When azoles cannot be used: the clinical effectiveness of intermittent liposomal amphotericin prophylaxis in haematology patients

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Abstract

**Background:** Patients unable to take azoles are a neglected group lacking a standardized approach to antifungal prophylaxis. We evaluated the effectiveness and safety of intermittent liposomal amphotericin (L-AMB) prophylaxis in a heterogenous group of haematology patients.

**Methods:** A retrospective cohort of all haematology patients who received a course of intravenous L-AMB defined as 1mg/kg thrice weekly, from 1 July 2013-30 June 2018 were identified from pharmacy records. Outcomes included breakthrough-invasive fungal disease (BIFD), reasons for premature discontinuation and acute kidney injury.

**Results:** There were 198 patients who received 273 courses of L-AMB prophylaxis. Using a conservative definition, the BIFD rate was 9.6% (n=19/198) occurring either during L-AMB prophylaxis or up to 7 days from cessation in patients who received a course. Probable/proven-BIFD occurred in 13 patients (6.6%, 13/198), including molds in 54% (n=7) and non-\textit{albicans} Candidaemia in 46% (n=6). Cumulative incidence of BIFD was highest in patients with acute myeloid leukaemia (6.8%) followed by acute lymphoblastic leukaemia (2.7%) and allogeneic stem cell transplantation (2.5%). The most common indication for L-AMB was chemotherapy or anticancer drug-azole interactions (75% of courses) dominated by vincristine or acute myeloid leukaemia clinical trials, followed by gut absorption concerns (13%) and liver function abnormalities (8.8%). Acute kidney injury using a modified international definition, complicated 27% of courses but was not clinically significant accounting for only 3.3% (9/273) of discontinuations.

**Conclusions:** Our findings demonstrate a high rate of BIFD among patients receiving L-AMB prophylaxis. Pragmatic trials will help find the optimal regimen of L-AMB prophylaxis for the many clinical scenarios where azoles are unsuitable, especially as targeted anticancer drugs increase in use.
Introduction

Azole antifungal drugs are the mainstay of prophylaxis used to prevent invasive fungal diseases (IFD) in patients with high risk haematological malignancies or haematopoietic stem cell transplant (HSCT) recipients. However, there are circumstances when patients may be unable to safely take azoles due to intolerance, toxicity, or drug-drug interactions. The latter is seen in patients undergoing treatment for acute lymphoblastic leukemia (ALL), where azoles can potentiate vincristine associated toxicity (1) and where tyrosine kinase inhibitors are used in Philadelphia positive disease (2), but it is increasingly seen in patients with acute myeloid leukaemia (AML) on targeted anticancer drugs (3).

The polyene antifungal, liposomal amphotericin B (L-AMB), has been evaluated over the years as an alternative to azoles with mixed results (4-10). L-AMB has several favourable characteristics that promote intermittent or extended interval dosing, including a long terminal half-life of 152 hours (11, 12), retention in tissues along with an absence of interactions with agents such as cyclosporine and tacrolimus (13). Unfortunately, the only placebo controlled randomised clinical trial in the modern era that used L-AMB 5mg/kg twice weekly in patients with ALL undergoing remission-induction chemotherapy, did not demonstrate a statistically significant reduction in short-term IFD rates (7.9% vs. 11.7%, p=0.24) (6). Despite this result, an unmet clinical need remains not only for ALL, but for a range of clinical scenarios. These have usually been disaggregated in studies of L-AMB prophylactic efficacy into either neutropenia (8, 10), transplant (7, 8) or acute leukaemia (4-6, 9) but are likely much wider given the heterogeneity of patients in clinical practice.

When azoles are avoided, several Australian centres including ours, have resorted to using intermittent L-AMB prophylaxis (12). The aim of this study was to describe the real-world clinical effectiveness and safety of intermittent L-AMB prophylaxis in a heterogeneous but contemporary
group of haematology patients. The clinical scenario described here represents the real-world challenges we navigate often without high quality evidence.

Methods

Study design and setting

This was a single-centre retrospective cohort study of haematology patients at The Alfred Hospital who received systemic L-AMB prophylaxis from 1 July 2013 to 30 June 2018. The Alfred Hospital is a 638-bed quaternary university-affiliated adult centre located in Melbourne, Australia, with trauma, heart/lung transplantation, allogeneic hematopoietic stem cell transplantation (HSCT), cystic fibrosis, burns, hyperbaric medicine and human immunodeficiency virus state-wide services.

Antifungal prophylaxis is prescribed according to an institutional protocol. Patients unable to take azole drugs have been given 1mg/kg of L-AMB (based on real body weight) intravenously three times per week on Monday, Wednesday and Friday both as inpatients and outpatients since 2012 with 250 mls of either 5% dextrose or saline pre-hydration. In the majority of patients this translates to 50 mg to 120 mg three times weekly but is usually either 50 mg or 100 mg thrice weekly depending on patient weight.

Chest computed tomography (CT) is performed for suspected IFD with bronchoalveolar lavage (BAL) or biopsy performed as tolerated. A surveillance driven approach using serum galactomannan or Aspergillus PCR surveillance is not routine, and when performed these are send-away tests performed as part of the diagnostic work-up when IFD is suspected. Empiric antifungal therapy is sometimes commenced in the presence of suspicious radiologic changes
while awaiting diagnostic investigations. Since April 2005, all patients have been treated in high efficiency particulate air-filtration rooms. An infectious diseases physician and registrar perform regular ward rounds on a referral basis for haematology patients.

**Patient Consent Statement**

Institutional ethics approval from Alfred Health with a patient waiver of consent was obtained (Project no. 104/17).

**Study criteria and clinical variables**

Haematology patients who received L-AMB for any indication were identified from pharmacy dispensing records. From these, all patients who received at least three consecutive alternate day doses of intravenous L-AMB typically on Monday, Wednesday and Friday, defining a course were identified. Patients were excluded if they did not receive a course of L-AMB prophylaxis, received treatment dosing, were administered different L-AMB dosing regimens (e.g. 5 mg/kg twice weekly) as part of a haematology clinical trial or were on more than one anti-fungal agent. Secondary courses of L-AMB were not excluded because we were interested in determining if any courses were complicated by additional breakthrough-invasive fungal disease (BIFD). Data collected included patient demographics, IFD details, outcomes including short-term mortality up to 12 weeks from the last L-AMB dose, adverse reactions, reasons for starting or premature discontinuation of L-AMB; renal function at baseline, weekly and up to 14 days after L-AMB cessation.

**Clinical definitions**

Our primary outcome was BIFD, classified by investigators (RB, BG, MAR) according to updated international consensus criteria (14) where a probable/proven case required fungal isolation while a
possible case lacked positive microbiology but satisfied radiographic and host criteria. Date of IFD diagnosis was defined as date of positive microbiology or supportive imaging, whichever came first.

BIFD was adjudicated by adapting published definitions to aid comparability with future studies. BIFD was defined using conservative, intermediate and broad criteria as follows. A conservative definition was IFD occurring during L-AMB prophylaxis or up to seven days from cessation similar to Ananda-Rajah et al. (15); while an intermediate definition was IFD occurring up to fifteen days post prophylaxis similar to Lerolle et al. (16) in patients who received at least one L-AMB course. We also included a broad modified intention-to-treat analysis, where BIFD at any time point after one dose of L-AMB was included. BIFD using all three definitions is reported, but the conservative definition by Ananda-Rajah et al. (15) was preferred as a post-prophylaxis interval of 7 days approximates the terminal half-life of L-AMB which is 152 hours (11, 12).

Duration of L-AMB prophylaxis was the number of days from date of commencement to completion inclusive of non-administered days (rather than days of therapy). Excess days of prophylaxis was calculated as the number of days L-AMB was continued (inclusive of non-administered days) after resolution of neutropenia (i.e. absolute neutrophil count <0.5 x 10^9/L), surmising that this may represent unnecessary L-AMB exposure.

Acute kidney injury (AKI) was recorded if it was documented in the medical record as the reason for discontinuation. AKI was also defined by modifying the Kidney Disease Improving Global Outcomes (KDIGO) criteria by retaining changes to serum creatinine up to 14 days post prophylaxis but excluding urine output which was not available for many patients (17).
Statistical analysis

The primary objective of this study was to evaluate the clinical effectiveness of L-AMB prophylaxis, defined as the incidence of BIFD. Secondary outcomes were renal toxicity and tolerability. Descriptive analyses were based on percentages and frequencies for categorical variables and for continuous variables, as means with standard deviation or medians with interquartile range (IQR), if the data were skewed. Creatinine values were plotted for each patient over time, by the number of weeks followed-up and those with levels above the upper limit of normal at baseline were grouped separately. Kaplan-Meier survival plots were used to display time to event data, with groups compared using the log-rank test. When comparing those who had BIFD to those who did not, the start of L-AMB prophylaxis was considered time zero and patients were assessed to date of death or, if still alive, censored at the maximum follow-up time. To quantify the effect of BIFD and other known risk factors on death, Cox regression analysis was used. Other known risk factors included acute disease (AML or ALL v. all other conditions), disease status at the start of the L-AMB course (i.e. active defined as partial remission, progressive or refractory disease; new or relapsed disease), HSCT type (allo-, auto- or none) and presence or absence of neutropenia. Cumulative incidence of BIFD from the time of haematological diagnosis (but excluding IFD occurring in the period prior to the start of L-AMB prophylaxis) was calculated at 3 years for the overall cohort and for AML, ALL and HSCT subgroups. P values were two-tailed with a value less than 0.05 considered statistically significant. Analyses were completed using Stata 15.1 software (Stata Corp, College Station TX, USA). Data were recorded onto a REDCap database.
Results

Clinical characteristics

We identified a total of 198 patients who received 273 courses of L-AMB prophylaxis from pharmacy dispensing records (Table 1). There was a male predominance (62%), mean age was 52 years (range 16-83 years) and 35% were aged 65 years or more. L-AMB was administered during remission induction chemotherapy for newly diagnosed disease or in patients with active (i.e. partial remission/progressive or refractory) disease in 45% and 40% of courses respectively (Table 2). Haematological malignancies accounted for the majority of underlying conditions (97%) dominated by acute myeloid leukaemia (AML) in 46% and acute lymphoblastic leukaemia (ALL) in 27% of patients. Allogeneic haematopoietic stem cell transplant (HSCT) accounted for the majority of HSCT recipients (27 of 29). Patient acuity and resource utilisation was high with 29% of patients requiring intensive care unit admission. Mortality at 30-days and 12-weeks from the end of prophylaxis was 17% and 22% respectively (Table 1).

Characteristics and indications for L-AMB prophylaxis

The median duration of L-AMB prophylaxis was 16 days (IQR 10-27 days) with a median of 7 doses (IQR 5-11) administered. The majority of courses were associated with neutropenia (87%) which was prolonged lasting 3 weeks or more in 45% of courses associated with neutropenia at L-AMB administration (Table 2). L-AMB prophylaxis was continued beyond neutrophil recovery in 23 (8.4%) courses. The median number of excess days of L-AMB administered was 10 (IQR 5-19) days, approximating 6 additional doses of L-AMB for these 23 courses.
Chemotherapy or anticancer drugs contraindicating azole antifungal prophylaxis was the most common indication for L-AMB prophylaxis accounting for 75% (n=206) of courses. This comprised vincristine-based treatment of ALL (93/206, 45%) and patients enrolled in clinical trials (80/206, 39%). The majority of L-AMB courses for anticancer drugs were for patients with AML (93%, n=74/80) receiving venetoclax (n=49 courses), sorafenib/placebo (n=7 courses) or a variety of other agents (Supplementary Fig 1). Other reasons for L-AMB use included impaired gastrointestinal absorption in 13% of courses (mostly due to GVHD and mucositis) and liver function abnormalities in 8.8%. Documented allergy or intolerance to azoles was uncommon accounting for 2.6% (n=7) of L-AMB courses.

**Breakthrough IFD**

Using a conservative definition adapted from (15), the BIFD rate was 9.6% (19/198) comprising 13 (68%) probable/proven IFD and 6 (32%) possible IFD episodes (Table 3). BIFD rates using intermediate and broad definitions were 12.1% (24/198) and 13.1% (26/198) respectively. This translated to 3.3, 4.2 and 4.6 BIFDs per 1000 L-AMB prophylaxis days for the conservative, intermediate and broad BIFD definitions. The respiratory tract (lung and sinus) accounted for 74% (14/19) of BIFDs followed by blood in 32% (6/19). Molds were slightly more common than *Candida* species (54% vs. 46%) among probable/proven cases comprising Paecilomyces in 1 and bronchoalveolar galactomannan in 6 cases. All proven episodes were caused by non-*albicans* Candidaemia. All mold infections were probable and mostly diagnosed by positive galactomannan on bronchoscopy (86%). No BIFD episodes complicated the two courses L-AMB given for secondary prophylaxis. Patient level data on BIFD is shown in a swimmers plot in Fig 1. Acute leukaemia was present in 12 of 19 BIFD patients (AML in 8, ALL in 4) with remission-induction chemotherapy in 9 (AML=6, ALL=3). The AML subgroup with BIFD also included 5 patients enrolled in a clinical trial.
Using intermediate (16) and broad definitions, the BIFD rate was 12.1% (24/198) and 13.1% (26/198) respectively.

Cumulative incidence curves showing time to BIFD using an intention-to-treat definition censored at 3 years from date of leukaemia diagnosis and date of HSCT, stratified by haematological conditions are shown in Fig 2. Overall cumulative incidence was 13.8% (95%CI, 9.53% to 19.9%). Corresponding cumulative incidence for AML, ALL and HSCT was 6.8% (95%CI, 3.9% to 11.7%), 2.7% (95%CI, 1.1% to 6.3%) and 2.5% (95%CI, 0.9% to 6.5%) respectively.

Outcomes

There were 84 deaths among 166 patients (Fig 3). Median survival was significantly lower in patients with BIFD who died earlier (62 days, versus 976 days, \( p = 0.0007 \)). Patients with BIFD had a significantly higher risk of death (unadjusted hazard ratio 3.0, 95% CI 1.7-5.1, \( p=0.001 \)). Survival at 100 days from start of L-AMB prophylaxis for those with BIFD was 45% (95% CI 24% to 64%) compared with 85% (95% CI 78% to 90%) in those patients without BIFD. After adjusting for acute leukaemia (AML, ALL vs others), active disease, HSCT and presence of neutropenia, BIFD remained significantly independently associated with death (adjusted HR 2.8, 95% CI 1.6 to 4.9, \( p<0.001 \)) in addition to new diagnosis of haematological condition, neutropenia and allo-HSCT (Table 4).

Safety and tolerability

L-AMB prophylaxis was well tolerated with few premature discontinuations (12.8%, 35/273, Table 5). These were due to IFD onset (7.7%, \( n=21 \)) followed collectively by acute kidney injury, lack of perceived efficacy, infusion related pain and liver function abnormality in 5.1% (\( n=14 \)). Acute kidney
injury according to a modified KDIGO criteria, occurring up to 2 weeks post prophylaxis, complicated 27% (n=75) of courses. KDIGO grade 3 occurred in 6.2% (n=17) of courses denoting an increase in serum creatinine to 3 times baseline or ≥ 353.6 mmol/L or initiation of renal replacement therapy. Median creatinine remained lower than baseline until after week 5 but did not exceed 50% of baseline, as shown in supplementary Fig 2. Weekly trends in serum creatinine per course indicated that patients who started with high values tended to remain high (Supplementary Fig 3).

Discussion

Intermittent L-AMB prophylaxis emerged historically in response to an unmet need among malignant haematology patients unable to take azole antifungals (12). This study demonstrates that the current 1mg/kg three times per week dosing strategy is associated with a high incidence of BIFD which was 9.6% using a conservative (15) definition. This was associated with a 3-fold higher mortality compared to patients without BIFD being most marked in the first 100 days from the start of prophylaxis. In our cohort, patients with acute leukemia were at highest risk for BIFD (Fig 2). AML and ALL patients accounted for 12 of 19 BIFD cases with AML responsible for 8 cases alone. Remission-induction chemotherapy was especially high risk for acute leukaemia patients, with BIFD complicating 9 cases (AML=6, ALL=3) overall. The most common reason for L-AMB prophylaxis was interactions with anticancer drugs or cytotoxic chemotherapy in 75% of courses. This subgroup was dominated by AML on investigational anticancer drugs (who tend to be at high risk for IFD due to chronic immunosuppression), and patients on vincristine-based therapy for ALL with lesser contributions from Burkitt’s lymphoma, NHL, CML. An evidence-based approach to managing patients unable to take azole prophylaxis is urgently needed noting that targeted anticancer drugs for AML are exploding (18) and a standardised approach to IFD prevention in ALL remains unresolved.
There remains considerable uncertainty regarding the appropriate regimen of L-AMB in the setting of prophylaxis particularly for ambulatory patients. Extended interval prophylaxis studies with either 7.5mg/kg (7), 10mg/kg (5) or 15mg/kg (4) once weekly of L-AMB were not powered for efficacy and were associated with dose limiting toxicity. Intermittent regimens of 2mg/kg (8) or 3mg/kg three times per week (9) and 5mg/kg twice a week (6) found no statistical difference in fungal infections compared to placebo (6, 8) or a combination of itraconazole and fluconazole (9). An exception to this pattern is a placebo-controlled study of alternate day L-AMB 50 mg in neutropenic patients with AML, ALL and non-Hodgkins lymphoma which was associated with a significant reduction in proven/probable IFD but was restricted to inpatients from a single centre (10). There is some in-human evidence to support weight-based dosing for L-AMB (19) and one possible explanation for our findings is that our patients simply received insufficient doses. However, plasma concentrations alone are not necessarily reflective of the biological activity of L-AMB in tissue and cellular compartments and distinguishing liposome associated, tissue-bound, protein-bound or free drug (13, 19), under variable immunosuppression conditions (e.g. neutropenia and non-neutropenia immunosuppressive states) is complex.

Uncertainties around L-AMB prophylaxis dosing risks undermining the anticipated gains of the anticancer precision drug era due to opportunistic infections like IFD. Our patients were enrolled in clinical trials for a variety of investigational drugs including inhibitors of BCL2, Pim/tyrosine kinase and hedgehog signalling pathways predominantly for AML but also for ALL, myelodysplasia, CML and multiple myeloma. Indeed, 5 of 19 patients who developed BIFD were enrolled in clinical trials, all for AML. Our previous study of invasive mold infections in unselected haematology patients from 2008-18, revealed that 1 in 5 patients were enrolled in clinical trials (20). It also highlighted the high fraction of BIFD using a definition by Lerolle et al. (16), which accounted for 60% of probable/proven infections (53/88) across a variety of antifungal prophylaxis regimens (20). Notably in that study,
intermittent L-AMB prophylaxis was associated with the highest incidence of BIFD in AML patients but direct comparisons between azoles and L-AMB are not possible given heterogeneity between groups.

Real-world approaches to antifungal prophylaxis are variable because no single antifungal regimen can cover all clinical scenarios. Azole prophylaxis was precluded due to organ dysfunction in 22% of courses, including gut absorption concerns and liver function abnormalities. Allergy or intolerance (hallucinations, nausea) to azoles and drug-azole interactions (e.g. prolonged QT) were rarely implicated. Our study emphasises that switching from azoles to L-AMB is not a trivial decision and all steps should be taken to mitigate this change with for example, therapeutic drug monitoring, intravenous azole formulations, dose adjustment of chemotherapy agents or use of azoles with less cytochrome P450 inhibition like isavuconazole. In 8.4% of courses, excess doses of L-AMB were administered beyond neutrophil recovery presenting a potential opportunity for antifungal stewardship, noting that there may have been other valid reasons to continue such as GVHD on immunosuppression. The issues of emerging groups at risk for IFD on targeted anti-cancer therapies and optimising antifungal stewardship in outpatients were corroborated by clinicians in a recent qualitative study on antifungal practice (21).

L-AMB prophylaxis was well tolerated with few premature discontinuations. Although the frequency of acute kidney injury was high at 27%, clinically significant nephrotoxicity resulting in premature discontinuation was rare at 3.3%. Severe nephrotoxicity developed in 6.2% of courses, suggesting that a cautious approach to patients with poor renal function at baseline is warranted or if prolonged courses greater than 5 weeks are anticipated (Fig 4).

Recent definitions for BIFD are an attempt to standardise reporting but we found the goodness of fit of these recommendations to be poor for L-AMB (22). The period of protection conferred by L-AMB following its discontinuation is difficult to quantify because its in-vivo behaviour is characterised by sequestration in specific organs and variable clearance depending on dose (13, 19), notwithstanding
the unknowns regarding its biologically active component. The preponderance of fluconazole resistant Candidaemia which comprised all proven BIFDs is concerning and consistent with the shift to non-
*albicans* Candida species in large epidemiological studies (23). It also underscores the importance of ongoing surveillance, audit and feedback of IFD to inform institutional policies as local “centre effects” dictate fungal epidemiology to a high degree (20, 24, 25).

The limitations of this study include its single centre focus, observational design and retrospective analysis of a heterogenous group of haematology patients. We did not capture all toxicities but rather focused on clinically relevant ones resulting in premature discontinuation. We provided a range of BIFD definitions to aid future comparisons but acknowledge the difficulties in application when scientific knowledge of the in-vivo behaviour of L-AMB is incomplete (19). Comparisons with triazole prophylaxis are difficult when there were legitimate reasons for avoiding them but a 9.6% BIFD rate does not compare favourably to possible/probable/proven BIFD on posaconazole of 3%\textsuperscript{15} and 4.7%\textsuperscript{16} respectively from real-world studies.

Evidence based alternatives to azole prophylaxis are urgently required for this increasingly large and complex group of haematology patients. We propose pragmatic trials for antifungal prophylaxis that can accommodate the heterogeneity of clinical practice spanning the microevolutionary changes in cancer care that are already upon us. Intermittent L-AMB will continue to have a role to play even when novel non-azole antifungals come online, but it deserves further study to optimise its efficacy.

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## Table 1. Clinical characteristics of patients receiving intermittent Liposomal amphotericin B prophylaxis

| Characteristic                                      | Patients, n=198, (%) |
|-----------------------------------------------------|----------------------|
| Male sex, no. (%)                                   | 123 (62)             |
| Age at diagnosis, mean (range)                      | 52 (16-83)           |
| Weight, mean (range, kg)                            | 74 (31-165)          |
| Ethnic origin, no. (%)                              |                      |
| Caucasian                                           | 166 (84)             |
| Asian                                               | 12 (6.1)             |
| Indian sub-continent                                | 7 (3.5)              |
| Pacific islander                                    | 3 (1.5)              |
| Middle eastern                                      | 6 (3.0)              |
| African                                             | 3 (1.5)              |
| Hispanic                                            | 1 (0.5)              |
| Comorbidities, no. (%)                              |                      |
| Diabetes                                            | 34 (17)              |
| Chronic kidney disease                              | 12 (6.1)             |
| Chronic liver disease                               | 13 (6.6)             |
| Underlying haematological disease, no. (%)          |                      |
| Acute myeloid leukaemia                             | 92 (46)              |
| Acute lymphoblastic leukaemia                       | 53 (27)              |
| Acute promyelocytic leukaemia                       | 16 (8.1)             |
| Non-Hodgkin's lymphoma                              | 13 (6.6)             |
| Multiple myeloma                                    | 10 (5.1)             |
| Chronic myeloid leukaemia                           | 4 (2.0)              |
| Chronic lymphocytic leukaemia                       | 1 (0.5)              |
| Myelodysplastic syndrome                            | 4 (2.0)              |
| Hodgkin's lymphoma                                  | 2 (1.0)              |
| Myelofibrosis                                        | 2 (1.0)              |
| Blastic plasmacytoid dendritic cell neoplasm        | 1 (0.5)              |
| HSCT recipients                                     | 29 (15)              |
| HSCT type, no. (%)                                  |                      |
| Allogeneic                                          | 27 (93)              |
| Autologous                                          | 2 (6.9)              |
| Allograft characteristics, n=27                     |                      |
| HLA matched                                         | 13 (48)              |
|                          |       |
|--------------------------|-------|
| Single antigen mismatch  | 1 (3.7) |
| Unrelated donor          | 13 (48) |
| Presence of GVHD         | 21 (78) |

**Clinical outcomes**

|                             |       |
|-----------------------------|-------|
| ICU admission               | 57 (29) |
| 30-day mortality from last dose, n=196<sup>1</sup> | 33 (17) |
| 12-week mortality from last dose, n=193<sup>1</sup> | 42 (22) |

GVHD, graft versus host disease; HLA, human leucocyte antigen; HSCT, haematopoietic stem cell transplant; ICU, intensive care unit.

<sup>1</sup>Two and five patients were lost to follow-up prior to 30 days and 12 weeks respectively.
Table 2. Characteristics of Liposomal amphotericin B prophylaxis courses

| Characteristic                                                                 | Total courses, n=273 (%) |
|--------------------------------------------------------------------------------|--------------------------|
| **Status of haematological disease at start of L-AMB prophylaxis**              |                          |
| New diagnosis, no prior treatment                                              | 123 (45)                 |
| Active disease                                                               | 110 (40)                 |
| Relapsed disease                                                             | 40 (15)                  |
| **Presence of neutropenia (<0.5 x 10^9/L) at start of L-AMB prophylaxis**     | 237 (87)                 |
| Neutrophil count, mean ± SD                                                  | 0.2 ± 0.15               |
| **Of those neutropenic, duration of neutropenia**                            |                          |
| >5 weeks                                                                     | 46 (19)                  |
| 3-5 weeks                                                                    | 62 (26)                  |
| 7 days – 3 weeks                                                             | 103 (43)                 |
| < 7 days                                                                     | 26 (11)                  |
| **L-AMB continued despite neutrophil count recovery**                        | 23 (8.4)                 |
| **Additional days of L-AMB prophylaxis, median (IQR)**                        | 10 (5-19)                |
| **Indication for L-AMB prophylaxis (may be >1)**                            |                          |
| Chemotherapy regimens contraindicating azole use                             | 206 (75)                 |
| ALL on vincristine in 93, dasatinib in 1                                      | 94 (46)                  |
| Enrolled in clinical trial                                                   | 80 (39)                  |
| APML in cycle 1                                                              | 16 (7.8)                 |
| Burkitt’s lymphoma on CODOX-M/IVAC                                           | 6 (2.9)                  |
| NHL on hyperCVAD regimen                                                     | 6 (2.9)                  |
| CML on vincristine (n=2) or dasatinib (n=1)                                  | 3 (1.5)                  |
| Blastic plasmacytoid dendritic cell neoplasm on hyperCVAD                    | 1 (0.5)                  |
| Condition                                              | Count (%) |
|--------------------------------------------------------|-----------|
| Gastrointestinal absorption concerns                   | 35 (13)   |
| Gastrointestinal GVHD                                  | 28 (82)   |
| Mucositis                                              | 6 (18)    |
| CMV colitis                                            | 1 (2.9)   |
| Liver function derangement                             | 24 (8.8)  |
| Allergy or intolerance to azoles                       | 7 (2.6)   |
| Drug interaction outside cytotoxic therapies           | 3 (1.1)   |
| Secondary prophylaxis for IFD                         | 2 (0.73)  |

**Dose and duration of prophylaxis courses**

| Measure                                                                 | Median (IQR) |
|------------------------------------------------------------------------|--------------|
| Duration in days of L-AMB prophylaxis per course                        | 16 (10-27)   |
| Number of doses of L-AMB per course                                    | 7 (5-11)     |
| Cumulative L-AMB dose per course adjusted for patient weight (mg/kg), | 8.6 (5.4 – 14) |
| median (IQR)                                                           |              |

L-AMB, Liposomal amphotericin B; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; APML, acute promyelocytic leukaemia; CMV, cytomegalovirus; CODOX-M/IVAC, cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate; GVHD, graft versus host disease; hyperCVAD, cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, cytarabine; IFD, invasive fungal disease; IQR, interquartile range; IVAC, ifosfamide, etoposide, high-dose cytarabine; NHL, non-Hodgkin lymphoma SD, standard deviation.

1. Course defined as receipt of at least three alternate day doses of L-AMB for prophylaxis.

2. Active disease defined as partial remission, progressive or refractory disease.

3. Underlying haematological malignancy in trial episodes: AML n=74, ALL and myelodysplasia n=2 each, CML and multiple myeloma, n=1 each.

4. Hallucinations to voriconazole in 4, unspecified allergy in 2, nausea in 1 course.

5. Two patients had 2 courses of L-AMB as secondary prophylaxis for previous possible and proven IFD respectively. Neither of these patients developed breakthrough IFD while on L-AMB prophylaxis.
Table 3. Characteristics of breakthrough invasive fungal disease

| Characteristic                                    | Patients, n=198 (%) |
|--------------------------------------------------|---------------------|
| BIFD<sup>1</sup>                                 | 19 (9.6)            |
| Proven/probable                                  | 13 (68)             |
| Possible                                         | 6 (32)              |
| Localized                                        | 12 (63)             |
| Disseminated                                     | 7 (37)              |
| Site of BIFD                                     |                     |
| Lung                                             | 13 (68)             |
| Bloodstream                                      | 6 (32)              |
| Sinus                                            | 1 (5.3)             |
| Skin                                             | 1 (5.3)             |
| Organism in probable/proven BIFD episodes, n=13  |                     |
| Candida species                                  | 6 (46)              |
| Candida glabrata                                 | 3 (50)              |
| Candida krusei                                   | 1 (17)              |
| Candida kefyr                                    | 1 (17)              |
| Candida guilliermondii                           | 1 (17)              |
| Mold species                                     | 7 (54)              |
| Positive BAL galactomannan                       | 6 (86)              |
| Paecilomyces                                     | 1 (14)              |
| Aspergillus PCR                                  | 3 (23)              |

BAL, bronchoalveolar lavage; BIFD, breakthrough invasive fungal disease; PCR, polymerase chain reaction.

<sup>1</sup>A conservative BIFD definition adapted from (15). Intermediate BIFD definition adapted from (16), n=24 (12.1%) and a broad definition according to a modified intention-to-treat analysis was n=26 (13.1%).
Table 5. Safety of Liposomal amphotericin B prophylaxis

| Characteristic                                                                 | Total courses, n=273 (%) |
|--------------------------------------------------------------------------------|--------------------------|
| Reason for cessation of L-AMB prophylaxis                                      |                          |
| Neutrophil count recovery                                                      | 154 (56)                |
| Treatment completed uneventfully                                              | 71 (26)                 |
| Due to IFD                                                                    | 21 (7.7)                |
| Palliation or death                                                           | 13 (4.8)                |
| Acute kidney injury                                                           | 9 (3.3)                 |
| Lack of perceived efficacy leading to commencement of other systemic antifungal therapy (excluding IFD) | 2 (0.7)                |
| Pain\(^1\)                                                                  | 2 (0.7)                 |
| Liver function derangement                                                    | 1 (0.4)                 |
| Acute kidney injury\(^2\)                                                     | 75 (27)                 |
| KDIGO Grade 1                                                                | 38 (14)                 |
| KDIGO Grade 2                                                                | 20 (7.3)                |
| KDIGO Grade 3                                                                | 17 (6.2)                |

IFD, invasive fungal disease; KDIGO, Kidney Disease Improving Global Outcomes; L-AMB, liposomal amphotericin B.

\(^1\)One each for gastrointestinal or musculoskeletal pain.

\(^2\)Maximal or worst KDIGO criteria during prophylaxis course and up to 2 weeks from L-AMB cessation.
Table 4. Risk factors for death (n=166 patients with known outcome)

| Risk factors                          | Adjusted hazard ratio (95% CI) | P value |
|---------------------------------------|--------------------------------|---------|
| Breakthrough IFD                      | 2.83 (1.64-4.87)               | <0.001  |
| Acute leukaemia (AML or ALL) vs all other conditions | 1.44 (0.81-2.56)               | 0.220   |
| Disease status¹                       |                                |         |
| Active disease                        | Reference                      |         |
| New haematological diagnosis         | 0.52 (0.29-0.91)               | 0.022   |
| Relapsed disease                     | 1.36 (0.72-2.59)               | 0.343   |
| HSCT status¹                         |                                |         |
| No HSCT                               | Reference                      |         |
| Allo-HSCT                             | 3.66 (1.37-9.73)               | 0.009   |
| Auto-HSCT                             | 1.44 (0.32-6.47)               | 0.636   |
| Presence of neutropenia               | 3.43 (1.13-10.4)               | 0.029   |

¹Risk is assessed against the reference group, e.g. the hazard ratio for allo-HSCT was 3.7 when compared with non-HSCT recipients.

IFD, invasive fungal disease; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; HSCT, haematopoietic stem cell transplant; Allo, allogeneic; Auto, autologous.
Figure legend

Figure 1. Swimmers plot demonstrating breakthrough-IFD relative to course of Liposomal amphotericin B prophylaxis shown by lanes, using three definitions (a, b, c). L-AMB, liposomal amphotericin; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BPDCN, Blastic plasmacytoid dendritic cell neoplasm; CML, chronic myeloid leukaemia; MM, multiple myeloma; Myelody, myelodysplasia; NHL, Non hodkings lymphoma; allo-HSCT, allogeneic-haematopoietic stem cell transplant.

BIFD (breakthrough invasive fungal disease) definitions are adapted from (15) in (a, a conservative definition); (16) in (a+b, an intermediate definition) and a modified intention to treat analysis in (a+b+c, a broad definition).

Figure 2. Cumulative incidence curves of time to breakthrough-invasive fungal disease (n=26) stratified by acute leukaemia and allogeneic HSCT status. AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; allo-HSCT, allogeneic haematopoietic stem cell transplant.

Taken from date of leukaemia diagnosis and date of HSCT to 3-year interval. Cumulative incidence in percentages with (95% confidence intervals) are: overall 13.8% (9.53 to 19.9); AML 6.75% (3.85 to 11.7); ALL 2.69% (1.13 to 6.34), Allo-HSCT 2.48% (0.93 to 6.51).

Figure 3. Kaplan Meier curve showing survival from start of liposomal amphotericin prophylaxis in patients with breakthrough-invasive fungal disease versus others with known outcome. Overall 84 deaths in 166 patients (17 with BIFD using intention-to-treat definition, 149 without BIFD). BIFD, breakthrough invasive fungal disease.
Figure 1

The figure shows the progression of patients with BFD over time from the start of L-AMB treatment. Different colors and symbols represent various patient groups and infection types:

- **ALL**: Green bars
- **AML**: Pink bars
- **Allo-HSCT**: Red bars
- **BPDCN**: Yellow bars
- **CML**: Blue bars
- **MM**: Purple bars
- **MEYELDYS**: Green triangles
- **NHL**: Orange bars
- **Possible mold**: Yellow triangles
- **Probable/Proven mold**: Black and white stars
- **Possible Candida**: Pink circles
- **Probable/Proven Candida**: Black and white circles

The x-axis represents the days from the start of L-AMB, ranging from 0 to 119 days. The y-axis lists the patients with BFD.
Figure 2

Disease type

Cumulative incidence

Weeks from haematological diagnosis

Overall
AML
ALL
Allo-HSCT
