# NON-INTERVENTIONAL (NI) STUDY PROTOCOL

## PASS Information

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|---|---|
| **Title** | A Retrospective Cohort Study of the Risk of Severe Hepatotoxicity in Hospitalized Patients Treated with Echinocandins |
| **Protocol version identifier** | Protocol, Version 3.0 |
| **Date of last version of protocol** | 9 October 2013 |
| **EU PAS register number** | Study not registered |
| **Active substance** | Anidulafungin (J02AX06)<br><br>Comparison: caspofungin, micafungin |
| **Medicinal product** | Ecalta |
| **Product reference** | EU/1/07/416/002 |
| **Procedure number** | EMEA/H/C/000788 |
| **Marketing authorisation holder(s)** | Pfizer Ltd. |
| **Joint PASS** | No |
| **Research question and objectives** | Objective: to quantify the risk for severe hepatotoxicity in users of anidulafungin |
| **Country(-ies) of study** | United States (no EU/EEG countries) |
| **Author** | Pfizer Ltd. (anonymous), Ramsgate Road, Sandwich, CT130NJ, UK |

## Marketing Authorisation Holder(s)

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| Marketing authorisation holder(s) | Pfizer Ltd. (anonymous), Ramsgate Road, Sandwich, CT130NJ, UK |
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## 2. LIST OF ABBREVIATIONS

| | |
|---|---|
| ALT | Alanine aminotransferase |
| AST | Aspartate transaminase |
| CCU | Critical Care Unit |
| CPT | Current Procedural Terminology |
| EMA | European Medicines Agency |
| ED | Emergency department |
| FDA | Food and Drug Administration |
| HCPCS | Healthcare Common Procedure Coding System |
| HIPAA | Health Insurance Portability and Accountability Act |
| ICD-9-CM | International Classification of Diseases, Ninth Revision, Clinical Modification |
| ICU | Intensive care unit |
| IV | Intravenous |
| LFT | Liver function test |
| MA | Massachusetts |
| MAH | Marketing authorisation holder |
| NDC | National Drug Code |
| PASS | Post-Authorization Safety Study |
| RR | Risk ratio (relative risk) |
| SAP | Statistical analysis plan |
| SGOT | Serum glutamic-oxaloacetic transaminase |
| SGPT | Serum glutamic-pyruvic transaminase |

## 3. RESPONSIBLE PARTIES

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Lisa Weiss, PhD, MPH (Pfizer Ltd.), Pfizer Contact

## 4. ABSTRACT

### Title

A Retrospective Cohort Study of the Risk of Severe Hepatotoxicity in Hospitalized Patients Treated with Echinocandins

Protocol, Version 3.0.

Main authors: Mei Sheng Duh, MPH, ScD, .; Francis Vekeman, MA.; Wendy Cheng, MPhil, MPH; Raluca Ionescu-Ittu, PhD; Yongling Xiao, PhD. All authors are employees of Analysis Group, Inc., Boston, Massachusetts (MA). Dr. Duh is also a visiting scholar with the Harvard School of Public Health, Boston, MA.

### Rationale and Background

Echinocandins are a class of antifungal medications that inhibit 1,3-β-D-glucan synthesis, an essential component of fungal cell walls. Available echinocandins in the market include anidulafungin, caspofungin, and micafungin. Hepatotoxicity was designated as an important identified risk during the anidulafungin clinical development program. To better understand the liver safety profile of anidulafungin relative to that of other echinocandins in real-world settings, a retrospective cohort study is proposed to assess the risk of severe hepatotoxicity among patients treated with echinocandins.

### Research Question and Objectives

The overall objective of the study is to estimate the risk of severe hepatotoxicity associated with exposure to echinocandins, and to compare the risk of severe hepatotoxicity in hospitalized patients treated with anidulafungin to that of hospitalized patients treated with other echinocandins in a real-world setting.

### Study Design

A retrospective observational cohort study to assess the association between echinocandins and severe hepatotoxicity.

### Population

Patients admitted to a hospital, with ≥1 dose of echinocandin antifungal medicines, and aged 18 and above at hospitalization admission will be included in the study.

### Variables

Exposure to echinocandins will be determined using HCPCS and NDC codes. The primary study outcome will be the occurrence of severe hepatotoxicity, which will be assessed using liver function lab tests (AST, ALT, and total bilirubin), diagnosis codes for hepatic diseases, and death due to hepatic causes.

### Data Sources

Data will be obtained from the US-based Humedica and Cerner electronic medical record databases.

### Study Size

Based on feasibility counts of patients with exposure to echinocandins within the Humedica and Cerner databases, there is at least 80% power to detect a risk ratio of 2.0 or larger for anidulafungin vs. micafungin/caspofungin in the study, assuming an incidence of severe hepatotoxicity risk of 2% in the micafungin or caspofungin groups.

### Data Analysis

Data analyses will consist of the following in support of the overall objectives of the study:

1. The absolute risk (cumulative incidence) and incidence rate of severe hepatotoxicity will be calculated for echinocandin (ie, anidulafungin, caspofungin, and micafungin) users. Stratified risk estimates will also be estimated by baseline liver function status. Adjusted risk estimates will be computed, controlling for demographic characteristics, baseline fungal infection severity, progression of fungal infection during hospitalization, baseline comorbidities (eg, Charlson comorbidity index), and other clinical characteristics.

2. Demographic and clinical characteristics of the three echinocandin cohorts will be described and compared to assess potential confounding factors unevenly distributed in anidulafungin vs. caspofungin and anidulafungin vs. micafungin groups. Multivariate comparison of the type of echinocandin received will also be conducted.

3. The association between the risk and incidence rate of a severe hepatotoxicity and exposure to various echinocandins will be compared using risk ratios and incidence rate ratios. Both crude and adjusted risk and rate ratios will be presented.

### Milestones

PASS study registration to be completed 2 weeks after EMA approval of the final protocol. Data will first be obtained for analysis 4 weeks after EMA approval of the final protocol, with analytic datasets created in 2 months. Analysis is expected to be completed 12 months after EMA approval of the final protocol and final study report will be submitted 14 months after EMA approval of the final protocol. Actual dates will be populated in the final approved protocol.

## 5. AMENDMENTS AND UPDATES

None

## 6. MILESTONES

| Milestone | Planned date (after EMA approval of final study protocol) |
|---|---|
| Registration in the EU PASS register | 2 weeks after EMA approval |
| Data obtained for analysis | 1 month after EMA approval |
| Creation of analytic dataset | 2 months after EMA approval |
| Completion of analysis | 12 months after EMA approval |
| Final report of study results | 14 months after EMA approval |

Final milestone dates to be populated based on approval data of final protocol.

## 7. RATIONALE AND BACKGROUND

Echinocandins are a class of antifungal medications that inhibit 1,3-β-D-glucan synthesis, an essential component of fungal cell walls. Anidulafungin (Eraxis/Ecalta) is one echinocandin marketed by Pfizer; other marketed echinocandins include caspofungin (Cancidas, MAH: Merck) and micafungin (Mycamine, MAH: Astellas Pharma).

Hepatotoxicity (or severe liver injury) was designated as an important identified risk during the anidulafungin clinical development program. The hepatic adverse events primarily consisted of hepatic enzyme elevations in healthy volunteers and in patients with severe underlying illness receiving multiple concomitant medications. Isolated cases of significant hepatic dysfunction or failure have been reported, but population-based evidence in real-world settings remains sparse.

To further evaluate the potential for severe hepatotoxicity among patients treated with echinocandins, a retrospective cohort study is proposed. Patients admitted to intensive/critical care units (ICUs/CCUs) or inpatient wards undergoing intravenous (IV) echinocandin will be followed until the earliest observation of a severe hepatotoxicity event, hospital discharge, or recorded death.

This study to assess the risk of severe hepatotoxicity among patients treated with echinocandins is designated as a Post-Authorization Safety Study (PASS) to the European Medicines Agency (EMA).

## 8. RESEARCH QUESTION AND OBJECTIVES

The primary objective of the study is to estimate the risk of severe hepatotoxicity associated with exposure to echinocandins, and to compare the risk of severe hepatotoxicity in hospitalized patients treated with anidulafungin to that of hospitalized patients treated with other echinocandins in a real-world setting. Specific aims are as follows:

1. To estimate the crude and adjusted risk estimates of severe hepatotoxicity in hospitalized patients treated with echinocandins (ie, anidulafungin, caspofungin, and micafungin);
2. To evaluate clinical and demographic features associated with the type of echinocandin received (ie, anidulafungin, caspofungin, and micafungin) during the hospitalization;

3. To assess the crude and adjusted risk ratios of severe hepatotoxicity in hospitalized patients treated with anidulafungin to that in hospitalized patients treated with caspofungin and/or micafungin.

The hypotheses to be tested are that the risk of severe hepatotoxicity in hospitalized patients treated with anidulafungin is not statistically different from that in hospitalized patients treated with caspofungin or micafungin. Specifically:

Hypothesis 1:

$H_0$: $P_{anidulafungin} = P_{caspofungin}$

$H_a$: $P_{anidulafungin} \neq P_{caspofungin}$

Hypothesis 2:

$H_0$: $P_{anidulafungin} = P_{micafungin}$

$H_a$: $P_{anidulafungin} \neq P_{micafungin}$

Where *P* is the incidence of severe hepatotoxicity in patients treated with echinocandins.

## 9. RESEARCH METHODS

### 9.1. Study Design

A retrospective cohort design is proposed to estimate the cumulative incidence (risk) of severe hepatotoxicity. Since the duration of echinocandin treatment and hospital length of stay may vary by patient, incidence density (incidence rate) of severe hepatotoxicity will also be calculated to account for differences in person-time of observation across patients. Patients will be observed during their hospitalization, including ICU/CCU stay within the hospitalization. Patients' liver function at hospital admission will serve as their baseline assessment and will be used for stratification and further adjustment. Additionally, approximately two-thirds of the patients in Humedica's database have both inpatient and outpatient records available (further discussed in Section 9.4 Data Sources). For these patients, medical history within 6 months prior to hospital admission will be assessed and used for adjustment.

To avoid the possibility of immortal person-time bias, the MAH will ensure only at-risk time periods are included in the denominator of the risk or rate calculation. The at-risk time period will be defined as from the initiation of the index echinocandin therapy (instead of from hospital admission) until the earliest observation of a severe hepatotoxicity event, hospital discharge, or recorded death. This design will ensure that patients will be at risk for the study outcome throughout the observation period.

### 9.2. Setting

Study Population

Patients admitted to a hospital, treated with an echinocandin for any reason, and meeting the eligibility criteria will be identified for the study. As described in detail in Section 9.4, data for the study will be collected from two US inpatient health care databases, Humedica (from 1 January, 2007–31 December, 2012) and Cerner Health Facts ("Cerner") (from 1 January, 2006-31 January, 2013). Given that some of the hospitals in the Humedica database are Cerner hospitals (approximately 20%), there are

duplicate records for select patients; methods will be applied to remove duplicate records (See 9.7 Data Analysis).

*Inclusion criteria*

The following criteria are used for inclusion in the cohort:

1. ≥1 hospitalization;

2. ≥1 dose of echinocandin antifungal medicines as defined by the Healthcare Common Procedure Coding System (HCPCS) and National Drug Code (NDC) codes:

    a. Anidulafungin:

    HCPCS code: J0348 and NDC codes: 00049011428, 00049011528, 00049011628, 00049101028;

    b. Caspofungin:

    HCPCS code: J0637 and NDC codes: 00006382210, 00006382310;

    c. Micafungin:

    HCPCS code: J2248 and NDC codes: 00469321110, 00469325010;

3. ≥18 years of age at hospitalization admission;

4. ≥1 liver function test (LFT) (ie, ALT, AST, total bilirubin) result prior to initiation of echinocandin (A sensitivity analysis to remove this criterion and to include all echinocandin patients regardless of the presence of LFT before starting therapy will be conducted; see Section 9.7);

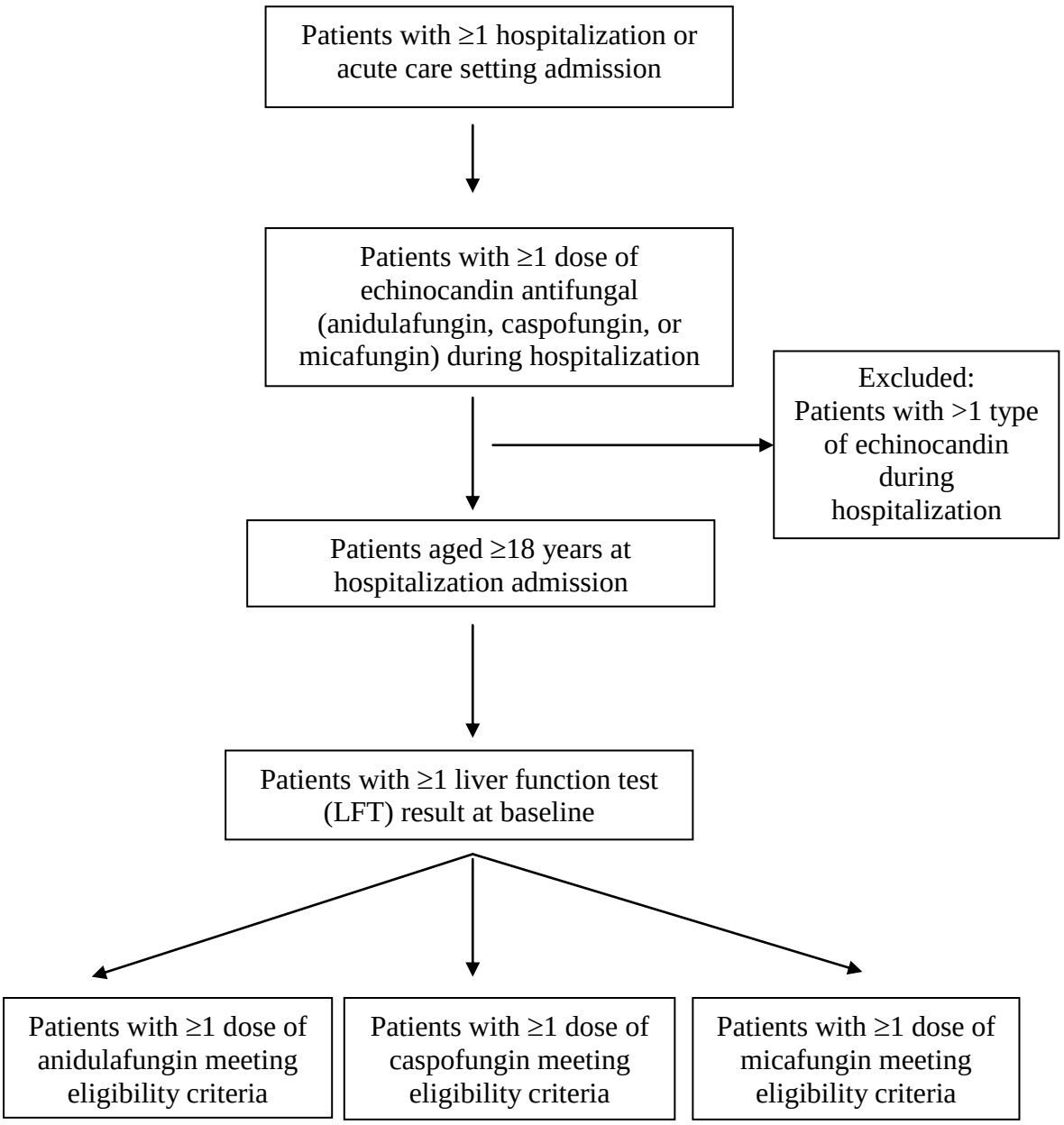
5. ≥1 LFT result following initiation of echinocandin (index date) during the study period (Since the outcome definition is based on LFT lab results, this criterion is important for identifying outcome cases. However, the absence of this criterion will be examined as a sensitivity analysis; see Section 9.3).

*Exclusion criteria*

Patients meeting the following criterion will not be included in the study:

1. Exposure to more than one type of echinocandin during hospitalization.

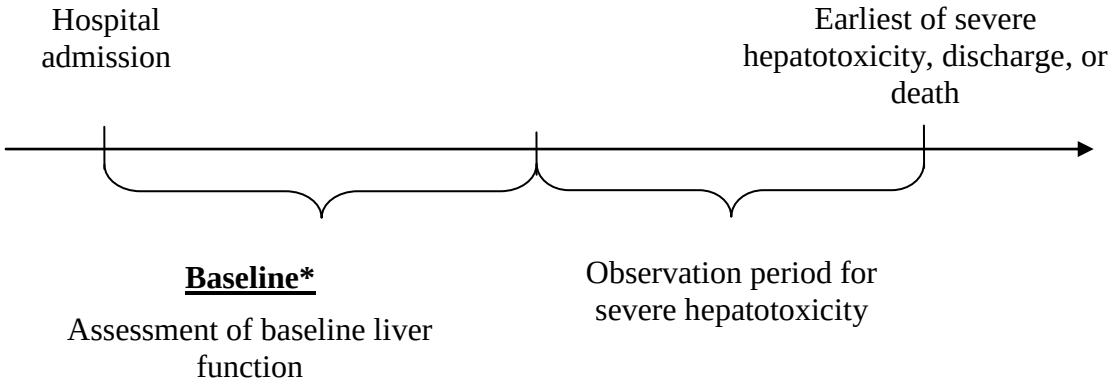
**Figure 1. Sample Selection Flow Diagram**



Study Design and Observation Period

Since the duration of echinocandin treatment and hospital length of stay varies by patients, an open-cohort design is proposed to calculate the risk of severe hepatotoxicity based on incidence rates. Patients will be observed during their hospitalization, including any ICU/CCU stay within the hospitalization. Patients' liver function at hospital admission will serve as their baseline value for stratification and further adjustment. Additionally, for patients in the Humedica database with outpatient data, their medical history within 6 months prior to hospital admission will be assessed and used for adjustment. Patients will be observed until the earliest of observation of an event of severe hepatotoxicity, hospital discharge, or death. The index date for exposure to echinocandins will be based on first report of administration of an echinocandin in the hospital record - considered to be the initiation of treatment.

**Figure 2. Study Design Scheme**




Note:

* For patients in the Humedica database with outpatient data, their medical history within 6 months prior to hospital admission will be assessed and used for adjustment.

The exposure time windows for echinocandins are constructed based on the bioavailability of IV injections of these drugs. No induction period is imposed, as $C_{max}$ is reached within 3-10 hours. Half-life ($t_{1/2}$) ranges from 9-50 hours;[1,2,3] hence, the MAH proposes to observe patients for the whole duration of hospitalization, instead of censoring at the end of treatment.

**Data Sources**

The MAH included the information on Data source in the Setting section. The assessment is provided in Section 9.4 Data Sources.

## 9.3. Variables

Exposure

Exposure to echinocandins will be determined using the following codes:

- Anidulafungin: HCPCS code: J0348 and NDC codes: 00049011428, 00049011528, 00049011628, 00049101028;
- Caspofungin: HCPCS code: J0637 and NDC codes: 00006382210, 00006382310;
- Micafungin: HCPCS code: J2248 and NDC codes: 00469321110, 00469325010.

The index date for exposure to echinocandins will be based on first report of administration of an echinocandin in the hospital record (initiation of treatment during a given hospitalization.

Moreover, because the risk of severe hepatotoxicity may increase with the exposure to echinocandins, exposure-specific variables will be created to control for the exposure duration by number of days (eg, 1-3, 4-7, 8+ days) and dosage (eg, high vs. low dose). The cutoffs of exposure duration will be determined based on the observed distribution of days of exposure to echinocandins. As each echinocandin has a different potency, the cutoff of high and low dose will differ by echinocandin and will be determined during the statistical analysis plan phase.

**Outcome**

The primary study outcome will be the occurrence of severe hepatotoxicity after initiation of an echinocandin treatment, regardless of whether there are known aetiologies involved, consistent with EMA guidelines.[4] Known aetiologies will be considered as confounders as described in the following subsection. Severe hepatotoxicity will be ascertained using liver function lab tests that are Grades 3, 4, and 5 based on the Terminology Criteria for Adverse Events in Trials of Adult Pancreatic Islet Transplantation (CIT-TCAE), which are modified standards of those set forth in the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE).[5] Specifically, the primary outcome is defined as follows:

*Liver function lab tests*

Severe hepatotoxicity will be ascertained using aspartate transaminase (AST or SGOT) or alanine aminotransferase (ALT or SGPT), and total bilirubin results. The grade of severe hepatotoxicity will be based on the highest grade of the three lab test results as defined per the CIT-TCAE and shown below.

| | Grade | | |
|---|---|---|---|
| | 3 | 4 | 5 |
| Aspartate transaminase (AST) or serum glutamic-oxaloacetic transaminase (SGOT)<br>Or<br>Alanine aminotransferase (ALT) or serum glutamic-pyruvic transaminase (SGPT) | >5.0 to 20.0 times the upper limit of normal (ULN) | Evidence of fulminant hepatic failure*, with international normalized ratio (INR) ≥2.5 and AST/ALT ≥20.0 x ULN | Death** |
| Total bilirubin | >3.0 – 10.0 x ULN | >10.0 x ULN | - |

*Fulminant hepatic failure will be ascertained using ICD-9-CM diagnosis code 572.2 for hepatic coma.

** Death will be defined as death due to hepatic causes. Only deaths occurring within a hospital facility are captured in the databases. Causes of death will be inferred from the primary and secondary discharge diagnoses.

A death will be considered due to hepatic causes if the primary or secondary discharge diagnoses contain at least one of the following codes for a severe acute hepatotoxicity (based on the narrow diagnosis code-based definition adopted by Observational Medical Outcomes Partnership):[6]

- Acute and subacute necrosis of liver (ICD-9-CM: 570);
- Hepatic coma (ICD-9-CM: 572.2);
- Hepatorenal syndrome (ICD-9-CM: 572.4);
- Other disorders of liver (ICD-9-CM: 573.xx), (specifically as pertains to acute liver disorders, and will be further defined in the planned statistical analysis plan).

As hepatotoxicity is primarily ascertained by the availability of LFT results, the core analyses will include patients with ≥1 LFT result following initiation of echinocandin (index date), and all hepatotoxicity cases based on LFT results meeting Grades 3, 4, or 5 criteria will be assessed. Known aetiology status will be considered as a potential confounder in adjusted analyses (see the Section Confounders and effect modifiers).

<u>Confounders and effect modifiers</u>

- Age;
- Sex;
- Race and/or ethnicity;
- Admission to acute care settings (eg, ICU, CCU);
- Use of other antifungal agents: amphotericin B, fluconazole, extended-spectrum azoles (itraconazole, voriconazole, posaconazole);

- Fungal infection severity indicators at baseline and during hospitalization (each independently assessed and to the extent that data are available):
    - Invasive (involving blood, internal organs such as liver, spleen, heart valve, lungs, brains, sinus) vs. non-invasive (involving mouth, urinary tract, skin, oesophagus, etc.) fungal infection vs. unknown (there are source and site data if blood cultures are obtained), and
    - Number of fungal infection sites.
- Risk factors for fungal infection (central venous catheter, catheter removed within 24 hours of hospitalization, broad-spectrum antibiotics, recent surgery, recent hyperalimentation, immunosuppressive therapy);
- Liver function test results at baseline;
- Sepsis or septic shock;
- Organ failures (eg, heart failure, kidney failure);
- Absolute neutrophil count (≤500 or >500 per cubic millimetre);
- Renal dysfunction (assessed by serum creatinine, glomerular filatration rate);
- Charlson comorbidity index;[7]
- Relevant specific comorbidities (eg, diabetes mellitus, hypertension, cardiovascular diseases, kidney diseases, obesity, esophageal varices, gastroesophageal reflux disease);
- Prior use of echinocandin based on available information from prior hospitalization;
- Known aetiology status for hepatotoxicity observed during the same hospitalization:[8]
    - Viral hepatitis (ICD-9-CM code: 070.xx)
    - Liver disease secondary to biliary pathologies (ICD-9-CM code: 574.xx, 575.xx, 576.xx)
    - Liver malignancy (ICD-9-CM code: 155.xx, 197.70)
    - Acute and subacute necrosis of liver associated with cardiovascular causes (ICD-9-CM code: 570.xx paired with codes for underlying diseases as listed below):
        - Right heart failure (ICD-9-CM code: 428.0);
        - Hypotension (ICD-9-CM code: 458).

- Hepatitis associated with viral infections (ICD-9-CM code: 573.1 paired with codes for underlying diseases as listed below):
    - Mononucleosis (ICD-9-CM code: 075.00);
    - Other viral infections (ICD-9-CM code: 072.71, 074.80, 078.50).
- Hepatitis in other infectious diseases classified elsewhere (ICD-9-CM code: 573.2 paired with codes for underlying malaria (ICD-9-CM: 084.9));
- Concomitant treatments of hepatotoxic drugs (including but not limited to below):[7]
    - Acetaminophen;
    - Chemotherapies (eg, methotrexate, azathioprine);
    - Non-steroidal anti-inflammatory drug (eg, diclofenac);
    - Antiretrovirals (eg, Nucleoside/nucleotide reverse transcriptase inhibitors such as zidovudine, didanosine, stavudine);
    - Psychotropics (eg, paroxetine, nefazodone, valproic acid);
    - Antibiotics (eg, amoxicillin, telithromycin);
    - Antimycobacterials (eg, isoniazid, rifampin);
    - Antidiabetics (Thiazolidinediones) (eg, rosiglitazone, pioglitazone).

### 9.4. Data Sources

As there are no known population-based databases in Europe with the necessary variables for this study, the MAH proposes to use two of the major US-based hospital databases: Humedica and Cerner databases. Two databases are used in order to obtain sufficiently large cohorts.

Humedica contains the following information:

- Demographic characteristics, type of healthcare provider (specialty), medical history and current diagnoses for all type of encounters, detailed area of care during hospitalization (ICU, ED, ward, etc., in-hospital procedures (ICD-9-CM, HCPCS and CPT-4 codes), inpatient medication including data for injectable and oral medications, pharmacy dispensing data, laboratory data (incl. date and time of observation results, value).

Approximately two-thirds of all patients in the Humedica database belong to an integrated delivery network, meaning that this subset of patients has both inpatient and outpatient records available. As such, a subset of the Humedica cohort who meets the study's eligibility criteria will have medical history data, which can be used for adjustment and stratified analyses.

Data is available from 2007 onward from Humedica and will be used for the study period 1 January, 2007 to 31 December, 2012.

The MAH has conducted a feasibility count for the exposure (Table 1).

**Table 1. Number of Patients ≥18 Years Old Who are Eligible for Inclusion Based on Their Exposure to Echinocandins in Humedica Database Between 1 January, 2007 to 31 December, 2012**

| | Unique patients | Unique patients with ≥1 liver function test |
|---|---|---|
| Anidulafungin | 1,659 | 1,567 |
| Caspofungin | 748 | 240 |
| Micafungin | 3,811 | 3,376 |

Cerner contains the following information:

- Demographic characteristics, medical history, comorbidities, in-hospital procedures (ICD-9-CM codes), comprehensive laboratory data, pharmacy dispensing data, in-hospital mortality, hospital characteristics.

Data is available from 2000 onward from Cerner and will be used for the study period 1 January, 2006 to 31 January, 2013.

**Table 2. Number of Patients ≥18 Years Old Who are Eligible for Inclusion Based on Their Exposure To Echinocandins in Cerner Database Between 1 January, 2006 to 31 January, 2013**

| | Unique patients | Unique patients with ≥1 liver function test |
|---|---|---|
| Anidulafungin | 612 | 590 |
| Caspofungin | 3,951 | 3,676 |
| Micafungin | 4,158 | 3,852 |

**Table 3. Total Number Of Patients ≥18 Years Old Who are Eligible for Inclusion Based On Their Exposure To Echinocandins in Humedica And Cerner Databases Combined Between 1 January, 2006 to 31 January, 2013***

| | Unique patients | Unique patients with ≥1 liver function test |
|---|---|---|
| Anidulafungin | 2,271 | 2,157 |
| Caspofungin | 4,699 | 3,916 |
| Micafungin | 7,969 | 7,228 |

* Note: Some of the hospitals in the Humedica database are Cerner hospitals (≈20%). Hence, duplicate records may exist. The current feasibility counts do not account for duplicates.

The Humedica and Cerner databases appear to provide sufficient sample size for the current study (sample size calculation is provided in Section 9.5 Study Size).

### 9.5. Study Size

Sample size calculations were conducted to assess the sample size needed in Objective 3 (See Section 9.7) to detect a range of risk ratios (RR) with at least 80% power and a 5% two-sided alpha assuming various risks of severe hepatotoxicity in the micafungin and caspofungin cohorts (Table 4).

**Table 4. Minimum Number of Patients Required in Each Cohort Given Various Relative Risks (RR) and Expected Incidence of Severe Hepatotoxicity Among Controls**

| Detectable RR | Incidence among caspofungin/micafungin users | | | | | |
|---|---|---|---|---|---|---|
| | 0.5% | 1.0% | 1.5% | 2.0% | 2.5% | 3.0% |
| 1.5 | 16,389 | 8,145 | 5,397 | 4,023 | 3,199 | 2,649 |
| 2.0 | 5,065 | 2,515 | 1,664 | 1,239 | 984 | 814 |
| 2.5 | 2,680 | 1,329 | 879 | 653 | 518 | 428 |
| 3.0 | 1,748 | 866 | 572 | 425 | 336 | 278 |

According to pooled estimates from a meta-analysis of clinical trials by Wang et al.,[9] 0.2% and 2.7% patients receiving caspofungin and micafungin had elevated liver enzymes that were on average ≥5 times the upper limit of normal and required treatment termination.[8] Given that for the current study, severe hepatotoxicity is defined based on either elevated liver enzymes (ALT or AST be >5 times the upper limit of normal, and total bilirubin >3 times the upper limit of normal) or hepatic system organ class conditions, or death due to hepatic causes — a broader definition than that used by Wang — it is expected that the observed incidence of severe hepatotoxicity will be higher. Assuming an incidence of severe hepatotoxicity of 2% in the micafungin or caspofungin cohorts, and a RR of 2.0, about 1,239 subjects per treatment group would be required to achieve 80% power. Given the counts of hospitalized patients receiving echinocandins in Humedica and Cerner as presented in Table 1-Table 3, the sample size is expected to be powered to detect a RR of 1.5-2.0. On the other hand, if we believe the baseline

incidence of severe liver disease is much lower at 0.5%, the current sample size is powered to detect a RR of 2.0-2.5.

### 9.6. Data Management

Data will be extracted electronically from the Humedica and Cerner databases and will be de-identified in compliance with HIPAA regulations and exempt Institutional Review Board approval for the study will be sought. Data extractions from the two databases will be conducted in-house within Humedica and Cerner. Data management and analyses will be conducted by investigators at Analysis Group, Inc. (lead investigator is Dr. Mei Sheng Duh, MPH, ScD, Managing Principal and Chief Epidemiologist at Analysis Group, Inc., also a visiting scholar at the Harvard School of Public Health, Boston, MA) using SAS release 9.2 (SAS Institute, Inc., Cary, NC).

### 9.7. Data Analysis

Merge of raw data from Humedica and Cerner databases

Data extracted from the two databases will first be cleaned separately. Patient-level analytical data files that contain all relevant variables will be created in each database; the variables will be formulated in uniform manners across the two databases. The analytical data files from the two databases will then be merged into one combined database with duplicates removed (see the following paragraph). Overall results using data from the combined dataset will be reported and considered as the core analyses. Differences in patient characteristics and results between the Humedica and Cerner databases will be evaluated; if major discrepancies are observed, independent analyses within each of the databases may be conducted and results discussed.

Handling of duplicate patient records in Humedica and Cerner databases

Given that some of the hospitals in the Humedica database are Cerner hospitals (≈20%), there exist duplicate records for selected patients. To ensure that such patients will not be counted twice and their records doubly analyzed, steps to remove duplicate records will be undertaken. Because the identities of patients and the hospitals at which they were treated are not available due to HIPAA protection and true service dates in the Cerner database are masked as an extra layer of protection to ensure patient confidentiality, duplicate patient and hospital records cannot be directly compared between databases and removed. Furthermore, as suggested by Cai and colleagues, in identifying duplicate records between two databases, it is more efficient to identify common hospitals as opposed to common patients from among the large underlying study population in the two databases.[9] The MAH therefore proposes to develop an algorithm to identify hospitals (and subsequently the patients within these hospitals) common to both the Humedica and Cerner databases by adapting the methods proposed by Cai and colleagues.[10] Specifically, the MAH proposes to execute two algorithms based on hospital-level data described below. Both algorithms are meant to identify indicators that suggest potential duplicate hospitals in the two databases. The rationale for using two algorithms is to improve the accuracy in identifying real matched pairs of any one algorithm.

*Algorithm 1:*

The first algorithm will employ hospital-level data based on three step-wise criteria:

1. Geographic region

   If the two databases utilize identical geographic region labels (eg, Northeast, South, West, Central), hospitals identified as being from the same regions in the two databases will be grouped together. If region labels differ for select regions between the two databases, hospitals falling under these regions will be grouped together as the "Other".

2. Total number of patients with a diagnosis of candidiasis (ICD-9 112.xx) (or another indication condition for echinocandins) in the medical history data at each hospital, ± 10%. For all hospitals within each group of region identified above, the total number of patients with at least one diagnosis of candidiasis based on medical history data will be determined.

3. Total number of patients prescribed marker drugs anidulafungin, caspofungin, and micafungin, separately, in each year during the study period, ±3 patients. For hospitals within each region with similar number of patients with candidiasis (or another indication condition selected in Step 2), ±10%, the total number of patients who were prescribed each of the echinocandins in each of the years within the study period will be assessed.

A matching score will be computed for each hospital and year that meet criterion 3, such that if a hospital between the two databases has a matching number of patients who were prescribed anidulafungin for Year 1, one point will be added to the matching score. If the hospital has matching numbers of patients for all three echinocandins for Year 1, three points will be added to the matching score. Depending on the number of years (*k*) that will be available for the observation period in the study, a total of *3k* points will be possible.

*Algorithm 2:*

The second algorithm allows for the identification of potential matched pairs by comparing the counts of the elderly population between the two databases, taking advantage of small counts among the elderly. Specifically, the following criteria will be determined by gender:

1. Total number of patients with birth year before 1900.

2. Total number of patients with birth year between 1900 and 1905.

3. Total number of patients with birth year between 1905 and 1910, and

4. Total number of patients with birth year before the 10th calendar year after the birth year of the oldest patient in the hospital (if different from Criterion 3).

A matching score is then calculated such that one point is given to a hospital with a potential matched pair if there exists the same number of patients for any one of the criteria, ±5% between the two databases. A total of 8 points will be possible (4 Criteria x 2 genders).

To ensure that duplicate patient records are identified and removed, patient-level data will be further used to validate and refine the above algorithms. For example, gender, birth year, hospital length of stay, liver function test results, and the order of hospital day for echinocandin administrations and liver function tests from the hospital admission day. The inclusion of a larger number of patient-level variables will allow for increased confidence in declaring a real match of patients between the two databases. However, the marginal gain in confidence decreases as too many patient-level variables are included. Hence, while additional patient-level data variables can be assessed to further assess level of match, a balance needs to be reached based on efficiency as well as data availability in the two databases.

Handling of missing values

Missing values will be assessed and compared across the three echinocandin cohorts. If the likelihood of missingness is not statistically significantly different across cohorts (ie, case of missing at random), and the proportion of missing is not so large as to affect the statistical power of the study, observations with missing information will be dropped. Otherwise, missing values will be imputed using the unconditional mean imputation approach.

Statistical analysis

*Objective 1 – To estimate the crude and confounder-adjusted risk of severe hepatotoxicity in hospitalized patients treated with echinocandins (ie, anidulafungin, caspofungin, and micafungin)*

The absolute risk (cumulative incidence) of severe hepatotoxicity will be calculated for echinocandin (ie, anidulafungin, caspofungin, and micafungin) users. In addition, the incidence rate of severe hepatotoxicity will be calculated to account for exposure time to echinocandin and the duration of ICU/hospital length of stay. Kaplan-Meier method will be used to assess time from the initiation of echinocandin to severe hepatotoxicity. The median time to severe hepatotoxicity will be reported by echinocandin.

The risk of severe hepatotoxicity assessed following the initiation of echinocandins, and time to severe hepatotoxicity will be further stratified by baseline liver function status (ie, abnormal or normal). Depending on the number of liver function measurements available prior to the initiation of echinocandins, baseline liver function status will be based on either the value upon hospital admission or the latest value prior to treatment initiation. The other potential confounders and effect modifiers listed on pages 14, 15 and 16 will also be used to conduct stratifications.

Adjusted risk and incidence rate will be computed and presented as the average predicted risk and incidence rate of severe hepatotoxicity for each echinocandin group based on coefficient estimates derived from Poisson regressions from Objective 3.

*Objective 2 – To evaluate clinical and demographic characteristics of echinocandin cohorts (ie, anidulafungin, caspofungin, and micafungin)*

First, demographic and clinical characteristics of the three echinocandin cohorts will be described. Comparisons of these characteristics will be made between the anidulafungin and caspofungin groups as well as between the anidulafungin and micafungin groups to assess potential confounders unequally distributed across the comparison groups of interest.

Second, multivariate comparison of the type of echinocandin received will also be conducted using two logistic regressions where the dependent indicator variable will be anidulafungin vs. caspofungin in the first model and anidulafungin vs. micafungin in the second. Characteristics of patients described in Section 9.3 (Variable – confounders and effect modifiers) will be entered in the models as predictors of the echinocandin received to study differences in likelihood of echinocandin exposure.

*Objective 3 – To compare the crude and confounder-adjusted risk of severe hepatotoxicity in hospitalized patients treated with anidulafungin to that in patients treated with caspofungin and/or micafungin.*

The association between the risk and incidence rate of a severe hepatotoxicity and exposure to various echinocandins will be compared using risk ratios and incidence rate ratios. Kaplan-Meier plot will be provided to illustrate the non-parametric time to severe hepatotoxicity. The median time to severe hepatotoxicity will be reported, and log-rank test will be conducted to compare the time to severe hepatotoxicity across the three echinocandins.

Given that anidulafungin is not metabolized by the liver and can be administered to patients with hepatic impairment, unlike caspofungin and micafungin, the association between anidulafungin and severe hepatotoxicity may be confounded by patients' baseline hepatic function as well as physicians' channelling bias (ie, confounding by indication). Adjustment for potential confounders to control for the confounding by indication bias will be conducted using multivariate regressions. Specifically, zero-inflated Poisson regressions will be conducted to assess the risk of severe hepatotoxicity between anidulafungin versus caspofungin and micafungin, while adjusting for confounders and assessing for potential effect modification as listed in Section 9.3. This method is particularly useful when the expected outcome event rate is low. In a regression where the number of outcome events is small, having too many covariates could produce spurious estimates. For that reason, the MAH will reduce the number of confounding factors included in the model by including a propensity score, modelled as a continuous covariate. This propensity score could thus be generated based on a wider set of covariates. Covariates for inclusion in the propensity score will be selected primarily based on clinical rationale. Covariates that are identified based on statistical significance will be separately evaluated for inclusion based on clinical grounds. Since the coefficient associated with the propensity score will represent the combined effect of the covariates considered in its calculation and thus will not be interpretable per se, different propensity scores based on more parsimonious and more inclusive sets of covariates will be tested as sensitivity analyses.

Incidence rate ratios and associated 95% confidence intervals based on Poisson distribution and robust variance estimates will be reported.

**Sensitivity Analyses**

The core analyses will include patients with ≥1 LFT result prior to the initiation of echinocandin; this approach will allow for the assessment and adjustment of prior liver function status to better isolate the etiological association of echinocandins with subsequent liver function results. However, because the immunocompromised conditions of these patients may necessitate immediate treatments, some patients may not have had opportunities to be tested for LFT before receiving echinocandins. Exclusion of such patients may hamper the external validity of the study. As such, a sensitivity analysis including all patients (ie, regardless of the availability of baseline LFT results) will also be conducted.

Detailed methodology for summary and statistical analyses of data collected in this study will be documented in a Statistical Analysis Plan (SAP), which will be dated, filed and maintained by the sponsor. The SAP may modify the plans outlined in the protocol; any major modifications of primary endpoint definitions or their analyses would be reflected in a protocol amendment.

### 9.8. Quality Control

Internal audits of all data collection, analytical modelling, and written materials will be conducted by the Analysis Group. Internal audits consist of a review of all final work product materials and the underlying analysis, including all programs, and supporting source documentation by a team member or another conflict-cleared employee who was not involved in the creation of the original work product. Quality review of all final deliverables will be documented and retained by a qualified individual independent of the writing team and incorporating the following steps:

1. Confirm that the source of the data and/or results has been documented and that results and data have been verified against the source.

2. Check the internal consistency of any data presented in the document.

3. Confirm that the conclusions are accurate, objective, balanced, and consistent with other published or released results.

4. Confirm that the format and content of the document are aligned with applicable external requirements.

### 9.9. Limitations of the Research Methods

First, given that data used in the study are hospitalization data (except for a subgroup of patients in the Humedica database who are in Integrated Network of Delivery system and who also have outpatient records), available patient medical history is only restricted to the time between hospital admission and first use of echinocandin. Hence, patients' full medical history are not available for the majority of the patients, and potential confounders, such as patients' prior exposure to echinocandins and prior medical history of liver diseases and other comorbidities, will not be completely controlled for in the

study. Hence, while efforts will be placed to use available data and methods to control for confounding, it is possible that residual confounding remains.

Second, confounding by indication may not be fully accounted for using just LFT results at admission. Treating physicians may channel patients predisposing to or at risk of hepatic impairments towards anidulafungin because anidulafungin does not metabolize through liver and is less prone for drug-drug interactions.[11] As such, patients on anidulafungin may be at a higher baseline risk of severe hepatotoxicity compared to patients on other echinocandins.

Third, official cause of death is not captured as part of the Humedica and Cerner databases. To the extent possible, cause of death will be inferred from the primary and secondary discharge diagnoses for patients whose deaths are recorded in hospital records. This misclassification may lead to either false positives or false negatives of attribution of deaths to hepatotoxicity.

Fourth, as aetiologies for hepatotoxicity may require workups and may not be completely identified during hospitalizations, covariates for known aetiologies are likely under-documented. The increased number of idiopathic hepatotoxicity cases may be falsely ascribed to the study drugs.

Fifth, given that only hospitalization data are studied, the observation period is expectedly short. Hence, it is likely that only acute liver injuries are included in the study. Drug-induced chronic liver cirrhosis is not accounted for in the risk estimates. However, we do not expect the under-estimation of hepatotoxicity is different across the three echinocandin groups.

Sixth, patients' underlying disease severity and progression during the hospitalization may affect the underlying risk of hepatotoxicity. Although efforts will be made to control for fungal infection severity and progression during the hospitalization, data from microbiology lab results may be incomplete since many fungal infection cases are treated empirically without labs ordered.

Seventh, a proposed sensitivity analysis is to include all patients receiving echinocandins regardless of LFT results prior to receiving the antifungal therapy to minimize the selection bias, as not all patients may have been tested for LFT before treatment initiation. On the other hand, this sensitivity analysis may introduce information bias in the etiological assessment of the role of echonicandin on liver toxicity if missing baseline LFT results are not random across the three echinocandin groups.

Eighth, in the US, hospital formularies drive the use of specific echinocandin. Hospital characteristics are not available in the databases to allow the assessment of hospital effects on the results.

Lastly, given the overlap in the hospitals covered by the Humedica and Cerner databases, steps will be taken to identify and remove possible duplicate patients. However, despite methodologies that will be applied to identify duplicate patients, all duplicate patient and hospital records may not be identified with complete certainty because: (i) in both databases, patients and hospitals are de-identified per HIPAA regulations and cannot be linked directly; and (ii) records from the Cerner database are date- and time-shifted to

further protect patient confidentiality. As such, residual duplicates of patient records may be possible.

### 9.10. Other Aspects

Not applicable

## 10. PROTECTION OF HUMAN SUBJECTS

### 10.1. Patient Information and Consent

Not applicable. All data provided will be de-identified in compliance with HIPAA regulations.

### 10.2. Patient Withdrawal

Not applicable.

### 10.3. Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

All data provided will be de-identified in compliance with HIPAA regulations and exempt Institutional Review Board approval for the study will be sought.

### 10.4. Ethical Conduct of the Study

The study will be conducted in accordance with legal and regulatory requirements, as well as with scientific purpose, value and rigor and follow generally accepted research practices described in *Good Pharmacoepidemiology Practices* (GPP) issued by the International Society for Pharmacoepidemiology (ISPE), European Medicines Agency (EMA) European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology, and FDA Guidance for Industry: Good Pharmacovigilance and Pharmacoepidemiologic Assessment, FDA Draft Guidance for Industry and FDA Staff: Best Practices for Conducting and Reporting of Pharmacoepidemiologic Safety Studies Using Electronic Healthcare Data Sets.

## 11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

This study uses patient-level electronic health related databases (e-HRD), in which it is not possible to link (ie, identify a potential association between) a particular product and medical event for any individual. Thus, the minimum criteria for reporting an adverse event (ie, identifiable patient, identifiable reporter, a suspect product, and event) are not available and adverse events are not reportable as individual AE reports.

## 12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

A study report which will include sections on background, methods, and results pertaining to each of the major study objectives listed in Section 8, discussion and conclusions, will be prepared at the study end.

Any additional dissemination of study results (eg, presentation at scientific conferences, publications) will be discussed by the Analysis Group and Pfizer Project Team.

## 13. REFERENCES

1. Anidulafungin [package insert]. New York, NY: Pfizer, Inc. 2012.

2. Caspofungin [package insert]. Whitehouse Station, MJ: Merck & Co., Inc. 2013.

3. Micafungin [package insert]. Northbrook, IL: Astellas Pharma US, Inc. 2012.

4. Non-clinical guideline on drug-induced hepatotoxicity. European Medicines Agency Committee for Medicinal Products for Human Use. 2008. Available from: http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003355.pdf. Accessed May 17, 2013.

5. Racoosin J and Ryan P. Implications of health outcomes of interest definitions: acute liver injury case study. Observational Medical Outcomes Partnership. 2011. Available from: http://75.101.131.161/download/loadfile.php?docname=HOI%20Definitions. Accessed June 3, 2013.

6. Terminology criteria for adverse events (TCAE) in trials of adult pancreatic islet transplantation. Version 4. 2007. Available from: http://www.isletstudy.org/CITDocs/CIT-TCAE%20V4.pdf. Accessed May 27, 2013.

7. Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. J Clin Epidemiol 1992;45:613-19.

8. Chen M, Vijay V, Shi Q, Liu Z, Fang H, Tong W. FDA-approved drug labeling for the study of drug-induced liver injury. Drug Discov Today. 2011 Aug;16(15-16):697-703.

9. Wang J-L, Chang C-H, Young-Xu Y, Chan KA. Systematic review and meta-analysis of the tolerability and hepatotoxicity of antifungals in empirical and definitive therapy for invasive fungal infection. Antimicrobial Agents and Chemotherapy, June 2010, p 2409-2419.

10. Cai B, Xu W, Bortnichak E, Watson D. An algorithm to identify medical practices common to both the General Practice Research Database and the Health Improvement Network database. Pharmacoepi and Drug Safety 2012;21:770-774.

11. Vazquez JA. Anidulafungin: a new echinocandin with a novel profile. Clin Ther. 2005 Jun;27(6):657-73.

## Appendix 1. LIST OF STAND-ALONE DOCUMENTS

None

## Appendix 2. ENCePP CHECKLIST FOR STUDY PROTOCOLS

See attached

## Appendix 3. ADDITIONAL INFORMATION

Not applicable