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Prospective Phase II Single-Center Study of the Safety of a Single Very High Dose of Liposomal Amphotericin B for Antifungal Prophylaxis in Patients with Acute Myeloid Leukemia

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Some preclinical and pharmacokinetic studies suggested the variable safety and the potential efficacy of an antifungal prophylaxis with a single high dose of liposomal amphotericin B (L-AmB) in high-risk patients. An open-label, prospective study was conducted with 48 adults receiving induction chemotherapy for acute myeloid leukemia (AML). Patients received a single infusion of 15 mg/kg of body weight L-AmB and, eventually, a second dose after 15 days of persistent neutropenia. The primary objective was tolerability and safety. Efficacy was also evaluated as a secondary endpoint. A pharmacokinetic study was performed with 34 patients in order to evaluate any association of plasma L-AmB levels with toxicity and efficacy. Overall, only 6 patients (12.5%) reported Common Toxicity Criteria (CTC) grade 3 hypokalemia, which was corrected with potassium supplementation in all cases, and no patient developed clinically relevant nephrotoxicity. Mild infusion-related adverse events occurred after 6 of 53 (11.3%) total infusions, with permanent drug discontinuation in only one case. Proven invasive fungal disease (IFD) was diagnosed in 4 (8.3%) patients. The mean AmB plasma levels at 6 h, 24 h, and 7 days after L-AmB administration were 160, 49.5, and 1 mg/liter, respectively. The plasma AmB levels were higher than the mean values of the overall population in 3 patients who developed CTC grade 3 hypokalemia and did not significantly differ from the mean values of the overall population in 3 patients who developed IFD. Our experience demonstrates the feasibility and safety of a single 15-mg/kg L-AmB dose as antifungal prophylaxis in AML patients undergoing induction chemotherapy.

Invasive fungal diseases (IFDs), in particular invasive aspergillosis (IA), are a leading cause of morbidity and mortality in patients with acute myeloid leukemia (AML) (1–4). Primary antifungal prophylaxis has been considered an appealing strategy, because the diagnosis of IFD is often difficult to obtain in time to implement an early and effective therapeutic intervention. However, despite the availability of various broad-spectrum antifungal drugs suitable for prophylaxis use, only oral posaconazole has proven to be effective in this setting and is actually recommended as the drug of choice for the prevention of IFDs in AML patients undergoing induction chemotherapy (5–12). However, a major problem with the use of oral posaconazole in patients treated with intensive chemotherapy is represented by the unpredictable gastrointestinal absorption, which may require monitoring of triazole plasma levels; the possible difficulty in taking oral medications; and some drug-drug interactions, which may limit the use of this drug, as well as of the other triazoles, in patients receiving certain antileukemic treatments (13–17). Therefore, other classes of antifungals may be considered prophylaxis in some particular settings.

The liposomal form of AmB (L-AmB) has been investigated in order to evaluate the feasibility of its use in primary prophylaxis of IFDs in patients with hematological malignancies receiving chemotherapy or stem cell transplant (SCT) (18–28). Some preclinical and pharmacokinetic studies suggested the relevance of a high-dose regimen of L-AmB (7.5 to 15 mg/kg of body weight) administered as long-interval infusions (once weekly) or as a single administration in the prophylaxis of IFDs (25–28). All these experiences demonstrated variable safety and clinical attractiveness of long intervals or single, high-dose, prophylactic schedules of L-AmB in certain high-risk populations, but this has not been adequately investigated for AML patients.

The aim of this pilot study was to evaluate the feasibility and tolerability of prophylactic administration of a single very high dose (15 mg/kg) of L-AmB in adult patients newly diagnosed with AML and undergoing induction chemotherapy.

MATERIALS AND METHODS

Study design. This study was a prospective, pilot, phase II, single-center trial. This study aimed to assess the feasibility and tolerability of a single very high dose of L-AmB (15 mg/kg/infusion) in 48 adult patients newly diagnosed with AML undergoing first-remission induction chemotherapy. Efficacy was also evaluated as a secondary endpoint. A pharmacokinetic study was also performed with 34 patients in order to evaluate any association of plasma L-AmB levels with toxicity and efficacy. Patients were enrolled between January 2004 and January 2011. The study was performed according to institutional guidelines and was approved by the local review board in compliance with good clinical practices and the Declaration of Helsinki. Written informed consent was obtained from each patient before any study procedure was performed.

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TABLE 1 Patient demographics and clinical characteristics

Characteristic	Value
No. (%) of male patients	29 (61.7)
Median age (yr) (range)	55 (32–78)
No. (%) of patients with type of leukemia	
Nonpromyelocitic AML	42 (87.5)
Acute promyelocitic leukemia	6 (12.5)
No. of patients in chemotherapy protocol[a]	
AML-12	14
AML-17	5
AML-19	2
AIDA	6
3 + 7	11
FLAG	9
No. (%) of patients with *de novo* AML	40 (83.3)
No. (%) of patients with secondary AML[b]	8 (16.7)

[a] See references 29–32.
[b] AML occurring after a myelodysplastic phase or other hematologic or solid tumor.

Patient population. Patients were eligible for the study if they were ≥18 years old, underwent first-remission induction chemotherapy, and had expected neutropenia of <500 neutrophils/ml for at least 2 weeks.

Patients should not have had any evidence of invasive mycosis at the onset of leukemia before the start of chemotherapy; therefore, all patients by protocol underwent a chest computed tomography (CT) scan as a baseline screening in order to exclude any pulmonary infection. Patients should not have received any other concomitant antifungal prophylaxis. Also, patients were not eligible for enrollment into the study if the serum creatinine level was >1.5 times the upper limit of our laboratory value and if the potassium serum level was <3.0 mEq/liter. Patients with severe cardiovascular disease, severe disease other than hematological disease, and pregnancy were not eligible. Patients were hospitalized in single-bed rooms with HEPA filtration and positive pressure. Antibacterial prophylaxis consisted of oral ciprofloxacin (500 mg/twice a day [b.i.d.]).

Chemotherapy procedures. Patients underwent induction chemotherapy protocols according to age (≤60 years or >60 years) and type of AML (most of the patients were enrolled in the EORTC-GIMEMA studies), as detailed in Table 1 (29–32).

Study drug administration. L-AmB (Ambisome; Gilead Sciences, Paris, France) was reconstituted, according to the manufacturer's instructions, to give a 2-mg/ml solution. Drug dilutions for injection were prepared as needed with 5% dextrose. All patients received a single intravenous L-AmB administration at a dose of 15 mg/kg as a 6-h infusion within 24 h after the stop of chemotherapy. A second 15-mg/kg dose was eventually administered to patients with persistent profound neutropenia (<100 neutrophils/μl) after 15 days from the first L-AmB dose. Premedication was not administered per protocol for infusion-related reactions prior to the study drug infusion.

Monitoring of safety and tolerability. In order to monitor the safety and tolerability of L-AmB infusions, patients were closely observed for infusion-related side effects during the administration of study drug and during the following 12 h. Serial vital signs during and after infusion as well as clinical signs and symptoms of toxicity were recorded. Pulse and blood pressure were monitored immediately before infusion, during infusion if necessary, and at the end of infusion. Signs, symptoms, and reported side effects associated with drug infusion or occurring at any time during the study period were documented and assessed for a relationship to the study drug. Routine laboratory examinations (blood counts and determination of serum creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, sodium, potassium, phosphate, calcium, magnesium, and glucose levels) for assessment of safety were performed daily for the first 15 days and then at least every other day until patient discharge. Safety and tolerability were assessed according to the incidence of grade 3 to 4 adverse events (AEs) based on the Common Toxicity Criteria (CTC) classification, reported as definitely, possibly, or probably related to the study drug. In particular, CTC grade 3 and grade 4 hypokalemia was defined as potassium serum levels of <3 mmol/liter and <2.5 mmol/liter, respectively, and CTC grade 3 and grade 4 acute kidney injury was defined as creatinine levels >3× baseline serum levels and dialysis indication, respectively. Any adverse event with onset during or within 1 h of completion of the study drug infusion was recorded as an infusion-related reaction.

Assessment of efficacy. Efficacy assessment was based on a clinically driven diagnostic workup. In the event of fever or other clinical signs potentially related to an underlying infection, patients underwent physical examination; serial blood cultures; cultures from other sites if indicated; and CT scan of the chest, paranasal sinuses, and abdomen. Bronchoalveolar lavage and biopsy of suspicious lesions/sites with microscopic evaluation and cultures were done at the discretion of the investigator. Since January 2007, serum *Aspergillus* galactomannan (Platelia Aspergillus) and *Candida* mannan (Platelia Candida) tests were also used in the diagnostic workup. Generally, patients who died underwent autopsy. Diagnosis of IFD was done according to the European Organization for Research and Treatment of Cancer-Mycoses Study Group (EORTC-MSG) criteria published in 2002 (33).

Pharmacokinetic study. Overall, 34 patients were evaluable for AmB pharmacokinetics, all after the first L-AmB infusion. Blood samples were taken immediately before L-AmB administration (T_0), at the end of the 6-h infusion (T_6), and at 24 h (T_{24}) and 7 days from the start of L-AmB administration. Amphotericin B concentrations were determined by high-performance liquid chromatography (HPLC) of plasma sample extracts.

Chromatography was performed with a Perkin-Elmer series 200 high-performance liquid chromatograph connected to a Perkin-Elmer series 200 fluorometer and autoinjector from Perkin-Elmer Instruments, Norwalk, CT. Data collection and processing were carried out by using a Totalchrome WS HPLC IPM chromatography data system (Perkin-Elmer Instruments, Norwalk, CT). The mobile phase consisted of acetonitrile–0.7 M EDTA (36:64, vol/vol) at a flow rate of 1.0 ml/min. Separation was performed by reverse-phase chromatography on a Bondclone C_{18} column (3.9 by 150 mm, 10 μm; Phenomenex) with a C_{18} guard cartridge packed with the same material. Detection was done by measurement of the absorbance at 405 nm.

An aliquot of plasma samples (500 μl) was extracted with acetonitrile (0.5 ml) containing 50 μl of the internal standard *N*-acetylamphotericin B at a 5-mg/liter final concentration. The mixture was vortex mixed briefly and centrifuged at 1,200 × *g* for 10 min twice.

The layer was transferred into clean tubes and was evaporated to dryness at 37°C. The residue was reconstituted in 500 μl of the mobile phase, and the components were mixed for 20 s; this was followed by centrifugation. The aqueous layer was transferred into injection vials, and 50-μl aliquots were injected into the HPLC system.

Standards were prepared by adding the diluted amphotericin B solution, and the internal standard, to appropriate volumes of plasma to give a total volume of 0.5 ml and concentrations of 0.15, 0.3, 0.6, 1.25, 2.5, 5, and 10 μg/ml. The standard curves for amphotericin B were constructed by weighted linear regression of the peak area-versus-concentration ratio and were linear in the concentration ranges. The lowest concentration of the prepared standards was used as the limit of quantification for each assay. If the concentration of a sample was above the range of the standard curve, a dilution of the sample was made in appropriate medium, and the sample was reassayed. The final concentration was calculated by multiplying the concentration of the diluted sample obtained by the assay by the dilution factor. When the concentration of a sample was below the detec-

TABLE 2 CTC AEs observed after 53 L-AmB infusions in 48 patients

CTC	No. (%) of L-AmB infusions	
	All CTC grade AEs	CTC grade 3–4 AEs
L-AmB infusions with at least one AE	19 (35.8)	6 (11.3)[a]
Infusion-related AEs		
Abdominal pain	1(1.9)	0
Lumbar pain	1 (1.9)	0
Pruritus	1 (1.9)	0
Skin rash	2 (3.8)[c]	0
Chills	2 (3.8)[c]	0
AEs subsequent to L-AmB infusion		
Serum creatinine level increase	4 (7.5)	0
Hypokalemia	14 (26.4)	6 (11.3)[a]
Infusion-related AEs that caused increase of infusion duration for better tolerance	4 (7.5)	0
Infusion-related AEs that caused permanent infusion discontinuation	1 (2.1)[b]	0

[a] All CTC grade 3, with 5 AEs after the first L-AmB infusion and 1 AE after the second one.
[b] The patient developed abdominal pain and refused to continue the infusion.
[c] One patient developed both chills and skin rash after the same infusion.

TABLE 3 Pharmacokinetic analysis

Time of blood sample collection after L-AmB infusion	Mean AmB plasma level (mg/liter) (SD)		
	Total patients ($n = 34$ for T_6 and T_{24}; $n = 27$ for day 7)	Patients who developed CTC grade 3 hypokalemia ($n = 3$)	Patients who developed invasive fungal disease ($n = 3$)
T_6	160 (72)	237 (60.3)	164 (19.7)
T_{24}	49.5 (19.1)	78 (6.0)	49.3 (9.0)
7 days from the start of infusion	1 (1.1)	1.1 (1.3)	0.9 (0.2)

tion limit, it was not used in the analyses. To investigate the accuracy and precision of each assay, quality control samples (e.g., standards prepared identically to, but independently from, those of the standard curve) were assayed with each standard curve in each assay run. The assay was linear over a concentration range of 0.15 to 10 μg/ml in plasma. Validation data for accuracy and precision were a coefficient of variation (CV) of between 2.7 and 9.5%, and intraday accuracy was in the range of 99.9 to 105.5%.

Analysis. The primary endpoints were tolerability and safety, defined by infusion-related side effects and the incidence of AEs occurring within 4 weeks from the administration of prophylactic treatment. The secondary endpoints were prophylaxis efficacy (incidence of proven and probable IFDs within 1 month from L-AmB administration), use of empirical antifungal therapy (in patients with persistent fever of unknown origin during postinduction chemotherapy), use of preemptive antifungal therapy (in patients with clinical and/or microbiological findings suspected to be related to an underlying IFD but not fulfilling the EORTC-MSG criteria for proven-probable IFD), and survival at 3 months. Death was attributed to IFD in patients who failed to respond to therapy (i.e., who had stable disease or disease progression) and in patients with a partial response to therapy who died as the result of an acute event involving any of the sites of infection or of an unknown cause.

Descriptive statistics included absolute and relative frequencies for categorical data and median, mean, and range for numerical measurements.

RESULTS

Demographic and clinical characteristics of patients are summarized in Table 1. All 48 patients received at least one prophylactic dose (median, 1,000 mg; standard deviation [SD], ±193 mg; range, 550 to 1,300 mg), whereas 5 patients with prolonged severe neutropenia received a second prophylactic dose (median, 900 mg; SD, ±185 mg; range, 900 to 1,300 mg).

Primary endpoints. Overall, 18 of the 48 (37.5%) patients experienced at least one AE (all CTC grade) after the first or the second L-AmB infusion, and only 6 of them (12.5%) reported a CTC grade 3 AE probably related to L-AmB administration.

The main AEs observed after 53 L-AmB infusions are detailed in Table 2.

CTC grade 3 hypokalemia was documented in 6 cases (11.3%) 3, 5, 5, 6, and 10 days from the first L-AmB infusion and 5 days from the second infusion, respectively. In one of these cases, CTC grade 3 hypokalemia was also associated with CTC grade 2 acute kidney injury. In all cases, hypokalemia was corrected with intravenous potassium supplementation. An increased infusion duration for better tolerance was required in 4 cases (7.5%), and in only 1 case (1.9%) was the infusion permanently discontinued due to the patient's refusal to continue the treatment after the development of abdominal pain. For this patient, no alternative antifungal prophylaxis was administered.

Secondary endpoints. Data on proven-probable IFDs documented within 1 month from L-AmB administration, use of empirical or preemptive antifungal therapy, and 3-month survival rate are detailed in Table 3. Proven IFD was diagnosed in 4 (8.3%) patients (2 cases of pulmonary aspergillosis, 1 case of pulmonary mucormycosis, and 1 case of disseminated *Geotrichum capitatum* infection). The first infectious clinical signs for the 4 proven IFDs were observed at 8, 8, 10, and 11 days from L-AmB administration, respectively. Empirical and preemptive (for a possible pulmonary IFD) antifungal therapy was administered to one patient each. Thirteen deaths (27.1%) occurred within 3 months from the first L-AmB administration; four deaths were attributed to a proven IFD.

Pharmacokinetic analysis. Plasma samples at T_0, T_6, and T_{24} were available for all 34 patients evaluated for pharmacokinetics, whereas plasma samples at day 7 were available for only 27 patients. The pharmacokinetic data are detailed in Table 3. The mean (±SD) AmB plasma levels at T_6, T_{24}, and day 7 were 160 (±72), 49.5 (±19.1), and 1 (±1.1) mg/liter, respectively.

Out of 34 patients considered for the pharmacokinetic evaluation, 3 developed CTC grade 3 hypokalemia. The plasma AmB levels were higher than the mean values of the overall population for all 3 patients at T_6 (300, 230, and 180 mg/liter, respectively) and at T_{24} (72, 84, and 78 mg/liter, respectively) and for 1 of them at day 7 (0.3, 2.6, and 0.4 mg/liter, respectively).

The plasma levels of 3 patients who developed IFD despite L-AmB prophylaxis did not significantly differ from the mean values of the overall population.

DISCUSSION

The aim of the present study was to investigate the safety and feasibility of a single very high dose of L-AmB administered as

TABLE 4 Review of prospective studies of primary antifungal prophylaxis with L-AmB in hematological and stem cell transplant populations[a]

Type of study (reference)	Population (no. of patients)	Schedule of L-AmB administration	Main toxicity and tolerability result(s)	Main efficacy result(s)
Prospective, double blind, multicenter, placebo controlled (18)	Adult neutropenic patients receiving allogeneic SCT (36 in the L-AmB arm and 40 in the placebo arm)	1 mg/kg/day	3 allergic reactions	Proven/probable IFDs, 2.8% in the L-AmB arm vs 7.5% in the placebo arm
Prospective, double blind, multicenter, placebo controlled (19)	Adult patients receiving chemotherapy or SCT for HM (74 in the L-AmB arm and 87 in the placebo arm)	2 mg/kg 3 times weekly during neutropenia	Treatment-related toxicity was modest, and no additional toxicity was observed in patients receiving L-AmB	Proven/probable IFDs, 0% in the L-AmB arm vs 2.3% in the placebo arm; 1 death attributed to IFD for each arm
Prospective, randomized, single center, open label, placebo controlled (20)	Pediatric patients receiving chemotherapy or autologous SCT for HM or solid tumor (16 in the L-AmB arm and 13 in the placebo arm)	1 mg/kg 3 times weekly during neutropenia	No difference in renal toxicity and hypokalemia; L-AmB was discontinued in 19% of cases due to infusion-related reactions	Proven/probable IFDs, 31.2% in the L-AmB arm vs 46.1% in the control arm; no death attributed to IFD
Prospective, randomized, single center, open label (21)	Adult AML patients undergoing induction chemotherapy (62 in the L-AmB arm and 67 in the control arm)	3 mg/kg 3 times weekly during neutropenia	The L-AmB-treated patients more often developed increased bilirubin levels, increased creatinine levels, and infusion-related symptoms	Proven/probable IFDs, 4.8% in the L-AmB arm vs 4.5% in the control arm
Prospective, randomized, single center, open label (22)	Adult patients receiving allogeneic SCT for HM (75 in the L-AmB arm and 57 in the control arm)	50 mg every other day during neutropenia	No CTC grade 3 or 4 toxicities were observed	Proven/probable IFDs, 6.7% in the L-AmB arm vs 35% in the control arm ($P = 0.001$); death attributed to IFD, 2.7% in the L-AmB arm vs 12.3% in the control arm ($P = 0.07$)
Single center, prospective, uncontrolled (23)	Pediatric and adolescent patients receiving allogeneic SCT for HM (51)	3 mg/kg daily until day 100 from SCT	Grade 3–4 side effects, hypokalemia in 4% of patients, nephrotoxicity in 12%; L-AmB was discontinued in 11% of patients due to toxicity	Proven/probable IFDs, 10%; no death attributed to IFD
Prospective, single center, historically controlled (24)	Pediatric patients receiving chemotherapy or autologous SCT for HM (187 prophylaxis courses in 44 patients)	2.5 mg/kg twice weekly during neutropenia	L-AmB was discontinued in 9% of patients due to allergic reactions; only 1 grade 3 reaction; grade 3–4 hypokalemia in 15.2% of prophylaxis courses	No breakthrough proven-probable IFD while on prophylaxis
Single center, prospective, pharmacokinetic (25)	Pediatric patients receiving allogeneic SCT (14)	10 mg/kg weekly for 4 wk	No significant change in serum creatinine level; none of the patients developed hypokalemia, hypomagnesemia, or increased alkaline phosphatase or transaminase levels; only 1 infusion toxicity requiring withholding of the wk 4 dose	Only 1 patient developed evidence of IFD
Single center, prospective, uncontrolled (26)	Adult neutropenic patients receiving allogeneic SCT and with GVHD (21)	7.5 mg/kg once weekly during treatment of GVHD	L-AmB was discontinued in 33% of patients, 19% due to nephrotoxicity and 9.5% due to infusion-related adverse events	Only one IFD; no death attributed to IFD
Multicenter, prospective, pilot, phase II (27)	Adult patients receiving chemotherapy for AL or allogeneic SCT (29)	10 mg/kg weekly for 4 wk in AL patients and for 8 wk in SCT patients	Grade 3–4 AEs, 2/21 AL patients and 6/8 SCT patients; enrollment of SCT patients was stopped	Proven/probable IFDs, 4/29 (13.8%); 1 death attributed to IFD
Single center, prospective, pharmacokinetic (28)	Adult patients receiving allogeneic or autologous SCT (21)	1 mg/kg/day for 15 days (7 patients), 7.5 mg/kg/wk for 2 wk (7 patients), single 15-mg/kg dose (7 patients)	For the daily dosing (1-mg/kg) group, 1 patient withdrew on day 1 due to sternal pain, and 1 patient developed CTC grade 3 hypokalemia; for the weekly dosing (7.5-mg/kg) group, 1 patient was withdrawn due bronchospam after the first dose, and 1 patient developed CTC grade 3 hypokalemia; for the single-dose (15-mg/kg) group, 2 patients developed CTC grade 3 and 1 patient developed CTC grade 4 hypokalemia	Not reported

[a] HM, hematologic malignancies; AL, acute leukemia; GVHD, graft-versus-host disease.

antifungal prophylaxis in AML patients undergoing first-remission induction chemotherapy. Overall, 48 patients were enrolled in the study, and this is the largest experience of high-dose L-AmB prophylaxis in this category of high-risk patients reported in the literature so far. The biological rationale of this schedule of L-AmB prophylaxis derives from previous studies in neutropenic animals suggesting that the administration of a single dose of L-AmB of up to 20 mg/kg of body weight can prevent infections due to yeasts or molds (34, 35). Higher L-AmB doses produce increased intravascular concentrations, which may facilitate its penetration into tissues with sustained concentrations sufficiently high to provide prophylactic efficacy against fungi for several weeks posttreatment (36). In a study on pharmacokinetics and safety of extended-interval dosing of prophylactic L-AmB in stem cell transplant recipients, different schedules of L-AmB administration were evaluated (28). A single L-AmB dose of 15 mg/kg produced mean plasma concentrations of >0.491 mg/liter for at least 7 days. Furthermore, measurement of buccal mucosal tissue concentrations showed that high levels of the drug, well in excess of the MICs reported in the literature for susceptible strains of *Candida* and *Aspergillus* spp., continued to be detected 2 weeks after a single 15-mg/kg dose of L-AmB. Of interest, the buccal mucosal concentrations increased 33% from day 7 to day 15. These pharmacokinetic data suggest that a single very high dose of L-AmB administered at the onset of the period of infectious risk may be considered antifungal prophylaxis with a presumable efficacy of at least 2 weeks.

The main result of our experience is represented by the favorable safety profile of the antifungal prophylaxis schedule. Although CTC grade 3 hypokalemia was observed after 11.3% of infusions, the electrolyte abnormality was still easily and quickly corrected with potassium supplementation and without clinically significant consequences. With regard to renal toxicity, only a mild and self-limiting increase of the serum creatinine level was observed. The continuous hydration with careful electrolyte monitoring usually performed at our institution for leukemic patients undergoing intensive chemotherapy may have contributed to the low toxicity of the treatment. Again, infusion-related reactions occurred after 6 infusions; the infusion duration was prolonged to achieve better tolerance in only 4 cases, and the infusion was stopped by decision of the patient in only 1 case. The low incidence of infusion-related side effects in our series may have been related to the longer infusion time (6 h) that we used than in previous studies reported in the literature (2 to 4 h) (25–28). Considering the data of the pharmacokinetic study performed with 34 patients, we observed that for the 3 patients who developed CTC grade 3 hypokalemia, the levels at T_6 and at T_{24} were higher than the mean values of the overall population. Presumably, the higher the peak level of AmB, the higher the distal tubular epithelial toxicity, with increased urinary potassium wasting and hypokalemia. This effect was observed within a few days after L-AmB infusion and rapidly disappeared.

Several studies reported in the last years, most of which were published since the initiation of the present pilot study, have shown a variable tolerance of L-AmB given as antifungal prophylaxis in neutropenic and SCT patients (Table 4). Various administration schedules in different hematologic populations have been used. When daily to weekly low or standard doses (from 1 to 3 mg/kg) of L-AmB were used, the toxicity profile was generally favorable (18–24). On the contrary, the use of high L-AmB doses (7.5 to 15 mg/kg) administered at weekly intervals or as a single administration was in some cases associated with severe adverse events, leading to the treatment discontinuation (25–28). In particular, a significant toxicity of high doses of L-AmB was observed in adult SCT patients (26–28). In the PROPHYSOME study, where 10-mg/kg L-AmB doses were planned to be administered weekly for 8 weeks in allogeneic SCT patients, 6 of the first 8 SCT patients enrolled in the study developed various CTC grade 3 to 4 AEs (dyspnea, thoracic pain, abdominal pain, tubulointerstitial nephritis, anuria, and anaphylactic shock), leading the independent data review committee to stop the inclusion of SCT subjects (27).

The toxicity observed in SCT patients was not confirmed in nontransplant neutropenic patient populations. In agreement with our experience, the PROPHYSOME study showed a good toxicity profile of high-dose L-AmB antifungal prophylaxis (10 mg/kg/week for 4 weeks) in acute leukemia patients undergoing intensive chemotherapy (27).

Our study was not primarily designed to measure prophylactic efficacy of a single 15-mg/kg L-AmB dose, and the 8.3% incidence of IFDs that we observed, although similar to or lower than that reported for real-life experiences of posaconazole prophylaxis in AML patients (6–8), does not allow any interpretation. However, in agreement with previous pharmacokinetic studies, the median day 7 plasma L-AmB concentration in our patients was within the range of MICs for susceptible strains of *Candida* (0.25 to 1 mg/liter) and at the lower limits for *Aspergillus* (0.5 to 2 mg/liter). In any case, it was difficult to find a correlation between AmB plasma levels and efficacy of the prophylaxis regimen considering the highly variable interpatient plasma concentration-time data; furthermore, the plasma levels of the 3 patients who developed IFD and were evaluated for pharmacokinetics did not significantly differ from the mean values of the overall population.

In conclusion, our prospective experience demonstrates the feasibility and safety of a single very high L-AmB dose as antifungal prophylaxis in AML patients undergoing induction chemotherapy. The challenging aspect of this particular schedule of mold-active prophylaxis is the single intravenous administration, which may be repeated in the event of prolonged neutropenia. It may be an alternative to oral triazoles (itraconazole, posaconazole, and voriconazole), which have the limits of gastrointestinal absorption, metabolic variability, and drug-drug interactions, or to other mold-active intravenous drugs (echinocandins, voriconazole, and itraconazole), which require daily administration during the entire at-risk period. The pharmacokinetic studies of patients receiving a single very high L-AmB dose demonstrated the achievement of prolonged serum and tissue therapeutic levels of the drug, and published uncontrolled clinical series, including the present one, suggest a promising clinical efficacy of this prophylactic schedule. Prospective, controlled clinical trials are needed to verify the potential efficacy of a single very high L-AmB dose as an alternative prophylaxis choice for acute leukemia patients undergoing intensive chemotherapy.

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