Non-Interventional Study Protocol
C3791008

Patterns of Real-World Isavuconazole Use, Effectiveness, Safety, and Health Care Resource Utilization—a Retrospective Chart Review Study of Patients With Mucormycosis or Invasive Aspergillosis (PRISMA)

Statistical Analysis Plan
(SAP)

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
CI	confidence interval
EDC	electronic data capture
EMA	European Medicines Agency
ER	emergency department
EU	European Union
FU	follow-up
HCRU	health care resource utilization
ICD-10	*International Classification of Diseases, Tenth Edition*
ICD-9	*International Classification of Diseases, Ninth Edition*
ICU	intensive care unit
KM	Kaplan-Meier
PRISMA	Patterns of Real-World Isavuconazole Use, Effectiveness, Safety, and Health Care Resource Utilization
RWE	real-world evidence
SAE	serious adverse event
SAP	statistical analysis plan
TDM	therapeutic drug monitoring
UK	United Kingdom

1 INTRODUCTION

Note: in this document any text taken directly from the non-interventional study protocol is ***italicized***.

1.1 STUDY BACKGROUND

Invasive aspergillosis and mucormycosis are rare, life-threatening fungal infections associated with high mortality.[1,2] Causative agents for fungal infections can vary across geographic locations; however, Aspergillus fumigatus *and* Rhizopus arrhizus *are associated with the majority (approximately 70%) of invasive aspergillosis and mucormycosis cases, respectively.[1] Although the epidemiology of rare fungal diseases is not well studied, the incidence rates (per 100,000 persons) for invasive aspergillosis and mucormycosis are estimated to be 4.6 and 0.09 in the UK, 2.8 and 0.04 in Spain, and 1.8 and 0.1 in France, respectively.[2] Incidence rates are difficult to estimate due to a lack of a reliable denominator.[2,5]*

Cresemba® (isavuconazole) is an antifungal agent that contains the active substance isavuconazole. Its mechanism of action involves blocking the production of a key component of the cell membrane of fungi, thus weakening the membrane and preventing fungal cell growth.[3] Isavuconazole can be administered orally or by infusion. Isavuconazole was approved by the EMA on October 15, 2015, for use in adults for the treatment of (1) invasive aspergillosis and (2) mucormycosis in patients for whom amphotericin B is inappropriate.

1.2 INVASIVE ASPERGILLOSIS

Invasive aspergillosis usually occurs in immunocompromised patients with hematological malignancies, oncology patients receiving chemotherapy, and immunosuppressed patients (e.g., as a result of taking immunosuppressive drugs after transplant surgery). Other risk factors include comorbidities such as diabetes, renal or liver dysfunction, chronic obstructive pulmonary disease, and neutropenic sepsis. Patients with comorbidities also are at higher risk for stays in the ICU.[6] Diagnosis of invasive aspergillosis typically is made by the combination of host status, imaging, and mycological findings.[1] Treatment of invasive aspergillosis usually includes antifungal medications such as voriconazole or isavuconazole.[1]

The SECURE clinical trial (NCT00412893) was a phase 3, randomized, double-blind, comparative-group study designed to compare the efficacy and safety of isavuconazole vs. voriconazole in the treatment of patients with invasive aspergillosis. The primary outcome measure was all-cause mortality at day 42. In the intention-to-treat population, all-cause mortality was 19% for isavuconazole and 20% for voriconazole, adjusted treatment difference = –1.0% (95% CI: –7.8, 5.7).[4] Patients treated with isavuconazole also had a lower frequency of hepatobiliary disorders (9% vs. 16%; P *= 0.016), eye disorders (15% vs. 27%;* P *= 0.002), and skin or subcutaneous tissue disorders (33% vs.*

42%; P = *0.037). Additionally, treatment-related AEs were reported in 42% and 60% of patients receiving isavuconazole and voriconazole, respectively (*P < *0.001). Guidelines therefore recommend isavuconazole and voriconazole for first-line treatment of invasive aspergillosis.*

1.3 MUCORMYCOSIS

The main risk factors for mucormycosis include diabetes mellitus, hematological malignancies, stem cell/organ transplantations, corticosteroid therapy, and neutropenia.[2] Mucormycosis usually is diagnosed through identification of causative agents by histopathological analysis of tissue specimens in patients showing symptoms of the infection.[1] However, diagnosis is challenging, and cultures are only occasionally positive. Initial treatment of mucormycosis typically includes surgery to remove infected tissues and antifungal treatment with amphotericin B.[1]

The VITAL clinical trial (NCT00634049) was a single-arm, open-label trial that included an assessment of the efficacy and safety of isavuconazole for treatment of mucormycosis in 37 patients. The primary outcome was independent data review committee-determined overall response (complete or partial response [treatment success] or stable or progressive disease [treatment failure]) at day 42, day 84, and end of treatment. The results demonstrated that by day 42, 11% of patients had a partial response, 43% had stable disease, 3% had disease progression, 8% had missing assessments, and 35% had died.[7] The efficacy of isavuconazole and amphotericin B also was compared in a matched case-control analysis.[7] At day 42, all-cause mortality was similar in cases with isavuconazole as the primary treatment and amphotericin B-treated matched controls (weighted all-cause mortality: 33% vs. 41%; P = *0.595).[7]*

Although the use of some antifungals are restricted in patients with renal impairment, in a pooled analysis of the SECURE and VITAL trials, isavuconazole was reported to be well tolerated in patients with renal impairment.[8]

1.4 STUDY RATIONALE

Isavuconazole obtained market authorization in the US and the EU in 2015. The generation of RWE will provide additional insight into isavuconazole use and related outcomes in clinical practice. The current study is a retrospective cohort study based on a multi-country chart review to be conducted in five European countries (France, Germany, Italy, Spain, and the UK), designed to obtain RWE of isavuconazole patterns of use, clinical response, HCRU, and safety in clinical practice.

2 STUDY DESCRIPTION AND OBJECTIVES

2.1 STUDY DESCRIPTON

This is an observational retrospective cohort study involving the review and abstraction of pertinent data from the medical records of patients diagnosed with invasive aspergillosis or mucormycosis who initiated treatment with isavuconazole (index event) between October 15, 2015, and June 30, 2019. The target sample size for this study is approximately 600 patients (with a minimum of 200) across 22 sites located in Europe.

De-identified data (i.e., anonymous to nonsite staff) on patient demographics, disease characteristics, isavuconazole treatment patterns, clinical response, safety outcomes, and HCRU will be abstracted from patient medical records and entered into an EDC system between August 2020 and March 2021 for Germany, Spain, and the UK, and between October 2021 and January 2022 for Italy. The differences in the data abstraction timelines are due to ethics and contracting processes in Italy taking between 6 to 12 months to complete, compared with an estimated maximum of 4 months in the remaining countries. As this study is retrospective, information pertaining to patient care will already be documented in patient medical records at the time of medical record abstraction.

A steering committee composed of experts within hematology and infectious diseases will provide input and guidance on the study design and implementation, as well as the interpretation of results.

2.2 STUDY OBJECTIVES

Primary Objectives

- *To document real-world clinical outcomes (all-cause mortality, overall response) of isavuconazole in patients with invasive aspergillosis or mucormycosis receiving treatment in routine clinical practice in Europe*
- *To assess real-world safety (isavuconazole-related AEs and SAEs) of isavuconazole in patients with invasive aspergillosis or mucormycosis receiving treatment in routine clinical practice in Europe*

Secondary Objectives

- *To describe demographic and clinical characteristics of patients with invasive aspergillosis or mucormycosis receiving isavuconazole in routine clinical practice in Europe*
- *To document treatment patterns among patients with invasive aspergillosis or mucormycosis receiving isavuconazole in routine clinical practice in Europe*
- *To quantify HCRU among patients with invasive aspergillosis or mucormycosis receiving isavuconazole in routine clinical practice in Europe.*

3 ANALYSIS POPULATION

The analysis population will consist of adult patients who received isavuconazole for the treatment of invasive aspergillosis or mucormycosis in the hospital setting. Patients who did not receive at least one dose of isavuconazole for treatment of invasive aspergillosis or mucormycosis within the eligibility period will not be considered for analysis.

Patients must meet all of the following criteria to be eligible for inclusion in the analysis:

1. *Patient must be aged ≥ 18 years at the time of isavuconazole initiation*
2. *Patient must have a record of a diagnosis of invasive aspergillosis or mucormycosis in their medical record at the time isavuconazole was initiated (regardless of whether this diagnosis is suspected or confirmed)*
3. *Patient must have received at least one dose of isavuconazole during the eligibility period (October 15, 2015, to June 30, 2019)*

All analyses will be performed on the analysis population. Analyses of the primary endpoints may also be performed on up to three subgroups, which will be selected from the following list and determined based on sample size:

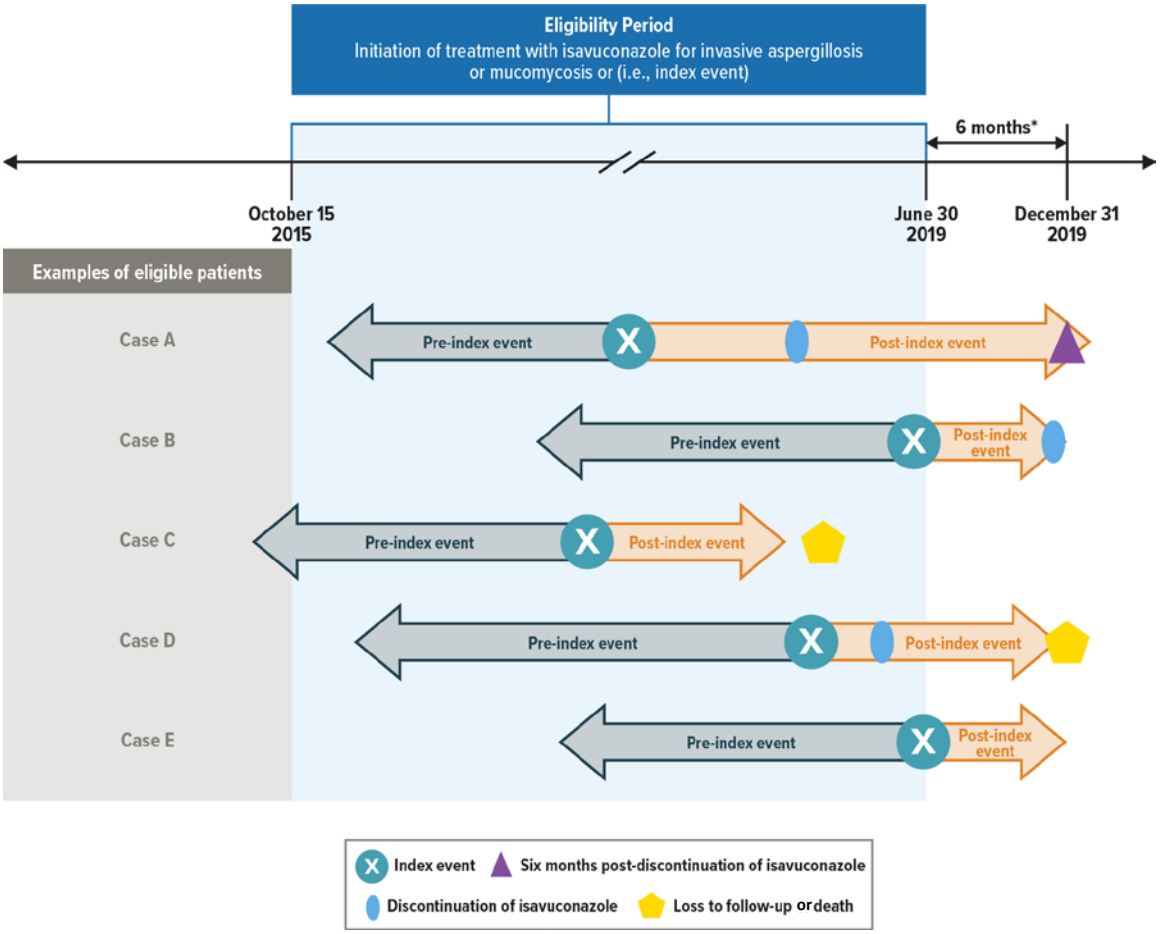
- Neutropenia and non-neutropenia patients
- Patients with central nervous system infections
- Age (e.g., <75 years vs. ≥75 years)
- Patients with corticosteroid use at baseline
- Patients with influenza at baseline
- Patients with ICU admission where isavuconazole is initiated
- Patients with history of use of biologics

4 STUDY DESIGN

4.1 SOURCE DATA

The source for all data collected will be patient medical records. Retrospective data will be abstracted from the medical records of patients and entered into an EDC system by trained local site staff. It is estimated that data abstraction will be completed in approximately 7 months from the point at which the first patient is included in the study for Germany, France, Spain, and the UK (estimated to be from August 2020 to March 2021). For Italy, data collection is anticipated to begin at a later date, and data abstraction will be completed in approximately 3 months from the point at which the first patient is enrolled (estimated to be from October 2021 to January 2022).

Figure 1 Overview of Study Periods and Examples of Eligible Cases



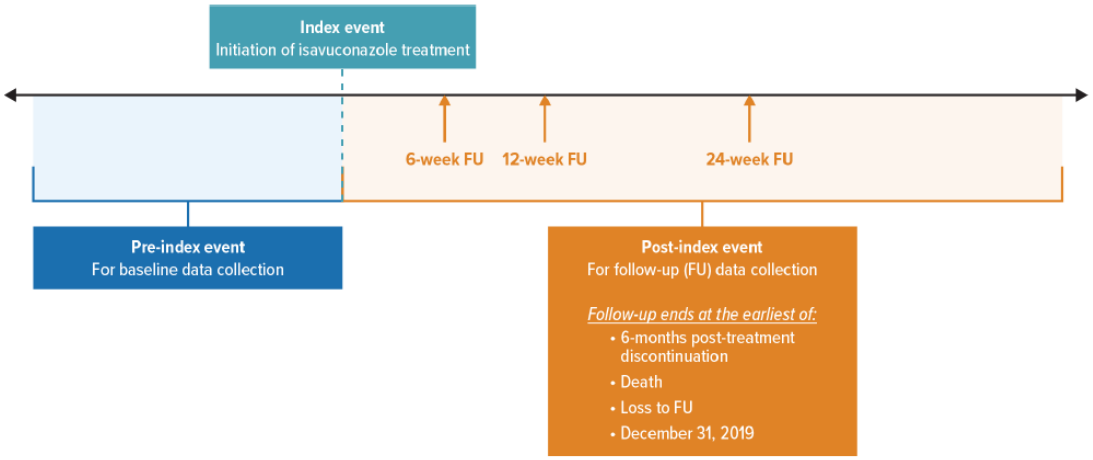
4.2 STUDY PERIODS

The index event is defined as the first initiation of isavuconazole treatment for the invasive fungal infection. The index event serves as the point at which (1) a patient

qualifies for study inclusion, (2) patient follow-up begins, and (3) the patient becomes at risk for experiencing study outcomes. Study periods for data extraction and variable assessment are defined relative to the index event (Figure 1).

- **Eligibility period:** The index date must occur within the eligibility period, which is the timeframe wherein potentially eligible patients are identified for enrolment. *The dates comprising the eligibility period will be October 15, 2015 (date of isavuconazole approval by the EMA in the EU), to June 30, 2019. The end date of the eligibility period ensures that the latest date of isavuconazole initiation will occur at least 6 months before the end of December 2019 (after which point the medical records may contain data related to the COVID-19 pandemic, which is not of interest for this study). This definition will allow for a minimum of 6 months of follow-up from the date of isavuconazole initiation unless the patient dies or is lost to follow-up within this timeframe.*
- **Pre-index period:** *Baseline data on demographics and medical history will be collected based on all available information in medical records before the index event.*
- **Post-index period:** *The timeframe for follow-up data collection will begin one day post-index treatment initiation and end at the earliest of the following: 6 months after post-index treatment discontinuation, death, or loss to follow-up (Figure 1 and Figure 2). Key timepoints of interest for assessment of clinical response and safety will be at 6 weeks, 12 weeks, and 24 weeks after initiation of index treatment (Figure 2).*

Figure 2 Data Collection Periods and Timepoints for Each Patient



FU = follow-up.

5 OUTCOME DEFINITIONS

Outcomes will be defined as presented in Table 1.

Table 1 Outcome Definitions

Variable	Definition	Timepoint
Death	Death as indicated on the medical record abstraction form.	Post-index period
Clinical response	**Clinical success:** Resolution or partial resolution of all attributable clinical symptoms and physical findings. **Clinical failure:** No resolution of any attributable clinical symptoms and physical findings and/or worsening. When medical record documentation is not clear to the abstractor as to which category should be specified, the investigator should review all available medical record documentation to determine classifications. If the investigator determines there is not enough medical record documentation to make the classification, he or she will indicate that the response is 'unknown'. Since clinical response classification is important to the study, participating sites will be selected for participation in the study based on their reporting whether they have enough medical record documentation to classify the response.	Post-index period
Radiological response	**Radiological success:** ≥ 50% improvement from initial assessment or improvement of at least 25% from the initial assessment for the follow-up at 6 weeks (i.e., day 42 [± 14 days]) or if end of treatment occurred before this time. **Radiological failure:** Failure to meet success criteria.	Post-index period

Variable	Definition	Timepoint
	When medical record documentation is not clear enough for the abstractor as to which category should be specified, the investigator should review all available medical record documentation to determine classifications. If the investigator determines there is not enough medical record documentation to make the classification, he or she will indicate that the response is 'unknown.' Since radiological response classification is important to the study, participating sites will be selected for participation in the study based on their reporting whether they have enough medical record documentation to classify the response.	
Mycological response	**Mycological success** will be defined as eradication or presumed eradication of the original causative organism cultured. **Mycological failure** will be defined as persistence or presumed persistence. When medical record documentation is not clear to the abstractor as to which category should be specified, the investigator should review all available medical record documentation to determine classifications. If the investigator determines there is not enough medical record documentation to make the classification, he or she will indicate that the response is 'unknown.' Since mycological response classification is important to the study, participating sites will be selected for participation in the study based on their reporting whether they have enough medical record documentation to classify the response.	Post-index period

Variable	Definition	Timepoint
Adverse events	Documentation of any adverse event in the medical record or as described by the ICD-9 or ICD-10 systems.	Post-index period
Serious adverse event	Documentation of any serious adverse event in the medical record or as described by the ICD-9 or ICD-10 systems.	Post-index period
Treatment patterns	Documentation of any of the following in the medical record: • Index treatment modification • Index treatment discontinuation • TDM • Switching to a new antifungal therapy after index treatment discontinuation • Use of subsequent antifungal therapy after end of index treatment • Index treatment administered as part of antifungal combination therapy • Other concomitant treatments not including combination antifungal therapy • Index treatment administered in the outpatient setting following initiation in the inpatient setting	Post-index period
Healthcare resource utilization	Documentation of any inpatient stay (including specialty wards, ICU, ER, general medicine ward, or other) or outpatient visit (not associated with a hospitalization) in the medical record.	Post-index period

ICD-9 = *International Classification of Diseases, Ninth Edition*; ICD-10 = *International Classification of Diseases, Tenth Edition*; ICU = intensive care unit; ER = emergency room; TDM = therapeutic drug monitoring.

6 STATISTICAL METHODS AND ANALYSIS

6.1 STATISTICAL CONVENTIONS

Categorical variables will be reported as counts and frequencies of nonmissing observations. Continuous variables will be reported using the number of nonmissing observations, mean, standard deviation, median, first quartile, third quartile, minimum value, and maximum value. Time-to-event outcomes will be assessed using the Kaplan-Meier (KM) method and will be reported as descriptive statistics with 95% CIs. Results will be presented for the overall group (both disease types) and separately for invasive aspergillosis and mucormycosis. Additionally, subgroup analyses may be performed. If so, log-rank tests will be reported, and Cox regression will be employed with presentation of hazard ratios (HRs) and 95% CIs.

Unless otherwise specified, the time between two dates of interest (including time-to-event analyses) will be computed as the difference between the two calendar dates (e.g., date 2 – date 1) and expressed in days.

No imputation for missing values will be performed. Criteria for censoring in KM analyses will be described for each time-to-event outcome analyzed.

Example tables and figure descriptions for analyses described in this section (analysis tables and analysis figures) are provided in Appendix 1.

6.2 CHARACTERIZATION OF STUDY POPULATION

Descriptive statistics will be used to summarize demographics and medical history assessed at the closest date available to the index date of the study population (Appendix 1, Analysis Table 1) and comorbid medical conditions assessed within 30 days prior to the index date (Appendix 1, Analysis Table 2). Because only year of birth was solicited on the data collection form, the calculation of exact age will not be precise and will be estimated as the year of the date of interest (e.g., data collection, death, last known follow-up visit) minus the year of birth. Height is collected in either feet and inches or centimeters; weight is collected as stone and pounds or kilograms. Weight and height will be summarized using kilograms and centimeters, respectively, for all analyses. Thus, height collected as feet and inches will be converted to centimeters [(12 × feet + inches) × 2.54], and weight collected as stone and pounds will be converted to kilograms [(14 × stone + pounds) ÷ 2.205]. To calculate body mass index, height in centimeters will first be converted to meters (centimeters ÷ 100), then the weight in kilograms will be divided by the square of the height in meters to yield a measurement with the units of kilograms per square meters.

Fungal disease characteristics (e.g., disease type, days from suspected diagnosis to initiation of isavuconazole, method of infection confirmation, localization) for the study population will be summarized from the closest assessment to the index date within the

pre-index period; however, if no pre-index period assessments are available, the closest assessment in the post-index period will be used (Appendix 1, Analysis Table 3). Antifungal drug use assessed within 1 year prior to index treatment and prior treatment history assessed within 30 days prior to the index date also will be summarized (Appendix 1, Analysis Table 4). Characteristics to be assessed include antifungal type, treatment switch to isavuconazole for the same infection, reason for switch to the index treatment, and other prior treatment history (including antibiotics, surgery, corticosteroids, and immunosuppressants).

6.3 ANALYSIS OF CLINICAL OUTCOMES

Each clinical outcome will be analyzed using counts and frequencies at weeks 6, 12, and 24, along with other descriptive information about the clinical outcome itself. Additionally, time-to-event analyses anchored on the index date of isavuconazole initiation will be performed using the KM method. For each clinical outcome, the time-to-event analyses will result in a KM plot with summary statistics, including survival-time quartiles and KM estimates at weeks 6, 12, and 24 with accompanying 95% CIs. All analyses will be performed for invasive aspergillosis patients, mucormycosis patients, and for the total study population. If subgroup analyses are pursued, Cox regression will be employed to derive HRs and accompanying 95% CIs.

For the death outcome, patients will be categorized as dead, alive, or unknown at each timepoint of interest, and primary cause of death will be tabulated (Appendix 1, Analysis Table 5.1). For KM analyses, a documented death will comprise the event, and patients will be censored at the date when they are last known to be alive (Appendix 1, Analysis Figure 1; Appendix 1, Analysis Table 5.2).

For the clinical response outcome, patients will be categorized as having clinical success, having clinical failure, or unknown response at each timepoint of interest will be tabulated (Appendix 1, Analysis Table 6.1). In addition, rates of reactivation of the same infection and rates of new infection will be documented overall and specific to whether these events occurred during isavuconazole treatment. For KM analyses, clinical response will comprise the event, and patients will be censored at the earliest timepoint of any of the following: loss to follow-up, end of study, or death (Appendix 1, Analysis Figure 2; Appendix 1, Analysis Table 6.2).

For radiological response, patients will be categorized as having a success, not having a response, unknown response, or not applicable (for the first assessment) at the relevant timepoints of interest (Appendix 1, Analysis Table 7.1). Additionally, radiological examination type and bodily location of the examination will be tabulated. For KM analyses, radiological response will comprise the event, and patients will be censored at the earliest timepoint of any of the following: loss to follow-up, end of study, or death (Appendix 1, Analysis Figure 3; Appendix 1, Analysis Table 7.2).

For the mycological response outcome, patients will be categorized having the infection eradicated, presumed eradicated, not eradicated (failure), unknown response, or not applicable (for the initial assessment) at relevant timepoints of interest. Additionally, mycological examination type and biomarker categories will be tabulated at post-baseline visits (Appendix 1, Analysis Table 8.1). For KM analyses, mycological response (eradication or presumed eradication) will comprise the event, and patients will be censored at the earliest timepoint of any of the following: loss to follow-up, end of study, or death (Appendix 1, Analysis Figure 4; Appendix 1, Analysis Table 8.2)

6.4 ANALYSIS OF SAFETY OUTCOMES

Descriptive statistics will be used to tabulate the occurrence of AEs at week 6 (Appendix 1, Analysis Table 9.1), week 12 (Appendix 1, Analysis Table 9.2), and week 24 (Appendix 1, Analysis Table 9.3). These tables will be repeated for SAEs (Appendix 1, Analysis Tables 10.1, 10.2, and 10.3, respectively). Additionally, these tables will summarize the occurrence of any AE, number of AEs, isavuconazole-related AEs, whether action was taken with isavuconazole, and the AE outcome.

KM plots for time to onset of the first AE will be generated (Appendix 1, Analysis Figure 5), and summary KM statistics will be tabulated (Appendix 1, Analysis Table 11). For KM analyses, onset of the first AE will comprise the event, and patients will be censored at the earliest timepoint of any of the following: loss to follow-up, end of study, or death.

6.5 ANALYSIS OF TREATMENT PATTERNS

Descriptive statistics will be used to tabulate characteristics of isavuconazole treatment at the index date, including setting of treatment administration, loading dose, and maintenance dose, administration mode, and therapy duration (Appendix 1, Analysis Table 12). Therapy duration will be calculated as the treatment modification date minus the isavuconazole initiation date among patients noted to have a frequency decrease and a frequency for initial treatment of every 8 hours. Treatment patterns during the post-index period also will be summarized with descriptive statistics. Characteristics related to treatment modification will include whether the modification occurred, days between index treatment and treatment modification, type and reason for therapy modification, and summaries of patients discharged from the hospital with an isavuconazole prescription (Appendix 1, Analysis Table 13). Days between index treatment and treatment modification will be calculated as the date of first modification minus the date of isavuconazole initiation for patients who did not receive a loading dose and as the date of the second modification minus the date of the first modification for patients who did receive a loading dose.

Similar descriptive summaries will be provided for treatment patterns in the post-index period related to combination therapy (Appendix 1, Analysis Table 14), therapeutic drug monitoring (Appendix 1, Analysis Table 15), isavuconazole discontinuation (Appendix 1, Analysis Table 16), and switching to a new antifungal agent (Appendix 1, Analysis

Table 17). Characteristics to be summarized will include occurrence of each item, days from isavuconazole initiation to the occurrence of each item, and other item-specific details among patients who experienced each item.

6.6 ANALYSIS OF HEALTH CARE RESOURCE UTILIZATION

Descriptive statistics will be used to tabulate HCRU during the post-index period (Appendix 1, Analysis Table 18). Characteristics will consist of items such as whether a hospitalization occurred; primary reason for the hospitalization; location (ward) of the hospitalization; use of a mechanical ventilator; days between isavuconazole initiation and hospital admission and discharge; and length of stay, which will be computed as the discharge date minus the admission date + 1.

Additionally, HCRU characteristics will be summarized for health care professional visits or referrals. Characteristics will consist of items such as the type and number of health care professional visits or referrals, primary reason for visit or referral, type of health care professional, and days between isavuconazole initiation and first visit or referral (Appendix 1, Analysis Table 19).

Potential analyses of subgroups may be performed but have not been defined explicitly in Appendix 1.

7 REFERENCES

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8 APPENDICES

8.1 APPENDIX 1: EXAMPLE ANALYSIS TABLES AND FIGURES

Please click on the Excel icon in the left column of this PDF to open up the tables.