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Background: Mucormycosis incidence is increasing and is associated with a high rate of mortality. Although lipid-based formulations of amphotericin B are the recommended first-line treatment, only one prospective trial in a limited number of patients has been performed to evaluate this regimen.

Methods: Patients with proven or probable mucormycosis were included between June 2007 and March 2011. Patients were scheduled to receive 10 mg/kg/day liposomal amphotericin B (L-AMB) monotherapy for 1 month and surgery was performed when appropriate. The primary outcome was response rate at week 4 or at the end of treatment (EOT) if before week 4, evaluated by an independent committee. ClinicalTrials.gov Identifier: NCT00467883.

Results: Forty patients were enrolled. Response was analysed in 33 patients at week 4. Most patients had a haematological malignancy as their primary underlying disease (53%). Seventy-one percent of patients underwent therapeutic surgery. The response rate at week 4 or at EOT was 36%, with 18% partial responses and 18% complete responses. The response rate at week 12 was 45%, with 13% partial responses and 32% complete responses. Overall mortality was 38% at week 12 and 53% at week 24. Serum creatinine doubled in 16 (40%) patients and returned to normal levels within 12 weeks in 10/16 (63%).

Conclusions: High-dose L-AMB for mucormycosis, in combination with surgery in 71% of cases, was associated with an overall response rate of 36% at week 4 and 45% at week 12 and creatinine level doubling in 40% of patients (transient in 63%). These results may serve as the basis for future clinical trials.

Introduction

Mucormycosis is a life-threatening fungal infection that is emerging worldwide,[1–6] occurring primarily in patients with haematological malignancies (HMs), diabetes mellitus (DM), solid organ transplantation and trauma with skin-penetrating injuries. Mucorales are ubiquitous filamentous virulent fungi intrinsically resistant to various antifungals. Moreover, high Mucorales angiotropism causes major necrosis,[7] which results in limited antifungal diffusion to the infection site. Amphotericin B, itraconazole, posaconazole and isavuconazole are the only antifungal agents active against Mucorales.[8,9] However, itraconazole, posaconazole and even isavuconazole are not active against all Mucorales species.[9] Therapeutic data on mucormycosis are scarce, limited to two prospective trials evaluating the efficacy of the combination of a polyene [liposomal amphotericin B (L-AMB)] and deferasirox as first-line treatment[10] and, recently, of isavuconazole as first-line or salvage treatment.[11] First results of this open-label, non-comparative study evaluating isavuconazole as first-line therapy in mucormycosis led to its recent FDA approval and EMA evaluation.[11] Amphotericin B deoxycholate treatment is complicated by unacceptably high rates of toxicity. Several retrospective trials have assessed the efficacy of lipid-based amphotericin B formulations in mucormycosis treatment, confirming their improved

safety profile.[12–17] In addition, a higher efficacy of high-dose L-AMB in mucormycosis mouse models has been shown: 10 mg/kg/day L-AMB was more effective than 5 or 1 mg/kg/day in reducing *Rhizopus arrhizus* fungal burden and led to higher lung tissue concentrations.[18] Furthermore, in a 44 patient Phase I–II trial comparing several dose regimens, i.e. 7.5, 10, 12.5 and 15 mg/kg/day L-AMB, the highest amphotericin B plasma concentration was obtained with 10 mg/kg/day as well as the highest mean AUC at 24 h.[19] While high-dose L-AMB is recommended for visceral leishmaniasis treatment,[20] it is associated with increased toxicity without better efficacy in invasive pulmonary aspergillosis.[21] Because of the severity of mucormycosis and cautiously promising evidence from pre-clinical data, in spite of the increased toxicity of a 10 mg/kg/day dose, we believed it was warranted to evaluate the efficacy and tolerance of high-dose L-AMB in the initial treatment of mucormycosis. We therefore conducted a prospective, multicentre, pilot trial to evaluate the efficacy and safety of high-dose (10 mg/kg/day) L-AMB monotherapy for initial mucormycosis treatment.

Methods

Trial design

The AmBizygo trial was a multicentre, national, prospective, pilot study conducted in 63 centres in France from June 2007 to July 2011 to evaluate the efficacy and safety of high-dose (10 mg/kg/day) L-AMB for the initial treatment of mucormycosis (NCT00467883). An ethics committee (CPP Comité de Protection des Personnes Ile de France II) approved the protocol. Written informed consent was obtained from each patient enrolled in the trial. Patient follow-up occurred at week 1, week 2, week 4 (W4), end of treatment (EOT) if treatment ended before W4, week 12 (W12) and week 24 (W24). Survival was assessed at 12 months.

Study participants

Eligible patients had proven or probable mucormycosis according to European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and NIAID Mycoses Study Group criteria without any age restrictions.[22] Mucormycosis was proven by the presence of large and non-/poorly septate hyphae consistent with Mucorales on tissue biopsy or Mucorales in culture from a sterile site. Mucormycosis was considered probable when Mucorales were found in culture associated with clinical or radiological abnormalities compatible with an invasive fungal infection, regardless of underlying disease of the patient. An independent expert committee (P. R., M. W. and S. P.) reviewed all charts and classified the cases by consensus into the above diagnostic categories. All the available isolates were sent to the National Reference Center for Invasive Mycoses and Antifungals, Institut Pasteur for phenotypic and molecular identification and *in vitro* susceptibility testing according to EUCAST methodology. Exclusion criteria were life expectancy <72 h, pregnancy, breast-feeding, severe allergy to polyene and a previous curative amphotericin B or posaconazole treatment >5 days during the previous month. An independent safety committee monitored the trial. Corticosteroids were considered a risk factor if patients received ≥ 0.3 mg/kg/day for >3 weeks and neutropenia was considered a risk factor if counts were <500 cells/mm^3 over 10 days in the previous month. Definition of the main risk factors and location of mucormycosis was as reported previously.[2]

Treatment

After inclusion, patients received L-AMB (AmBisome®, Gilead Sciences, Boulogne-Billancourt, France) at high dose (10 mg/kg/day) for 4 weeks along with surgical therapy when deemed necessary. Surgical procedures were designed according to local extent of disease and decision of the surgeon. L-AMB was given as an infusion over ≥ 2 h. If any reaction was noticed, infusion duration could be extended. No recommendation on fluids or pre-medication was given. In patients in whom there was doubling of serum creatinine levels as compared with baseline, it was recommended that the L-AMB dose be reduced to 7.5 mg/kg/day. If creatinine values did not improve within 3 days, L-AMB dosing was further reduced to 5 mg/kg/day. Safety monitoring included serum creatinine, magnesium, potassium, AST, ALT, GGT and blood cell counts (baseline, twice weekly for the first 2 weeks and then weekly) as well as possible concomitant nephrotoxic drugs. Creatinine values were monitored at 3 months. Treatment was stopped in cases of anaphylactic reactions or if there was an increase in liver enzymes >20 times normal levels.

Primary and secondary outcomes

Clinical, radiological and mycological responses were assessed at W4 or at EOT if it occurred before W4. Response was also evaluated at W12. A centralized analysis of all radiological studies was performed. Overall response was evaluated according to the previously described Herbrecht criteria[23] and the Segal criteria,[24] published after the beginning of the present study. Response was assessed by the previously mentioned independent expert committee. Complete and partial responses were considered favourable responses, whereas stable disease and response failure were considered unfavourable. Death of any cause was considered a failure. Patient follow-up lasted for 48 weeks. Disseminated infection was defined by at least two non-contiguous sites of infection.

The primary endpoint was the percentage of overall favourable responses (complete or partial response) at W4 or before if treatment ended earlier, according to the Herbrecht evaluation criteria. The endpoint was chosen because the study treatment duration was 4 weeks. Non-evaluable patients were not included in response evaluation. Secondary endpoints were treatment efficacy at W12, survival and relapse at 6 and 12 months and safety. Mucormycosis-related deaths were defined as deaths occurring when the mucormycosis infection was still considered to be progressing and when there was no alternative cause, as decided by the independent expert committee.

Statistical analyses

Sample size was calculated based on an estimate of the efficacy of L-AMB monotherapy in the treatment of mucormycosis. On the basis of an expected primary event rate of 30%, we estimated that enrolment of 36 patients would provide a precision equal to $\pm 15\%$. To take into account the possibility of non-evaluable patients, the initial sample size was increased to 44 subjects. The efficacy analysis was performed on all evaluable patients for whom diagnosis was confirmed by the expert panel and who had not received previous antifungal treatment with anti-Mucorales activity as per the exclusion criteria. The safety analysis was performed on all included patients. Efficacy was expressed as a percentage with the 95% CI calculated using a binomial method. Relationships between categorical variables were tested with the χ^2 test or Fisher's exact test. Student's *t*-test was used to compare continuous variables between two groups. Survival time was measured from the date of entry into the study to the date of death or last follow-up. Survival curves were estimated using the Kaplan–Meier method. Prognostic effect of clinical or biological factors was estimated using the log-rank test or univariate Cox model. All statistical analyses were performed using R software (http://cran.r-project.org).

Results

Patients

Forty patients were included between June 2007 and March 2011, including two children under the age of 16 years. Six patients were

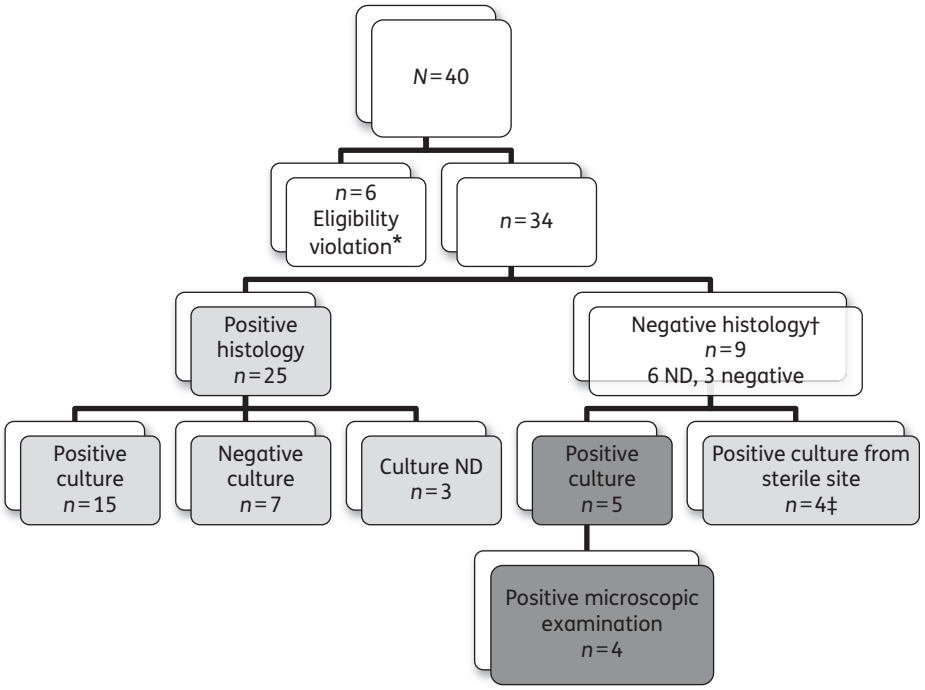

Figure 1. Mucormycosis diagnosis. *Recent antifungal treatment active against Mucorales ($n=1$) or mucormycosis diagnosis excluded according to the expert panel evaluation ($n=5$). †Negative or not done (ND). ‡Bile duct, muscle, bone or pleural fluid. Light grey shading indicates proven mucormycosis and dark grey shading indicates probable mucormycosis.

excluded because they did not meet the eligibility criteria, i.e. recent antifungal treatment active against Mucorales ($n=1$) or mucormycosis diagnosis excluded by the expert panel evaluation ($n=5$). Thirty-four patients had mucormycosis and all inclusion criteria. Twenty-nine patients (85%) had proven infection (25 with positive histology and 4 with positive Mucorales culture from a sterile site) (Figure 1). Five patients had probable infection with positive culture from a non-sterile site with signs of infection (four positive cultures were associated with positive microscopic examination). The patient characteristics are detailed in Table 1. Most patients had an underlying HM as their main risk factor (53%). In the patients with HM, 28% underwent HSCT, 17% had graft versus host disease, 44% received corticosteroids and 33% had recent neutropenia. Eighteen percent of patients had DM (two patients had type I DM and four had type II DM) and in 9% trauma was the main risk factor. Nine patients had BMI >25 kg/m^2 and 2 had BMI >30 kg/m^2.

Infection location included lung only (29%), rhino-orbito-cerebral only (26%) or skin only (18%) and was disseminated in 18% of cases. Other locations included gastrointestinal ($n=1$), liver ($n=1$) and larynx ($n=1$). Mucormycosis location varied with underlying disease: HM was associated with disseminated or lung infections and DM with rhino-orbito-cerebral infections ($P=0.018$). Among rhino-orbito-cerebral infections, 3/9 patients had DM and 5/9 had HM; among lung infections, 7/10 had HM and 2/10 DM; and among disseminated infections, 4/6 had HM. Eight patients had developed another invasive fungal infection during the 3 months prior to mucormycosis diagnosis [invasive candidiasis ($n=4$) and invasive aspergillosis ($n=4$)]. Twenty-nine patients had received at least one antifungal drug during the month before diagnosis [posaconazole ($n=6$), polyenes ($n=21$) for <5 days, fluconazole ($n=7$),

Table 1. Characteristics of the 34 analysed patients

Patients' characteristics	No. (%) or median (range)
Male	21 (62)
Age (years)	53 (0.50–78.10)
Time from symptom onset to treatment (days)	46 (4–344)
Underlying disease	
HM	18 (53)
haematopoietic stem cell transplant	5/18 (28)
GVHD	3/18 (17)
neutropenia	6/18 (33)
corticosteroids	8/18 (44)
DM	6 (18)
trauma	3 (9)
solid organ transplant	3 (9)
other[a]	4 (12)
Site of infection	
lung	10 (29)
rhino-orbito-cerebral	9 (26)
skin	6 (18)
disseminated	6 (18)
other[b]	3 (9)

GVHD, graft versus host disease.
[a]Prematurity ($n=1$), cancer ($n=1$), no risk factor ($n=1$) and diabetic ketoacidosis ($n=1$).
[b]Gastrointestinal ($n=1$), liver ($n=1$) and larynx ($n=1$).

voriconazole ($n=11$) and echinocandin ($n=13$)]. Thirteen patients had breakthrough infections: nine occurred in patients treated with voriconazole and four with caspofungin. No patients presented reverse halo sign on chest CT scan.

Phenotypic and molecular characterization of Mucorales strains was performed for 23 strains out of 24. The main species identified were *Lichtheimia corymbifera* (22%), *Lichtheimia ramosa* complex (35%) and *R. arrhizus* (18%). Other species identified were *Rhizomucor pusillus* (9%), *Saksenaea vasiformis* (4%) and *Cunninghamella elegans* (4%). No molecular identification could be performed for one strain.

Treatment

Study treatment is detailed in Table 2. Twenty-four patients (71%) underwent surgical procedures (diagnostic surgery not included), including 18 (53%) before initiation of L-AMB therapy. Twelve patients had one therapeutic surgical procedure, six had two surgical procedures and six had three surgical procedures. All nine patients with rhino-orbito-cerebral infection underwent surgery, two (20%) with lung infections, five (83%) with skin infections and five (83%) with disseminated infections [kidney resection ($n=2$), soft tissue resection ($n=2$) and digestive tract and liver surgery ($n=1$)]. Surgical procedures were mainly performed before L-AMB treatment: 67% in rhino-orbito-cerebral cases, all cases of lung infection, 60% for skin infections and 80% for disseminated infections. One patient with laryngeal infection as well as a patient with liver involvement underwent surgery. Median time between first symptoms and L-AMB initiation was 46 days (4–367). One patient had telluric trauma 367 days before mucormycosis diagnosis and was infected by *S. vasiformis*. Twenty-nine (85%) patients received 10 mg/kg L-AMB on the first day of study inclusion and 21 patients (62%) received 10 mg/kg/day during the first 7 days. Median L-AMB monotherapy duration was 21 days (0–28). One patient received 5 days of L-AMB before inclusion and a combination of L-AMB and posaconazole after inclusion. Median cumulative L-AMB dose was 161 mg/kg (0–320). Median dose per treatment day was 9.5 mg/kg/day (5–11.4). L-AMB was administered at a 10 mg/kg/day dosing for a median of 13.5 days (0–28).

Table 2. Study treatment

Treatment	No. (%) or median (range)
L-AMB monotherapy duration (days)[a]	21 (0–28)
L-AMB monotherapy cumulative dose (mg/kg)	161 (0–320)
L-AMB daily dose (mg/kg)	9.5 (5.0–11.4)
10 mg/kg/day L-AMB duration (days)	13.5 (0–28)
Surgical procedure[b]	24 (71)
Surgical procedure before L-AMB treatment[b]	18 (53)
Number of surgical procedures[b]	
one	12 (50)
two	6 (25)
three	6 (25)

[a]One patient received additional treatment with posaconazole after inclusion.
[b]Count excluding diagnostic surgery.

Primary endpoint

Treatment responses according to Herbrecht criteria are detailed in Table 3. Thirty-three out of the 34 patients were evaluated at W4 or at EOT when it occurred earlier. One patient was considered non-evaluable because they had no evaluation of radiological response at W4, whereas the clinical response was complete. Twelve patients (36%, 95% CI 20%–55%) had a favourable response at W4 or at EOT if earlier. Six patients (18%) had a complete response and 6 a partial response; 4 patients had stable disease (12%), 10 failed treatment and survived (30%) and 7 died (21%). Focusing specifically on the L-AMB dose, the W4 response rate was 43% (9/21) in patients who received 10 mg/kg/day during the first week compared with 25% (3/12) in patients who did

Table 3. Treatment response according to Herbrecht and Segal criteria

	Herbrecht, W4 or EOT if before ($n=33$)[a]	Segal, W4 or EOT if before ($n=32$)[b]	Herbrecht, W12 ($n=31$)[c]	Segal, W12 ($n=31$)[c]	W24
Favourable response	12/33 (36%)	10/32 (31%)	14/31 (45%)	15/31 (48%)	NA
partial response	6/33 (18%)	4/32 (13%)	4/31 (13%)	6/31 (19%)	NA
complete response	6/33 (18%)	6/32 (19%)	10/31 (32%)	9/31 (29%)	NA
Failure	21/33 (64%)	22/32 (69%)	17/31 (55%)	16/31 (52%)	NA
stable	4/33 (12%)	7/32 (22%)	2/31 (6%)	1/31 (3%)	NA
failure without death	10/33 (30%)	8/32 (25%)	2/31 (6%)	2/31 (6%)	NA
death[d]	7/34 (21%)	7/34 (21%)	13/34 (38%)	13/34 (38%)	18/34 (53%)
related to mucormycosis	5/34 (15%)		9/34 (26%)		10/34 (29%)
not related to mucormycosis	2/34 (6%)		4/34 (12%)		8/34 (24%)

NA, not available.
[a]Response was evaluated for 33 patients as 1 patient was non-evaluable at W4.
[b]Response was evaluated for 32 patients as 2 patients were non-evaluable at W4.
[c]Response was evaluated for 31 patients as 3 patients were non-evaluable at W12.
[d]Death was evaluated for the 34 patients with mucormycosis.

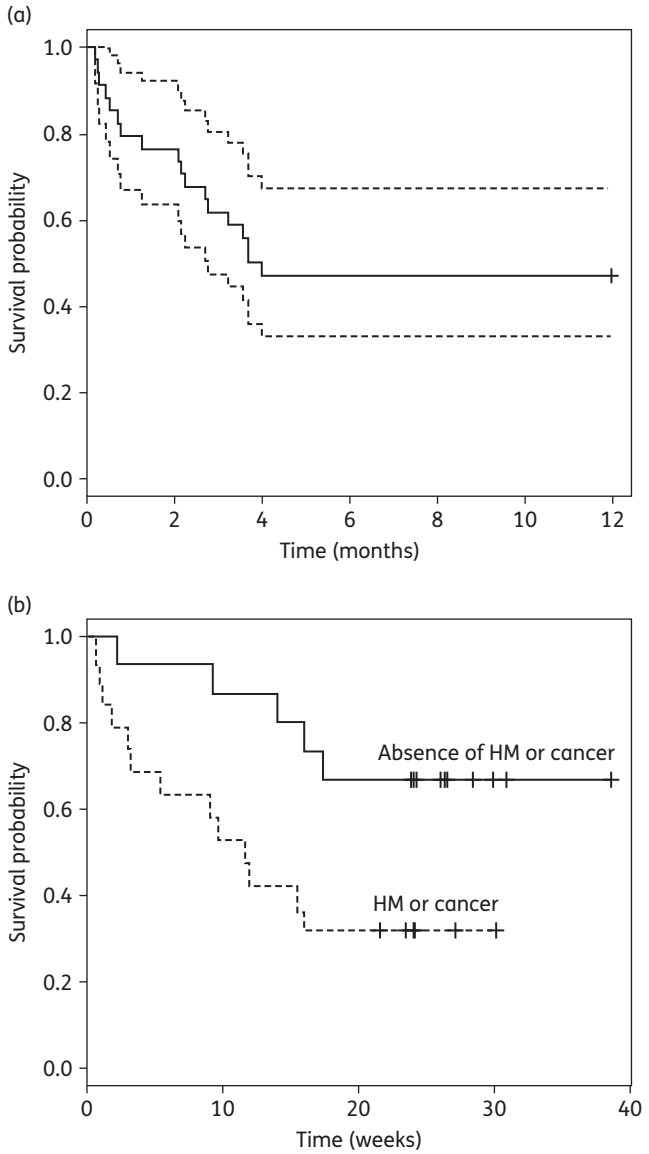

Figure 2. Survival: Kaplan–Meier curve. (a) 1 year survival. (b) Impact of underlying condition on 1 year survival.

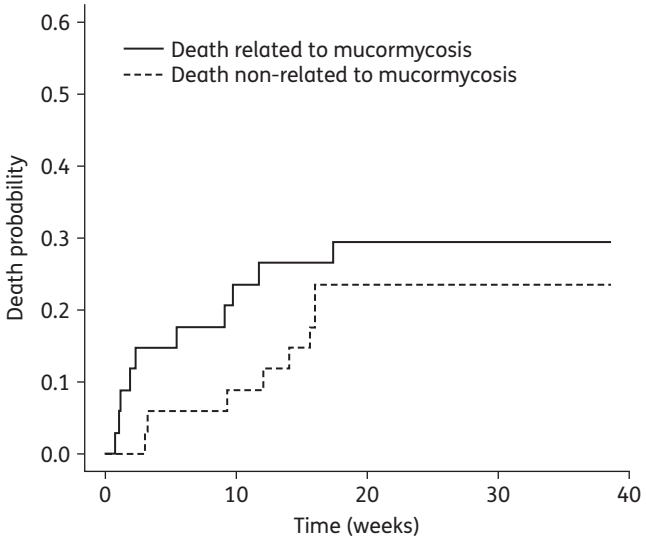

Figure 3. Mucormycosis-related deaths (Kaplan–Meier curve).

not. Although not statistically significant, the W4 response rate was 43% (12/28) in patients who received ≥7.5 mg/kg/day during the first week compared with 0% (0/5) in patients who did not. Age, gender, risk factor, location, risk factor number, surgery, weight and delay between first symptoms and treatment were not associated with response at W4 or at EOT when it occurred earlier.

Secondary endpoints

Fourteen out of 31 evaluable patients (45%, 95% CI 27%–64%) had a favourable response at W12. Three patients were considered non-evaluable because radiological evaluation was not performed; however, clinical response was complete. These three patients were therefore not included in the response analysis. Ten patients had a complete response (32%), 4 had partial responses (13%), 2 were stable, 2 failed but survived and 13 patients died, 6 between W4 and W12. Treatment response evaluated according to Segal's criteria was 31% (16%–50.0%) at W4 (32 evaluable patients) and 48% (31%–67%) at W12 (31 evaluable patients).

At W24, survival was 47% (95% CI 33%–67%) (Figure 2a); 18 patients died, 10 with mucormycosis (Figure 3). Mortality rates at W4, W12, W24 and 12 months were 21%, 38%, 53% and 53%, respectively. No deaths or relapses were observed between W24 and 12 months. Among age, gender, weight, infection location, risk factor number and duration between first symptoms and treatment, the only factor associated with mortality was HM or cancer [HR=3.15 (1.12–8.91), P=0.02; Figure 2b]. Patients who received 10 mg/kg/day L-AMB during the first 7 days compared with those who did not had similar mortality rates [1.07 (0.40–2.85), P=0.91]. No difference was observed for those who received ≥7.5 mg/kg/day during the first 7 days of treatment compared with those who did not [HR=0.67 (0.19–2.33), P=0.52]. No deaths were observed between W24 and W48.

Safety

The safety analysis population was 40 patients. Adverse events related to L-AMB are detailed in Table 4. Creatinine levels doubled compared with baseline in 40% (16/40) of patients, leading to dose reduction in 7 patients, treatment interruption in 5 and definitive treatment discontinuation for 1 patient. Creatinine doubling occurred in 58% (7/12) of patients with DM compared with 32% (9/28) of patients without DM (P=0.17). Three months after EOT, among the 16 patients who doubled their creatinine levels, 10 (63%) recovered normal kidney function, 4 (25%) died before 3 months and 2 (12%) did not recover. Patients presented with the following adverse events: 2 (5%) had hypokalaemia <2.5 mmol/L and 16 (40%) <3 mmol/L, 10 (25%) had gastrointestinal side effects, 5 (12%) exanthema, 4 (10%) transaminase elevation, 6 (15%) cholestasis, 1 (2%) lumbar pain, 3 (7%) low

Table 4. Adverse events related to L-AMB

	No. (%)	Dose reduction	Treatment interruption	Definitive treatment interruption	Serious adverse events
Doubling of creatinine level[a]	16 (40)	7 (17)	5 (12)	1 (2)	4 (10)
Potassium level					
<2.5 mmol/L	2 (5)	0	0	0	1 (2)
<3 mmol/L[b]	16 (40)	1 (2)	0	0	2 (5)
Gastrointestinal	10 (25)	0	2 (5)	0	1 (2)
Rash	5 (12)	1 (2)	0	0	1 (2)
Elevation of liver enzymes[c]	4 (10)	1 (2)	1 (2)	0	0
Cholestasis	6 (15)	1 (2)	1 (2)	1 (2)	1 (2)
Lumbar pain	1 (2)	1 (2)	0	0	1 (2)
Hyperglycaemia	1 (2)	0	0	0	1 (2)
Catheter thrombosis	1 (2)	0	0	0	1 (2)
Low blood pressure	3 (7)	0	0	0	0
Fever	3 (7)	0	1 (2)	0	0
Cytopenia	7 (17)	1 (2)	0	1 (2)	1 (2)

[a]Creatinine level two times the baseline level.
[b]Includes those with hypokalaemia <2.5 mmol/L.
[c]Defined by the investigator.

blood pressure during infusion, 3 (7%) fever and 7 (17%) cytopenia. Ten adverse events resulted in treatment interruption. Thirty-seven severe adverse events were reported from 26 patients; 14 were considered related to L-AMB.

Discussion

We report the largest prospective clinical trial performed to date in the treatment of mucormycosis. Most data available currently in the field of mucormycosis treatment are retrospective and have several limitations, i.e. inclusion criteria, confounding bias and response evaluation. In the present, trial inclusion criteria were not selective and all forms of mucormycosis were included, regardless of the underlying disease, patient age and severity. This study has several methodological strengths: (i) all diagnoses were confirmed and all evaluations made by an independent expert committee; (ii) most (85%) of the mucormycosis cases were proven; (iii) patient characteristics and sites of infections were representative of those found in recent retrospective studies (indeed, underlying disease and location were similar to those obtained during the nationwide retrospective RetroZygo study conducted in France between 2005 and 2007, which included 101 patients[2]); and (iv) identification to the genus and species level was assessed using molecular tools and morphology for 96% of the isolates in the national reference centre. The main Mucorales species identified in this study were *Lichtheimia* spp. compared with *R. arrhizus* in the RetroZygo study.[2] *Lichtheimia* spp. represented 29% of strains in the latter study. The high number of patients with proven mucormycosis, the absence of reverse halo sign and the limited number of patients that received mucormycosis treatment during the first 2 weeks after the occurrence of first symptoms probably depict the delayed diagnosis.

Our main finding was that a therapeutic strategy combining high-dose L-AMB and surgery (performed in 71% of patients) was associated with a 36% response rate at W4. These results can be compared with the Defeat study, which is the only published prospective therapeutic trial in mucormycosis[10] where 20 patients were treated with L-AMB at a minimum dose of 5 mg/kg with either deferasirox or placebo, in combination with surgical debridement in all patients. W4 response rates were similar in both studies with a 36% response rate in the AmBizygo study and 40% global success in the Defeat study. W12 response rates were higher in the AmBizygo study with a 45% response rate compared with 35% global success reported in the Defeat study. W12 mortality was 42% PP in the Defeat study compared with 38% in the AmBizygo study. More recently, the first results of an open-label, non-comparative study evaluating isavuconazole as first-line therapy in 21 patients with mucormycosis showed a response in 31.6% at EOT while survival was 66.7% at week 6 (W6) and 57.1% at W12.[11] Based on those data, isavuconazole was approved by the FDA as first-line treatment of mucormycosis. Therefore, despite the lack of any comparative study, the W12 response rate was 45% with high-dose L-AMB in the AmBizygo trial compared with 31.6% at end of first-line treatment with isavuconazole (median time of 102 days) in the VITAL study.[11] Furthermore, isavuconazole MIC values for Mucorales species are higher than those of amphotericin B[25] and, finally, data on isavuconazole penetration in the CNS are scarce. Among the 18 patients who died during study follow-up, mucormycosis-related

mortality occurred mainly during the first 3 months after diagnosis (10 patients died from mucormycosis: 5 during the first month; 4 between months 1 and 3; and 1 between months 3 and 6). These results are concordant with those reported by Xhaard *et al.*,[26] who reported 59% mortality occurring before day 115 among 29 cases of mucormycosis in allo HSCT patients.

Several methodological conclusions, potentially useful for further trials, can be drawn from our results. Interestingly, for the first time, we had the opportunity to evaluate patient responses using both Herbrecht[23] and Segal[24] criteria. W4 response was 36% according to Herbrecht criteria versus 31% according to Segal criteria, while W12 response was 45% versus 48%, respectively, thereby showing concordant results between the two criteria.

The optimal evaluation timepoint for assessment of antifungal therapy in mucormycosis has not been defined. According to Segal *et al.*,[24] the optimal timepoint for evaluation in the treatment of invasive aspergillosis is W6. In a study comparing amphotericin B deoxycholate versus voriconazole in invasive aspergillosis treatment, survival difference reached significance at W6.[23] W6 has also been chosen as the key timepoint in a recent trial comparing voriconazole versus voriconazole combined with anidulafungin in invasive aspergillosis.[27]

Response criteria are a composite of clinical, radiological and mycological response. For lung lesions, CT scanning has a key role. CT scan follow-up was well evaluated in invasive aspergillosis with stable lesions at week 2 compared with initial CT.[28] Legouge *et al.*[29] evaluated intensive CT follow-up in 16 patients with proven pulmonary mucormycosis and found that mucormycosis lung lesions continued to increase through day 26. Mucormycosis radiological response is therefore slower than aspergillosis. Our evaluation timing was chosen at W4 to coincide with the end of study treatment. However, because mucormycosis radiological evolution is slower than aspergillosis, the optimal timepoint to evaluate response in mucormycosis most likely should be later than for invasive aspergillosis and, based on our experience, would be at W8. Therefore, stability or regression below 25% of a lesion should be considered a positive response at W4.

Radiological evaluation in patients with rhino-orbito-cerebral infections is difficult in patients who have undergone surgery as it is challenging to distinguish active mucormycosis lesions from surgical sequelae. In these cases, we found the systematic evaluation of treatment response via naso-fibroscopic evaluation and biopsies of great utility in assessing clinical, endoscopic and mycological response. This management will be evaluated prospectively in a national research project (NCT02226705).

We also evaluated patient tolerance of the 4 week regimen of high-dose L-AMB, which is twice the duration used in the AmBiLoad trial. As expected, we report greater toxicity, with higher rates of plasma creatinine level doubling (40% versus 31%). Of interest, renal toxicity tended to be more frequent in patients with DM, probably related to diabetic nephropathy. However, only 12% had persistent renal impairment 3 months after the end of L-AMB therapy.

In conclusion, results of the AmBizygo study provide evidence that high-dose L-AMB for mucormycosis, in combination with surgery in 71% of cases, was associated with an overall response rate of 36% at W4 and 45% at W12 and creatinine level doubling in 40% of patients (transient in 63%). These results may serve as the basis for future clinical trials.

Acknowledgements

We thank Assistance Publique-Hôpitaux de Paris, promoter of the study. We thank URC Paris Descartes Necker (Beatrice Barbier) for the implementation, monitoring and data management of the study.

We also thank Eric Dannaoui and Michel Huerre for their help in isolate collection and pathological slide reviews and Susan DeWolf for her helpful comments prior to submission.

We thank Muriel Vray, Michel Tod, Vincent Jullien, Agnes Lefort and Caroline Charlier-Woerther for their participation in the safety committee.

Other members of the French Mycosis Study Group

We thank the following principal investigators of the French Mycosis Study Group, who contributed to the current study. Institut Pasteur: Francoise Dromer and Damien Hoinard (Centre National de Référence Mycoses Invasives et Antifongiques, Molecular Mycology Unit), and Fabrice Chretien and Gregory Jouvion (Human and Animal Model Histopathology Unit). Those outside Paris (in alphabetical order by city) are as follows: Amiens (J. Maizel and T. Chouaki), Angers (M. Hunault and J. P. Bouchara), Annecy (M. Sirodot, J. Gaillat and X. Blanc), Avignon (Q. Gobert), Besançon (J. Leroy, F. Legrand, J. Navallou, F. Grenouillet, L. Million and G. Blasco), Bordeaux (D. Malvy and I. Accoceberry), Brest (S. Ansart and E. Moalic), Caen (O. Reman, R. Verdon and J. Bonhomme), Dijon (D. Caillot, P. Charles, P. Chavanet and F. Dalle), Grenoble (J. Stahl, O. Epaulard, A. Thiebaut-Bertrand and M. Cornet), Lille (B. Guery and B. Sendid), Lyon (T. Ferry, T. Baudry, M. Michallet, M. Moutran, P. Miailhes and A. Bienvenu), Marseille (C. Faucher, S. Gayet and S. Ranque), Montpellier (O. Jonquet and Y. Sterkers), Nantes (P. Moreau and F. Morio), Nice (P. M. Roger, P. Dellamonica and M. Gari-Toussaint), Poitiers (A. Veinstein, J. Saulnier and C. Kauffmann), Rennes (C. Arvieux, M. Bernard, V. Gandemer and J. P. Gangneux), Rouen (C. Joly, G. Pfister, B. Veber and L. Favennec), Saint Etienne (C. Cazorla and H. Raberin), Strasbourg (V. Letscher-Bru and M. Sabou) and Toulouse (M. Alvarez, A. Huynh and S. Cassaing). Those in Paris are as follows: Ambroise Paré (E. Giroux, Leprieur and J. Dunand), Avicenne (J. M. Naccache, C. Gardin, O. Freynet and S. Brun), Saint Louis (E. Azoulay, C. Lacroix and E. Raffoux), Bichat (V. Joly and C. Chochillon), Cochin (J. Charpentier and A. Paugam), Foch (E. Rivaud and P. Cahen), HEGP (A. Novara and E. Dannaoui), Henri Mondor (C. Cordonnier, A. Mekontso Dessap, F. Botterel and S. Bretagne), Kremlin Bicêtre (N. Anguel and A. Angoulvant), Necker Enfants Malades (F. Suarez, F. Lacaille, M. Oualha and M. E. Bougnoux), Pitié Salpétrière (A. Combes and P. Buffet), Raymond Poincaré (B. Clair and J. Dunand), Saint Antoine (E. Maury and C. Hennequin), Trousseau (M. D. Tabone) and Villejuif (B. Wyplosz, M. Merad and L. Mihaila).

Funding

This work was supported by a grant from Gilead Sciences, but the sponsor took no part in the scientific evaluation. L-AMB was kindly provided by Gilead Sciences.

Transparency declarations

F. L. is on the speaker bureau for Gilead Sciences, Merck and Novartis. S. P. is on the speaker bureau for Gilead Sciences and Pfizer. R. H. is a consultant, advisory board member or on the speaker bureau for Pfizer, Gilead Sciences, Merck, Schering-Plough and Astellas, and has received a research grant from Pfizer. M. W. is an advisory board member for Gilead Sciences, AztraZeneca and MSD, and is on the speaker bureau for Pfizer. P. R. is an advisory board member for Pfizer and Merck, and is on the speaker bureau for Pfizer, Merck and Gilead Sciences. O. L. is an advisory board member for Pfizer, Merck, Astellas and Gilead Sciences, is a consultant for Gilead

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