CLINICAL TRIAL PROTOCOL

A PHASE IV, INTERVENTIONAL, NON-BLINDED, RANDOMIZED CONTROLLED MULTICENTER STUDY OF POSACONAZOLE PROPHYLAXIS FOR THE PREVENTION OF INFLUENZA-ASSOCIATED ASPERGILLOSIS (IAA) IN CRITICALLY ILL PATIENTS

POSA FLU

Version number: v 1. – Date *25-9-2017*

Version number: v IV. – Date 03-01-2018

EudraCT Nr: *2017-003270-14*

Sponsor

Universitaire Ziekenhuizen Leuven (UZ Leuven)

Herestraat 49, 3000 Leuven

Coordinating Investigator

Nationale coördinator voor POSA-FLU studie

Prof. Dr. Joost Wauters Intensivist, UZ Leuven

Herestraat 49, 3000 Leuven, Belgium

+32-16-344275

joost.wauters@uzleuven.be

Prof. Dr. Paul Verweij Microbiologist, UMC Radboud Nijmegen

P.O. box 9101, 6500 HB Nijmegen, the Netherlands

+31-24-3614356

Paul.verweij@radboudumc.nl

Nationale coördinator voor PK substudie

Prof. Apr. Isabel Spriet Clinical pharmacist, UZ Leuven

Herestraat 49, 3000 Leuven, Belgium

+32 16/34 12 61

isabel.spriet@uzleuven.be

Prof. Apr. Roger Bruggemann Clinical pharmacist, Radboudumc
Geert Grootplein Zuid 10, 6525 GA Nijmgen
+31 24361 6405
roger.bruggemann@radboudumc.nl

Principal Investigators (participating sites)

Prof. Dr. Pieter Depuydt	Intensivist, UZ Gent
Dr. Marc Bourgeois	Intensivist, AZ Sint-Jan Brugge
Prof. Dr. Bart Rijnders	Microbiologist, Erasmus MC Rotterdam
Prof. Dr. G. Kampinga	Microbiologist, Groningen

CLINICAL TRIAL PROTOCOL HISTORY

CSP / Amendment#	Date	Reason for amendment
Clinical Trial Protocol v1	25-9-2017	NA
Clinical Trial Protocol vIV	3-1-2018	PK substudy

SIGNATURES

Title: A PHASE IV, INTERVENTIONAL, NON-BLINDED, RANDOMIZED CONTROLLED MULTICENTER STUDY OF POSACONAZOLE PROPHYLAXIS FOR THE PREVENTION OF INFLUENZA-ASSOCIATED ASPERGILLOSIS (IAA) IN CRITICALLY ILL PATIENTS

Protocol: POSA FLU

The undersigned confirm that the following protocol has been agreed and accepted and that the Principal Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the requirements for the conduct of clinical trials in the EU as provided for in "Directive 2001/20/EC", and any subsequent amendments, GCP guidelines, the Belgian law of May 7th 2004 regarding experiments on the human person, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the trial publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies from the trial as planned in this protocol will be explained.

Coordinating Investigator

.....	.....	.....
Name & Title	Signature	Date

Principal Investigator (participating site)

.....	.....	.....
Name & Title	Signature	Date

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Definition
(e)CRF	(electronic) Case Report Form
AB	Antibiotics
AE	Adverse Event
AR	Adverse Reaction
BA	Bronchial Aspirate
BAL	Bronchoalveolar Lavage
CM	Concomitant Medication
DMC	Data Monitoring Committee
EC	Ethical Commission
ECG	Electrocardiogram
FAMHP / FAGG	Federal Agency for for medicines and health products
FPFV	First Patient First Visit
GCP	Good Clinical Practice
HSCT	Hematopoietic Stem Cell Transplanation
IAA	Influenza-Associated Aspergillosis
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
LPLV	Last Patient Last Visit
MIC	Minimal Inhibitory Concentration
PD	Pharmacodynamics
PI	Principal Investigator
PRO	Patient Reported Outcome
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOC	Standard Of Care
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction

FUNDING AND SUPPORT

Funder	Kind of Financial or Non-Financial Support
MSD	MSD provides posaconazole and partially funds study-related work

CONFLICT OF INTEREST

Investigator	Kind of Conflict of Interest

ROLES AND RESPONSIBILITIES

The Principal Investigator is accountable for the conduct of the trial at his/her site and must ensure adequate supervision of the conduct of the trial at the trial site. If any tasks are delegated, the Principal Investigator must maintain a log (link) of appropriately qualified persons to whom he/she has delegated specified trial-related duties. The Principal Investigator must ensure that there is adequate and documented training for all staff participating in the conduct of the trial. (It is the Principal Investigator's responsibility to supervise the conduct of the trial and to protect the rights, safety and well-being of the subjects.)

TRIAL SYNOPSIS

Title of clinical trial	A PHASE IV, INTERVENTIONAL, NON-BLINDED, RANDOMIZED CONTROLLED MULTICENTER STUDY OF POSACONAZOLE PROPHYLAXIS FOR THE PREVENTION OF INFLUENZA-ASSOCIATED ASPERGILLOSIS (IAA) IN CRITICALLY ILL PATIENTS
Protocol Short Title Acronym	POSA FLU
Trial Phase	phase IV
Sponsor name	Universitaire Ziekenhuizen Leuven (UZ Leuven)
Coordinating Investigator	Prof. Dr. Joost Wauters, Intensivist, UZ Leuven Prof. Dr. Paul Verweij, Medical microbiologist, UMC Radboud Nijmegen
Contact Adress	UZ Leuven, Herestraat 49, 3000 Leuven, Belgium
Contact Email	joost.wauters@uzleuven.be
Contact Phone	+32-16-344275
EudraCT number	2017-003270-14
Other public database nr	
Prinicpal Investigators and Participating Sites	Prof. Dr. Joost Wauters, Intensivist, UZ Leuven Prof. Dr. Paul Verweij, Medical microbiologist, UMC Radboud Nijmegen
Medical condition or disease under investigation	Influenza-associated pulmonary aspergillosis (IAA)
Purpose of clinical trial	Study the effect of antifungal prophylaxis on the incidence of IAA in critically ill patients with severe influenza.
Primary objective	Study if antifungal prophylaxis can decrease the incidence of IAA compared to SOC in critically ill patients with severe influenza pneumonia.
Secondary objective(s)	
Trial Design	Interventional non-blinded randomized controlled multicenter study
Endpoints	The IAA incidence at ICU discharge is the **primary endpoint** and will be compared in patients receiving posaconazole prophylaxis with those receiving SOC. **Secondary outcomes** will be time to IAA diagnosis, length of ICU stay, length of hospital stay, 30-day mortality and 90-day mortality
Sample Size	50 patients in each arm
IMP, dosage and route of administration	Posaconazole, 2*300mg/d on day 1, followed by 1*300mg/d from day 2 untill day 7, intravenously administrated
Active comparator product(s)	None, SOC
Maximum duration of treatment of a Subject	7 days of prophylaxis

Maximum duration of Trial	4 influenza seasons (the first two influenza seasons will be supported my MSD)
Anticipate First Patient First Visit (FPFV)	Expected 01/12/2017
Anticipate Last Patient Last Visit (LPLV)	Expected 30/03/2021

TRIAL FLOWCHART

Schedule of Events – Trial specific Procedures / Assessments

EVENTS	Screening	Randomization	Treatment Period	ICU discharge	Follow-Up Visits (phone call)
Timing (weeks)		Day 1	Day 2 - ICU discharge		Day 30 and day 90
Informed consent [a]	X				
PCR-confirmed influenza [b]	X				
Inclusion/Exclusion	X	X			
Demographics; Medical history	X				
Symptom directed physical examination	X	X	X	X	
Weight / Height	X				
Vital Signs	X	X	X	X	
Randomization		X			
Microbiology sampling	X	X [c]	X [d]	X	
Chemistry : laboratory measurements	X	X	X	X	
Radiological Assessments (CT / RX)		X	X		
Trial drug administration (max 7 days)		X	X		
IAA mycological evidence [e]	X	X	X	X	X
IAA signs and symptoms assessment	X	X	X	X	
Survival status		X	X	X	X
Adverse event (AE) assessment [f]	→	→	→		
Serious Adverse Event (SAE) assessment	→	→	→	→	
Concomitant Medication (CM)	→	→	→		

a: Informed Consent process should take place prior to all other trial-related procedures at the screening visit
b: On NS, BA or BAL within the last 7 days before ICU admission or within 2 days after ICU admission; or based on a positive rapid test if PCR not available
c: BAL GM, BAL culture and serum GM within 48h after influenza diagnosis to collect mycological evidence for IAA-infection
d: BAL GM, BAL culture and serum GM between day 2 after randomization and ICU discharge, at the discretion of the treating physician, or later during ICU admission in case of clinical respiratory deterioration
e: Provided by either (A) a positive culture of *Aspergillus* cultured from BAL, (B) ≥2 positive cultures of *Aspergillus* cultured from a sputum or a bronchial aspirate or (C) a galactomannan (GM) optical density of ≥1 in BAL or ≥0.5 in serum
f: Study drug related adverse events

1 Background and Rationale

Invasive aspergillosis has been regarded as an infectious complication of specific immunocompromised patients, which affects a relatively small group of patients. However, in recent years aspergillus disease has become a prominent problem in ICU-patients.

Influenza-associated aspergillosis (IAA) was recently found to be a frequent and severe complication of influenza pneumonia in critically-ill patients.[1] A retrospective observational study during the 2015/2016 influenza season indicated that among 144 patients admitted to the ICUs of all eight Dutch University Medical Centres with influenza, 23 (16%) developed IAA.[2] The underlying diseases in these patients were diverse, and 7 patients had been previously healthy. A 61% mortality rate was observed, including 5 of 7 previously healthy patients who died.[2] It is thus clear that IAA is an early and frequent complication of severe influenza pneumonia, and that delay in diagnosis and treatment increases the probability of death in these critically ill patients. Early diagnosis is difficult due to unspecific clinical and radiological presentation. [1,2]

As influenza virus infection leads to lysis of infected cells, we believe that a severe influenza infection causes cytopathology of the epithelial lining of the trachea and bronchi, providing *A. fumigatus* the opportunity for invasive growth. Many patients developed invasive *Aspergillus* tracheobronchitis, which is a manifestation of invasive aspergillosis that is difficult to diagnose due to lack of specific radiologic features.[3] Furthermore, influenza may contribute to a hyperinflammatory status known as macrophage activation syndrome that contributes to pulmonary damage.[4] Disruption of anatomical barriers and the dysregulated host defense provides an opportunity for *A. fumigatus* to cause fulminant invasive disease. Although biomarkers, such as galactomannan, in BAL and serum are useful tools in diagnosing IAA, early diagnosis proved difficult in the above-mentioned Dutch observational study. Moreover, the median time to initiation of antifungal therapy after influenza diagnosis in survivors and non-survivors was two days versus nine days ($P<0.06$), which underscores that early intervention is critical for survival. Finally, a high azole resistance rate of 29% was observed in these Dutch ICUs, which further complicates patient management.[2,5]

Therefore we propose to perform **an interventional non-blinded randomized controlled multicentric Dutch-Belgian proof-of-concept interventional study** in patients with severe influenza admitted to the ICU, randomizing patients either to oseltamivir therapy with standard care for diagnosis and treatment of secondary infectious complications or to oseltamivir plus posaconazole prophylaxis.

Currently there is no mold-active antifungal agent licensed for prophylaxis of invasive mold disease in ICU patients. The antifungal azole posaconazole is the drug of choice for antifungal prophylaxis in neutropenic patients with acute myeloid leukemia and those with graft-versus host disease following allogeneic HSCT.[6,7] These studies showed that posaconazole prophylaxis reduced the prevalence of invasive aspergillosis to below 2% in these high risk patients. Posaconazole is however not licensed for prophylaxis in other patient groups, i.e. critically ill patients. Based on the experience in patients with hematological

malignancy we anticipate that posaconazole prophylaxis will be effective to reduce the IAA incidence and eventually outcome in patients at risk for IAA.

Although the planned study is a multicenter Dutch-Belgian study, a specific concern in Dutch ICUs is the high rate of azole resistance.[2,8] Although the efficacy of triazoles, including posaconazole, is reduced in experimental models of azole-resistant invasive aspergillosis,[9,10] these animal experiments do indicate that posaconazole prophylaxis is effective to *prevent* infection due to azole-resistant *A. fumigatus.*[11] Infection was prevented in animals challenged with *A. fumigatus* with a posaconazole MIC of 0.5 mg/l, which represents the vast majority of resistant isolates that are encountered. There is already substantial experience with posaconazole in terms of target PD, toxicity and drug interactions, which will facilitate a rapid implementation into clinical practice if this and future studies demonstrate the efficacy of the intervention.

2 Trial Objectives and Design

2.1 Trial objectives

Our **hypothesis** is that antifungal prophylaxis can reduce the IAA incidence in critically ill patients with influenza pneumonia.

The **objective** of this study is to deliver proof of concept that antifungal prophylaxis in addition to SOC in comparison with SOC alone can reduce the IAA incidence in ICU patients with severe influenza.

2.2 Primary Endpoints

The incidence of IAA-infection at ICU discharge is the **primary endpoint** and will be compared for patients receiving posaconazole prophylaxis with those receiving standard of care.

A patient with **IAA-infection** is defined as a patient having mycological evidence of *Aspergillus* and at least one *Aspergillus* compatible sign or symptom and radiological abnormalities (other than interstitial infiltrates) based on chest CT, or chest X-Ray when CT imaging is not available. Mycological evidence can be provided by either (A) a positive culture of *Aspergillus* cultured from BAL sample, (B) ≥2 positive cultures of *Aspergillus* cultured from a sputum or a bronchial aspirate or (C) a galactomannan (GM) optical density of ≥1 in BAL or ≥0.5 in serum. Galactomannan antigen detection is conducted through sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia *Aspergillus*; Bio-Rad Laboratories, Marnes-la-Coquette, France) and will be performed according to the instructions of the manufacturer. For the isolation of fungi, lower tract respiratory samples will be inoculated on a Sabouraud agar (2 days at 37°C and another 19 days at 30°). *Aspergillus* species are identified by their culture characteristics and morphologies.

Aspergillus compatible signs or symptoms are defined as at least one of the following:

- Worsening respiratory insufficiency in spite of proper antibiotic and ventilator support
- Dyspnea
- Haemoptysis

- Fever refractory to at least 3 days of appropriate antibiotic therapy. Recrudescent fever after a period of defervescence of at least 48 h while still on AB without apparent cause.

The presence of hyphae in lung biopsy or autopsy are also considered as sufficient evidence for IAA.

An independent review committee, consisting of a clinician and a microbiologist with substantial expertise in invasive fungal infections, will evaluate at the end of the study whether IAA-infection is present, based on the above mentioned criteria.

2.3 Secondary Endpoints

Secondary outcomes will be time to IAA diagnosis, length of ICU stay, length of hospital stay, 30-day mortality and 90-day mortality.

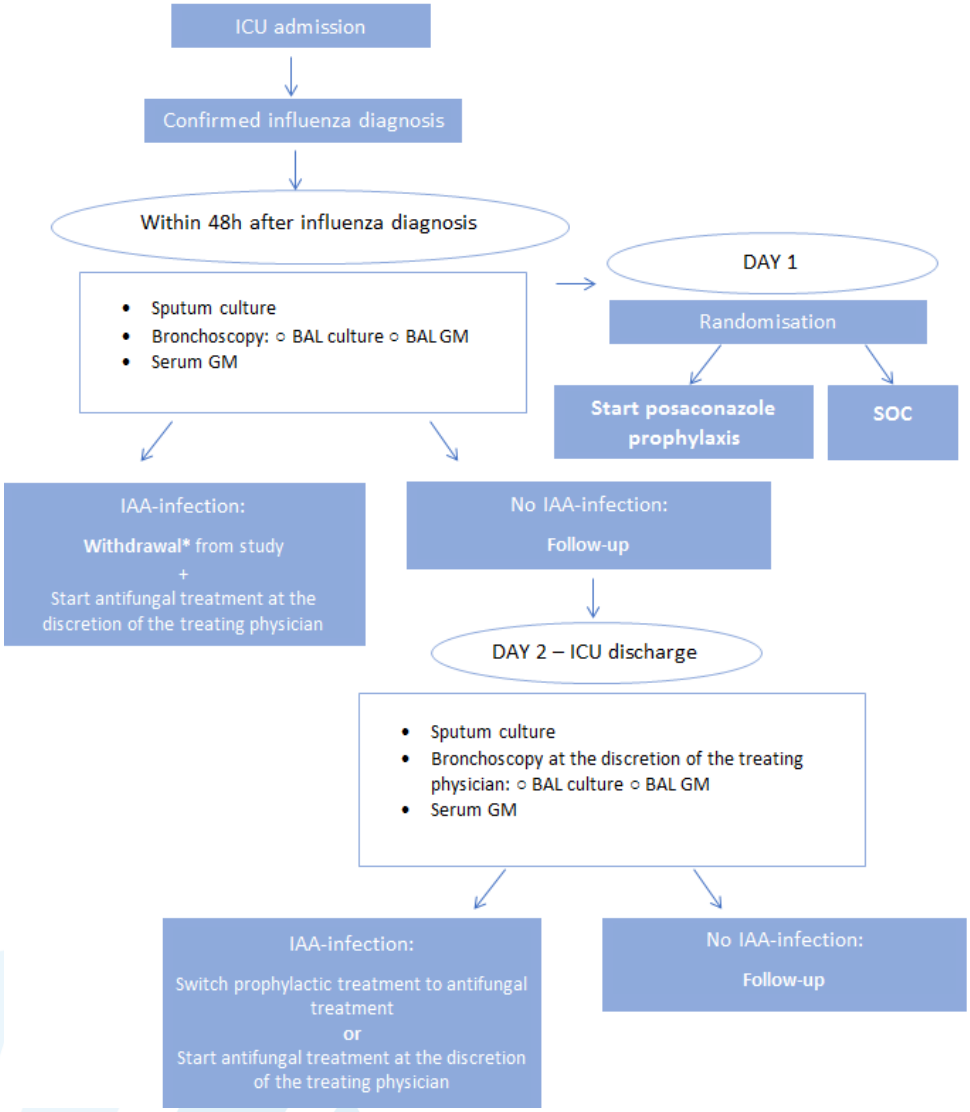
2.4 Trial Design

This study is an interventional non-blinded randomized controlled multicenter study.

- Critically ill patients with PCR-confirmed influenza (for details: see inclusion criteria) are eligible for inclusion in this study and will be randomized to or the posaconazole prophylaxis group or the SOC group. Within 48 hours after influenza diagnosis with a bronchoscopy with BAL (minimum volume of 2x20mL, 0.9% NaCl) and a with a serum galactomannan will be performed as part of routine ICU care in this type of patients. As any bronchoscopy, this bronchoscopy will only be performed if the safety of the patient will not be compromised. If an IAA-infection is suspected based on the result of this BAL ((A) *Aspergillus* cultured from BAL, or (B) a galactomannan (GM) ≥1 in BAL or ≥0.5 in serum), the patient will be withdrawn from the study and antifungal treatment will be started. The type and duration of antifungal therapy will be at the discretion of the treating physician.

- SOC further includes:
    - The treating physician can decide to perform an additional bronchoscopy with BAL between day 2 after randomisation and ICU discharge in case of respiratory deterioration and clinical suspicion of an IAA-infection. Any bronchoscopy will only be performed if the safety of the patient will not be compromised. In case of positive mycological evidence of an IAA-infection, a CT thorax without IV contrast is performed if possible. If an IAA-infection is documented, antifungal treatment will be started and - in case the IAA-infection is diagnosed before the ending of the study drug - the patient will be withdrawn from the study. Again, type and duration of antifungal therapy will be at the discretion of the treating physician.

- Oseltamivir (non-IMP) will be started at the discretion of the treating physiscian from the first day of ICU admission as 2*150 mg/day for a duration of 10 days. If oseltamivir had already been started up before ICU admission, oseltamivir treatment will be continued up to a total of 10 days.



*If *Aspergillus* is ***only*** cultured from sputum (and not from BAL) within the first 48 hours after the influenza diagnosis, the patient will not be witdrawn from the study

3 Trial Medication / Drug

Drug Name	IMP or non-IMP
Posaconazole	IMP

3.1 Investigational Medicinal Product and Dosing Regimen

If a patient is randomized non-blinded to the **posaconazole prophylaxis** group, posaconazole (Noxafil, MSD) will be started intravenously from day 1 of randomization (2*300mg/d on day 1, followed by 1*300mg/d from day 2 for 7 days). Noxafil concentrate injections (vials made of type I glass containing 18mg posaconazole /mL, 300mg posaconazole/vial in total) for intravenous use will be provided by MSD.

In both patient groups (prophylaxis and SOC) **oseltamivir** (non-IMP) will be started at the discretion of the treating from the first day of ICU admission as 2*150 mg/day for 10 days. If oseltamivir had already been started up before ICU admission, oseltamivir treatment will be continued up to a total of 10 days.

3.2 Drug Accountability and Compliance

For each randomized patient 8 drug preparations (posaconazole or placebo) will be delivered by the hospital pharmacy. The amount of used and non-used posaconazole vials will be stated in a drug accountability log.

3.3 Concomitant medication / Prohibited Medication

Medication for the treatment of common adverse events related to posaconazole treatment such as headache, abdominal pain, nausea, diarrhea, constipation, vomiting, cough and dyspnoea are permitted concurrently with the trial medication. Other treatments related to standard of care of critically ill influenza patients are allowed during treatment with posaconazole, taking in to account the precautions below.

Patients being treated with the following medication should be continuously monitored for AEs: sirolimus, CYP3A4 substrates (pimozide, quinidine), HMG-CoA Reductase Inhibitors Primarily Metabolized Through CYP3A4 (simvastatin), ergot alkaloids (ergotamine and dihydroergotamine), vincristine, terfenadine, cisapride, ebastine, astemizole, cimetidine, rifampin, carbamazepine, phenytoin, rifabutin, barbiturates, isoniazid and anthracyclines.

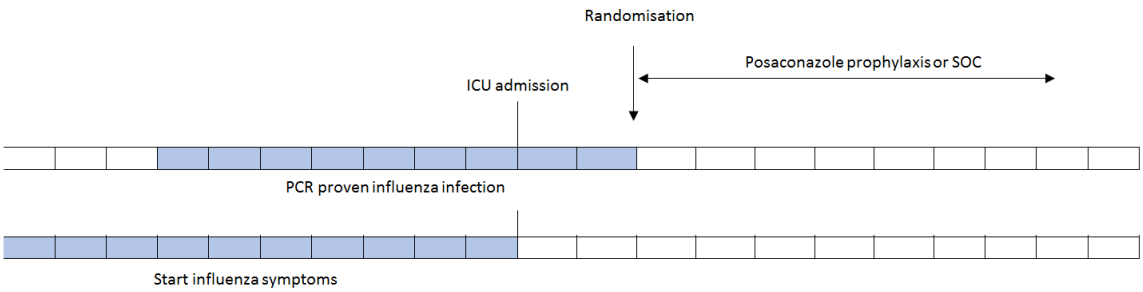
In patients being treated with calcineurin-inhibitors (cyclosporine or tacrolimus) frequent monitoring of cyclosporine and tacrolimus whole blood concentrations should be performed during and at discontinuation of posaconazole treatment since elevated concentrations of these drugs can lead to nefrotoxicity and leukoencephalopathy. Cyclosporine an tacrolimus dosing should be adjusted according to their whole blood concentrations.

4 Eligibility Criteria

4.1 Inclusion criteria

Patients eligible for inclusion in this trial have to meet **all** of the following criteria:

1. Written informed consent must be obtained from the patient or his/her legal representative prior to any study procedures
2. Adult patient (≥ 18 years)
3. PCR-confirmed influenza based on nasopharyngeal swab (NS), bronchial aspirate (BA) or broncho-alveolar lavage (BAL) within 7 days before ICU admission or within 48 hours after ICU admission. If PCR is not available a positive result of a rapid test is required (a negative rapid test does not imply absence of influenza and thus requires confirmation by PCR)
4. Influenza symptoms present for no more than 10 days before ICU admission
5. Respiratory distress as the main reason for ICU admission. Respiratory distress will be defined as tachypnea with an respiratory rate ≥ 25x/min and a paO2/fiO2-ration ≤ 300 with or without (bilateral) infiltrates.



4.2 Exclusion criteria

Patients eligible for this trial must not meet any of the following criteria:

1. Patients with age < 18 years
2. Pregnant women (based on a positive serum sample)
3. Expected survival on ICU admission ≤ 48h
4. Patients having influenza symptoms for more than 10 days before ICU admission
5. Patients being transferred from another hospital ward or another hospital who already have mycological evidence for an IAA-infection (based on sputum, BA or BAL culture, BAL or serum GM)
6. Patients with known intolerance or hypersensitivity to posaconazole or other azole antifungal agents
7. Patients that are being treated actively with antifungal agents for invasive aspergillosis
8. Patients with a QTc interval ≥500 msec
9. Patients with liver cirrhosis (Child C)
10. Participation in another interventional clinical trial

11. Any disorder, which in the investigator's opinion might jeopardise subject's safety or compliance with the protocol
12. Patients or their legal representatives who did not sign the informed consent form

4.3 Criteria for premature discontinuation of trial product

- Safety concerns related to trial product (see paragraph 6) or unacceptable intolerability.
- Included in the trial in violation of the inclusion and/or exclusion criteria
- Pregnancy
- Simultaneous participation in another clinical trial of an approved or non-approved investigational medicinal product (IMP)

4.4 Withdrawal criteria

The subject may withdraw consent at will at any time. The subject's request to withdraw from the trial must always be respected. Patients will also be withdrawn from the study when an IAA-infection is diagnosed based on clinical and mycological evidence with radiological abnormalities, between day 1 and day 7 of posaconazole prophylaxis. At that time antifungal treatment will be started. The type and duration of antifungal therapy will be at the discretion of the treating physician.

5 Trial Procedures

5.1 Selection of Participants / Recruitment

Patients admitted to ICU with a severe influenza pneumonia will be screened by the study nurse. When all eligibility criteria are met, the PI or the treating physician will approach the patient or his/her relatives in order to give study information and request informed consent.

5.2 Consent

The Principal Investigator (PI) retains overall responsibility for the informed consent of participants at their site and must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki. If delegation of consent is acceptable then details should be provided. Adequate documention will be performed.

Informed consent must be obtained prior to the participant undergoing procedures that are specifically for the purposes of the trial and are out-with standard routine care at the participating site (including the collection of identifiable participant). All obtained original informed consent forms must remain at the site and cannot be destroyed (even when a scanned copy is available).

The right of a participant to refuse participation without giving reasons must be respected. The participant must remain free to withdraw at any time from the trial without giving reasons and without prejudicing his/her further treatment and must be provided with a contact point where he/she may obtain further information about the trial. Where a participant is required to re-consent or new information is required to be provided to a participant it is the responsibility of the PI to ensure this is done in a timely manner.

The PI takes responsibility for ensuring that all vulnerable subjects are protected and participate voluntarily in an environment free from coercion or undue influence. Where the participant population is likely to include a significant proportion of participants who cannot read or write, require translators or have cognitive impairment, appropriate alternative methods for supporting the informed consent process should be employed. This may include allowing a witness to sign on a participant's behalf (in the case of problems with reading or writing), designate a legal representative , or providing Participant Information Sheets in other languages or in a format easily understood by the participant population (in the case of minors or cognitive impairment) providing they are approved by the EC.

5.3 Expected Duration of the Trial

The expected duration of the trial will be 90 days. If the patient is not hospitalized any more at the 90th day after randomization, the patient or his/her relatives will be contacted by telephone in order to obtain 90-day mortality data.

5.4 Randomisation Procedure / Code Break / Allocation concealment

Patients will be randomly assigned to the posaconazole prophylaxis group or the SOC group by using the Sealed Envelope randomization procedure (www.sealedenvelope.com). Block randomization will be used.

5.5 Visit schedule and assessments

5.5.1 Screening / Baseline

After eligibility screening and signing the trial ICF, the following screening assessments will be done:

- Patient demographics and baseline parameters (SOC)
- Medical History (SOC)

5.5.2 Treatment Visits

Day 1 = day of randomization:

- Bronchoscopy with BAL sampling, serum galactomannan (SOC)
- Pregnancy screening negative
- Start study medication
- Gather baseline clinical data including demographic, biochemical, microbiological and radiological data (SOC)

Day 2 – ICU discharge:

- Withdrawal of patients from study if mycological evicdence of IAA-infection on BAL of day 1
- Continuation of study medication up to day 7
- Gather clinical data including demographic, biochemical, microbiological and radiological data (SOC)
- Assess presence of IAA-infection (SOC)
- Assess outcome parameters: ICU mortality, IAA-infection (SOC)

30 days after randomization:

Assess outcome parameters: hospital and/or 30-day mortality and a retrospective assessment of IAA-infection (SOC)

90 days after randomization:

- Assess outcome parameters: hospital/or and 90-day mortality and a retrospective assessment of IAA-infection (SOC)

5.5.3 Discontinuation of Treatment – End of Trial Visit – Withdrawal of Consent

Patients may voluntarily discontinue from the trial treatment for any reason at any time. If a patient decides to discontinue from the trial treatment, the investigator must make a reasonable effort (e.g. telephone, e-mail, letter) to understand the primary reason for this decision and record this information in the patient's chart and on the appropriate CRF pages. They may be considered withdrawn if they state an intention to withdraw, fail to return for visits, or become lost to follow-up for any other reason. The investigator should discontinue trial treatment for a given patient if, he/she believes that continuation would be detrimental to the patient's well-being.

Patients may voluntarily withdraw consent to participate in the trial for any reason at any time. Withdrawal of consent occurs only when a patient does not want to participate in the trial any longer, and does not want any further visits or assessments, and does not want any further trial related contact. Sponsor will continue to retain and use all research results that have already been collected for the trial evaluation. All biological samples that have already been collected may be retained and analyzed at a later date (or as required by local regulations). If a patient withdraws consent, the investigator should make a reasonable effort (e.g. telephone, e-mail, letter) to understand the primary reason for this decision and record this information. Trial treatment must be discontinued and no further assessments conducted. Further attempts to contact the patient are not allowed unless safety findings require communication or follow up.

5.5.4 Follow-up Visits

A follow-up visit will be perfomed at day 90 by phone in case of discharge. Patient data after the ICU discharge will be gathered through the patient medical file .

6 Adverse Event Reporting

6.1 Definitions

6.1.1 Adverse Event

This study uses posaconazole, which is an antifungal agent licensed for prophylaxis as well treatment of invasive fungal infections. The clinical indications and dosages used in this trial are similar to the licensed indications and dosages or lower; therefore no potential harmful risks are expected in this cohort. Furthermore, the study medication, posaconazole, given as antifungal prophylaxis, is given on top of standard intensive diagnostic work-up to detect a fungal infection as soon as possible.

We strongly believe the burden for the patient as well as the risk for severe adverse events is reduced to an absolute minimum. The risk-classification is assessed as negligible to the patient population receiving study drug at the current regimens.

An Adverse Event (AE) is any untoward medical occurrence in a patient or subject during an experiment, and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a product, whether or not considered related to the product. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE.

The following AEs should be reported:

- New ALAT elevation after the start of posaconazole to >10x ULN, not being explained by another overt reason
- QTc interval prolongation (QTc > 500 msec)

These adverse events are monitored as a part of SOC on ICU.

6.1.2 Serious Adverse Event

A Serious Adverse Event (SAE) is any untoward medical occurrence that results in any of the following:

- Death
- A life-threatening[a] experience
- In-patient hospitalisation or prolongation of existing hospitalisation
- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- Important medical events that may be considered an SAE when - based on appropriate medical judgement - they may jeopardise the subject and may require medical or surgical intervention to prevent one of the above outcomes

[a] *The term "life threatening" in the definition of SAE refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe.*

6.1.3 Adverse Events of Special Interest

The following events should be reported within the same timelines as SAEs:

- Overdose
- Misuse/abuse
- Medication error

6.2 Adverse Events that do not require Reporting

In general, the following should not be reported as AEs:

- Pre-existing conditions, including those found as a result of screening (these should be reported as medical history or concomitant illness.
- Pre-planned procedures unless the condition for which the procedure was planned has worsened from the first trial-related activity after the subject has signed the informed consent.

Adverse events other than the above mentioned AE that should be reported, will be reported at the discretion of the treating physician.

Although these events should not be reported to the sponsor, these should be recorded in the patient's medical notes according to routine practice.

The following events not to be considered as SAEs are hospitalisations for :

- Pre-planned hospitalisation unless the condition for which the hospitalisation was planned has worsened from the first trial-related activity after the subject has signed the informed consent.
- A standard procedure for protocol therapy administration. However, hospitalisation or prolonged hospitalisation for a complication of therapy administration will be reported as an SAE.
- Hospitalisation or prolongation of hospitalisation for technical, practical, or social reasons, in absence of an AE.

6.3 Recording and Reporting of Averse Events

Investigators will seek information on AE at each patient contact. All events, whether reported by the patient or noted by trial personnel, will be recorded in the patient's medical record and in the (e)CRF within a reasonable time after becoming aware. If available, the diagnosis should be reported on the AE page, rather than the individual signs or symptoms. If no diagnosis is available, the investigator should record each sign and symptom as individual AEs using separate AE forms.

6.3.1 Assessment

All AEs must be evaluated by an Investigator as to:

- **Seriousness:** wether the AE is an SAE. See above for the seriousness criteria.

- **Severity:**

- If applicable: grading must be evaluated by an Investigator according to the current active minor version fo the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE, version 4.0) **OR**
- If applicable: severity must be evaluated by an Investigator according to the following definitions:
  - Mild – no or transient symptoms, no interference with the subject's daily activities
  - Moderate – marked symptoms, moderate interference with the subject's daily activities
  - Severe – considerable interference with the subject's daily activities, unacceptable

- **Causality:**
  - None – An AE which is not related to the trial product or experiment
  - Unlikely – An AE for which an alternative explanation is more likely (e.g. concomitant medication(s), concomitant disease(s)), and/or the relationship in time suggests that a causal relationship is unlikely
  - Possible – An AE which might be due to the use of the trial product or the experiment. An alternative explanation is inconclusive. The relationship in time is reasonable; therefore the causal relationship cannot be ruled out.
  - Probable - An AE which might be due to the use of the trial product or the experiment. The relationship in time is suggestive (e.g. confirmed by dechallenge). An alternative explanation is less likely.
  - Definitely – An AE which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation. The relationship in time is very suggestive (e.g. it is confirmed by dechallenge and rechallenge).

6.3.2 Timelines for reporting

- After informed consent has been obtained but prior to initiation of trial treatment, only serious adverse events caused by a protocol-mandated intervention (e.g…….) should be reported
- After initiation of trial treatment, adverse events will be reported as follows:
  - All adverse events will be reported during study treatment
  - Serious adverse events and adverse events of special interest will continue to be reported until 90 days

All SAEs/AESI as defined in the protocol must be reported to the Sponsor within 24 hours of the research staff becoming aware of the event.

Describe here the details of how the SAE must be reported by the Investigator to the Sponsor:

- By completing the SAE form in the (e)CRF

If an authorised Investigator from the reporting site is unavailable, initial reports without causality and expectedness assessment should be submitted to the Sponsor by a healthcare professional within 24 hours of becoming aware of the SAE, but must be followed-up by medical assessment as soon as possible thereafter.

6.3.3 Follow-up

The Investigator must record follow-up information by updating the medical records and the appropriate forms in the (e)CRF. The worst case severity and seriousness of an event must be kept throughout the trial.

SAE follow-up information should only include new (e.g. corrections or additional) information and must be reported within 24 hours of the Investigator's first knowledge of the information. This is also the case for previously non-serious AEs which subsequently become SAEs.

- All **SAEs** must be followed until the outcome of the event is 'recovered', 'recovered with sequelae', 'not recovered' (in case of death due to another cause) or 'death' (due to the SAE) and until all related queries have been resolved.
- **Non-serious AEs** must be followed unil the end of the follow-up period, and until all related queries have been resolved.

SAEs after the end of the trial: If the Investigator becomes aware of an SAE with suspected causal relationship to the trial product or experiment after the subject has ended the trial, the investigator should report this SAE within the same timelines as for SAEs during the trial.

6.3.4 Pregnancy

Female subjects must be instructed to notify the investigator immediately if they become pregnant during the trial. The investigator must report any pregnancy in subjects who have received trial product(s), as soon as possible using an SAE form.

6.3.5 Technical Complaints

Technical complaints should be reported to the Market Authorisation Holder.

6.4 Reporting requirements to Ethics Committee's and Health Authorities

The investigator is responsible for ensuring that all safety events are recorded in the (e)CRF and reported to the Sponsor in accordance with instructions provided below:

Who reports	What	To	Within which period
Investigator	AE	(e)CRF	Cfr protocol

Investigator	SAE (including death) AESI Pregnancy	Coordinating Investigator / sponsor	Immediately (within 24 hours of becoming aware of the event) Exceptions: cfr protocol
PI /sponsor	Each death of a subject from a Belgian site	Central EC	Immediately
PI (Belgian site)	Each death of a subject from an external Belgian site	Local EC	Immediately
PI (non-Belgian site)	Each death of a subject from an external non-Belgian site	According to local legislation	According to local legislation
PI / sponsor	Any SUSAR that is fatal or life-threatening	- Competent Authorities - All other member states if applicable - EC - Investigators - Marketing Authorisation Holder if applicable	Within 7 calendar days, every additional information within additional 8 calendar days
PI / sponsor	All other SUSAR's		Within 15 calendar days
PI / sponsor	Report of all SAE's	Competent Authorities	Annually or final report
		All other member states if applicable	Annually or final report
		EC	Annually a summary of the past year with a safety analysis and risk-benefit evaluation

The Sponsor will promptly evaluate all serious adverse events and adverse events of special interest against experience to identify and expeditiously communicate possible new safety findings to investigators, ECs and applicable health authorities based on applicable legislation.

7 Statistics and Data Analysis

7.1 Sample Size

Based on an estimated IAA incidence of 25% and considering a 80% reduction of the incidence (to 5%) caused by posaconazole prophylaxis and a power of 80%, a sample size calculation showed that 47 patients in each study arm are needed. Because it is impossible that the use of posaconazole will increase the

incidence of IA, a one-sided p-value was used for this sample size calculation. To include at least 100 patients in the MITT endpoint analysis, 110 patients will be included in the study (55 in each arm).

7.2 Data Analysis

Appropriate statistical tools will be used to test for significance with respect to the primary and secondary endpoints.

Categorical variables will be reported as a percentage and continuous variables as mean ± standard deviation (SD) or median and interquartile range (IQR), as appropriate. Differences in categorical variables will be assessed using the Chi squared test or the Fischer's Exact test and for the analysis of continuous variables the Student's t test or the Mann-Whitney U test will be used, where appropriate. P values <0.05 are considered significant. Patients that die during the trial duration will also be included in the statistical analysis.

The primary endpoint analysis will consist of a one-sided fisher-exact test in which the proportion of cases of IAA will be compared between both groups. The population included in this analysis will consist of a modified intent to treat population. This MITT population consists of all patients that received at least 1 posaco/placebo administration but excluding the patients in which a diagnosis of IAA was made on the basis of the BAL sample taken on day 1 because these patients in fact had an IAA at the day of inclusion.

8 Data handling

Patient data will be encoded: which means that personal data can only be related to an identified or identifiable person by means of a code.
Demographic, biochemical, microbiological and radiological data will be collected prospectively as a part of clinical routine. Samples will be obtained for characterization of the influenza type and for *Aspergillus* diagnosis including culture, galactomannan and PCR. These samples are part of daily routine, no additional samples for this study will be taken. All *Aspergillus* colonies will be screened for azole resistance, using an agar-based selection tool, and relevant samples, i.e. BAL fluid, will be stored for direct detection of resistance mutations by PCR. Clinical *A. fumigatus* isolates will be sent the National Mycology Reference Laboratory in Leuven (Belgium) or Nijmegen (the Netherlands) for MIC-testing and analysis of azole-resistance mutations. A blinded data review committee following a pre-defined case definition will classify patients with evidence for primary and breakthrough IAA-infection.

8.1 Data Collection Tools and Source Document Identification

Source data will be collected and recorded in the trial participants files/medical records. They will be kept on a secured location at all times. The collection and processing of source data (from subjects enrolled in this trial) will be limited to those data that are necessary to fulfill the objectives of the trial. These data

must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Personnel whose responsibilities require access to personal data agree to keep the data confidential.

Documentation of source data is necessary for the evaluation and validation of clinical findings, observations and other activities during a clinical trial. Source documentation serves to substantiate the integrity of trial data, confirms observations that are recorded and confirms the existence of trial participants. Furthermore source documentation must be available for the following to confirm data collected in the (e)CRF: subject identification, eligibility, and trial identification; trial discussion and date of informed consent; dates of visits; results of safety and efficacy parameters as required by the protocol; record of all adverse events and follow-up of adverse events; concomitant medication; drug receipt/dispensing/return records; trial drug administration information; and date of trial completion and reason for early discontinuation of trial drug or withdrawal from the trial, if applicable.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the investigator. The investigator will maintain complete and accurate documentation for the trial. All source documents will be reviewed by the clinical team to ensure that they are accurate and complete.

As defined in section 1.52 of the ICH Guideline for Good Clinical Practice (latest version of ICH E6) source documents may include: original documents, data and records (e.g. hospital records, clinical and office charts, laboratory notes….)

Case report forms

CRFs are provided for each subject in electronic/paper format. The trial data will be transcribed on a regular basis by trial personnel from the source documents onto an (e)CRF in a pseudonymised manner, and transmitted in a secure manner to the PI within the timeframe agreed upon between PI and the sites. The file will be considered to be the (e)CRF.

Worksheets may be used for the capture of some data to facilitate completion of the (e)CRF. Any such worksheets (including but not limited to copies of the (e)CRF) will become part of the trial participant's source documentation. All data relating to the trial must be recorded in (e)CRFs prepared by the investigator. Data must be entered into (e)CRFs in English. Designated site personnel must complete the (e)CRF as soon as possible after a subject visit, and the forms should be available for review at the next scheduled monitoring visit.

All subjective measurements (e.g., dairies, questionnaires, or …) will be completed by the same individual who made the initial baseline determinations whenever possible. The investigator will ensure that data are recorded on the (e)CRFs as specified in the trial protocol and in accordance with the instructions provided.

All (e)CRF entries, corrections, and alterations must be made by the investigator or other authorized trial-site personnel. Proper audit trails are available to demonstrate the validity of the trial data. A certified copy of the completed (e)CRFs will be archived at the trial site.

Data handling and record keeping

The Investigator and the Participating Site shall treat all information and data relating to the Trial disclosed to Participating Site and/or Investigator in this Trial as confidential and shall not disclose such information to any third parties or use such information for any purpose other than the performance of the Trial. The collection, processing and disclosure of personal data, such as patient health and medical information is subject to compliance with applicable personal data protection and the processing of personal data .

The investigator will maintain a certified copy of (e)CRFs and all source documents that support the data collected from each trial participant, as well as all trial documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all trial documents as specified by the applicable regulatory requirement(s). A Certified copy as specified in addendum 1.63 in ICH/GCP is a copy (irrespective of the type of media used) of the original record that has been verified (i.e. by a dated signature or by generation through a validated process) to have the same information, including data that describe the context, content and structure, as the original. The investigator will take measures to prevent accidental or premature destruction of these documents.

If data need to be transferred, this will be performed via a secured method of transfer taking into account all applicable security arrangements and regulations (such as the Belgian Privacy Act of 8 December 1992 on the protection of privacy in relation to the processing of personal data and the European Data Protection Act). Receiving party will agree to keep the transferred data confidential at all times.

8.2 Access to Data, Monitoring, Audit and Inspection

The investigator will permit trial-related monitoring, audits, EC review and regulatory inspection, providing direct access to all related source data / documents.

(e)CRFs and all source documents, including progress notes and copies of laboratory and medical test results must be available at all times for review by the sponsor's clinical trial monitor, auditor and inspection by health authorities. The accuracy of the data will be verified by review of the source documents.

8.3 Archiving

As specified in in ICH/GCP Section 8.1 Addendum the sponsor and investigator/institution should maintain a record of the location(s) of their respective essential documents including source documents. The storage system used during the trial and for archiving (irrespective of the media used) should provide for document identification, version history, search and retrieval.

Essential documents for the the trial should be supplemented or may be reduced where justified (in advance of trial initiation) based on the importance and relevance of the specific documents to the trial.

The sponsor should ensure that the investigator has control of and continuous access to the CRF data reported to the sponsor. The sponsor should not have exclusive control of those data. When a copy is used to replace an original document (e.g. source documents, CRF), the copy should fulfill the resuirements for certified copies. The investigator/institution should have control of all essential documents and records generated by the investigator/institution before, during and after the trial.

The Sponsor is responsible for archiving trial specific documentation (such as but not limited to protocol, potential amendments, copy of (e)CRF burned on CD, final report and database) for at least twenty years. Site-specific trial documents (such as but not limited to ICF) will be archived locally on site according to local practice and guidelines. Archived data may be held on electronic record, provided that a back-up exists and that hard copies can be obtained, if required. Destruction of essential documents will require authorisation from the Sponsor.

9 Ethical and Regulatory Considerations

9.1 Ethics Committee (EC) review & reports

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (most recent version), the principles of GCP and in accordance with all applicable regulatory requirements. Before the start of the trial, this protocol, the informed consent forms and other related documents e.g. advertisements and general practisioners information letters, will be submitted for review to the EC and to the Federal Agency for for medicines and health products (FAMHP) for Clinical Authorization (the below mentioned obligations shall only apply to the extent applicable). The trial shall not commence until such approvals have been obtained.

Any subsequent protocol amendments will be submitted to the EC and Regulatory Authorities for approval. No substantial amendment that require review by EC will be implemented until the EC grants a favorable opinion for the trial. The CI acknowledges that amendments may also need to be reviewed and accepted by the FAMHP before they can be implemented in practice at sites.

The trial can and will be conducted only on the basis of prior informed consent by the trial participants, or their legal representatives, to participate in the trial. Extensive discussion of risks and possible benefits of participation will be provided to the patients and/or their families. The participating site shall obtain a signed informed consent form for all trial participants prior to their enrollment and participation in the trial in compliance with all applicable laws, regulations and the approval of the (local) Ethics Committee, if required. The participating site shall retain such ICFs in accordance with the requirements of all applicable regulatory agencies and laws. All correspondence with the EC/FAMHP shall be retained in the Trial Master File/Investigator Site File.

The CI acknowledges that it is his responsibility to produce annual progress reports (APR) and he will do so by submitting to the EC/FAMHP within 30 days of the anniversary date on which the favorable opinion was given, and annually until the trial is declared ended.

The PI shall notify the EC/FAMHP of the end of the Trial. Should the trial be ended prematurely, the PI will notify the EC/FAMHP and include the reasons for the premature termination. The PI will submit a final report with the results, including any publications/abstracts, to the EC/FAMHP.

9.2 Peer review

This trial protocol was peer reviewed by certain independent experts. Peer review was conducted by expert referees to the professional and scientific standards expected for clinical studies.

9.3 Regulatory Compliance

Before the start of the trial, this protocol and other related documents will be submitted for review to the Federal Agency for medicinal products for Clinical Trial Authorisation (FAMHP). The trial shall not commence until such approvals have been obtained.

This trial protocol and the conduct of the trial in general is in compliance with applicable law, including but not limited to the Belgian law of May 7th 2004 regarding experiments on the human person and any relevant amendments.

9.4 Protocol/GCP compliance

The CI is responsible for ensuring that the clinical trial is performed in accordance with the protocol, current ICH guidelines on GCP, and applicable regulatory and country-specific requirements. GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of trial participants are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the clinical trial data are credible.

It is acknowledged and agreed that prospective, planned deviations or waivers to the protocol are not allowed under applicable regulations on clinical studies and must not be used. However, should there be an accidental protocol deviation, such deviation shall be adequately documented on the source documents and on the relevant forms and reported to the Chief Investigator and Sponsor immediately. Protocol deviations which are found to frequently recur, will require immediate action. CI acknowledges that such recurring protocol breaches could be potentially classified as a serious violation.

It is understood that "a serious violation" is likely to effect to a significant degree

- the safety or physical or mental integrity of the participants of the trial; or
- the scientific value of the trial

9.5 Data protection and patient confidentiality

The trial will be conducted in compliance with the requirements of the Belgian Privacy Act of 8 December 1992 on the protection of privacy in relation to the processing of personal data and the European Data Protection Act. Any collection, processing and disclosure of personal data, such as patient health and medical information is subject to compliance with the aforementioned personal data protection laws.

Any personal data shall be treated as confidential at all times including during collection, handling and use, and that the personal data (including in any electronic format) shall be stored securely at all times and with all technical and organizational security measures that would be necessary for compliance with data protection legislation. The Sponsor shall take appropriate measures to ensure the security of all personal data and guard against unauthorized access thereto or disclosure thereof or loss or destruction while in its custody.

The personal data of trial participants will be encoded, which means that they can only be related to an identifiable person by means of a unique code. The unique code will only be in the possession of the members of the trial team who are in direct contact with the trial participants. In no event will the coded personal data include personal identifiers, including any Trial participant's initials. Such coded personal data can only be traced or linked back by said trial team members, and said trial team members shall treat these codes as strictly confidential.

All trial related data and documents will be stored for at least twenty (20) years, in accordance with Belgian legislation.

9.6 Indemnity

The Sponsor shall throughout the duration of the trial effect and maintain with a reputable insurance company a policy or policies of insurance providing an adequate level of cover in respect of all risks which may be incurred by the Sponsor arising out of the Sponsor 's performance of the trial, including the insurance that is required to be taken out as sponsor of the trial as set out in the Law of 2004.

In accordance with the Belgian Law relating to experiments on human persons dated May 7, 2004, Sponsor shall assume, even without fault, the responsibility of any damages incurred by a Trial Patient and linked directly or indirectly to the participation to the Trial, and shall provide compensation therefore through its insurance.

The terms or the amount of cover of any insurance shall not relieve the Sponsor of any liabilities under the clinical trial agreement.

9.7 Amendments

In accordance with the Belgian law of May 7th 2004 regarding experiments on the human person, the Sponsor may make a non-substantial amendment at any time during a trial. If the Sponsor wishes to make a substantial amendment to the clinical trial agreement or the documents that supported the original

application for the CTA, the Sponsor must submit a valid notice of amendment to the licencing authority (FAMHP; if applicable) for consideration. If the Sponsor wishes to make a substantial amendment to the EC application or the supporting documents, the Sponsor must submit a valid notice of amendment to the EC for consideration. The FAMHP and/or the EC will provide a response regarding the amendment within 28 days of receipt of the notice. It is the Sponsor's responsibility to decide whether an amendment is substantial or non-substantial for the purposes of submission to the FAMHP and/or EC (if applicable).

9.8 Post trial care

Not applicable to this study.

10 Publication Policy

Publications will be coordinated by the Investigator of Sponsor. Authorship to publications will be determined in accordance with the requirements published by the International Committee of Medical Journal Editors and in accordance with the requirements of the respective medical journal.

For multi-centric trials, it is anticipated that the results of the overall Trial shall be published in a multi-centre publication, involving the data of all clinical sites participating in the Trial.

Participating Site is not allowed to publish any data or results from the Trial prior to the multicentre publication, provided however that Participating Site is allowed to publish the results generated at the Participating Site if the multicentre publication has not occurred after 12 months from Trial database lock.

Any publication by Participating Site will be submitted to the Sponsor for review at least thirty (30) days prior to submission or disclosure. Sponsor shall have the right to delay the projected publication for a period of up to three (3) months from the date of first submission to the Sponsor in order to enable the Sponsor to take steps to protect its intellectual property rights and know-how.

11 References

1. Wauters J, Baar I, Meersseman P, et al. Invasive pulmonary aspergillosis is a frequent complication of critically ill H1N1 patients: a retrospective study. Intensive Care Med. 2012;38:1761-8.
2. van de Veerdonk FL, Kolwijck E, Lestrade PPA, et al. Invasive pulmonary aspergillosis complicating influenza in critically ill patients: a nationwide retrospective observational cohort study. AJRCCM 2017; doi: 10.1164/rccm.201612-2540LE. [Epub ahead of print]
3. Patterson TF, Thompson GR 3rd, Denning DW, et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. ClinInfect Dis. 2016; 63:e1-e60.

4. Schulert GS, Zhang M, Fall N, et al. Whole-exome sequencing reveals mutations in genes linked to hemophagocytic lymphohistiocytosis and macrophage activation syndrome in fatal cases of H1N1 influenza. J Infect Dis. 2016;213:1180-8.
5. Verweij PE, Chowdhary A, Melchers WJG, Meis JF. Azole resistance in Aspergillus fumigatus: can we retain the clinical use of mold-active antifungal azoles? Clin Infect Dis 2016;62:362-8
6. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med. 2007;356:348-59.
7. Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med. 2007;356:335-47
8. van Paassen J, Russcher A, in 't Veld-van Wingerden AW, Verweij PE, Kuijper EJ. Emerging aspergillosis by azole-resistant Aspergillus fumigatus at an intensive care unit in the Netherlands, 2010 to 2013. Euro Surveill 2016;21:30.
9. Seyedmousavi S, Brüggemann RJM, Meis JF, Melchers WJG, Verweij PE, Mouton JW. Pharmacodynamics of isavuconazole in an Aspergillus fumigatus mouse infection model. Antimicrob Agents Chemother 2015;59:2855-66.
10. Seyedmousavi S, Brüggemann RJM, Melchers JWG, Mouton JW, Verweij PE. The role of azoles in the management of azole-resistant aspergillosis: from the bench to the bedside. Drug Resist Updat 2014;17:37-50.
11. Seyedmousavi S, Mouton JW, Melchers WJG, Verweij PE. Posaconazole prophylaxis in experimental azole-resistant invasive pulmonary aspergillosis. Antimicrob Agents Chemother 2015;59:1487-94.

PHARMACOKINETICS OF POSACONAZOLE AS PROPHYLAXIS FOR INVASIVE FUNGAL DISEASE ON ICU

De farmacokinetiek van posaconazole als profylaxe voor invasieve schimmelinfecties op Intensieve Zorgen

This study is an **amendment** on the principal study "A phase IV, interventional, non-blinded, randomized controlled multicenter study of posaconazole prophylaxis for the prevention of influenza-associated aspergillosis (IAA) in critically ill patients" (S60744, coordinating investigator Joost Wauters, MD PhD).

1. RESEARCH TEAM

Ruth Van Daele, PhamD	Clinical pharmacist (PhD-student), UZ Leuven & Clinical Pharmacology and Pharmacotherapy, KU Leuven
Isabel Spriet, PharmD, PhD	Clinical pharmacist, UZ Leuven & Clinical Pharmacology and Pharmacotherapy, KU Leuven
Joost Wauters, MD, PhD	Intensivist, UZ Leuven & Laboratory for Clinical Infectious and Inflammatory Disorders, KU Leuven
Roger Brüggemann, PharmD	PhD Clinical pharmacist and clinical pharmacologist, Radboudumc & Radboud Institute for Health Sciences

UZ Leuven, Herestraat 49, 3000 Leuven, Belgium

Isabel Spriet +32 16/34 12 61 isabel.spriet@uzleuven.be

Joost Wauters +32 16/34 03 85 joost.wauters@uzleuven.be

Radboudumc, Geert Grootplein Zuid 10, 6525 GA Nijmegen, The Netherlands

Roger Brüggemann +31 24361 6405 roger.bruggemann@radboudumc.nl

This study is supported by DB-MSG.

2. GENERAL BACKGROUND, OBJECTIVES AND AIMS

Fungal infections cause a serious threat to critically ill patients. The triazole antifungal drugs are the most important drugs for managing infections due to aspergillus moulds and the principal study (S60744) will investigate the role for those drugs in prophylaxis. Adequate treatment and prophylaxis of IAA is of the greatest essence but regrettably failure to therapy can occur. One of the shortcomings in previous studies is that pop-PK in critically ill patients haven't been investigated. As a consequence, solid recommendations of regimens other than the once investigated in hematology patients cannot be drawn based on previous models.

With this research we believe we can resolve multiple critical questions/objectives

1) Document pharmacokinetics of posaconazole in a cohort of critically ill patients on IV posaconazole to help determine the PK
2) Determine the impact of known and unknown covariates on posaconazole PK

The derived PK model can be used for modeling and simulation purposes to demonstrate target attainment in a wide distribution of patients.

As a general statement, we hypothesize that altered clearance is more likely in critically ill patients than altered clearance in hematology patients. We expect that a difference in clearance is largest in critically ill patients. Thus if we want to identify changes in PK of posaconazole, this is the cohort that is best used to address the question.

3. PATIENTS AND METHODS

3.1. Study characteristics

Open label, multiple dose, pharmacokinetic study on intravenous posaconazole.

3.2. Inclusion and exclusion criteria

Patients who are randomized in the posaconazole prophylaxis-group in de principal study (S60744) are automatically eligible for this PK study.

3.3. Study design

1) Posaconazole sampling

a. Group I: extensive sampling (UZ Leuven and Radboudumc)

- Plasma
  - A full PK curve will be taken on two extensive sampling days: day 2 and day 5. Intravenous administration of posaconazole sampling scheme: t = 0 (pre-dose), 1.5 (= end of infusion), 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours post infusion (11 samples).
  - On non-PK days (until day 7), samples will be taken daily just prior to the daily infusion therapy. The exact times of sampling will be recorded in the case report forms.
  - In the full PK group a maximum of 30 PK plasma samples will be taken. The total volume associated with this setup is 60 ml. No problems for the patients are expected with this limited amount of blood taken. In previous studies similar setup was used and approved by the ethics committee.
  - Together with the posaconazole concentration, the concentration of the glucuronide metabolite will be reported (no extra sample).
- BAL (bronchoalveolar lavage) – only in patients with mechanical ventilation and when medically indicated
  - The BAL fluid and alveolar cells from the BAL carried out 48 hours after influenza diagnosis (see principal study (S60744)) will be analyzed to measure the posaconazole concentration. Bronchoscopy will only be performed if the safety of the patient will not be compromised.
  - If other BALs are carried out by the treating physician, a part of that BAL fluid and alveolar cells will be analyzed to measure the posaconazole concentration.
  - BAL sampling by mini broncho-alveolar lavage (2 x 20 mL NaCL 0.9%) and a plasma sample at the same time.
  - Urea is determined simultaneously on BAL fluid and in plasma to correct for the dilution with NaCL 0,9%.

b. Group II: limited sampling (other investigation sites)

- Plasma
  - A limited sampling strategy will be deployed taking samples on the same days (day 2 and 5) but at less frequent time points : between 1.5 – 3 hours ; between 4-8 hours ; between 8-12 hours and between 12-24 hours (4 samples in total).
  - On non-PK days (until day 7), samples will be taken daily just prior to the daily infusion therapy. The exact times of sampling will be recorded in the case report forms.

2) Other data:

a. Data on critical illness, albumin, renal and liver function and albumin will be used for covariate analysis of posaconazole pharmacokinetics. One extra whole blood cell sampling tube will be used for genotyping (UGT1A4).

b. The clinical response of the posaconazole prophylaxis will be evaluated in the principal study (S60744).

3.4. Sample analysis and quantification

The samples will be analyzed in Radboudumc, Nijmegen.

3.5. Pharmacokinetic and statistical analysis of the data

To assess the sampling design for development of a population pharmacokinetic model, we evaluated a design using an 11 point sampling pharmacokinetic study. This was performed by means of stochastic simulation and estimation (SSE) of 500 virtual pharmacokinetic studies, based on a previously developed pharmacokinetic model for concentrations of posaconazole patients [Dolton and Bruggemann et al.] and this was performed with the SSE tool in Perl Speaks NONMEM and the non-linear mixed effects modelling program NONMEM V7.3.

3.6. Sample size

We will include all patients who are eligible for the principal study (S60744) and of who Informed Consent was attained. Intensive sampling will be done for all patients in UZ Leuven and Nijmegen and for the remainder of patients the limited sampling strategy will be used.