	yes (central randomization centre)	2% (702 enrolled, 687 analysed)
Walsh 2002[14]	no	yes (computer-generated randomization system)	1% (849 enrolled, 837 analysed)
Walsh 2004[15]	yes (double)	unclear (randomization performed at each site)	2.5% (1123 enrolled, 1095 analysed)
White 1998[16]	yes (double)	unclear (randomization performed at each site using randomization table)	9% (213 enrolled, 193 analysed)
Wingard 2000[17]	yes (double)	unclear	2% (250 enrolled, 244 analysed)
Studies assessing the impact of pre-emptive treatment strategies (Analysis 2)			
Cordonnier 2009[19]	no	yes (computer-generated randomization scheme)	0% (293 enrolled, 293 analysed)
Hebart 2009[18]	no	yes (patients were randomized centrally)	NA (NA enrolled, 403 analysed)
Studies assessing the impact of directed therapy (Analysis 3)			
Bowden 2002[9]	yes (double)	unclear	40% (174 enrolled, 103 analysed)
Herbrecht 2002[10]	yes (double)	yes (central randomization)	29% (391 enrolled, 277 analysed)
Leenders 1998[11]	no	unclear	38% (106 enrolled, 66 analysed)

NA, not available.

trial was terminated prematurely due to a low incidence rate of IFI in both groups. Randomization was done centrally, although there is no description of the method used to generate the randomization sequence. There was blinded assessment of the radiological findings, but it is uncertain if there was overall independent adjudication of outcome. Intention-to-treat analysis involving 403 participants suggested that there was no difference in the incidence of IFI and survival 100 days post-transplant.

The quality of each trial included is assessed in Table 6.

Comparison of anti-mould agents used in the empirical treatment of invasive fungal infections (Analysis 1)

MTC results

Survival in studies using empirical therapy Six trials were included which met the inclusion criteria and reported data on survival.[8,13–17] The network of included trials describing the relevant randomized comparisons, the number of patients randomized and number of deaths is depicted in Figure 1(a).

The mixed treatment comparison results were described in comparison with AmB (Figure 1b). Caspofungin was the only agent associated with a significantly higher rate of survival than AmB. Caspofungin was also statistically significantly superior to L-AmB [odds ratio 0.661, 95% confidence interval (CI) 0.434–0.997], caspofungin was superior to voriconazole (odds ratio 0.479 95% CI 0.24–0.938), and caspofungin was also superior to ABLC (odds ratio 0.238 95% CI 0.068–0.77). No other comparisons were statistically significant.

Response to treatment in studies using empirical therapy Seven trials were included which met the inclusion criteria and provided data for the outcome of response.[8,12–17] The numbers of trials, patients and patients responding are described in Figure 2(a).

No agents showed statistically significant differences in treatment response to AmB (Figure 2b). There were also no statistically significant differences between the other agents.

Effectiveness of pre-emptive as compared with empirical anti-mould treatment strategies (Analysis 2)

The characteristics of the two randomized trials are described in Table 3. Pooling the results from the two trials using conventional meta-analysis methods, we found no difference in all-cause mortality (see Figure 3).[18,19]

Comparison of antifungal agents used in the directed treatment of invasive aspergillosis infections (Analysis 3)

Survival in studies using directed therapy

Three trials met the inclusion criteria and provided data on survival.[9–11] The numbers of trials, deaths and number of subjects randomized by agent are described in Figure 4(a).

Voriconazole was statistically significantly superior to AmB on overall survival (see Figure 4b). There were no other statistically significant differences between the agents.

Response to treatment in studies using directed therapy

Three trials met the inclusion criteria and provided data on survival.[9–11] The number of trials, patients responding and

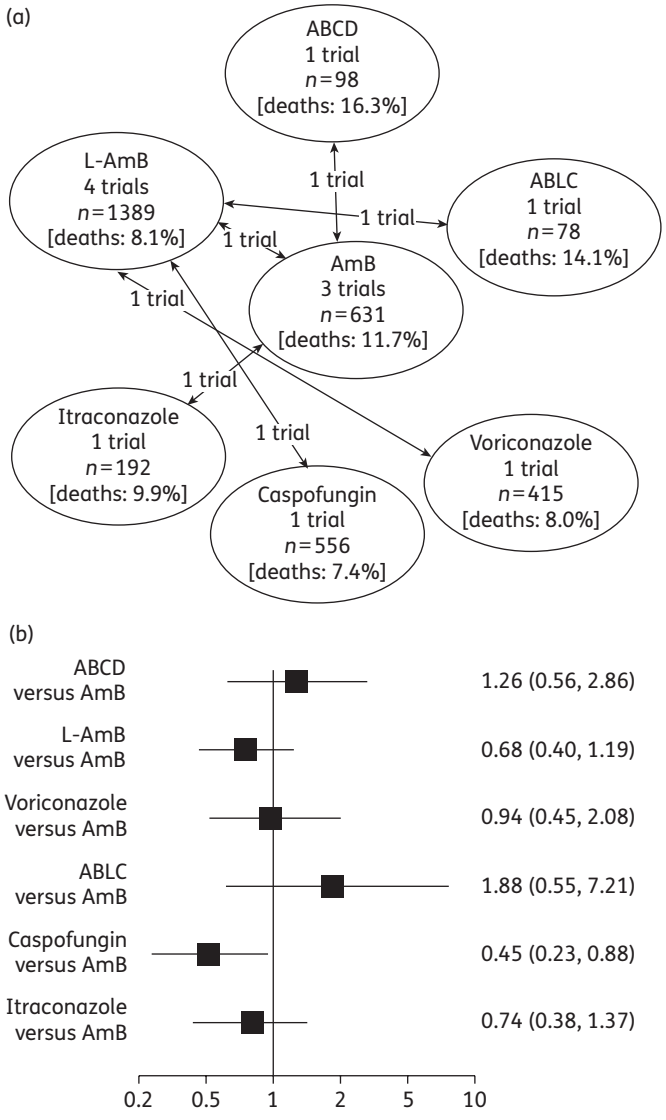

Figure 1. (a) Network of trials describing survival in empirical therapy. Each agent is identified within an ellipse in the diagram, which also includes the number of trials randomizing subjects to that agent, the number of patients (*n*) randomized to that agent and the percentage of subjects randomized to that agent who died. (b) Treatment compared with amphotericin B, effect on survival, odds ratio and 95% CI. Odds ratio >1 indicates benefit to amphotericin B.

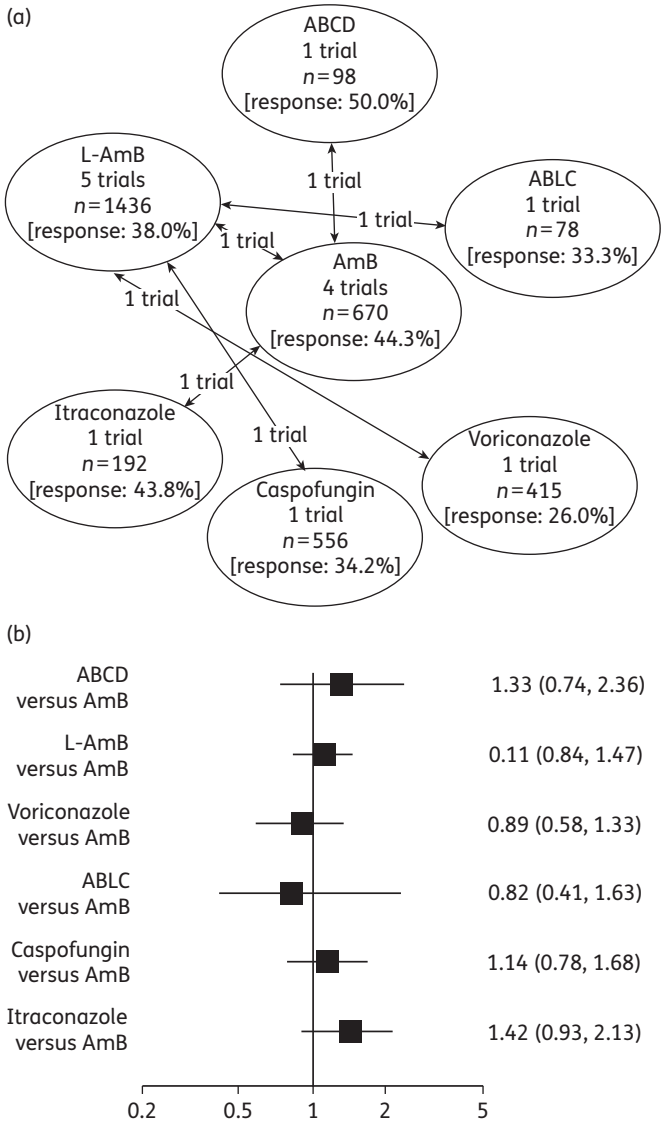

Figure 2. (a) Network of trials describing empirical therapy response. Each agent is identified within an ellipse in the diagram, which also includes the number of trials randomizing subjects to that agent, the number of patients (*n*) randomized to that agent and the percentage of subjects randomized to that agent who died. (b) Treatment compared with amphotericin B, effect on response, odds ratio and 95% CI. Odds ratios <1 indicate advantage to amphotericin B.

number of subjects randomized by agent are described in Table 7.

The network of trials is described in Figure 4(a). Both voriconazole and L-AmB were significantly better than AmB on the outcome of response (Figure 4c). In addition, L-AmB was significantly superior to ABCD (odds ratio 3.695, 95% CI 1.019–14.28) and voriconazole was superior to ABCD (odds ratio 2.411, 95% CI 1.071–5.324). There was no statistically significant difference between voriconazole and L-AmB.

Discussion

The time period between the biological start of a fungal infection and the appearance of clinical signs and symptoms represents a window of opportunity that, if identified through prospective screening, may allow earlier therapeutic intervention, and may potentially improve outcome. This ‘pre-emptive’ strategy would rest on better identification of those patients who are at the highest risk of fungal infections, through rapid diagnostic approaches, who would benefit from more targeted treatment delivered at a time when it can have most clinical value.

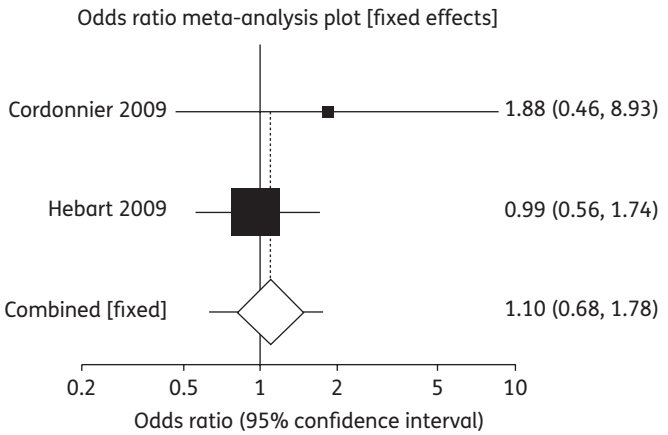

Figure 3. Pre-emptive versus empirical mortality, odds ratios and 95% CI. Odds ratio <1 demonstrates a benefit for pre-emptive therapy.

We have conducted a mixed treatment comparison of drugs for IMD, comparing pre-emptive and empirical strategies, and the efficacy of different agents for empirical treatment and directed treatment strategies. We have examined the outcomes of survival for all analyses, and therapeutic response for the comparison of therapeutic agents. In the comparison of pre-emptive versus empirical therapy, we found no benefit on survival for the pre-emptive strategy. In the comparison of different agents, for the outcome of survival, caspofungin appears most effective for empirical therapy, and voriconazole appears most effective for directed therapy. For response, there is no agent that appears significantly better or worse out of those examined in empirical therapy, but for directed therapy both L-AmB and voriconazole appear significantly better than AmB.

The mixed treatment effects approach has a number of advantages and limitations. First, the approach enables conditional estimates of treatment response, allowing us to generate comparative estimates for any agents included in the network. Second, the approach enables us to overcome the limitations of the network of trials through generating indirect comparisons through the network of direct comparisons, and borrowing weight from indirect comparisons.

There are also a number of limitations. First, the mixed treatment approach is not a substitute for large, well-designed randomized trials examining the questions of interest. It is, however, arguably the best we can do with the available data and quantifies the kind of informal narrative approach that is the only alternative. Second, although we selected the fixed effects methodological approach in an *a priori* manner, and the DIC comparing the fixed and random models appeared to confirm that this approach was optimal, the evaluation of model fit by DIC is limited as the metric only applies when comparing models that address the data in a realistic manner. Thus, if our underlying assumptions were flawed, then the DIC should give us little reassurance. However, the assumption of a common treatment effect is not a strong one when examined on a ratio scale, and indeed is the common assumption applied within all the included trials. Third, the mixed treatment approach, while efficient in deriving treatment estimates, is only as good as the data that are included. These were quite limited for some agents. For example, in the comparison of survival by agent in empirical therapy, it may be surprising to learn that voriconazole is not statistically significantly different from ABCD. This, however, is because of the paucity of data for ABCD. Further, only four agents are examined in the directed

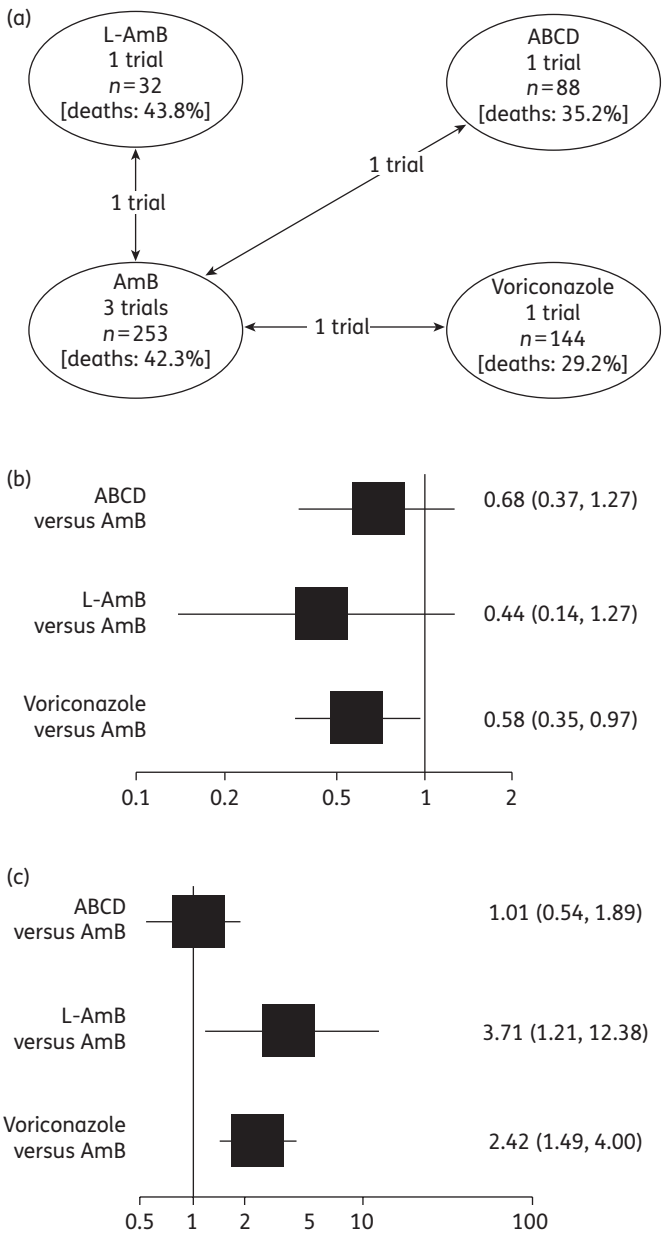

Figure 4. (a) Network of trials describing survival and response in directed therapy. Each agent is identified within an ellipse in the diagram, which also includes the number of trials randomizing subjects to that agent, the number of patients (*n*) randomized to that agent and the percentage of subjects randomized to that agent who died. (b) Treatment compared with amphotericin B, effect on survival, odds ratio and 95% CI. Odds ratio >1 indicates benefit to amphotericin B. (c) Treatment compared with amphotericin B, effect on response, odds ratio and 95% CI. Odds ratio <1 indicates benefit to amphotericin B.

Table 7. Categorization of included patients by treatment (directed therapy)

Treatment	Response	Trials (*n*)
AmB	78/253	3
ABCD	31/88	1
L-AmB	14/32	1
Voriconazole	76144	1

ABCD, amphotericin B colloidal dispersion; AmB, conventional amphotericin B; L-AmB, liposomal amphotericin B.

therapy trials, limiting the opportunity to extrapolate findings to other agents.

Funding

Authors attended a meeting in Amsterdam to discuss the content for this article, for which they received financial compensation for their time and expenses. No reimbursement was received for drafting or reviewing the article. Logistical support and assistance with styling and submission of the manuscript was provided by HealthCare21 Communications Ltd and was funded by Pfizer International Operations.

Transparency declarations

This article is part of a Supplement sponsored by Pfizer Inc.

N. F. has received funding for research, consulting and travel from a range of pharmaceutical companies that manufacture treatments for invasive mould disease, including Pfizer Inc., who provided financial support for the analysis described in this article. The analysis was designed, executed and interpreted by the authors without input from the sponsor.

P. T. received payment for conducting the systematic review and statistical analysis for this manuscript.

R. H. has been a consultant for Astellas, Basilea, Gilead, Merck and Pfizer, is a member of speakers bureaus for Gilead, Merck and Pfizer and has received a research grant from Pfizer.

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