NON-INTERVENTIONAL STUDY REPORT

Study Title:	A Multi-country, Non-interventional, Retrospective Drug Utilisation Study in Haematological Malignancy Patients Treated for Probable or Proven Invasive Aspergillosis
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EU PAS Register Number:	EUPAS104818

Indication:	Fungal infection Treatment of proven or probable invasive aspergillosis
Active Substance:	Anatomical Therapeutic Chemical Code J02AA01 Liposomal amphotericin B
Medicinal Product:	AmBisome liposomal amphotericin B 50 mg, powder for dispersion for infusion
Study No.:	GS-EU-131-6385
Product Reference	Belgium BE166257, France 562 408 2 or 34009 562 408, Germany 34231.00.00, Spain 61.117, United Kingdom 16807/0001
Joint PASS:	No
Research Question and Objectives	To evaluate the drug utilisation in haematological malignancy patients treated for probable or proven invasive aspergillosis and to generate real-world evidence on current treatment patterns/sequences, treatment outcomes, and adverse events of special interest. CCI [redacted]
Countries of Study	Belgium, France, Germany, Spain, UK
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CONFIDENTIAL STATEMENT

2. ABSTRACT

Study GS-EU-131-6385
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Study title: A Multi-country, Non-interventional, Retrospective Drug Utilisation Study in Haematological Malignancy Patients Treated for Probable or Proven Invasive Aspergillosis

Keywords: AmBisome, Liposomal Amphotericin B, voriconazole, invasive aspergillosis, high risk haematology patients, invasive fungal infections, drug utilisation, real-world evidence

Rationale and background: Opportunistic invasive fungal infections (IFIs) are a major cause of morbidity and mortality in haematology patients. Invasive aspergillosis (IA) is a particular problem, despite the widespread use of mould-active prophylaxis in vulnerable populations. International clinical guidelines recommend voriconazole and isavuconazole as the first-line treatment (FLT) for IA; amphotericin B and its various lipid formulations are recommended as an alternative. This is based partly on the results of the study conducted by {Herbrecht 2002a}, which compared conventional amphotericin B to voriconazole and concluded that voriconazole resulted in improved survival with decreased adverse events (AEs) compared with conventional amphotericin B. This has been subsequently extrapolated to suggest that liposomal formulations of amphotericin B such as AmBisome® would perform in the same way without the necessary evidence to support this extrapolation.

Due to the lack of real-world data evaluating both AmBisome and voriconazole in the targeted treatment setting, this study undertook a retrospective chart review to generate evidence associated with the characterisation of AmBisome- and voriconazole-treated patients, including effectiveness and adverse events of special interest (AESIs). This study was based on data from high-risk haematological malignancy patients, defined as adult patients who had undergone allogeneic haematopoietic stem cell transplantation (allo-HSCT) or patients with acute myeloid leukaemia (AML), myelodysplastic syndromes (MDS), or acute lymphoblastic leukaemia (ALL).

Research objectives:

Primary objective

- To describe demographic, clinical, and treatment patterns/sequences in high-risk haematological malignancy patients treated either with AmBisome or voriconazole as primary treatment for the index proven or probable IA according to {De Pauw 2008}

Secondary objectives

- To determine 42-day overall survival (OS) in high-risk haematological malignancy patients with IA treated either with AmBisome or voriconazole as primary treatment
- To estimate the percentage of AmBisome- and voriconazole- treated patients who experience AESIs, in particular nephrotoxicity and hepatotoxicity, and any AEs leading to treatment discontinuation or modification
- To estimate the time to first nephrotoxicity and/or hepatotoxicity during the follow-up period (in patients without kidney or liver impairment at index date)

CCI [redacted]

Study design: This was a real-world, non-interventional, multi-country, retrospective chart review study using patient medical record data collected from 15 hospitals in five European countries (Belgium, France, Germany, Spain, and UK). High-risk haematological malignancy patients (allo-HSCT, AML, MDS, and ALL) with a diagnosis of documented probable or proven IA, who initiated primary treatment of AmBisome or voriconazole between 01 January 2014 and 31 December 2019 were included in the study.

All treatments were prescribed in accordance with local routine clinical practice. There were no additional samples, visits, diagnostic, or monitoring procedures required in the study.

Setting: The study population consisted of high-risk haematological malignancy adult patients (allo-HSCT, AML, MDS, and ALL) who received at least one dose of AmBisome or voriconazole as primary treatment for probable or proven IA, at any time from 1 January 2014 to 31 December 2019.

Inclusion criteria

- Adult patients (age ≥18 years) with haematological malignancies who have undergone allogeneic HSCT or patients with AML, MDS, or ALL
- Patients with proven or probable IA according to {De Pauw 2008}, for whom the treating physician initiated treatment with either AmBisome or voriconazole
- Patients who received at least one dose of AmBisome or voriconazole as primary treatment for probable or proven IA at any time from 01 January 2014 to 31 December 2019 (Primary antifungal therapy is defined as the first antifungal treatment that was administered for at least 5 consecutive days)

Exclusion criteria

- Patients who were treated with AmBisome or voriconazole as part of a clinical trial during the observational period
- Patients treated jointly with any combination of AmBisome or voriconazole, and/or other mould-active azoles and/or echinocandins in primary setting

Variables: Each patient's data were pseudonymised and collected from the start of AmBisome or voriconazole treatment for the index proven or probable IA. Available routine clinical data were extracted until the end of follow-up period.

Baseline (defined as start of AmBisome or voriconazole as primary treatment for the index proven or probable IA)

- Demographics, clinical variables, and treatment patterns

Follow-up period (until 84 ±7 days after start of primary treatment)

- IA treatment and outcome variables

Safety information: Any AEs that led to AmBisome or voriconazole dose modification or discontinuation, as well as AESIs (nephrotoxicity, hepatotoxicity) (safety events were only collected for first-line AmBisome and voriconazole treatments)

Data sources: Data was collected from 15 participating sites across Belgium, France, Germany, Spain, and the UK. The primary data source was the patients' medical records. Reviews of electronic or paper medical records, data identification, abstraction, and data transcription were performed by trained site personnel. All data were pseudonymised and collected for patients meeting all eligibility criteria. Each patient was identified by a unique electronic case report form generated patient identifier.

Study size: The primary objective of the study was the characterisation of AmBisome- and voriconazole treated high-risk haematological malignancy patients with respect to their demographics, clinical characteristics, and treatment patterns. The target sample size was approximately 200 patients in each arm (AmBisome and voriconazole).

Data analysis/methods:

Descriptive analysis

Descriptive statistics were tabulated for the demographic and clinical characteristics and outcome variables. In all cases, point estimates as well as the corresponding two-sided 95% CIs were presented. No missing value imputation was performed.

Treatment sequence analysis

Treatment sequence was visualised through a Sankey diagram based on the FLT.

OS and Time-to-event analysis

The secondary objective was to estimate the 42-day OS of patients treated with AmBisome and of patients treated with voriconazole. The 84-day OS was also estimated in these patients. Time to first nephrotoxicity and hepatotoxicity were described using Kaplan-Meier (KM) methods and reported using descriptive statistics with 95% CIs and survival curves.

Results:

The study sites identified 372 potentially eligible patients. Eight patients were excluded from the analysis due to protocol deviations related to eligibility criteria. An additional five patients were excluded from the analysis due to incomplete data entry either on the inclusion/exclusion form (n=1) or on the study completion form and AE form (n=4) at the time of database lock. Following these patient exclusions, the FLT cohort comprised 359 patients.

This study included 127 patients treated with first-line AmBisome and 232 patients treated with first-line voriconazole. The AmBisome arm had 39 (30.7%) patients with a proven IA diagnosis and the voriconazole arm had 33 (14.2%) patients with a proven IA diagnosis. In the AmBisome arm, 41.7% of patients (n=53) had used antifungal prophylaxis within 1 week before primary treatment, as did 31.9% patients (n=74) in the voriconazole arm.

Voriconazole was administered through IV in 163 (70.3%) patients and orally in 68 (29.3%) patients in the FLT (1 missing, 0.4%). The mean duration of treatment was 18.7 days in the AmBisome arm and 25.9 days in the voriconazole arm. There were 67 (52.8%) patients in the AmBisome arm and 96 (41.4%) patients in the voriconazole arm who were documented to have switched to another treatment (second-line or prophylaxis). Neutropenia at the time of index date was documented in 62 (48.8%) patients in the AmBisome arm and in 114 (49.1%) patients in the voriconazole arm.

Nephrotoxic medications were concomitantly used in 37 (29.1%) patients in the AmBisome arm of the FLT cohort and in 62 (26.7%) patients in the voriconazole arm of the FLT cohort. Hepatotoxic medications were taken concomitantly by patients in the AmBisome arm of the FLT cohort (n=23, 18.1%) and in the voriconazole arm of the FLT cohort (n=41, 17.7%).

The analyses related to nephrotoxicity and hepatotoxicity excluded patients with liver or kidney dysfunction up to 3 months before index. Nephrotoxicity events during treatment were recorded in three (1.7%) patients in the voriconazole arm, none considered treatment-related, and in one (1.0%) patient in the AmBisome arm, considered treatment-related. Hepatotoxicity AEs occurred among 10 (5.6%) patients in the voriconazole arm, eight considered treatment-related and in one (1.0%) patient in the AmBisome arm, not considered treatment-related.

Nephrotoxicity led to treatment modification in one (1.0%) patient in the AmBisome arm, whereas no primary treatment modifications were caused by nephrotoxicity in the voriconazole arm. Few hepatotoxic and nephrotoxic events were documented. Hepatotoxicity led to treatment discontinuation in four (2.2%) patients in the voriconazole arm and no patients in the AmBisome arm.

Other AEs that led to primary treatment discontinuation in more than one patient in the voriconazole arm were hepatic cytolysis (n=4, 1.7%), visual hallucinations (n=2, 0.9%), and hyperbilirubinemia (n=2, 0.9%). AEs that led to primary treatment discontinuation in more than one patient in the AmBisome arm were renal failure (n=2, 1.6%) and worsening of aspergillosis (n=2, 1.6%). Other AEs that led to treatment modification in more than one patient in the voriconazole arm were hallucinations (n=3, 1.3%), elevated liver function tests (n=2, 0.9%), and visual hallucinations (n=2, 0.9%). No AEs led to primary treatment modification in more than one patient in the AmBisome arm.

Evaluating patients based on the first drugs which they received for 5 consecutive days (three arms; AmBisome, IV voriconazole, oral voriconazole); there were 108 patients treated with AmBisome, 121 patients treated with IV voriconazole, and 65 were treated with oral voriconazole. The AmBisome arm had 36 (33.3%) patients with proven IA diagnosis, where there were 10 (8.3%) patients in the IV voriconazole arm, and 16 (24.6%) patients in the oral voriconazole arm. Further, 41.7% (n=45), 34.7% (n=42), and 16.9% (n=11) of patients in the AmBisome, IV voriconazole, and oral voriconazole arms, respectively, had used antifungal prophylaxis within one week before primary treatment. Approximately half of the AmBisome (50.9%, n=55) and IV voriconazole arms (50.4%, n=51), and 36.9% (n=24) of the oral voriconazole arm had neutropenia at the time of their IA diagnosis.

Focusing on the IV treatments, at day 42, 57.4% (n=62) of patients in the AmBisome arm, 63.6% (n=77) of patients in the IV voriconazole arm were alive. Among those patients who died, the mean (SD) time to death from index date was 29.5 (23.57) and 25.7 (17.52) days in the AmBisome and IV voriconazole arms respectively. Of patients who died, 54.1% (n=33) of the AmBisome arm and 64.7% (n=33) of the IV voriconazole arm died during primary therapy.

Discussion:

This real-world study has demonstrated the complex and diverse nature of high-risk haematology patients undergoing treatment for an IFI, which better represents the real life setting due to less strict exclusion criteria compared to clinical trials and absence of treatment randomization. A higher number of patients were included in the voriconazole arm (n=232 on voriconazole, n=127 on AmBisome), which can be linked to voriconazole being the recommended treatment according to the guidelines, with AmBisome being the alternative recommendation.

Baseline differences between the two FLT arms of this retrospective chart review study included the proportion of patients with underlying allo-HSCT (46.5% in the AmBisome arm, 35.8% in the voriconazole arm). The AmBisome arm had a higher proportion of patients with a “proven” (rather than probable) IA diagnosis (30.7%) than the voriconazole arm (14.2%). The AmBisome arm also had a higher proportion of patients using antifungal prophylaxis within 1 week before index date than the voriconazole arm (41.7% vs. 31.9%). IFIs occurring on prophylaxis are classified as breakthrough infections and can be considered more difficult to diagnose and treat {Boutin 2024}.

Rates of use of concomitant nephrotoxic and hepatotoxic medications were similar between the AmBisome treatment arm (29.1% and 18.1%, respectively) and the voriconazole treatment arm (26.7% and 17.7% respectively) of the FLT cohort. Despite the high rate of concomitant nephrotoxic medications in the AmBisome arm, this rarely translated into renal toxicity for AmBisome-treated patients. Nephrotoxicity was uncommon in both groups, a finding that is especially useful to address potentially remaining misconceptions about the beneficial renal AE profile of AmBisome versus conventional amphotericin B and other lipid formulations {Botero Aguirre 2015}. This real-world evidence demonstrates that there do not seem to be clear differences in the renal AE profiles of AmBisome and voriconazole in the real-life setting, despite the baseline presence of renal disease in 12.6% and use of concomitant nephrotoxic medication in 29.1% of AmBisome-treated patients. It appears that hepatotoxicity

was more common in patients with voriconazole, which is in line with available evidence {Levin 2007}

For the 42-day OS evaluation in the primary treatment cohort, while statistical comparisons were not performed, patients treated with oral voriconazole had a lower percentage of proven IA and, a longer antifungal treatment duration. Furthermore, fewer patients treated with oral voriconazole had neutropenia at baseline, or hepatic and renal disease at baseline, and fewer patients in this group received antifungal prophylaxis prior to study treatment start. This could potentially indicate an 'easier to treat patient population'.

In the AmBisome and IV voriconazole arms of the primary treatment cohort, 42-day OS were similar (57.4% of AmBisome, 63.6% of voriconazole arms). This provides new evidence that adds support to the use of AmBisome compared to conventional amphotericin B or voriconazole in the treatment of invasive aspergillosis. Data from a previous trial comparing conventional amphotericin B with voriconazole, demonstrating a higher efficacy and better safety profile for voriconazole, have been extrapolated to AmBisome liposomal formulation despite questions raised about the methodology and that a comparative trial between voriconazole and AmBisome was never performed, as only very few small real-world datasets were available.

Despite thorough review of the data and data querying, a few unresolvable raw data entry issues persisted at the time of database lock, which are presented in section 9.2. Some limitations are inherent to the retrospective chart review study design, such as selection bias, the variability in reporting practices of physicians. These limitations were mitigated by instructing sites to identify and enrol all eligible patients, by using standard measurement instruments such as electronic case report forms, and by ensuring appropriate training of site staff who were involved in data abstraction and entry into the study electronic data capture. In addition, this is a real-world study, with more potential confounding factors that can be more difficult to control, compared to clinical trials.

Differences in severity between AmBisome-treated patients and voriconazole-treated patients are an important limitation and were masked by combining oral and IV voriconazole in the FLT analysis as compared to AmBisome. Further, information on route of administration and dose of follow-up treatments was not collected, which makes it difficult to interpret reasons for treatment switch.

Lastly, the laboratory values at the time of switch to the second-line treatment should be interpreted with caution. The measurement at the time of switch to the second-line treatment was defined as the measurement closest to the date of the switch. Therefore, the laboratory value may have been measured before or after switch.

Conclusion: This retrospective chart review is the largest available real-world analysis evaluating the use of voriconazole and AmBisome for the targeted treatment of proven or probable invasive aspergillosis in the high-risk haematology setting. It demonstrates the diverse treatment patterns used and the complex nature of the patient populations, including unique patient characteristics for those first treated with IV versus oral antifungal treatment. While multivariable statistical comparisons between the two antifungal treatments were not

performed, effectiveness in terms of day 42 overall survival was numerically similar in the AmBisome and IV voriconazole arms of the primary treatment cohort.

This study confirms evidence generated in earlier clinical trial programs for both AmBisome and voriconazole, but it also creates an opportunity to address potential misconceptions about the AE profile of AmBisome, which was demonstrated to have a very mild AE profile in this complex patient population, with very few treatment related clinically relevant renal adverse events.