WHISCON World Health Information Science Consultants, LLC

Study Protocol
Amended Version as of 19 July 2012

A Multicenter Cohort Study of the Short and Long-term Safety of Micafungin and Other Parenteral Antifungal Agents

Astellas Protocol Number: ISN 9463-CL-1401

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This study protocol was developed by WHISCON for Astellas Pharma Europe (Lovett House, Lovett Road, Staines, Middlesex, TW18 3AZ, United Kingdom).

This study protocol follows the Good Pharmacoepidemiology Practice guidance by the International Society for Pharmacoepidemiology.

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1. Executive Summary

1.1. Objective

Echinocandins have shown hepatic toxicity in clinical trials and observational studies, but their profile of renal toxicity appears to be superior to that of older agents.[1,2,3] Micafungin carries a black box warning in Europe, but not elsewhere, because of tumors observed in rat models.[4] There remains little information on the frequency of these effects in routine care in comparison to other parenteral antifungal agents. Human carcinogenicity has not been observed or formally investigated.

This multicenter observational cohort study proposes to establish the risks of short and long-term outcomes in users of parenteral micafungin and in users of other parenteral antifungal agents from 2005 through 2011 or later.

Three 30-day outcomes (a, b, and c) will be identified retrospectively within the cohort during the 30 days following treatment, and one long-term outcome (d) will be identified both retrospectively and prospectively in the same cohort for up to 13 years following treatment.

a. Treatment-emergent hepatic injury or dysfunction
b. Treatment-emergent renal failure or dysfunction
c. Rehospitalization for the parenteral treatment of fungal infections
d. Death from hepatocellular carcinoma (HCC)

1.2. Rationale

Extending knowledge about the safety of micafungin with respect to hepatotoxic outcomes including liver injury and hepatocellular carcinoma requires detailed clinical information on the patients' health state before treatment initiation, precise definitions of outcomes, large numbers of patients, and an opportunity for long-term follow-up. After substantial review of the research options and two feasibility studies, and after review of parenteral antifungal utilization patterns, we have concluded that the most effective path forward would be to utilize electronic medical record (EMR) databases of a small number of major tertiary care centers in the US.

The clinical details available in EMRs will allow us to identify study patients and balance users of the various parenteral antifungal agents with regard to their baseline risk. The fact that most patients receiving parenteral antifungal medications are very sick and are mostly treated in tertiary care centers ensures that a few centers will be sufficient to identify large numbers of study patients in their retrospective EMR data. 30-day outcomes, such as renal failure, can be identified with high accuracy in such data. Each center can link patients to the US National Death Index (NDI) for long-term mortality follow-up. This study approach can be flexibly adapted to extend the enrollment period beyond 2011 and/or incorporate additional centers if needed to provide adequate sample size for the study, and since micafungin has been on the US market since 2005 it will

permit a retrospective cohort analysis of 30-day effects as well as a bidirectional cohort study (retrospective and prospective) for long-term follow-up of HCC.

1.3. Data Sources

Data for this study will be derived from the longitudinal electronic medical records of multiple hospitals. These records will be used to identify patients who received parenteral antifungals, assess eligibility for cohort entry, characterize patients' health state at entry, and evaluate 30-day outcomes. For the long-term mortality follow-up, a search of the US NDI will be conducted among patients in the cohort using patient identifying information and within patient privacy safeguards. These are both well-tested data sources and frequently used approaches in drug safety research. [5, 6]

1.4. Exposure Groups

The study cohorts will consist of at least 7,000 patients who received micafungin and a combination of patients who received another intravenous antifungal (table) up to a total of 35,000 patients from 2005 (when micafungin entered the US market) through 2011 or later within the partner hospitals.*

Group	Generic Name	Brand Name	IV form US Approval
Primary Exposure	Micafungin	Mycamine	March 2005
Comparator	Caspofungin	Cancidas	January 2001
Comparator	Anidulafungin	Eraxis	February 2006
Comparator	Fluconazole	Diflucan, Others	January 1990
Comparator	Itraconazole	Sporanox	March 1999
Comparator	Voriconazole	VFend	May 2002
Comparator	Amphotericin B	Fungizone	Prior to January 1982
Comparator	Liposomal Amphotericin B	Ambisome, Abelcet, Amphotec	August 1997, November 1995, November 1996

*: The enrollment period can be extended by one or more months into 2012 and beyond to the extent that is needed to obtain the required number of patients.

1.5. Analysis

Patient identification, eligibility for cohort inclusion, and cohort characteristics will be described. Study outcomes will be identified in the short term (up to 30 days) and the long term (up to 13 years).

Survival analysis will compare the occurrence of each 30-day outcome among the parenteral antifungal agents and will employ multivariate propensity score methods to adjust for possible confounding effects of age, gender, race, and comorbid conditions as available in the EMR. A further assessment of potential for residual effects due to patient characteristics obtained through chart review of cases and a random sample of the cohort will be conducted. The occurrence of the long-term outcome (HCC mortality) will analogously employ survival analysis and propensity score techniques.

As the outcome with the lowest expected frequency of occurrence, HCC mortality is the limiting outcome for considerations of study power. If patients who receive parenteral antifungals have a risk of HCC mortality similar to the United States population as a whole, the proposed research has a power of 90 percent to detect a relative risk of HCC mortality of 3.5. Since patients receiving parenteral antifungals have characteristics that are likely associated with considerably elevated risk of HCC mortality, a more likely scenario involves a ten-fold risk of HCC mortality providing the study a 90 percent power to detect a relative risk of 1.7.

1.6. Study Milestones, tasks and timeline

This table identifies the project milestones, content and scheduled dates.

Year	Milestone	Content	Date
2011	Interim Status Report	Update on progress	June 30, 2011
	Draft Protocol	Research plan	July 7, 2011
	Final Protocol	Revised Research plan	August 2011
	Annual Status Report	Update on progress	November 1, 2011
2012	Interim Report	Update and preliminary counts	June 30, 2012
	Annual Report	Update on accrual and 30-day outcomes as available	November 1, 2012
2013	Interim Report and updated HCC power considerations	Update on accrual and 30-day outcomes as available	June 30, 2013
	Annual Report	Update on accrual and 30-day outcomes as available	November 1, 2013
2014	Interim Report	Update on accrual and 30-day outcomes as available	June 30, 2014
	Annual Report and manuscript on 30-day outcomes	Update on accrual, 30-day outcomes	November 1, 2014
2015	Mortality Report	HCC mortality through 2012	June 30, 2015
	Status Report	Update on study status.	November 1, 2015
2016	Mortality Report	Update on HCC mortality through 2013.	June 30, 2016
	Status Report	Update on study status.	November 1, 2016
2017	Mortality Report	Update on HCC mortality through 2014	June 30, 2017
	Status Report	Update on study status.	November 1, 2017
2018	Mortality Report	Update on HCC mortality through	June 30, 2018

		2015	
	Status Report	Update on study status.	November 1, 2018
2019	Mortality Report	Update on HCC mortality through 2016	June 30, 2019
	Status Report	Update on study status	November 1, 2019
2020	Status Report	Update on project status	March 2020
	Draft Final Report	Full report including HCC mortality through 2017	September 2020
	Final Report and Manuscript	Revised report including HCC mortality, manuscript describing all study findings	November 2020

2. Background

2.1. Parenteral Antifungal Medications

Invasive fungal infections and fungemia are caused by a variety of fungal pathogens including Candida species, Aspergillus species, and others. Ma of these infections occur in patients with substantial comorbidity such as those in intensive care or those with neutropenia, and are associated with high mortality either from the infection or the underlying conditions.[7]

Treatment for patients with confirmed or suspected invasive fungal infections involves parenteral antifungal therapy. The mainstay of antifungal therapy for many years has been amphotericin B either as a deoxycholate or more recently in lipid formulations. The triazole antifungals available for parenteral therapy include fluconazole, itraconazole and voriconazole. Another class of parenteral antifungal medications consists of the echinocandins: caspofungin, micafungin and anidulafungin.[8]

2.2. Micafungin

Micafungin is an echinocandin used in the EU in adult and pediatric patients. Micafungin has been approved for marketing in the U.S. since March 2005.

Micafungin is indicated in the U.S. for:

Adults, adolescents ≥16 years of age and older:

- Treatment of patients with candidemia, acute disseminated candidiasis, *Candida* peritonitis and abscesses
- Treatment of patients with esophageal candidiasis
- Prophylaxis of *Candida* infections in patients undergoing hematopoietic stem cell transplantation

The U.S. approved indication for micafungin is largely similar to that of the European Medicines Agency except that micafungin is in the EU also approved for the treatment of

invasive candidiasis and prophylaxis of *Candida* infection in children and adolescence younger than 16 years of age. In addition according to the label in the EU, Micafungin should only be used if other antifungals are not appropriate. This restriction is due to foci of altered hepatocytes (FAH) and hepatocellular tumours observed in rats after a treatment period of 3 months or longer.

Micafungin is generally used in the context of severe co-existent illness, whose signs and symptoms may contribute to and/or aggravate adverse events. Other parenteral antifungals used for prophylaxis[9] and treatment of similarly severe fungal infections have their own adverse event issues, including the azoles[10], caspofungin[11] and anidulafungin[12].

2.3. Unknown short-term Toxicity: Liver and Kidney Injury

Short-term hepatic and renal toxicity have been seen with the echinocandins in clinical trials. There are insufficient available data on the frequency of these effects in routine care in comparison to other parenteral antifungal agents.

In a retrospective cohort study of hospitalized users of parenteral micafungin and other parenteral antifungals we had assessed 30-day hepatotoxic and nephrotoxic effects as well as rehospitalization for renewed parenteral antifungal therapy using linked outpatient and inpatient administrative healthcare data. We found no increase in nephrotoxicity of micafungin compared with other antifungals and concluded that an analysis of liver injury was not possible without more detailed clinical data. Residual confounding and insufficient specificity of endpoint definition most likely had biased the study. The need for more detailed clinical data motivated the current and improved study design.

2.4. Unknown Long-term Toxicity: Hepatocellular Carcinoma

Known risk factors account for variations in the risk of HCC that range over at least three orders of magnitude.

HCC accounts for 85-90% of all primary liver cancers. In Europe, the incidence of liver cancer varies from low in the North (<5.0/100,000 annually in males and 1.1/100,000 in females) to medium in the South (7.5/100,000 in males and 2.4/100,000 in females in Spain).[13] The 15-year period prevalence from 1990-2004 was 57/100,000. The age-standardized mortality rates in 2003 were 4.1/100,000 for men and 1.7/100,000 for women. A meta-analysis performed at the University of Brescia in Italy associates higher incidence of HCC in the Mediterranean Europe with increased alcohol consumption and intermediate levels of HCV and HBV infections (1–3% of population infected by each virus); alcohol, HBV and HCV together account for about 85% of the total HCC cases in those countries.[14]

The incidence rates in the US appear to be higher than those in Europe. In 2004, the annual incidences were 8.7 and 3.0 per 100,000 in males and females, respectively, with increases in incidence by age and substantial association with race.[15]

The reported incidence of liver cancer is increasing over time. The incidence rate for cases of cancer of the liver and intrahepatic bile ducts reported in the UK grew from 1351 cases in 1991 to 2708 cases in 2005.[16] In the United States, incidence rates are rising at

approximately five percent per year[17] possibly late consequences of earlier epidemics of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV).

Globally, HBV is the most frequent underlying cause of HCC, with an estimated 300 million persons with chronic infection worldwide. Case-control studies have shown that chronic HBV carriers have a 5- to 15-fold increased risk of HCC compared with the general population. The great majority, between 70% and 90%, of cases of HBV-related HCC develop in patients with cirrhosis. However, HBV is a notorious cause of HCC in the absence of cirrhosis.

Chronic HCV infection is a major risk factor for the development of HCC. Markers of HCV infection are found in a variable proportion of HCC patients; for example, 44-66% in Italy, 27-58% in France, 60-75% in Spain, and in 80-90% of HCC patients in Japan.[13].

Recent studies have shown an increased risk for liver cancer in HIV carriers and other immunosuppressed patients.[18,19,20,21] These studies found standardized incidence ratios (SIR) for liver cancer ranging from 9.4 to 29.43 in individuals with HIV. In immunosuppressed patients, the SIR ranged from 3.2 to 4.1. A recent meta-analysis found a pooled SIR of 5.22 for liver cancer in HIV patients, and in transplant patients this figure was 2.13. In Europe, HCC and end-stage liver disease account for 15% of non-AIDS related mortality in AIDS patients.[22]

Some of the chronically ill patients who may be at risk for candidemia or invasive candidiasis may be at extraordinarily high risk for HCC. Between 2002 and 2004, among 8,566 patients awaiting orthotopic liver transplantation in the United States, typically for less than a year, 1,167 (14 percent) developed apparently *de novo* hepatocellular carcinoma. Age and liver compromise due to HCV and HBV were associated with increased risk, but even in the absence of HCV, the crude risk of HCC was eight percent.[23]

The wide variability in estimates of the incidence of HCC presents a challenge to the accurate prediction of the incidence of HCC in patients receiving intravenous antifungal therapy. The conduct of this study will describe the demographics and comorbidities of patients receiving intravenous antifungals in the United States, facilitating this prediction so that power estimates can be revisited as they become available. The US population incidence rate is 10 per 100,000 per year. The final rate in the study population could prove to be ten or even 100 times as high. Differences in compared populations, particularly with respect to comorbidities, may distort comparisons between recipients of different antifungals and need to be addressed with comprehensive confounding adjustment methodologies.

Survival of patients with HCC is approximately 25% at one year in the United States.[24] SEER estimates 1-year survival as 25% if localized at diagnosis, 8% regional, 3% distant. This has the effect of making HCC mortality a reasonable proxy for HCC incidence.

3. Objectives

This multicenter cohort study investigates the risks of the following 30-day and long-term outcomes in users of parenteral micafungin and in users of other parenteral antifungal agents:

30-day outcomes include the following events occurring between the cohort-defining initiation of parenteral antifungal treatment and 30 days after the last recorded administration of that same agent:

a. Treatment-emergent hepatic failure or dysfunction
b. Treatment-emergent renal failure or dysfunction
c. Rehospitalization for the parenteral treatment of fungal infections

Long-term outcome:

d. Death from hepatocellular carcinoma (HCC)

4. Specific Aims

1. Establish a network of six or more tertiary care centers in the US that have electronic medical records systems and constitute a scientific advisory board (SAB).
2. Characterize the use of the parenteral antifungal agents micafungin, caspofungin, anidulofungin, fluconazole, itraconazole, voriconazole and amphotericin B in six tertiary care centers in the US.
3. Identify groups of recipients of parenteral antifungal agents, closely balanced with respect to indication, concomitant medication, disease severity and prior health conditions using propensity score methods.
4. Determine the occurrence of outcomes during the index hospitalization and up to 30-days following discharge.
    a. Treatment-emergent hepatic failure or dysfunction
    b. Treatment-emergent renal failure or dysfunction
    c. Rehospitalization for the parenteral treatment of fungal infections
5. Analyze the risk of outcomes as a function of antifungal agent used and of other patient characteristics.
6. Determine the occurrence of death due to liver cancer on a long-term basis (up to 13 years; 2005 through 2017) and analyze the risk of liver cancer as a function of antifungal agent used and of other patient characteristics.

5. Data and Data Sources

5.1. Electronic Medical Records

Cohort eligibility requirements, baseline covariates, and 30-day outcomes will be identified on the basis of hospital-based electronic medical records.

Large tertiary care centers in the US routinely capture all their medical records in electronic form. This includes the prescribing of medications through a computerized order entry system. The advantages are that any medication order has to be recorded in electronic form and all procedures, test results, notes, and diagnoses are electronically recorded. The fact that data are available in computerized form will facilitate the accurate and expedited identification of the study cohort based on antifungal use and will allow the recording of patient characteristics and outcomes retrospectively without having to retrieve any paper records. These data systems exist independently in different medical centers, and a multicenter study will require that data extraction and supervision occur in parallel at all the participating sites.

The data elements in EMRs exist in both structured and unstructured formats. Structured data exist in a relational database with "tables" corresponding to different kinds of information on individual subjects. Structured data include administrative information such as the date and nature of all care, associated diagnoses, laboratory test results including liver and kidney function tests, some physical measurements (such as weight and blood pressure), and medications dispensed or prescribed. A database specialist can retrieve structured data according to specified rules and can arrange the resulting information into files suitable for analysis.

Unstructured data are embedded in text information recorded in the process of care. While the location of text can be specified within the relational database, its information content is accessible only to a clinically informed reader. Many aspects of presentation, history of the present illness, personal habits, family history and social factors as relevant to care will be accessible only through text retrieval by trained chart abstractors.

Although individual electronic medical records (EMR) systems differ in appearance, the underlying data structure is very similar. Systems have in common that all information relevant to the treatment of a patient is recorded in EMRs; no paper-based records exist. Because of that it is likely all relevant patient information for the current study will be captured in such electronic medical records. Since most if not all patients receiving parenteral antifungal medications are expected to be very sick, these patients will likely return to the index hospital center for their follow-up care in their outpatient clinics and would be admitted to the same tertiary care centers for any relapses. We expect information on these patients to be essentially complete for the 30 days of follow-up after discharge from the first recorded hospitalization with parenteral antifungal use (index hospitalization). All data elements are recorded longitudinally with an automatic date and time stamp for each entry.

Key data elements that are of interest for this study include:

	Data Element	Available in Structured Form
Patient demographics	Age	Yes
	Gender	Yes
	Race/Ethnicity	Sometimes
	Payer information	Yes
	Marital status	Yes
Hospital episode of care	Admission date, time, source (and re-admissions)	Yes
	Detailed medical assessment of patient health state at admission, including medical history and anthropometric measures, BMI, blood pressure etc., as well as relevant life style factors, like alcohol use, smoking etc.	No
	All diagnoses related to hospitalization with clear identification of the primary problem(s)	Yes
	All laboratory test results related to the hospitalization (including liver and renal function tests, microbiology results etc.)	Yes
	All procedures related to the hospitalization (including liver biopsy with histology result)	Yes
	Operating room procedures	Yes
	Radiological procedures and their results and contrast agents used	Sometimes
	Discharge disposition	Yes
	Detailed discharge summary	No
Drug specific information	Prescribing physicians	No
	Generic and brand name of drug	Yes
	Indication for drug use	Sometimes
	Strength	Yes
	Dosage form	Yes
	Date and time administered	Yes
	Route of administration	Yes
	Treatment dose	Yes

	Data Element	Available in Structured Form
Outpatient episode of care	Treatment duration	Sometimes
	Visit date	Yes
	Medical assessment of patient health state at visit	No
	All diagnoses related to visit with clear identification of the primary problem(s)	Yes
	All laboratory test results related to the hospitalization (including liver and renal function tests, microbiology results etc.)	Yes
	All procedures related to the hospitalization (including liver biopsy with histology result)	Yes
	Primary and consulting physicians	Yes

5.2. US National Death Index[25]

Long-term follow-up will be accomplished through the US NDI, a central computerized index of death records in the United States. The National Center for Health Statistics (NCHS) established the US NDI as a resource to aid researchers with their mortality ascertainment activities. Records are available to investigators solely for statistical purposes in medical and health research.

A national file of identifying death record information (beginning with 1979 deaths) has been compiled from computer files submitted by vital statistics offices of the individual states. Death records are added to the US NDI file each year in July or August for the second preceding year. Decedents are identified by as many of the following data items as possible: first and last name, middle initial, father's surname, social security number, month, day, and year of birth, race, sex, marital status, state of residence, and state of birth. The US NDI-Plus system includes full death certificate information, including identification of the individual and the date, place and causes of death, including underlying causes and an index of the certainty of the match reflecting the number of identifying elements matching between the submitted record and the presumed matching death record. The US NDI has consistently indicated very high sensitivity and specificity for identification of the deaths of US residents showing sensitivity and specificity in the high ninety percents.[26,27,28,29,30,31] (More extensive material is available at http://www.cdc.gov/nchs/ndi.htm.)

To facilitate proper identification of decedents, NDI users are encouraged to submit as many of the following data items as possible for each study subject: first and last name, middle initial, father's surname, social security number, month, day, and year of birth, race, sex, marital status, state of residence, and state of birth. In our earlier study in patients who used parenteral antifungal agents and were comparable to the current study population the sensitivity of the NDI linkage was 95% and the specificity was 99%. The

sensitivity was lowest in children and infants with 90%.[32] As we had experienced from our NDI linkage feasibility study, apart from race all this identifying information is routinely available in the administrative databases of any tertiary care hospital.

As discussed above, three-quarters of patients with hepatocellular carcinoma in the United States die within a year of diagnosis. Since the US NDI has essentially complete ascertainment of all deaths in the United States, it is an effective system for identifying hepatocellular carcinoma in a US cohort.

5.3. Candidate US Tertiary Care Centers

The WHISCON investigator team is itself based in the middle of the Harvard Medical Campus and through their personal contact with many major medical centers in the US will establish a network of participating hospitals. We plan to approach as many of the following tertiary care medical centers as is required to identify six that meet the cohort size requirements:

Back-up hospital centers will be approached if any of the primary centers prove to have unexpectedly low utilization of micafungin:

We will establish access to these data sources by contracting with each partner hospital and identifying a qualified hospital Principal Investigator. The hospital PI may be an infectious disease specialist or any other type of internist or otherwise qualified to run the site-specific tasks related to this project. The Site PI will identify and involve the necessary medical expertise, including infectious diseases, immunology, nephrology and gastrointestinal oncology. These specialties are all available in the tertiary care centers listed above.

The site PIs together with the PI and Senior Scientist from WHISCON will form the Scientific Advisory Board SAB (see Section 7.3). The SAB will serve as the endpoint committee (see Section 6.7).

6. Study Design

6.1. Study Cohort

The study cohort will be derived from patients hospitalized and treated with a parenteral antifungal medication for the first time in that medical center from 2005 and through 2011 or later at a participating medical center (defined as the index hospitalization). Follow-up starts on the day of the first administration of the cohort defining parenteral antifungal medication.

The primary analysis will not consider patients with any diagnosis of chronic renal or chronic hepatic disease

1. At any hospitalization preceding the index hospitalization
2. At any outpatient visit preceding the index hospitalization
3. As a chronic disease discharge diagnosis on the index hospitalization

or

4. Who had received parenteral antifungal therapy during the 6 months prior to the index hospitalization.

Patients will only be included in the primary analysis if liver function tests (LFT), specifically serum ALT and AST levels and a creatinine level are available during the index hospitalization on the day of or before parenteral antifungal therapy was started. Patients will be excluded if either the ALT levels are 5 times the upper normal level or more than 300 U/L, or AST levels are 5 times the upper normal level or more than 200 U/L, or creatinine is higher than 2.0 mg/dL (150 μmol/L) at least once during the index hospitalization preceding the initiation of parenteral antifungal therapy (see Section 13.1 for details).

Patients that are identified as participating in a randomized trial involving parenteral antifungal therapy will be excluded.

Patients who received parenteral antifungal treatment but who do not meet inclusion/exclusion criteria will be excluded from the primary analysis of short-term endpoints and HCC, but their data will be collected and their follow-up experience regarding death from HCC will be recorded.

6.2. Exposure status

Each patient's exposure status will be determined during the index hospitalization. Parenteral administration of at least one dose of the following antifungal agents marketed in the U.S. will characterize the patient's exposure status.

Group	Generic Name	Brand Name	IV form Approval
Primary Exposure	Micafungin	Mycamine	March 2005
Comparator	Caspofungin	Cancidas	January 2001
Comparator	Anidulafungin	Eraxis	February 2006

Comparator	Fluconazole	Diflucan, Others	January 1990
Comparator	Itraconazole	Sporanox	March 1999
Comparator	Voriconazole	VFend	May 2002
Comparator	Amphotericin B	Fungizone	Prior to January 1982
Comparator	Liposomal Amphotericin B	Ambisome, Abelcet, Amphotec	August 1997, November 1995, November 1996

First-line treatment cohort: Patients will be classified as primary recipients of one of the above antifungal medications based on the first antifungal administration during the index hospitalization. In our previous analysis of a large sample of US hospitals, multiple simultaneous initiations were extremely rare, as was second-line therapy following any of the antifungal agents except fluconazole. If utilization analyses in the study cohort indicate substantial second-line use of micafungin following some parenteral antifungal other than fluconazole then patients receiving earlier therapy with these other parenteral antifungals may be included. Otherwise, patients receiving more than one study drug other than fluconazole as well as the few patients who initiate antifungal therapy with two or more agents simultaneously will be identified, set aside, and their follow-up experience regarding death from HCC will be recorded.

Second-line treatment cohort: Among patients who were started on fluconazole during the index hospitalization we will also include second-line treatments in the analysis. Exposure status will be defined based on the subsequent antifungal use second to fluconazole during the index hospitalization. For example, patients who were initiated on fluconazole and later received micafungin as additional therapy or switch will be categorized as micafungin users within the second-line treatment cohort. Many patients initiated on fluconazole will contribute in the first-line as well as the second-line treatment cohort.

6.3. Patient Characteristics from Structured Portions of the EMR

Using the data available from hospitalizations and outpatient visits during the 6 calendar months before admission to the index hospitalization as well as during the calendar month of hospitalization, but preceding hospitalization, we will ascertain a list of covariates describing patient characteristics.

We will develop center-specific procedure documents that will operationalize how the varying EMR systems will map to the covariates of interest. This will involve the center study coordinator and the WHISCON senior project manager (Ms. Lisa Weatherby, MS) with support by the overall study PI (Dr. Schneeweiss) and will be approved by the site PI as well as the overall study PI. The center-specific procedure documents will be annexed to the protocol. Upon completion, the WHISCON senior project manager or overall study PI will visit each center and evaluate the abstraction, coding and storing of covariate information in the study database.

All patient characteristics will be assessed before the index hospitalization. Factors recorded as discharge diagnoses during the index hospitalization will only be considered if they are chronic conditions undoubtedly present before the hospitalization.

During 6 months before the index hospitalization

Recorded diagnoses at prior hospitalization or outpatient visits:
Cancer type (none, myeloma, leukemia [ALL, AML, CLL, CML, ATL], NH lymphoma, Hodgkin's disease, any solid cancer), graft-versus-host disease, organ transplantation, agranulocytosis, neutropenia, pancytopenia, HIV/AIDS, congenital immunodeficiency, MI, old MI, CABG surgery, percutaneous coronary procedure, thrombolysis, heart failure, diabetes, hypertension, COPD/asthma, stroke, old stroke, peripheral artery disease, cholecystitis, gastritis, peptic ulcer disease, hemostatic disorder (idiopathic thrombocytopenia, hemophilia, protein S deficiency, protein C deficiency)

Procedures or medications at prior hospitalization or outpatient visits:
Immunosuppressant therapy (cyclosporine, sirolimus, tacrolimus, mycophenolate, azathioprine, cyclophosphamide, clorambucil, methotrexate), G-CSF, long-term corticosteroid use.

During the index hospitalization

- Socio-demographic factors:
  - Age, sex, race,
  - Low-income status (Medicaid or indigent),
  - Marital status (living with partner),
  - Month and year of index admission,
  - Admission type.
  - Intensive care unit stay on the day of antifungal initiation
- Medications
  - Number of different medications received
  - Oral antifungal therapy
  - Receipt of potentially nephrotoxic or hepatotoxic medications before or during antifungal therapy
  - Number and type of antibiotic received at index hospitalization
  - Individual medications received (for all medications received by at least 5 percent of the study population)
- Discharge diagnoses (other than diagnoses of acute or chronic renal and hepatic disease)
  - Antifungal use for prophylaxis versus treatment
  - Cancer type (none, multiple myeloma, aplastic anemia, leukemia [ALL, AML, CLL, CML, ATL], NH lymphoma, Hodgkin's disease, any solid cancer)
  - Graft-versus-host disease
  - Organ transplantation
  - Agranulocytosis, pancytopenia, neutropenia
  - HIV/AIDS
  - MI, old MI

  - Heart failure
  - Diabetes
  - Hypertension
  - COPD/asthma
  - Stroke, old stroke
  - Peripheral artery disease
  - Hemostatic disorder (idiopathic thrombocytopenia, hemophilia, protein S deficiency, protein C deficiency)
  - RA and other collagen vascular diseases
  - Gastritis, peptic ulcer disease
  - Inflammatory bowel disease, including Crohn's disease and ulcerative colitis.
- Procedures
  - CABG surgery
  - Percutaneous coronary procedure
  - Transplantation (other than liver)

6.4. Forming Balanced Cohorts

We will use propensity-score matching to select a subset of eligible patients into comparison groups that will be balanced with respect to patient characteristics as represented in the structured data elements. This methodology has been shown to achieve excellent balance of all component covariates in sufficiently large studies.[33] The technique is frequently used in pharmacoepidemiologic studies using electronic healthcare utilization databases.[5,34,35]

For recipients of first-line and second-line therapy separately, we will match micafungin recipients, who form the common reference group, with recipients of any of the comparator exposures, within categories defined by calendar year, a method developed by Drs. Seeger and Walker.[36] Propensity score matching takes the drug (micafungin versus comparator) as the dependent variable in a logistic regression in which the independent or predictor variables consist of all the baseline patient and hospital characteristics. The resulting fitted probability of receiving micafungin vs. the comparator antifungal is called the propensity score. We will match micafungin users with comparator patients who were close in propensity score at a fixed ratio, using a "nearest neighbor matching" algorithm allowing for a caliper of up to 1 decimal.[37] Matching on the propensity score produces balance in the factors that make up the score.

As the choice between echinocandins is generally made at the level of the hospital formulary we anticipate that the average difference between patients will not be large and that essentially all micafungin patients will be matched to non-micafungin antifungal agents.

It is currently planned to match each micafungin case with 4 control cases. However, the ratio of controls: micafungin may be amended if the proportion of micafungin cases is markedly different from current expectations.

An indicator term for elevated LFT results will be included in the propensity score model as well as interaction terms between LFT and major potential confounders. Elevated LFT is defined as the highest ALT or AST measurement at the index hospitalization but before initiation of antifungal therapy of ≥3 times normal to <5 times upper normal level. Patients with admission ALT or AST levels of 5 times upper normal level and higher will be excluded (see Section 6.1). This will allow the use of propensity score matched subjects in subgroup analyses and ensuring balance in strata of LFT results.

We expect that several centers have formularies in place that will strongly favor one or two parenteral antifungal agents and will not allow the use of others. This has two implications: (1) Formularies may change over time within the same institution so that calendar time becomes an important treatment predictor. We have anticipated that and therefore will stratify the propensity score estimation by calendar year. (2) There may be greater treatment variation between centers than within centers for any calendar year. We will therefore pool data from all centers and estimate the PS disregarding the center ID. Similar to an instrumental variable analysis,[38,39] this method exploits the variation in treatment choice that is observable between hospitals to admit patients with similar distributions of baseline characteristics into the different treatment groups.[40,41] The technique assumes that centers are comparable with regard to the probability of treatment outcomes, apart from the effects of antifungal agents under study. This assumption is made more reasonable within the context of this study since the initial selection criterion for hospitals (tertiary care centers of large academic institutions that have electronic medical records in place) will enhance the similarity of the hospitals.

We will present the comparability of treatment groups by tabulation of patient characteristics between antifungals in the matched comparison cohort and present propensity score diagnostics such as the model c-statistic and a plot of the propensity score distributions among micafungin and comparator groups. Patient characteristics that are found to be out of balance (unexpectedly due to the propensity score matching) will be included as adjustment factors in the outcome analyses.

6.5. Patient Characteristics from Unstructured Data

For a 10 percent simple random sample of PS-matched patients and for all case-defining events of 30-day hepatic or renal failure/dysfunction or HCC among them, the unstructured data will be reviewed through electronic access of the text record. To this end, each center will develop site-specific protocols for text access, and a research nurse or physician will fill out a standard abstract form. Data will be used for supplementary analyses described in Section 6.8. As long-term HCC endpoints may occur years after patient accrual the raw electronic medical patient records at the point of cohort entry of each patient will be stored in a separate electronic archive by each site PI for easy retrieval at a later point in time. Only the site PI and his/her staff within the partner hospital will have access to these raw electronic records.

Medical Centers will vary as to which of these factors is available in structured or unstructured format, so the abstraction process will be tailored to each site. We will therefore develop center-specific sub-protocols that will operationalize how the varying

EMR systems will map to the covariates of interest. This will involve the center study coordinator and the WHISCON senior project manager (Ms. Lisa Weatherby, MS) with support by the overall study PI (Dr. Schneeweiss) and will be approved by the site PI as well as the overall study PI. The center-specific procedure documents will be annexed to the protocol. Upon completion, the WHISCON senior project manager or overall study PI will visit each center and audit the abstraction, coding and storing of covariate information in the study database.

The unstructured data collection instrument will seek to record, as available, the following characteristics.

From up to 6 months before the index hospitalization

Medical history:
Severity of underlying disease at admission, severity of comorbidities
Relevant medical history not captured in the structured variables

Life-style factors:
History of smoking, history of alcohol use, height, weight, Body Mass Index

During index hospitalization

Socio-demographic factors:
Race, low income status as reflected by insurance payor

Hospital characteristics:
Service of admission (general internal, surgery, oncology, infectious diseases etc), intensive care unit stay, admitting physician, attending physician

Specific conditions:
Viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatonecrosis (NASH), hemochromatosis and Wilson's disease, known aflatoxin exposure, alpha 1 antitrypsin deficiency, intrahepatic cholestasis, bile acid synthesis disorders, tyrosinemia type I, defects in carbohydrate metabolism, porphyrias, cystic fibrosis, alagille's syndrome, linked sideroblastic anaemia, Fanconi anaemia, hereditary fructose intolerance, hereditary hemorrhagic teleangiectasia, history of anabolic steroid use and Hormone Replacement Therapy use.

Discharge medications:
Oral antifungal therapy, receipt of potentially nephrotoxic or hepatotoxic medications

6.6. 30-day Outcomes

Using data from the index and subsequent hospitalizations and from outpatient visits following the index hospitalization, we will ascertain the following outcomes during the period between the first day of the cohort-defining parenteral antifungal exposure and 30 days following last recorded administration (discontinuation) of that same agent:

- Treatment-emergent hepatic injury or dysfunction
- Treatment-emergent renal failure or dysfunction
- Rehospitalization for the parenteral treatment of fungal infections

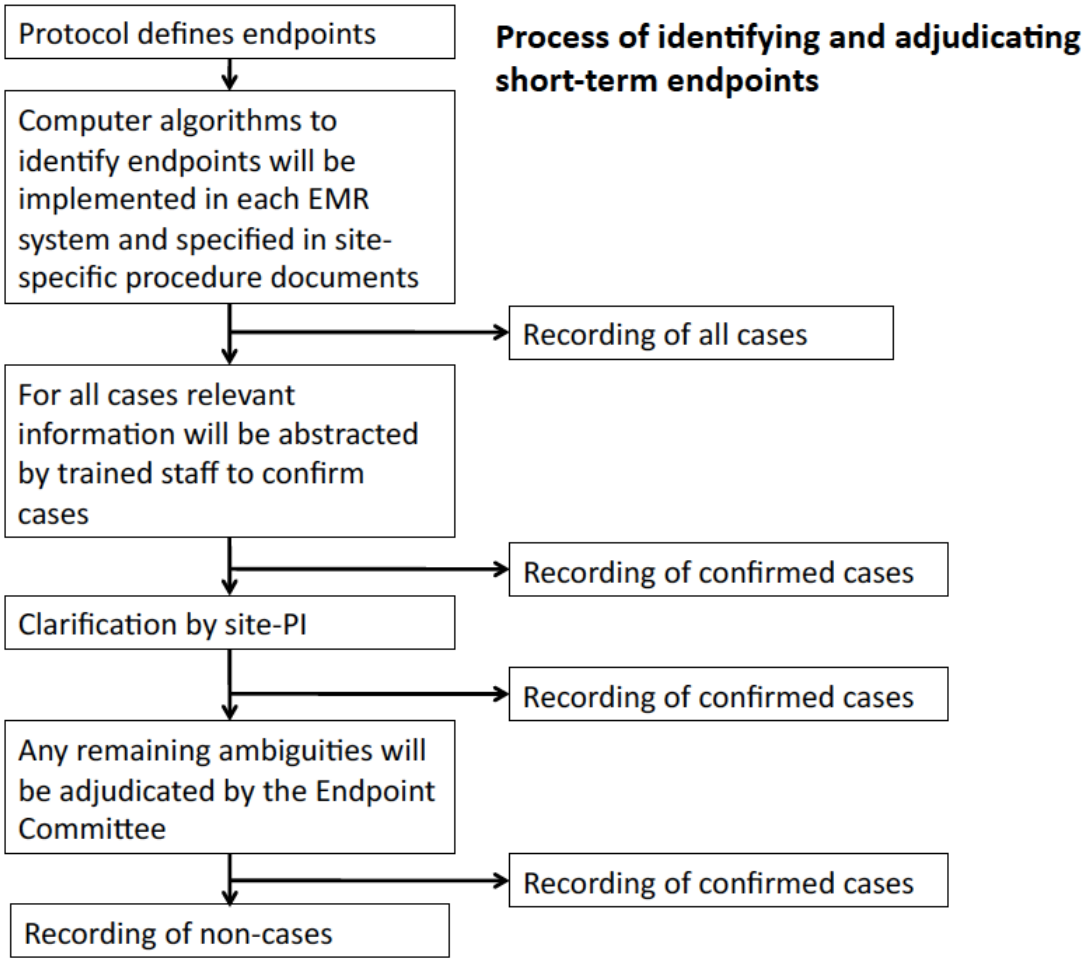
The initial outcome definitions are provided in Section 13.5. The endpoint committee consisting of the PI and investigators from the participating centers will finalize operational definitions for these outcomes based on EMR information including lab test results and diagnostic data. These operational definitions will be implemented in computer algorithms that will search the electronic medical records of study patients.

All endpoints that will be identified through these computer algorithms will be recorded in the study database. For each of these cases information that is relevant for defining a case is recorded on a paper-based form by trained study staff. The form is developed by the study investigators and approved by the endpoint committee. If the recorded information confirms the case then such cases will be marked in the study database as confirmed cases.

Sometimes clarification on the medical interpretation of EMR information is necessary in order to confirm a case. In such cases the trained study staff will consult with their respective site PI. If the clarified information confirms the case then such cases will be marked in the study database as confirmed cases.

The few remaining cases that cannot be confirmed by the above process will be brought forward to the endpoint committee at the annual meeting. The medical history and clinical development of each such case will be presented by the PI of the site that recorded the case. The PI presenting a case will avoid mentioning the specific parenteral antifungal agent if at all possible. The endpoint committee will determine the case status by consensus. Cases will be marked in the study database as confirmed cases. If consensus cannot be reached within the endpoint committee the patient will be classified as a non-case.

The flow-chart below illustrates the process.



6.7. Statistical Analyses for 30-day Outcomes

Characteristics of parenteral antifungal utilization

We will tabulate all the identified patient and hospital characteristics against antifungal use in the starting population, separately for first-line and second-line treatment.

We will examine the degree of clustering of the agents within partner hospital to identify settings in which agent choice appears to be driven by differing local protocols and formularies. After propensity score matching (see Section 6.4) we will plot hospitals according to the proportion of each specific intravenous antifungal agent (y axis) used in each hospital (x-axis) by calendar year. Hospitals will be sorted by increasing proportion of use of a specific agent. Plots will be produced for each agent stratified by first-line and second-line treatment.

Characteristics of study population

We will similarly tabulate all identified patient and hospital characteristics in the balanced study population stratified by first-line and second-line treatment.

Outcome risks

As explained at length in Section 6.4 we will balance the micafungin patient population and the comparator population using multivariate propensity score fixed ratio matching. All covariates and potential confounders listed in Section 6.3 will enter into the propensity score model. There will be no variable selection as it is a distinct advantage of propensity score methods that many covariates along with their 2-way interactions, including highly correlated factors, can be balanced simultaneously without the usual restrictions we know from maximum likelihood estimation in outcome regression models.

We will tabulate the frequency of occurrence of each of the outcomes, calculating 30-day risks and relative risks comparing the two propensity score balanced exposure groups with the corresponding 95% percent confidence intervals. Since in this cohort study analysis covariates are balanced via a fixed ratio propensity score matching no conditional analyses are necessary.[42]

Analyses will initially be stratified by first-line and second-line treatment. If effect estimates are not substantially modified by first-line vs. second-line treatment status we will calculate combined relative risk estimates. Since these observations are dependent this may lead to falsely small standard errors. We therefore propose to estimate standard errors robustly using generalized estimating equation methods.[43] In theory a matched cohort analysis, e.g. conditional logistic regression or stratified Cox regression would be more efficient but it will lack transparency. Once NDI matching becomes available (beginning in 2013) we will also compute person-time denominators censoring cohort members at the time of recorded death and the corresponding incidence rates for each exposure group and outcome (Note that the first set of NDI results in 2013 will be necessary to evaluate the 30-day outcomes because of high anticipated mortality). We will then complete a proportional hazards analysis to estimate rate ratios and 95% confidence intervals.

Additionally, we will perform the above analyses separately in the following subgroups:

- Groups of age, sex, race, principal diagnosis of index hospitalization (grouping depending on observed occurrences in the study cohort)
- Days of antifungal therapy (calculated as days with at least one iv antifungal infusion during the index hospitalization)
- Receipt of hepatotoxic medications and nephrotoxic medications before or during the parenteral antifungal medication use during the index hospitalization

Analysis of unstructured EMR information

After the data extraction from the unstructured record is complete, we will compare the prevalence of each of the data elements from the unstructured record between groups formed by propensity matching on the structured record. If we can demonstrate that information from the unstructured data is balanced between treatment groups we will conclude that the propensity score matching based on structured information only was sufficient to make exposure groups comparable and the analysis is complete.

If there is meaningful residual imbalance between groups, the proportional hazards analysis will be repeated using the full information from the 10 percent of sampled records, in what becomes a "case-cohort" analysis.[44] In this analysis, all the sampled members of each case's risk set are matched to the case, and the determinants of case status are evaluated by conditional logistic regression. To account for the repeated appearance of individuals in multiple matched sets, we will use robust variance estimates.[45]

6.8. Mortality from Hepatocellular Carcinoma (HCC)

Using data from a linkage of the study cohorts to the US National Death Index, the following outcome will be ascertained over a period of up to 13 years following hospital discharge:

- Death from hepatocellular carcinoma (HCC)

There will be no long-term endpoint adjudication for HCC as the NDI death reports come from the state coroner offices and are informed by the medical information available at the point of death. It is highly like that patients dying of HCC are undergoing medical/surgical care and their diagnosis is well known to the patient, physicians and family members. This would make the cause of death by HCC highly specific.

6.9. Statistical Analysis for HCC Mortality

Counting death and the end of study period (December 2017) as censoring events we will compute for each patient the person time contributed to the analysis and calculate the rate of HCC mortality for each of the exposure groups. Through propensity score balancing, the study cohorts are expected to be empirically balanced at baseline so that no further adjustment will be necessary. We will perform a Kaplan Meier analysis to graphically display the survival functions for micafungin and the comparison group and perform a log-rank test.

Survival time will be calculated from the date of first PAF dosing in the index hospitalization until date of HCC death or censoring by death from other causes or data-cutoff.

We will perform a proportional hazards analysis to estimate hazard rate ratios and their 95% confidence intervals. Subsequently we will perform separate analyses for patients

who had 30 days or longer of parenteral antifungal therapy during the index hospitalization and those who had less than 30 days of therapy.

If the earlier comparison of characteristics derived from the unstructured text data has indicated meaningful differences, the analysis will be extended, as for 30-day outcomes, in a case-cohort analysis (see Section 6.7).

In a separate descriptive analysis we will tabulate the numbers of patients, person-time, and HCC death rates of all patients excluded from the primary analysis at various steps during the study. Such exclusions are necessary to ensure the comparability of the exposure groups in the primary analysis.

6.10. Study Size and Power

Below are counts of individual patients receiving either caspofungin or micafungin study drugs over a period of 6 years at one tertiary care center that will be invited to be a partner hospital.

	2005	2006	2007	2008	2009	2010	**2005-2010**
Caspofungin	509	554	42	3	<3	<3	**1,083**
Micafungin	<3	89	701	754	758	873	**3,026**
Fluconazole	467	378	357	441	436	728	**2,781**

In this tertiary care center about 3,000 patients were treated with micafungin and about 3,800 with either caspofungin or fluconazole. Caspofungin was substantially restricted starting 2007 either by a change in formulary or by new local treatment guidelines.

From this it is expected that the study will identify about 2,000 individual micafungin users per hospital. Due to exclusion criteria we assume that about 1,200 new micafungin users will participate on average from each hospital during the study period. Therefore, among the participating centers 7,000 micafungin users are estimated to be eligible for the study. In the feasibility analysis of the Premier data, we noted a substantial excess of users of other antifungal agents, so that we anticipate that it will be possible to create a larger comparison group. We have listed back-up hospitals in case micafungin use proves to be less frequent than assumed from the pilot data or entirely absent in any of the centers.

6.10.1. 30-day outcomes

We used the two-group continuity corrected Chi^2 test of equal proportions to assess the expected power for a comparison of micafungin versus a comparison group, conservatively assuming equal group sizes[46] and informed by the range of event risks (1% and 4%) observed in our preliminary study based on Premier data:[47]

Baseline event rate = 1%

Odds ratio	1.5	2.0	2.5	3.0	3.5
Power (%)	85	99	99	99	99
n per group	7,000	7,000	7,000	7,000	7,000

Baseline event rate = 4%

Odds ratio	1.5	2.0	2.5	3.0	3.5
Power (%)	99	99	99	99	99
n per group	7,000	7,000	7,000	7,000	7,000

There is ample statistical power to demonstrate even small differences in 30-day event rates between groups. The main reason for this high power is that the study size is powered towards identifying difference in the risk of hepatocellular cancer, a much less frequent outcome.

It is planned to reassess power considerations for 30-day outcomes by the end of 2011. If the power calculation indicates insufficient users of parenteral micafungin or the comparison agents then the enrollment period will be extended and/or additional hospitals will be recruited.

6.10.2. Long-term outcomes

The study was originally designed with the expectation that the 35,000 patients planned for the cohort would consist of 7,000 eligible users of micafungin and 28,000 users of comparator agents. Review of sales data and preliminary inquiries with potential partner hospitals suggest that the available number of micafungin users may be larger, so that the cohort could consist of up to 9,000 users of micafungin, with correspondingly smaller numbers of users of comparators to maintain the same the overall cohort size of 35,000.[1]

The expected number of person-years of follow-up in the micafungin users will be governed by the distribution of calendar years of entry into the cohort and the expected survival through 2018. Because the comparator cohort is to be selected with matching on calendar year of entry, the expected number of person years in comparators is the expected number in micafungin users, multiplied by the matching ratio, assuming the null hypothesis and a balanced distribution of risk factors for death in the compared groups.

[1] Note that for a fixed total cohort size, the power will increase with increasing numbers of micafungin users, up to the point at which the expected number of cases in micafungin users on the alternative hypothesis equals the expected number in the comparators, that is where the ratio of comparators to micafungin users equals the relative risk.

For the purposes of planning, the expected survival is based on the empirical experience of 845 recipients of amphotericin B, as previously reported.[2] The table below gives the observed survival in those patients and the survival that has been assumed for the present analysis. Note that the survivals beyond seven years represent guesses that the annual late mortality might be on the order of 5-10 percent per year.

Table of mortality observed in amphotericin B users 2000-2001 and assumed mortality for the present study in relation to years of follow-up

Years of Follow-up	At Risk at Start of Interval	Interval Deaths	Interval Mortality	Cumulative Deaths	Cumulative Mortality	**Assumed Cumulative Mortality**
1 yr	845	423	0.501	423	0.501	**0.50**
2 yr	422	43	0.102	466	0.551	**0.55**
3 yr	379	20	0.053	486	0.575	**0.58**
4 yr	359	18	0.050	504	0.596	**0.60**
5 yr	341	15	0.044	519	0.614	**0.61**
6 yr	326	17	0.052	536	0.634	**0.63**
7 yr	309	3	0.010	539	0.638	**0.64**
8 yr	306	0	0.000	539	0.638	**0.66**
(9 yr)						**0.68**
(10 yr)						**0.70**
(11 yr)						**0.72**
(12 yr)						**0.74**

We have used US sales data from Astellas for calendar years 2005 through 2010 to estimate the temporal distribution of micafungin entrants into the cohort. In the table below "Number of entrants" for each calendar year is calculated as 7000 (the originally planned cohort size) multiplied by the fraction of 2005-2010 sales. The cohorts were then aged through 2018, assuming for the purposes of calculation that all entries took uniformly through each calendar year and that all deaths were evenly distributed through each follow-up year. Total follow-up was summed over all years of entry and observation.

[2] WHISCON. Empirical Feasibility Analysis of Using the United States National Death Index for the Surveillance of Hepatocellular Carcinoma in Patients Identified through the Premier Perspective Comparative Database. Prepared for Astellas Pharma R&D. 15 November 2010

Table of estimated distribution of cohort entrants, and person years of follow-up, by calendar year assuming a total of 7,000 eligible micafungin users

Year	2005	2006	2007	2008	2009	2010	Total
Sales[a]	0.22	0.98	1.12	1.00	1.76	1.96	7.03
Entrants	220	971	1,116	995	1,752	1,947	**7,000**
Year ending in June							
2006	165						165
2007	104	728					832
2008	96	461	837				1,393
2009	90	422	530	747			1,789
2010	87	398	485	473	1314		2,757
2011	83	383	457	433	832	1460	3,649
2012	80	369	441	408	762	925	2,985
2013	77	354	424	393	718	847	2,813
2014	72	340	407	378	692	798	2,688
2015	68	320	391	363	666	769	2,577
2017	64	301	368	348	639	740	2,460
2018	59	282	346	329	613	710	2,339
Dec2018	30	141	173	164	307	355	1,169
Total	1075	4499	4859	4037	6543	6604	27,617
Average	4.9	4.6	4.4	4.1	3.7	3.4	3.9

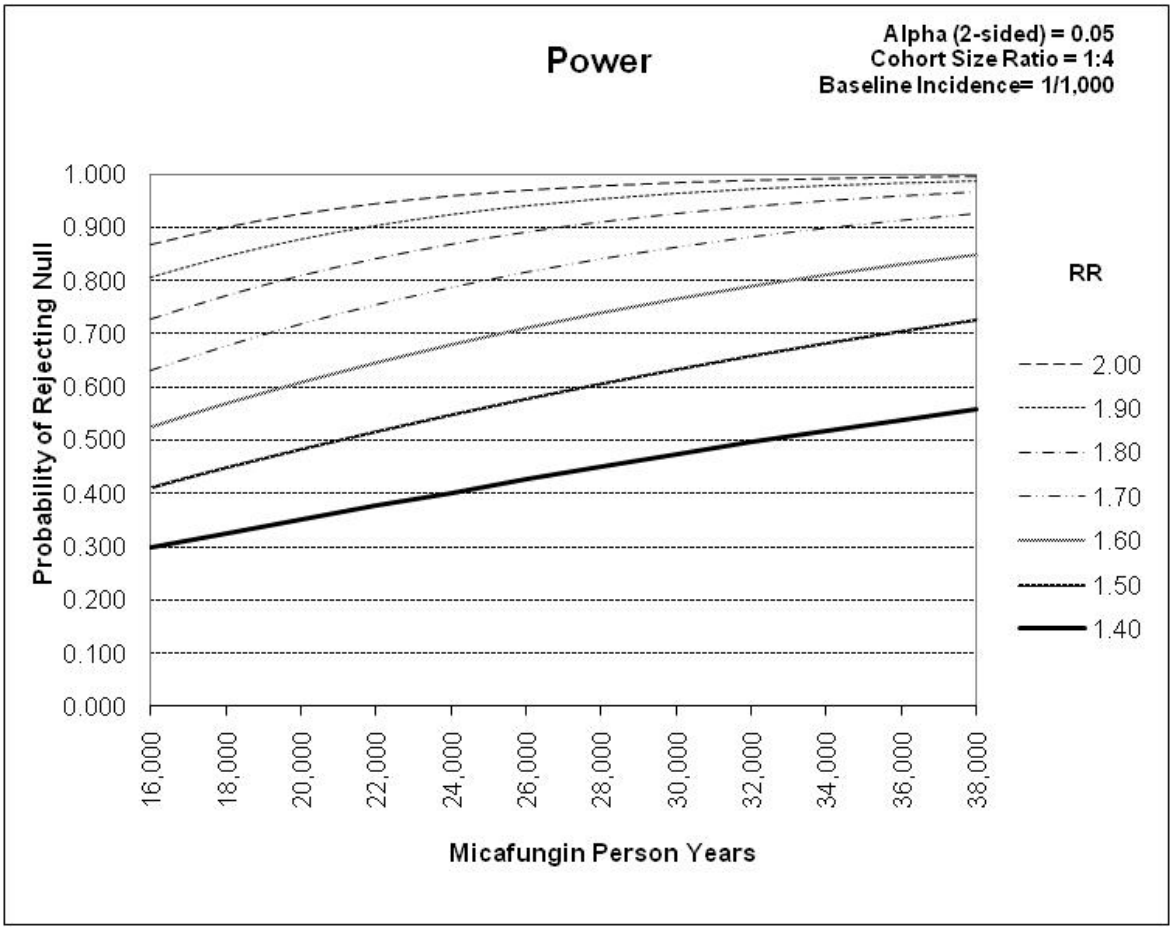
[a] Relative sales, using 2008 as a reference year, for hospitals being considered for inclusion in the cohort. Choice of the reference year does not affect the expected distribution of cohort entrants.

Under the initial study assumptions, there would be just under 28,000 person years of expected follow-up. Because of the fixed administrative censoring date at the end of 2018, the average follow-up decreases from 4.9 years for the 2005 cohort to 3.4 for the 2010 cohort with an overall average of 3.9 years.

The incidence of HCC in US residents around the anticipated age of cohort members is about 10 per 100,000 persons per year. The patients themselves are likely to derive in part from extraordinarily high-risk segments of the population, with risks as much as 100 times higher. We have provisionally put the expected incidence of HCC at 100 per 100,000 persons per year. If patient ineligibility reduces the size of the study cohorts power will be diminished correspondingly. The figure shows that a loss of 1,000 micafungin patients (about 4,000 micafungin person-years) would result in a power of >90% to detect an RR of 1.9.

The first figure below gives the power to detect various levels of relative risk of HCC in a study population compared against a four-fold larger comparison as a function of the number of person-years observed in the micafungin group. The anticipated study of 7,000 micafungin recipients (28,000 person years) and 28,000 comparators (112,000 person years) would have greater than 90 percent power to detect relative risks of 1.8 or higher at a two-sided alpha level of 0.05.

Power to detect various levels of relative risk (RR) of HCC in a population with an expected incidence of HCC of 100/100,000 per year and a 1:4 ratio of comparators to micafungin.[48,49]



As mentioned above, preliminary indications are that the recruitment of micafungin users might exceed the originally planned goal of 7,000. With an increasing number of eligible micafungin users the statistical power of this study will slightly increased even if the overall study size remains constant at 35,000 patients.

If survival exceeds that of amphotericin B users in 2000-2001, the anticipated number of person years of observation in the cohorts will increase and the power of the study will be correspondingly higher.

It is planned to revisit the power considerations for studying HCC mortality in 2013 after the first NDI linkage has been completed. A lower-than-expected mortality through 2011 (considering a 2+ year lag time for NDI data) would increase the power of the study of long-term HCC incidence. A lower mortality rate than predicted from our feasibility study would be compatible with progress in the medical care of these patients. A higher-than-expected mortality will reduce power. Information on the number of eligible micafungin and control patients will also then be available. If necessary, we will address reduced power by extending accrual of subjects from the already participating hospitals.

If this strategy is not providing sufficient power, Astellas will take measures to increase the number of participating hospitals.

The NDI feasibility study also indicated that the median survival of the 2000-2001 amphotericin B patients was one year. This means that a substantial fraction of all the person time of observation will occur very early after cohort entry. The implications of this for study power depend on the anticipated model of carcinogenesis and the corresponding time course of risk.

Some cancer-causing agents induce cancers rapidly. Lymphoma associated with immunosuppressant therapy appears within the first or second year of treatment, and those associated with at least one agent (OKT3) appear within months. Under a multistage model of carcinogenesis, these are termed late-stage promoters. If the effect of any of the parenteral antibiotics is to promote the appearance of tumor from already transformed precursors, by promoting cell division, one might expect an early effect. In this case the early years of follow-up are fully informative about risk, and the study power is unaffected by competing mortality.

Most documented carcinogens have substantially longer periods between exposure and first appearance of tumor. If the postulated mechanism of carcinogenicity leads to delayed effects, competing mortality poses a serious threat to investigation of carcinogenicity, as the relevant years of experience, long after exposure, may be only a small fraction of the total follow-up. In this context, the seven-year survival of 34 percent in the NDI feasibility study may indicate that there will only be a third of the relevant person-years of follow-up available long after exposure.

Excerpt from the NDI Feasibility study report:[32]

"Overall person-time in cohort was calculated from cohort enrollment to death or end of study, which was December 31, 2007. Many deaths occurred after the last known follow-up within Premier, and these were included in the survival analysis, so that overall there were a total of 555 valid deaths out of 845 patients resulting in a seven-year risk of death of 66 percent. The cohort follow-up included 2,486 patient-years resulting in an all cause mortality rate of 22.3 deaths per 100 person years. Although the potential follow-up extended from 2000-2001 through the end of 2007, because of the high mortality rate, the mean follow-up to death or end of 2007 was 2.9 years and the median follow-up was 0.84 years.

*The majority of deaths occurred within the first year after discharge of the index hospitalization (**Figure 5.1**). There was a slightly higher risk of death among women compared to men (**Figure 5.2**) and the risk of death increased with increasing age (**Figure 5.3**).*

Figure 5.1: Kaplan-Meier plot of all cause death after index hospitalization Years Following Receipt of Amphotericin B

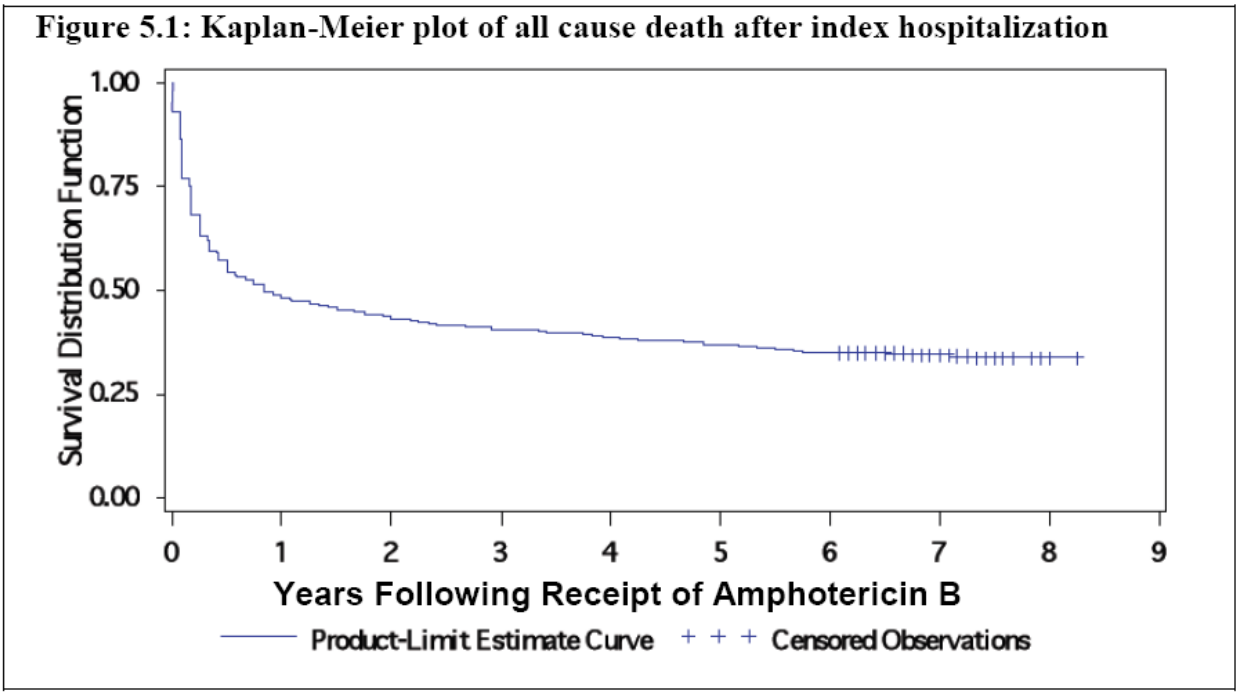


Figure 5.2: Kaplan-Meier plot of all cause death after index hospitalization by patient sex Years Following Receipt of Amphotericin B Male Female

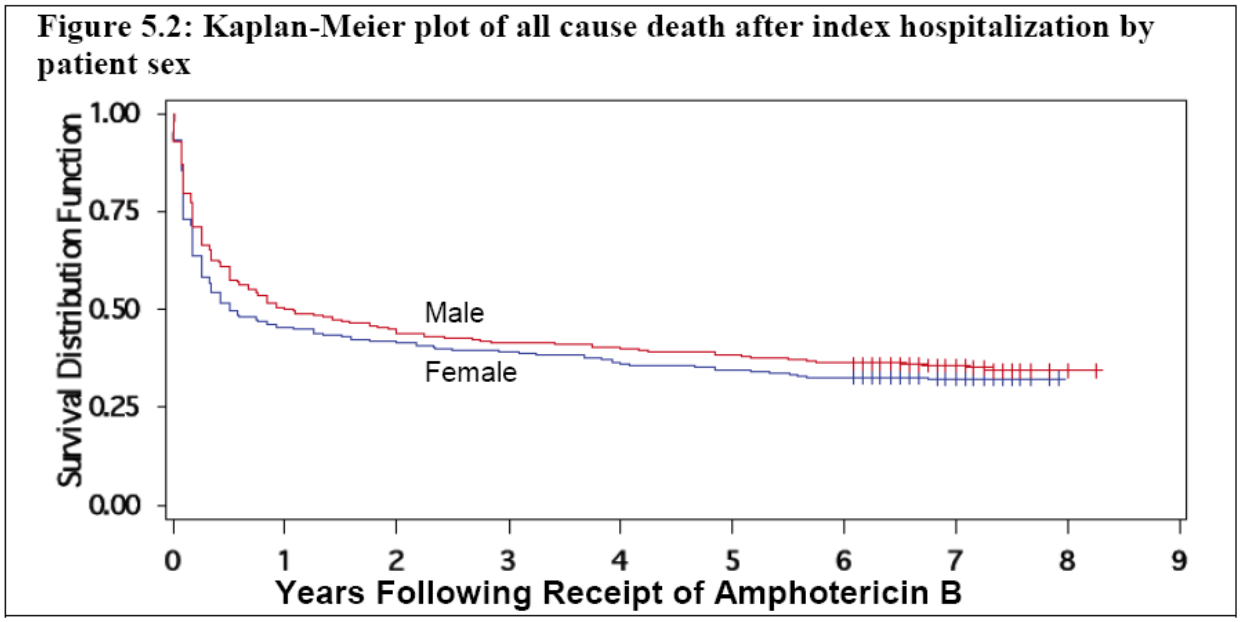
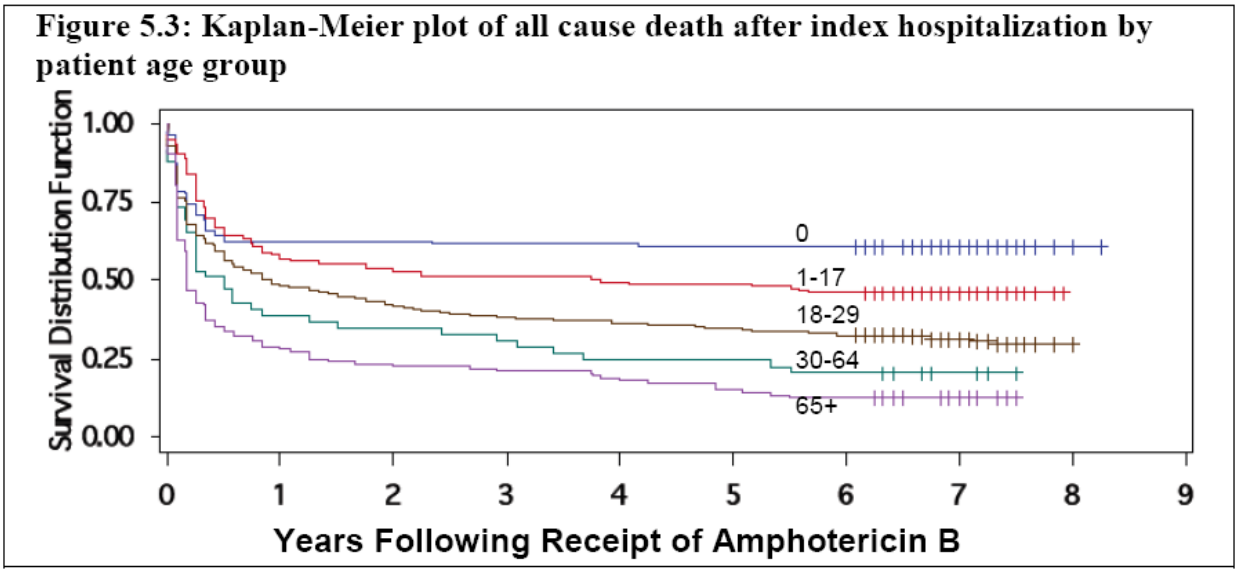


Figure 5.3: Kaplan-Meier plot of all cause death after index hospitalization by patient age group Years Following Receipt of Amphotericin B 0 1-17 18-29 30-64 65+



The study patients, identified by the amphotericin B use in 2000-2001 had a mortality rate of 22 per 100 patient-years and a seven-year risk of dying of about 66% with 50% of patients dying in the first year of follow-up. Among the deaths there were none whose primary or contributing cause of death was primary liver cancer.

Causes of death in the cohort were predominantly attributable to the immunocompromised conditions that presumable had made patients susceptible to systemic fungal infections."

End of excerpt from NDI feasibility report.

7. Limitations of Study Design

As with any study, there are limitations to the design described in this protocol. Since patients will be selected from among those admitted to one of the participating centers, it is possible that their characteristics may differ from recipients of micafungin and other parenteral antifungal medications in general. These differences could reduce the generalizability of the study results. To address this limitation, we will provide a description of the study cohorts that will permit assessment of their similarity or difference from other populations.

Exposure categorization will derive from hospital records that are used for routine patient care, so that their accuracy is expected to be high. However misclassification of exposure cannot be completely ruled out if, for example, patients receive parenteral antifungal therapy outside of the hospital without a record of such exposure.

The 30-day study outcomes assessment (hepatic injury or dysfunction, renal failure or dysfunction, and rehospitalization for the parenteral treatment of fungal infections) should be identified with high specificity within the hospital records, but the sensitivity of outcome identification will likely be incomplete since patients could receive care for one of these outcomes at another hospital. High specificity will be further assured through the adjudication process for the 30-day outcomes hepatic injury or dysfunction, and renal failure or dysfunction. High specificity of study outcome ascertainment should result in minimal bias in study effect estimates.

Confounding could result if recipients of micafungin differ from recipients of other parenteral antifungal medications in ways that are prognostic of the study outcomes. This study will involve extensive confounder control through design (propensity score) and includes an assessment of additional confounding through medical record review.

It is possible that the projected sample size is not reached or that other assumptions used is estimating the sample size turn out to be incorrect. If either of these were to happen, the statistical power of the study could be reduced so that it addresses the objectives with less certainty. Through the periodic reports, the accrual of patients according to exposure group and the occurrence of outcomes among them will be closely monitored and the study can be modified to address study size issues if needed.

8. Data Privacy and Data Use Agreements

8.1. Ethical Approval

Each Site PI will be responsible for obtaining Institutional Review Board approval at his or her institution in addition to an overall study IRB that WHISCON will obtain through the New England Institutional Review Board (NEIRB). The project does not involve patient contact, but does involve the use of protected health information in order to create the de-identified data sets that are to be pooled.

8.2. Data Use

This is a fully passive reporting system, involving the examination of existing medical records and the use of publically available information source. Hospital-specific data remains the property of each partner hospital and is shared with the data coordinating center at WHISCON in a de-identified format for the purpose of the study analysis and reporting, as specified in and governed by each institution's IRB.

The pooled data source is only to be used by WHISCON investigators for the purpose of this study. Its components continue to belong to the contributing medical centers and the pooled data source will be destroyed 5 years after submission of the final study report. For purposes of quality control and audit regulatory agencies, including the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA), and the study sponsor will be allowed to audit these data residing at WHISCON.

9. Human Subjects

9.1. Benefits of this Research

Hepatic and renal toxicity have been seen with the echinocandins in clinical trials. There are insufficient available data on the frequency of these effects in routine care in comparison to other parenteral antifungal agents.

This multicenter cohort study proposes to establish the risks of 30-day liver and nephrotoxicity and long-term risk of hepatocellular carcinoma for periods of up to 13 years in users of parenteral micafungin and in users of other parenteral antifungal agents.

The insights of this study will lead to a better understanding of the safety of these products and ultimately to better patient care. We will publish the findings of this study in the peer-reviewed literature so that the medical profession and ultimately our patients will benefit from this study.

9.2. Risks of this Research

This study does not involve any risks to patients since it is not intervening on or contacting any patient, but is only performing retrospective analyses using previously collected health care utilization data stored in the electronic medical records database. As described in the protocol above, we have several safeguards in place to protect patients' privacy to the highest possible level. The WHISCON PI will never be in the possession of identifiable patient information. For purposes of audits and quality control WHISCON staff will visit the partner hospitals and together with the hospital staff will observe the abstraction process from the original electronic medical records that may contain identifiable information. Such information will not at any time be retained by WHISCON staff. Identifiable information that is necessary for linkage with the National Death Index is only transferred between the partner hospitals and the NDI directly and without any attached health information to protect patient privacy. The risk of disclosure of individual identities is extremely low.

This protocol describes a retrospective study that is based on previously recorded electronic medical records and death certificates. In these records there will be no attribution of causality to the events under study nor will there be an identifiable reporter of a potential adverse drug reaction. Therefore there is no need for expedited reporting of potential adverse drug reactions according to current guidelines.

9.3. Risk/Benefit Assessment

Given the benefits of better understanding the safety and effectiveness of parenteral antifungal medication use and the non-existent or less than minimal risks for patients we conclude that the risk/benefit balance is strongly in favor of the study's benefits.

9.4. Waiver of Informed Consent

We request a waiver of informed consent for this retrospective study based on previously collected data. It would be impractical to receive informed consent in retrospect for large numbers of patients, some of which will be deceased. The risks of this research are less than minimal as described above and the benefits strongly outweigh such risks. A waiver of informed consent will not affect the rights and welfare of the research subjects. This research is not intervening or contacting patients but is a retrospective analysis of previously collected data. Any findings from this study will ultimately benefit patient care.

9.5. Jurisdiction of Parent Ethics Approval by the New England IRB

The proposed study is a multi-center effort. While all analyses are performed at WHISCON, LLC, which is directly covered under the NEIRB approval, each partner hospital has its own human subjects committee that is responsible for the ethical conduct of the local research activities.

9.6. WHISCON's Relationship with the Partner Hospitals

WHISCON will have a contractual relationship with all the partner hospitals. This includes strict regulations on data confidentiality and regulations on handling identifying information between WHISCON and the partner hospitals. The following aspects are relevant for this proposal:

1) Partner hospitals never release identifiable information to WHISCON. Any data delivered to WHISCON will be HIPAA compliant.

2) In order for the partner hospitals to link data to the National Death Index - the long-term outcome of this research proposal - patient identifying information is needed. As outlined above, this identifying information will at no point be passed on to WHISCON or any other entity other than the National Death Index, a trusted federal agency and part of the Centers for Disease Control (CDC). The only purpose is to identify the date and cause of death in patients who have passed away. Any identifying information will be deleted after the NDI information has been retrieved by the partner hospitals. Only information of date and cause of death as well as NDI's information on the likely linkage quality will be passed on to WHISCON investigators.

10. Data Quality/Integrity

The source EMR data is used by participating hospitals for clinical care of patients and is maintained at a high quality level for that purpose. Information that is derived from the EMR (as will be used for cohort formation) will depend on the reliability of the data transformation process. Since the transformation of EMR data to study data will be conducted by each of the participating centers, we will standardize this process as much as possible across centers. We will conduct internal checks for quality and consistency of all data received from the participating centers. These checks will be conducted both

cross-sectionally and longitudinally to detect variances within and between participating centers.

From the 10 percent sample of patients for whom unstructured EMR data will be obtained, we will further select a 10 percent sample for re-abstraction to evaluate the reproducibility of the EMR extraction process. Reproducibility metrics will be developed for variables extracted from the EMR individually and collectively.

11. Dissemination

The protocol and amendments will be posted at clinicaltrials.gov and at the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (encepp.eu).

Study reports will be made according to the schedule in the timeline and milestones section.

Astellas may use and disseminate all reports as it sees fit.

WHISCON will submit two manuscripts based on this work for publication in the scientific literature (see time line in Section 1.6). These publications will acknowledge Astellas support. Authorship will follow the usual rules for scientific publication, and may include WHISCON investigators, members of the Scientific Steering Committee (that is, the local PI's), and Astellas employees, as appropriate. WHISCON and Astellas may jointly decide on additional publications.

12. Key Personnel

12.1. Principal Investigator Sebastian Schneeweiss, MD, ScD

Sebastian Schneeweiss, M.D., Sc.D., is an Associate Professor of Medicine at Harvard Medical School, and Director for Drug Evaluation and Outcomes Research and Vice Chief of the Division of Pharmacoepidemiology and Pharmacoeconomics at Brigham and Women's Hospital. A physician and pharmacoepidemiologist, his research centers on the safety, effectiveness, and economics of pharmaceuticals and biologics.

Dr. Schneeweiss received his medical degree from the University of Munich and a doctoral degree in Epidemiology from Harvard, where he is an Associate Professor of Epidemiology in the Department of Epidemiology. His current NIH-funded research focuses on the comparative safety and effectiveness of medications and clinical and economic consequences of drug reimbursement restrictions using observational and randomized designs. Developing and testing new pharmacoepidemiologic methods using large computerized claims databases is an important part of his research and teaching. Dr. Schneeweiss is Principal Investigator of the BWH DEcIDE Research Center on Comparative Effectiveness Research funded by AHRQ. He is Past President of the International Society for Pharmacoepidemiology.

12.2. Senior Scientist: Alexander M. Walker, MD, DrPH

Alexander M. Walker, MD, DrPH, a Principal at WHISCON, is Adjunct Professor of Epidemiology at Harvard School of Public Health, where he was formerly a professor and Chair of the Department of Epidemiology. His research encompasses the safety of drugs, devices, vaccines and medical procedures. Current studies include post-marketing safety studies for recently approved drugs, natural history of disease studies to provide context for Phase III clinical trials, studies of the impact of drug labeling and warnings on prescribing behavior, and determinants of drug uptake and discontinuation. Additional areas of research and expertise include health effects of chemicals used in the workplace and statistical methods in epidemiology. Dr. Walker received an MD degree from Harvard Medical School in 1974, and a doctorate of Public Health in Epidemiology from the Harvard School of Public Health in 1981. Dr. Walker is associate editor of Pharmacoepidemiology and Drug Safety and is on the Board of Directors of the International Society for Pharmacoepidemiology, which he also served as President in 1995-1996. He was a statistical consultant for the *New England Journal of Medicine* from 1992 through 1996 and a Contributing Editor of *The Lancet* from 1999 through 2001. From 2000 through 2007, he served as Senior Vice President for Epidemiology at Ingenix. Dr. Walker has written or contributed to over 275 peer-reviewed articles in drug safety, epidemiology and occupational health, and is the author of a book of essays, *Observation and Inference: an Introduction to the Methods of Epidemiology*.

12.3. Other Personnel

The principal investigator and senior scientist will be assisted by other WHISCON staff and consultants along with External investigators.

Deborah Hennessey, Principal at WHISCON. Ms. Hennessey is responsible for contracting and administrative support. She works closely with Drs. Schneeweiss and Walker.

Lisa Weatherby, MS, Senior Project Manager: Ms. Weatherby is responsible for coordinating the research activities across all centers. She works closely with Dr. Schneeweiss and the participating centers.

John Seeger, PharmD, DrPH, Senior Pharmacoepidemiologist: Dr. Seeger is Lecturer in Medicine, Brigham and Women's Hospital and Harvard Medical School with 15 years experience in pharmacoepidemiolgy study design and analysis. He will support the project team with a broad range of pharmacoepidemiology expertise.

Local site investigators from each of the participating centers (to be determined) will also be involved in the conduct of this study.

13. Appendix

The codes and terms used here are provisional, and may be modified after review with the clinical leaders and inspection of the respective EMR databases.

13.1. Appendix 1: Definition of pre-existing liver and kidney diseases

Diagnoses and procedures marking pre-existing liver disease:

Hepatitis A, B, C, D, E

Non-virus hepatitis

Cirrhosis, including ascites, intraabdominal venous shunt, esophageal varices

Congenital liver disorders, including Wilson's disease, hemochromatosis, Gilbert's syndrome, Glycogen storage disease, Amyloid degeneration

Liver cancer, any type including metastases of other origin

Jaundice 277.3

ICD-9: 070.x (Viral hepatitis)

ICD-9: 570.x through 574.x

ICD 570 Acute and subacute necrosis of liver
ICD 571 Chronic liver disease and cirrhosis
ICD 572 Liver abscess and sequelae of chronic liver disease
ICD 573 Other disorders of liver

ICD-9: 456.0-456.2x, (Esophageal varices)

ICD-9: 39.1 (Intra-abdominal venous shunt)

ICD-9: 42.91 (Ligation of esophageal varices)

ICD-9: 155.0 (primary cancer of liver)
ICD-9: 155.1 (cancer of intrahepatic bile ducts)
ICD-9: 155.2 (cancer of liver not specified as primary or secondary)

ICD-9: 277.3 (Amyloid degeneration),

ICD-9: 782.4 (jaundice unspecified not of newborn)

Laboratory test results marking pre-existing liver dysfunction:

ALT larger than 5 times the upper normal level or more than 300 U/L on any day of the index hospitalization the preceded the initiation of parenteral antifungal therapy. We focus on ALT only since chances are high that every patient in this sick patient

population will have ALT tests done during the admission process and before initiating antifungal therapy.

Diagnoses and procedures marking kidney disease:

Dialysis (hemo, peritoneal, filtration)

Chronic kidney disease, kidney failure, acute kidney failure

Nephritis, glomerulonephritis

Renal cancer

ICD-9: 580.x through 589.x (acute and chronic kidney diseases),

- ICD 580 Acute glomerulonephritis
- ICD 581 Nephrotic syndrome
- ICD 582 Chronic glomerulonephritis
- ICD 583 Nephritis and nephropathy not specified as acute or chronic
- ICD 584 Acute renal failure
- ICD 585 Chronic kidney disease (CKD)
- ICD 586 Renal failure unspecified
- ICD 587 Renal sclerosis unspecified
- ICD 588 Disorders resulting from impaired renal function
- ICD 589 Small kidney of unknown cause

ICD-9: V56.x, (history of procedures related to dialysis)

- ICD V56.0 (Extracorporeal dialysis)
- ICD V56.1 (Fitting and adjustment of extracorporeal dialysis catheter)
- ICD V56.2 (fitting and adjustment of peritoneal dialysis catheter)
- ICD V56.8 (peritoneal dialysis)

ICD-9: 39.27, 39.42, 39.93-39.95, 54.98 (Dialysis etc.)

- ICD 39.27 (Arteriovenostomy for renal dialysis)
- ICD 39.42 (Revision of shunt)
- ICD 39.93 through 39.95 (Hemodialysis)

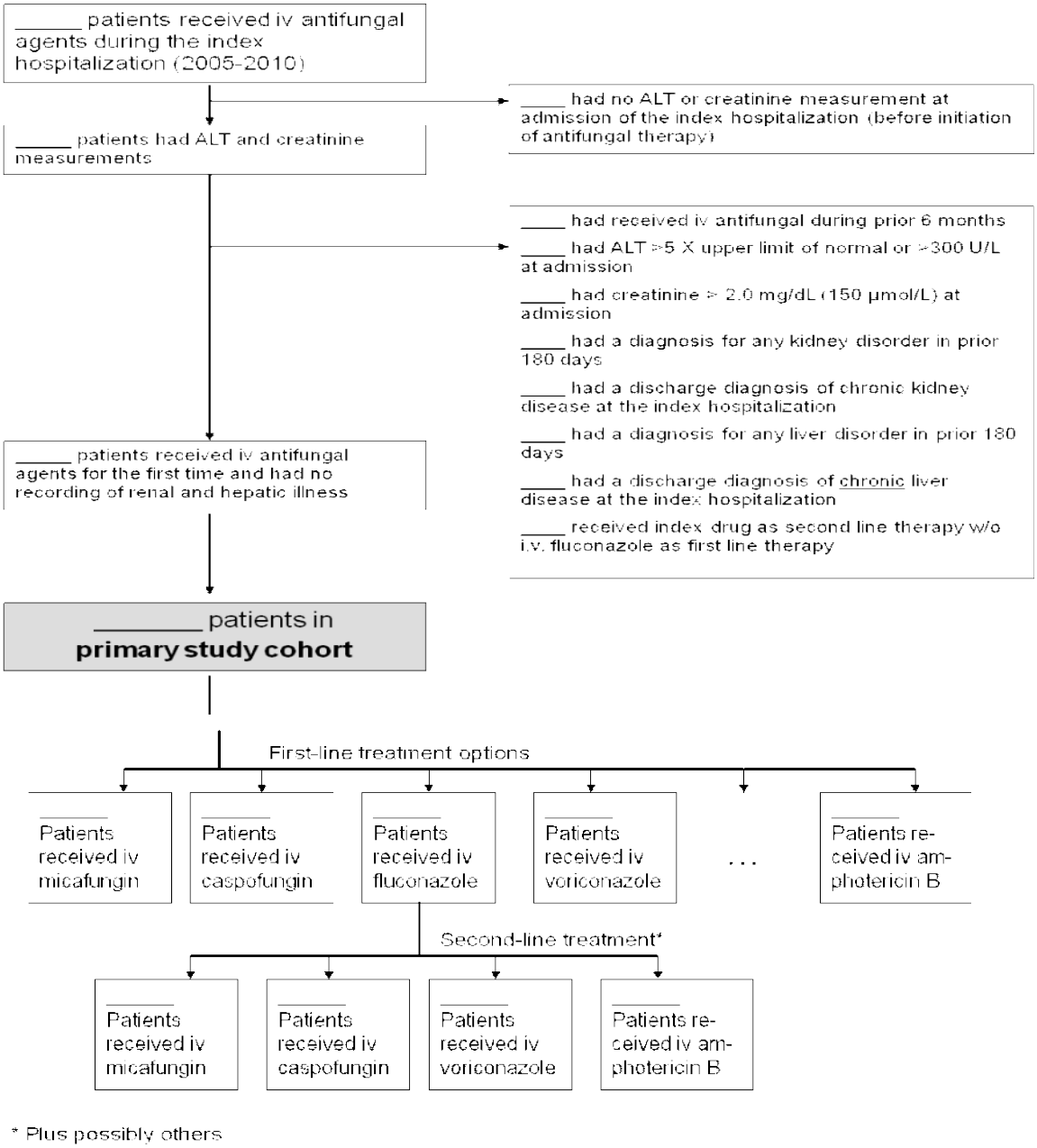
ICD 54.98 (Peritoneal Dialysis)

ICD-9: 189.0 (cancer of kidney except pelvis)
ICD-9: 189.1 (cancer of renal pelvis)

Laboratory test results marking pre-existing renal dysfunction:

Serum creatinine of 2.0 mg/dL (150 μmol/L) or higher. We focus on creatinine since chances are high that every patient in this sick patient population will have creatinine tests done during the admission process and before initiating antifungal therapy.

13.2. Appendix 2: Patient flow chart



* Plus possibly others

Note: Patients that do not fit the study inclusion criteria and therefore not contribute to the primary study analysis will be identified, set aside, and described regarding their baseline characteristics and risk of death by HCC.

13.3. Appendix 3: Exposure definitions

Generic injectable	Brand injectable	Description
Micafungin	Mycamine	MICAFUNGIN, MYCAMINE INJ 50MG
		MICAFUNGIN, MYCAMINE INJ 100MG
Caspofungin	Cancidas	CASPOFUNGIN, CANCIDAS VL 50MG
		CASPOFUNGIN, CANCIDAS VL 70MG
Anidulafungin	Eraxis	ANIDULAFUNGIN, ERAXIS INJ 50MG
Fluconazole	Diflucan	FLUCONAZOLE, DIFLUCAN IV PREMIX 100MG
		FLUCONAZOLE, DIFLUCAN IV PREMIX 200MG
		FLUCONAZOLE, DIFLUCAN IV PREMIX 400MG
		FLUCONAZOLE, DIFLUCAN VL 100MG 50ML
		FLUCONAZOLE, DIFLUCAN VL 200MG 100ML
		FLUCONAZOLE, DIFLUCAN VL 400MG 200ML
Voriconazole	Vfend	VORICONAZOLE, VFEND VL 200MG
Amphotericin B	Fungizone,	AMPHOTERICIN B(CHOL), AMPHOTEC VL 100MG
	Amphotec,	AMPHOTERICIN B(CHOL), AMPHOTEC VL 50MG
	Abelcet,	AMPHOTERICIN B(LIPID), ABELCET VL 100MG
	Ambisome	AMPHOTERICIN B(LIPID), ABELCET VL 50MG
		AMPHOTERICIN B(LIPO), AMBISOME VL 50MG
		AMPHOTERICIN B, FUNGIZONE IVPB 50MG
		AMPHOTERICIN B, FUNGIZONE VL 100MG
		AMPHOTERICIN B, FUNGIZONE VL 50MG
Anidulafungin	Eraxis	ANIDULAFUNGIN, ERAXIS INJ 50MG
Itraconazole	Sporanox	ITRACONAZOLE, SPORANOX INJ KIT 10MG/ML IVPB

13.4. Appendix 4: Covariate definitions

All patient characteristics will be assessed before the index hospitalization. Factors recorded as discharge diagnoses during the index hospitalization will only be considered if they are chronic conditions undoubtedly present before the hospitalization.

Characteristics	**Notes re. variable definition**	**Specific codes and definitions**
Age	Predefined age categories	0 to 17 years old 18 to 40 years old 40 to 65 years old Over 65 years old Also code as continuous: age + age^2 terms
Sex		Sex Code = M and F
Race/ethnicity		1 = White = reference 2 = Black 3 = Other (Hispanic, American Indian, Asian, Pacific Islander)
Smoking	Any smoking	ICD V15.82 (History of tobacco use)
Admission Year	In calendar years	2005, 2006, 2007, 2008, 2009, 2010
Admission type	Emergency vs. elective	UB-92 Admission Types Emergency = 1 or 2 Non-emergency = all other codes
Low Income Status	Medicaid, indigent	UB-92 Payor Code = 330, 340, 350, 400
Marital Status	With partner vs. single	UB-92 Marital Status 1, 7 = with partner all other codes = no partner
Primary d/c/ Dx of index hospitalization	Grouping dependent on occurrence	
Days of therapy with antifungal agent	See definitions of agents in Appendix 3	<30 days, 30 days or more
Preexisting Percutaneous coronary procedures	HX of percutaneous procedure or procedures during index hospitalization but before CABG Surgery	ICD V45.82 (History of PTCA, at any time) ICD 36.01 through 36.09 (before index date)
Diabetes	Discharge diagnosis of diabetes or antidiabetic drug use on more than 2 days during the hospital stay	ICD 250.x Diabetes medications: Insulin; metformin; sulfonylureas (including chlorpropamide, acetohexamide, tolazamide, tolbutamine, glipizide, glimepiride, glyburide); acarbose, miglitol; meglitinides (repaglinide, nateglinide); thiazolidinediones (rosiglitazone, pioglitazone); glucagon.
Hypertension	Discharge diagnosis	ICD 401.x (Primary HTN) ICD 405.x (Secondary HTN)
COPD/asthma	Discharge diagnosis	ICD 490.x, 491.x, 492.x, 493.x, 496.x

Characteristics	Notes re. variable definition	Specific codes and definitions
Cancer	Discharge diagnosis	ICD 140-208 (excluding 173) ICD V10.x (History of personal malignant neoplasms)
Old MI	Discharge diagnosis	ICD 412.x, 411.0 (post myocardial infarction syndrome)
Old Stroke	Discharge diagnosis	ICD V12.59
Endocarditis	Discharge diagnosis	ICD 421.x
Peripheral artery disease	Discharge diagnosis	ICD 443.9, 440.2
Hemostatic disorder (Dx of idiopathic thrombocytopenia, hemophilia, protein S deficiency, protein C deficiency, factor Leiden, or leukemia)	Discharge diagnosis	ICD 287.3 (primary ITP), 286.0-286.4 (hemophilias), 289.81 (primary hypercoagulable state), 208 (acute, chronic leukemia)
Angina	Drug (nitrate)	Nitrates: Amyl nitrite, isosorbide dinitrate, isosorbide mononitrate, nitroglycerin
Heart Failure	Drug (loop diuretic, digoxin)	Dopamine, dobutamine, digoxin, digitoxin, furosemide
Being on warfarin	Warfarin	Warfarin, Coumadin, Jantoven, Marfarin
Being on heparin	Heparin	Unfractionated heparin, low molecular weight heparin
Fibrinolytic medications or direct thrombin inhibitors	Fibrinolytics or direct thrombin inhibitors prior to index surgery or on the day of index surgery	ICD 36.04 (Thrombolysis) **OR** Meds: streptokinase, alteplase, anistrplase, tissue plasminogen activator (TPA), reteplase, tenecteplase, urokinase, lanoteplase, hirudin, bivalirudin, argatroban, melagatran
Use of clopidogrel or glycoprotein 2b/3a inhibitors		Meds: Clopidogrel (Plavix), abciximab (ReoPro) eptifibatide (Integrilin), tirofiban (Aggrastat)
Use of plasma expander	Plasma expander on the day(s) before or during index CABG	Meds: Albumin, hetastarch, pentastarch, dextran
Use of radiologic contrast media		
Arrhythmia	Antiarrhythmic drug (consider separate variables: beta-blocker, others)	Antiarrhthymics: amiodarone, dofetelide, disopyramide, flecanide, moricizine, procainamide, propaphenone, quinidine, and sotalol, Beta blockers: Acebutolol, esmolol, propranolol, Calcium channel blockers: Verapamil, diltiazem, bepridil.

Characteristics	Notes re. variable definition	Specific codes and definitions
Hospital		
Hospital size	Reported by the institution	# of beds <400 400-649 650+
Hospital antifungal volume	According to our study cohort	# of study subjects who received iv antifungal therapy admitted to each hospital per year 0-49 50-100 >100*

* the final categories will be defined after inspection of antifungal utilization patterns in the partner hospitals.

Hepatotoxic drugs:

We will define a binary covariate for use of potentially hepatotoxic medications at the index hospitalization before or during antifungal therapy. The following medications are identified as hepatotoxic and will be included in our definition:

According to Lee:[50]

isoniazid, trazodone, diclofenac, nefazodone, venlafaxine, lovastatin, chlorpromazine, estrogen, oral contraceptives, erythromycin, phenytoin, sulfamethoxazole, diltiazem, quinidine, didanosine, tetracycline, aspirin, valproic acid, amiodarone, tamoxifen, nitrofurantoin, methyldopa, minocycline, methotrexate, nicotinic acid, androgens, amoxicillin-clavulanate, carbamazepine, cyclosporine, methimazole, troglitazone, acetaminophen, bromfenac, cyclophosphamide

According to Navarro and Senior:[51]

Acarbose, acetaminophen, allopurinol, amiodarone, baclofen, bupropion, fluoxetine, HAART drugs, isoniazid, ketoconazole, lisinopril, losartan, methotrexate, NSAIDs, omeprazole, paroxetine, pyrazinamide, rifampin, risperidone, sertraline, statins, tetracyclines, trazodone, trovafloxacin, valproic acid, amitriptylene, azathioprine, captopril, carbamazepine, clindamycin, cyproheptadine, enalapril, flutamide, nitrofurantoin, phenobarbital, phenytoin, sulfonamides, trazodone, trimethoprim-sulfamethoxazole, verapamil, amoxicillin-clavulanate, anabolic steroids, chlorpromazine, clopidogrel, oral contraceptives, erythromycins, estrogens, irbesartan, mirtazepine, phenothiazines, terbinafine, tricyclics

Nephrotoxic drug use:

We will define a binary covariate for use of potentially nephrototoxic medications at the index hospitalization before or during antifungal therapy. The following medications are listed by Hoffman et al. and Guo and Nzerue:[52,53]

Antihypertensives: Methyldopa, captopril,
Aminoglycoside antibiotics: streptomycin, gentamycin,
Anticonvulsants: Carbamazepine, phenobarbital, phenytoin, trimethadione, paramethadione
Anesthetics: Methoxyflurane, halothane, enflurane
Antineoplastics: Cisplatin, methotrexate, mithramycin, ifosfamide, streptozotocin, cisplatin, nitrosoureas, mitomycin C
Antiviral agents: Acyclovir, indinavir
Lithium
Interleukin-2
Immunosuppressants: Cyclosporine, tacrolimus, azathioprine, tacrolimus
Radiocontrast agents: High-osmolal and ionic agents

13.5. Appendix 5: 30-day outcome definitions

Treatment emergent liver disease:

LFT elevation, specifically ALT more than 5 times norm

Non-virus hepatitis

Acute necrosis of liver

Liver failure, hepatic coma

Liver transplant

Jaundice

ICD discharge diagnoses at the end of the index hospitalization: ICD 570 (Acute and subacute necrosis of liver) ICD 572.2 (Hepatic coma) ICD 573.3 (Hepatitis unspecified, non infectious) ICD 782.4 (jaundice unspecified not of newborn) **OR**
ICD procedure codes at the end of the index hospitalization: ICD 50.5x Liver transplantation

Treatment emergent kidney disease:

Creatinine increase, specifically creatinine increase by more than 20% from pre-treatment level

Dialysis (hemo, peritoneal, filtration)

Kidney failure, acute kidney failure, NOT chronic kidney disease (ICD-9 585)

Nephritis, glomerulonephritis,

ICD discharge diagnoses at the end of the index hospitalization: ICD 580 (Acute glomerulonephritis) ICD 581 (Nephrotic syndrome) ICD 583 (Nephritis and nephropathy not specified as acute or chronic) ICD 584 (Acute renal failure) ICD 586 (Renal failure unspecified)
ICD procedure codes at the end of the index hospitalization: ICD V56.0 (Extracorporeal dialysis) ICD V56.1 (Fitting and adjustment of extracorporeal dialysis catheter) ICD V56.2 (fitting and adjustment of peritoneal dialysis catheter) ICD V56.8 (peritoneal dialysis) ICD 39.93 through 39-95 (Hemodialysis)

ICD 54.98 (Peritoneal Dialysis) ICD 39.27 (Arteriovenostomy for renal dialysis) but NOT ICD 39.42 (Revision of shunt) **OR** dialysis AFTER the first administration of an iv antifungal agents:

Subsequent hospitalization for the treatment of fungal infection

ICD discharge diagnoses at the end of a new hospital admission within 30 days after discharge from the index hospitalization: ICD 112.4 (Candidiasis of lung) ICD 112.5 (Disseminated candidiasis) ICD 112.8 (Candidiasis of other specified sites) ICD 114 (Coccidioidomycosis) ICD 115 (Histoplasmosis) ICD 116 (Blastomycotic infection) ICD 117 (Other mycoses) ICD 118 (Opportunistic mycoses) **OR**
Charge codes for any iv antifungal agents during a new hospital admission within 30 days after discharge from the index hospitalization

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