Azole-echinocandin combination therapy for invasive aspergillosis. A randomized pragmatic superiority trial (DUET)

My mama always said, life is like a box of chocolates.
You never know what you're gonna get. (Forrest Gump)

RESEARCH PROTOCOL

Studying the relevance of antifungal combination therapy for invasive aspergillosis. (IA-DUET)

Short title	**IA-DUET study / HOVON 502**
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
A. fumigatus	Aspergillus fumigatus
A. species	Aspergillus species
AE	Adverse Event
AML	Acute Myeloid Leukemia
AR	Adverse Reaction
BAL	Bronchoscopic Alveolar Lavage
BID	Twice daily
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CRF	Case Report Form
CT	Cycle Treshold
CV	Curriculum Vitae
D	Day
DSMB	Data Safety Monitoring Board
DDI	Drug-drug interaction
EORTC	European Organisation for Research and Treatment of Cancer
EORTC/MSG	European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
GVHD	Graft Versus Host Disease
HSCT	Hematopoietic stem cell transplantation
IA	Invasive Aspergillosis
IB	Investigator's Brochure
IC	Informed Consent
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
iMTA	institute for Medical Technology Assessment
IRB	Institutional review board
IV	Intravenous
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MITT	Modified intention to treat
MSG	Mycoses Study Group
PCR	Polymerase Chain Reaction
PO	Per os (oral intake)
QALY	Quality-adjusted life year
RAMS	Azole reistance associated mutations
RT	Resistance

(S)AE	(Serious) Adverse Event
SOC SOT SPC	Standard of care Solid Organ Transplant Summary of Product Characteristics
Sponsor	The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor but referred to as a subsidizing party.
SUSAR SWAB	Suspected Unexpected Serious Adverse Reaction Stichting Werkgroep Antibioticabeleid (in Dutch)
TR	Tandem Repeat
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale

Patients with underlying haematological malignancies or immunocompromised for various other reasons, are prone to fungal infections. Invasive aspergillosis (IA) is a common complication during remission inducing chemotherapy for acute leukemia or other hematological malignancies, as well as those who have undergone allogeneic hematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT). For more than 15 years voriconazole, a drug of the triazole class, has been the recommended treatment for this life-threatening infection after a pivotal randomized trial showed an improved survival with voriconazole compared with amphotericin B deoxycholate. However, also with voriconazole the overall 6-week mortality is still unacceptably high at 25-30% (Herbrecht et al., 2002[1]). Therefore, a randomized controlled trial assed the efficacy of voriconazole with or without anidulafungin for the treatment of IA in haematology patients to prove that combination therapy can improve outcome.[2] Among the 277 patients with IA in this study, the 6-week mortality with combination therapy was 30% lower (19.3%) than with monotherapy (27.5%), p=0.087. In a post-hoc analysis of the 222 patients with radiographic abnormalities and a positive galactomannan antigen test, a statistically significant difference in mortality was observed (p=0.037). Though, this study did not result in conclusive evidence in favor of combination therapy, it is a credible study which adds to the already existing in vitro and animal studies in support of echinocandin triazole combination therapy for IA and thus paves the way for a second larger and pragmatic clinical trial. Another important and new consideration about the management of IA is the upcoming of infections with triazole-resistant A.*fumigatus*. This is increasingly becoming a worldwide problem and leads to longer hospital stay, higher costs and is associated with a very high mortality. It is very likely that the exces-

sive use of antifungals of the triazole class in agriculture has formed the basis of this problem. Since 2018 the Dutch Working Party on Antibiotic therapy (SWAB) guideline on the management of invasive fungal infections therefore recommends upfront combination therapy (azole plus echinocandins or liposomal-amfotericine B) until resistance can be excluded as one of the treatment options for IA.

Given the evidence in favor of voriconazole-echinocandin combination therapy as well as the increasing incidence of voriconazole-resistant *A. fumigatus* in Belgium and the Netherlands, a large clinical study on the value of combination therapy is urgently needed.

Objectives

Primary objective

1. Evaluate if the survival in patients with a triazole susceptible IA can be improved when the initial therapy consists of triazole and echinocandin combination therapy instead of triazole monotherapy. This objective is captured in the primary endpoint as well as secondary endpoints 1 to 6)

Secondary objectives

1. Evaluate if a triazole/echinocandin combination therapy improves the overall quality of life and if it is a cost-effective intervention (these objectives are captured in secondary endpoint 11 and 12)
2. Evaluate the outcome of patients in which a triazole-resistant *A. fumigatus* is detected in relation to the initial antifungal therapy they had received (i.e. triazole monotherapy or combination therapy). This objective translates into secondary endpoint 7 an 8.
3. Evaluate the outcome of patients in which resistance testing is unsuccessful in function of the antifungal therapy they received. This translates into secondary endpoint 10.
4. Evaluate if the baseline serum galactomannan value and the serum galactomannan kinetics are predictive of overall 6-week survival. This translates into secondary endpoint 3 and 9.

Study design and intervention: The study is designed as a large pragmatic clinical trial to facilitate enrolment as much as possible. In particular, we want to leave the choice of the triazole (voriconazole or isavuconazole or posaconazole IV or oral) to the treating physician. This will not only lead to less patients being excluded but also allow the clinician to switch from one drug to another (within the same class) in case of treatment limiting toxicity. With the unbiased endpoint of overall survival, we consider a pragmatic approach that allows for easy recruitment of a sufficient number of patients

more important than the use of one specific drug within a class or the use of a placebo. Combination therapy will be discontinued after 28 days in all patients in which triazole susceptibility has been documented but when a treatment response is observed before day 28, the echinocandin can be discontinued as from day 7.

Study population

Immunocompromised patients who fulfill the EORTC/MSG host factor and mycological criteria of invasive aspergillosis ICU patients with influenza who fulfill a definition of IA specific for this population

Primary endpoint

Primary endpoint

Overall survival 42 days after the start of antifungal therapy in the MITT population

Secondary endpoints

1. Overall aspergillus attributable mortality 12 weeks after the start of antifungal therapy.
2. Overall survival 12 weeks after the start of antifungal therapy in the MITT population
3. Overall survival 6 weeks after the start of therapy in the subgroup of patients in the MITT population with a positive serum galactomannan test at baseline.
4. Overall survival 6 weeks after the start of therapy in the subgroup of non-ICU patients who fulfill the EORTC/MSG probable or proven definition (MITT population).
5. Overall survival 6 weeks after the start of therapy in the subgroup of non-ICU patients with an underlying haematological disease (MITT population)
6. Overall survival 6 weeks after the start of therapy in the subgroup of non-ICU patients without an underlying haematological disease (MITT population)
7. Overall survival 6 weeks after the start of therapy in patients that started with triazole monotherapy and in which triazole resistance is detected during follow-up (MITT population)
8. Overall survival 6 weeks after the start of therapy in patients that started with triazole-anidulafungin combination therapy and in which triazole resistance is detected during follow-up (MITT population)
9. In the subgroup of patients with a positive serum galactomannan; Kinetics of serum galactomannan levels with combination versus monotherapy
10. Outcome of patients in which resistance testing was unsuccessful
11. Time to hospital discharge (in the MITT subgroup of patients admitted to the hospital at baseline)
12. Cost-effectivity of azole-anidulafungin combination therapy

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The safety of this combination therapy has previously been demonstrated in a large randomization clinical trial (Marr et al., 2015).[2] As a result of the underlying disease as well as the chemotherapy, serious adverse events are very frequently observed in this patient population (e.g. bleeding, life threatening infections, death due to progression of the underlying disease). The study will comprise of 4 study visits and as most patients will be hospitalized at the start of therapy few of these will be additional hospital visits on top of the standard of care.

This video presentation describes the study in more detail as well

https://www.youtube.com/watch?v=Knq58Zar4hY

1. INTRODUCTION AND RATIONALE

Invasive aspergillosis (IA) is a common complication during remission inducing chemotherapy for acute leukemia as well as in those who have undergone allogeneic hematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT). For more than 15 years voriconazole, a drug of the triazole class, has been the recommended treatment for this life-threatening infection after a pivotal randomized trial showed an improved survival with voriconazole compared with amphotericin B deoxycholate. However, also with voriconazole the overall 6-week mortality is still unacceptably high at 25-30% (Herbrecht et al., 2002[1]). Voriconazole blocks the synthesis of ergosterol, a part of the fungal membrane, while antifungals from the echinocandin class block the synthesis of ß-(1,3)-D glucan, a component of the cell wall. Both drugs may therefore work synergistically as suggested by in vitro studies, neutropenic animal models of IA and case series (Philip et al., 2005[3]; Petraitis et al., 2003[4]). This synergistic effect was the hypothesis of a randomized trial that assessed the efficacy of voriconazole with or without anidulafungin for the treatment of IA in haematology patients (Marr et al., 2012[2]). Among the 277 patients with IA in this study, the 6-week mortality with combination therapy was 30% lower (19.3%) than with monotherapy (27.5%), p=0.087. In a post-hoc analysis of the 222 patients with radiographic abnormalities and a positive galactomannan antigen test, a statistically significant difference in mortality was observed (p=0.037). These results were clearly promising and although we agree that in real-life in haematology patients a diagnosis of IA is indeed very often based on the combination of a positive galactomannan and pulmonary abnormalities, formal conclusions on the value of combination therapy cannot be based on a post-hoc analysis from a single clinical trial. This is the reason why, despite the 30% relative reduction in mortality that was observed, combination therapy has not been included as preferred

first choice therapy for all patients with IA in the 2017 ESCMID guideline nor in the 2017 Dutch SWAB guideline. Therefore, a second clinical trial is needed to confirm this finding.

Another important reason to study upfront combination therapy for patients with IA in the Netherlands and Belgium is the increasing incidence of triazole-resistant *A. fumigatus*. Indeed, from a global perspective the highest prevalence of triazole resistance has been documented in the Netherlands. It increased from 0% before the year 2000 to 5.3% in 2009, and further increased to 15% in 2017. Unfortunately, more recently triazole resistance was observed in 5% of IA cases in Belgium as well and in 2017 researchers from the Erasme hospital in Brussels even reported a prevalence of 13% (Vermeulen et al., 2015[5]; Montesinos et al., 2017[6]). It has also clearly been demonstrated that the overall mortality becomes very high (50-88%) when patients infected with a triazole-resistant *A. fumigatus* initially receive inappropriate voriconazole therapy and therapy is only changed at a time when it has become clinically obvious that the IA is progressing (Lestrade et al., 2018[7], van der Linden et al., 2015[8]; Chong et al., 2015[9]). These important observations recently led to a change in the treatment recommendations of the 2017 Dutch SWAB guideline on fungal infections. In the absence of any evidence from prospective studies on the treatment of IA in regions with a high prevalence of azole resistance, this guideline recommends 2 possible strategies. The first is upfront combination therapy (triazole combined with an echinocandin or liposomal amphotericin B) until resistance can be excluded. The second option, which should only be considered in non-critically ill patients and in centers that are able to perform real-time PCR as well as cultured based resistance testing on BAL samples, is to start with voriconazole monotherapy while waiting for the resistance test (Kullberg et al., 2018[10]). Unfortunately, resistance testing will not lead to an interpretable result in approximately 35% of the patients with IA. Indeed, fungal cultures remain negative in the majority of the patients with IA and PCR testing for CYP51 resistance associated mutations is not always successful either. For this subgroup of patients, the SWAB guideline recommends switching from triazole monotherapy to combination therapy as soon as it becomes clear that no resistance result will become available. The latter recommendation has been criticized when it relates to patients that are not very sick and have an infection that is limited to the lungs and that is not widespread. Indeed, some clinicians argue that close monitoring for treatment progression is a valid option as well because the poor outcome of azole resistant IA has not (yet) been convincingly demonstrated for culture negative cases.

The goals of this study are therefore 3-fold.

First, the main study and the primary endpoint will evaluate if the overall mortality can be decreased with initial azole-echinocandin combination therapy compared with triazole monotherapy in patients with IA and documented *voriconazole susceptibility.*

Second, the study design described below will also allow to study several other sub-populations; Indeed, the outcome of the following subgroups will be evaluated as well; *a*. Patients starting azole monotherapy but who switch to directed therapy when it has become clear that the infection is caused by an azole resistant A. fumigatus. *b*. patients in which eventually no resistance data become available in relation to the treatment they received.

Third, we want to evaluate what the outcome is of patients that turn out to be infected with a triazole resistant A. fumigatus who started with a triazole-echinocandin combination therapy.

Please note that a 20-minute presentation with illustrated slides has been put together in order to explain the background and the design of the study. This presentation is available via this URL: https://www.youtube.com/watch?v=Knq58Zar4hY and may help to understand the design and logistics of the study as an introduction to the full protocol

2. OBJECTIVES

Primary objective

1. Evaluate if the survival in patients with a triazole susceptible IA can be improved when the initial therapy consists of triazole and echinocandin combination therapy instead of triazole monotherapy. This objective is captured in the primary endpoint as well as secondary endpoints 1 to 6)

Secondary objectives

1. Evaluate if a triazole/echinocandin combination therapy improves the overall quality of life and if it is a cost-effective intervention (these objectives are captured in secondary endpoint 11 and 12)
2. Evaluate the outcome of patients in which a triazole-resistant *A. fumigatus* is detected in relation to the initial antifungal therapy they had received (i.e. triazole monotherapy or combination therapy). This objective translates into secondary endpoint 7 an 8.
3. Evaluate the outcome of patients in which resistance testing is unsuccessful in function of the antifungal therapy they received. This translates into secondary endpoint 10.
4. Evaluate if the baseline serum galactomannan value and the serum galactomannan kinetics are predictive of overall 6-week survival. This translates into secondary endpoint 3 and 9.

3. STUDY DESIGN

A **non-blinded phase 3 multicenter randomized pragmatic clinical trial**.
Intervention: Add anidulafungin IV to the standard of care therapy that consists of a triazole (voriconazole or isavuconazole or posaconazole).

In both the intervention and control group, the triazole will be given for at least 6 weeks. In the intervention group the echinocandin will be discontinued after 28 days but when a treatment response is observed and the patients can be discharged from the hospital, the echinocandin can be discontinued from day 7 onwards according to the choice of the treating physician. Figure 1 illustrates the study design and the patient flow in the study. The youtube presentation https://www.youtube.com/watch?v=Knq58Zar4hY also clearly explains this flowdiagram.

4. STUDY POPULATION

4.1. Population (base)

The study population will consist of patients age 18 or older that fulfill the host-factor definition of the EORTC/MSG[11] in combination with any pulmonary infiltrate and a positive fungal culture from a bronchoalveolar lavage or positive serum (optical density ≥0.5) or BAL galactomannan (optical density ≥1.0). This not only includes patients with proven or probable IA but also patients with a pulmonary infiltrate that does not comply with the EORTC radiological criteria (halo, nodule, cavitary lesion). We consider the inclusion of this latter patient population important as well because not only the mortality of these patients is comparable to patients with an EORTC/MSG probable IA but clinicians also uniformly treat these patients in the same way as they treat patients with probable IA (Nucci et al., 2010[12]).

The host-factor definition of the EORTC/MSG implies that not only patients with an underlying haematological disease can be included but any patients that is sufficiently immunocompromised to fulfil the host-factor definition. Furthermore, ICU patients with a predicted mortality not exceeding 50% and admitted with influenza and respiratory insufficiency can be included as well as this has recently been described as an important risk factor for IA by Schauwvlieghe et al[13].

4.2. Inclusion criteria

Patients should fulfill the following inclusion criteria:

1. 18 years or older

2. Have started or will start voriconazole or isavuconazole (or posaconazole if voriconazole or isavuconazole cannot be given as per treating physician's decision) as antifungal therapy on the baseline visit.
3. For all patients: presence of one of the EORTC/MSG host factors as defined in appendix 1 or being admitted to the ICU with influenza
4. For non-ICU patients or ICU patients without influenza: Meet the EORTC/MSG clinical criterium (appendix 1)
5. For non-ICU patients or ICU patients without influenza: Meet the mycological criterium (appendix 1) or fulfil inclusion criterium 7
6. For ICU patients with influenza we consider an isolated positive sputum culture for Aspergillus spp. insufficient as a mycological criterium. Therefore, in these patients only one of the following mycological criteria are acceptable; Serum galactomannan □0.5, BAL galactomannan □1.0 or *Aspergillus* spp. cultured in BAL fluid.
7. Please note that patients with AML receiving chemotherapy or patients with ALL receiving or having received corticosteroid therapy within the last 4 weeks in the context of their pre-phase, induction, consolidation, intensification or interphase treatment as well as patients receiving systemic immunosuppressive therapy for GVHD can be included before the mycological criterium is fulfilled on condition that they fulfill the EORTC/MSG lung CT radiology criteria (halo sign, well-described nodule, cavity as described in appendix 1) at the time of inclusion and as long as the mycological test results are expected to become available within 96 and no later than 7 days after inclusion. If these test results turn out to be negative, the patient will be withdrawn from the study and further treatment is at physician's discretion.

4.3. Exclusion criteria

1. Known history of allergy, hypersensitivity or serious reaction to azole or echinocandin antifungals;
2. Patients with chronic invasive aspergillosis or a chronic non-invasive aspergillus infection (e.g. aspergilloma) defined as the clinical or radiological sign of infection being present for >28 days.
3. Receipt of itraconazole, voriconazole, posaconazole or isavuconazole as prophylaxis for at least 7 days in the 14 days preceding the date of the first radiological signs of the *Aspergillus* infection. Patients in which the most recent serum level of the triazole given as prophylaxis was subtherapeutic can be included [(*)].
4. Receipt of echinocandin prophylaxis for >96 hours in the preceding 7 days
5. Receipt of systemic antifungal treatment with an echinocandin or an azole for the current episode of invasive aspergillosis for a duration of > 96 hours.

6. For patients in the Netherlands only: Diagnostic testing to exclude azole resistance will not be possible (sputum cultures are negative and BAL sampling will not be performed)
7. ICU patients only: Patients with a sequential organ failure assessment (SOFA) score >11 at the time of screening for the study are excluded. If randomization is done >24 hours after screening the calculation should be repeated before the patient can be randomized (appendix 3)
8. ICU patients only: Patients in which weaning from the ventilator or ECMO system is deemed unlikely due to irreversible lung damage
9. Patients with any condition which, in the opinion of the investigator, could affect patient safety, preclude evaluation of response (e.g. because survival beyond 6 weeks is unlikely due to the underlying disease status)
10. Patient previously included in this study

(*) Subtherapeutic levels are defined as itraconazole (parent compound only) <0.5 mg/L or posaconazole <0.7mg/L or voriconazole <1.0mg/L or isavuconazole <1.0mg/L

5. TREATMENT OF SUBJECTS

5.1. Investigational product/treatment

Anidulafungin

Detailed information on anidulafungin, the investigational product used in this study can be found in the SPC. A short summary is given here. The information currently available on combination therapy with a triazole and an echinocandin for the treatment of IA is described below (6.2).

Mechanism of action

Anidulafungin is a semi-synthetic echinocandin, a lipopeptide synthesised from a fermentation product of Aspergillus nidulans. Anidulafungin selectively inhibits 1,3-ß-D glucan synthase, an enzyme present in fungal, but not mammalian cells. This results in inhibition of the formation of 1,3-beta-D-glucan, an essential component of the fungal cell wall. Anidulafungin has shown fungicidal activity against Candida species and activity against regions of active cell growth of the hyphae of Aspergillus fumigatus.

Chemical Name

Anidulafungin has the chemical name 1-[(4R,5R)-4,5- Dihydroxy-N2- [[4”-(pentyloxy) [1,1’:4’,1”-terphenyl]- 4- yl]carbonyl]- Lornithine]echinocandin B.

Name of medicinal product

ECALTA 100 mg powder for concentrate for solution for infusion

Method of administration

For intravenous use only.

ECALTA should be reconstituted with water for injections to a concentration of 3.33 mg/mL and subsequently diluted to a concentration of 0.77 mg/mL.

Dosage & duration

Adult patients receive a loading dose on day-1 of 200mg IV, followed by 100 mg daily thereafter.

Anidulafungin will be given for at least 7 days but no longer than 28 days. Please note that this refers to the MITT primary endpoint population. In patients in which triazole resistance is demonstrated or in which the results of the resistance test are inconclusive, the treatment duration of combination therapy can be much longer. These patients are not included in the MITT primary endpoint population.

Intervention

Treatment with a triazole (voriconazole or isavuconazole or posaconazole) + anidulafungin IV. The triazole is administered for at least 6 weeks while anidulafungin is given for at least 7 and a maximum of 28 days.

Comparator

Treatment with a triazole (voriconazole or isavuconazole or posaconazole) for at least 6 weeks.

In both groups, the route of administration is according to the choice of the treating physician as well as the decision to perform or not perform therapeutic drug monitoring of the triazole drug.

5.2. Use of co-intervention (if applicable)

Not applicable

5.3. Resistance testing

At the end of 2017, the Dutch SWAB guideline on the treatment of invasive fungal infections was updated. To take the increasing incidence of triazole resistance in the Netherlands into account, this guideline describes 2 preferred treatment strategies for patients with invasive aspergillosis. One strategy is to start with combination therapy (azole in combination with echinocandin or azole with liposomal amphotericin-B) until resistance test results become available. This is an option for critically ill patients or in

a setting where real-time resistance testing is not readily available. In a setting where real-time and state-of-the-art resistance testing is available, treatment with triazole monotherapy is recommended as an alternative strategy.

To allow for the treatment with triazole monotherapy in the Dutch patient population of this study, resistance testing will be done in all patients included in the Netherlands at the study site or at Erasmus MC if not part of the standard local diagnostic work-up. Furthermore, all Belgian sites in which triazole resistance testing is not the current standard of care, will be given the opportunity to send BAL samples to a central lab in Belgium (UZ Leuven or AZ Sint-Jan in Brugge) for real-time resistance testing free of charge. All participating centers will also receive the lab tools to perform in-house phenotypic resistance testing using VIPcheck™ free of charge (see below).

Resistance testing will be performed using a combination of phenotypic as well as genotypic resistance tests. *Phenotypic resistance testing* means that the fungus is cultured in the presence and absence of triazole drugs to observe suppression of growth in the presence of triazole drugs. It is currently certainly not the standard of care diagnostic procedure in all Belgian centers. Centers where this is not a standard procedure will receive the necessary tools to implement phenotypic resistance testing with the use of the VIPcheck™. This test was developed and validated by Radboud UMC.[6,14] The VIPcheck™ is a test consisting out of a 4-well plate in which three of the four wells contain agar supplemented with an azole (voriconazole, itraconazole and posaconazole) and the fourth functions as a growth control. If an *Aspergillus* strain grows in a well with an azole, it is very likely that this strain is azole-resistant. This test will be performed locally at the sites where this is the standard of care and the presence of resistance will be confirmed with the European Committee on Antibiotic Susceptibility Testing (EUCAST) method at the reference mycology lab of the Netherlands or Belgium (Radboud UMC and UZ Gasthuisberg Leuven). Sites that are currently not using the VIPcheck but are willing to use it can contact the study team to get the test sent to them. *Genotypic resistance testing* means that a PCR test is used to document the absence or presence of certain mutations in the DNA of the fungus that are known to result in phenotypic resistance. For this purpose, 1.5 to 2ml of the BAL sample of each patient will be submitted to the central lab to test for the presence of with the commercially available AsperGenius®. This PCR allows for the simultaneous detection of *Aspergillus* species and identification of the most common mutations circulating in Belgium and the Netherlands (TR_{34}/L98H or TR_{46}/T289A/Y121F) in the *A. fumigatus Cyp51A* gen by using melting curve analysis.

With this state-of-the-art diagnostic approach, it is safe to start with azole monotherapy for the treatment of invasive aspergillosis and the treating physician will not be tempted to change therapy from azole monotherapy to combination therapy because of fear for resistance.

5.4. Escape medication (if applicable)

Not applicable

6. INVESTIGATIONAL PRODUCTS

See also 5.1 and the SPC for more details on anidulafungin, the IP used in this study. Here we describe the findings from non-clinical and clinical studies on combination therapy with a triazole and an echinocandin as combination therapy for the treatment of IA.

6.1. Summary of findings from non-clinical studies concerning triazoles and echinocandins as combination therapy

Findings from non-clinical studies concerning the investigational products show a positive effect of combination therapy in the treatment of IA. A study by Kirkpatrick et al.[15] in which the efficacy of caspofungin and voriconazole combination therapy was evaluated in an immunocompromized guinea pig model of IA, showed a reduced mortality in the combination group compared to the single therapy dosage. In the combination therapy the colony counts were reduced compared to those obtained with either Amfo B alone or voriconazole alone. Only combination therapy resulted in more sterile cultures of organs 96 h after completion of therapy than those achieved with the other therapeutic regimens examined in these experiments. The authors concluded that the combination therapy has relevant clinical importance and the need for further clinical studies to investigate the use of combination therapy for invasive aspergillosis. In a neutropenic rabbit model of invasive pulmonary aspergillosis, the combination of voriconazole and anidulafungin was superior to single agent therapy with respect to mean pulmonary fungal burden and survival, among other measures.[16]

6.2. Summary of findings from clinical studies regarding triazoles and echinocandins as combination therapy.

A non-randomized observational study by Singh et al., 2006[17], in which the efficacy of combination of voriconazole and caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients was assessed, showed that the survival at 90 days was 67,5% (27/40) in the combination group compared to 51% (24/47) in the L-AmB group, a difference that was not statistically significant. In transplants recipients with renal failure and in those with A. fumigatus infection, combination therapy was independently associated with an improved 90-day survival in multivariate analysis. This study has important limitations as the control group did not receive triazole monotherapy but rather liposomal amphotericin-B as they were treated in the years 1999

and 2002 when voriconazole was not yet available. The only randomized clinical trial in which triazole monotherapy was compared with triazole-echinocandin combination therapy is the study by Marr et al., 2015, in which the efficacy of voriconazole with or without anidulafungin for the treatment of IA in haematology patients was assessed.[2] Patients were 16 years or older, had an underlying hematologic malignancy and/or had undergone a hematopoietic cell transplantation and were diagnosed with probable or proven IA according to the EORTC/MSG criteria. Patients received either voriconazole and a placebo or voriconazole in combination with anidulafungin. The primary end point was all-cause mortality at 6 weeks after inclusion and one of the secondary endpoints was the 12-week overall mortality. 459 patients were enrolled and were randomly assigned to one of the treatment arms. The miTT population included 277 patients who had confirmed proven or probable IA by the end of the first study week; 135 patients received combination treatment and 142 received monotherapy. The median duration of combination treatment was 14 days (range, 1 to 29); the median duration of voriconazole treatment was 42 days (range, 1 to 48 days). The mortality at 6 weeks in the miTT population was 19.5% (26 of 135) for combination treatment and 27,8% (39 of 142) for monotherapy (with a difference of -8.2%; 95% CI, -,19.0 to 1.5; 2-sided P= 0.087). The mortality at 12 weeks was 29.3% (39 of 135) for the combination treatment and 39.4% (55 of 142) for monotherapy (difference -10.1%; P=0.077). A post-hoc analysis of mortality in the patients with confirmed diagnosis of probable IA that was based on radiographic findings and galactomannan antigen positivity in serum or BAL was performed. All-cause mortality was 15,7% (17 of 108) in the combination therapy group compared with 27,3% (30 of 110) in the monotherapy group (p=0.037). These results were clearly promising and we agree that in real-life a diagnosis of IA in hematology patients is indeed very often based on the combination of a positive galactomannan and pulmonary abnormalities. However, formal conclusions on the value of combination therapy cannot be based on a post-hoc analysis from a single clinical trial. The authors concluded that though results do not provide a conclusive evidence of superiority they add to the support of combination therapy for IA.

6.3. Summary of findings from clinical studies regarding triazole monotherapy

Voriconazole, available as an oral and IV formulation has been the first-line standard of care therapy for patients with invasive aspergillosis since 2003. Indeed, in a pivotal randomized clinical trial in which voriconazole was compared with IV amphotericin-B deoxycholate, the overall 12-week survival was significantly higher in patients treated with voriconazole (71% versus58%).[1]

Isavuconazole, available in capsule and IV formulation, is another triazole registered for the treatment of IA. In the large randomized SECURE trial, 527 patients with inva-

sive mold infection were randomized to receive either IV isavuconazole followed by IV or oral (PO) isavuconazole versus IV voriconazole followed by IV or PO voriconazole. The majority of patients in both groups had underlying hematologic malignancy (82% in the isavuconazole group versus 86% in the voriconazole group). The primary endpoint was all-cause mortality at 6 weeks. At 6 weeks, 19% of patients in the isavuconazole group died compared to 20% in the voriconazole group, a difference that did not meet statistical significance. Drug-related adverse events were significantly higher in the voriconazole group compared to the isavuconazole group (60% versus 42%, $p < 0.001$), and permanent drug discontinuation was lower in the isavuconazole group compared to the voriconazole group (8% versus 14%).[18]

Posaconazole, available as tablets and IV formulation, is approved for the use as prophylaxis against invasive fungal infections in patients with acute myeloid leukemie and GVHD. It's in vitro activitity against *Aspergillus species* is comparable to isavuconazole. A phase III study in which posaconazole is compared with voriconazole is fully enrolled and results are expected in the near future. In patients who cannot tolerate voriconazole and/or isavuconazole, posaconazole is used off-label for the treatment of IA and this is allowed in this trial if the treating physicians things that posaconazole is the best treatment option available for the patient.

6.4. Summary of known and potential risks and benefits

Anidulafungin has an excellent safety profile with reduced toxicities, compared to other licensed antifungal agents. Adverse events that were observed in clinical trials are described in the SPC.

6.5. Description and justification of route of administration and dosage

Anidulafungin will be used at the licensed dose of a 200mg IV loading dose on day 1 and 100mg QD IV thereafter. No dose adjustment is needed in patients with renal or hepatic insufficiency of any grade.

6.6. Dosages, dosage modifications and method of administration

Triazoles

All patients will receive triazole therapy as per standard of care and this will be initiated by the treating physician before the patient is included in the study. Therefore, the triazoles (voriconazole or isavuconazole or posaconazole) are not considered study drugs.

Voriconazole or isavuconazole or posaconazole will be dosed according to the SPC and according to the route of administration (IV or orally) that is preferred by the treating physician. However, the dose may be changed based on therapeutic drug monitoring levels according to the local standard of care.

Anidulafungin:
Anidulafungin (Ecalta) is available as an intravenous formulation only. It will be used at the licensed dose of a 200mg loading dose on day 1 and 100mg QD thereafter. No dose adjustment is needed in patients with renal or hepatic insufficiency of any grade.

6.7. Preparation and labeling of Investigational Medicinal Product

The investigational product will be labeled at the Erasmus MC trial pharmacy according to the relevant good manufacturing practice (GMP) guidelines and with the use of a study label compliant with the Annex 13 EU directive. The investigational products will be shipped to the study sites after labeling and resupplies can be ordered at Erasmus MC.

6.8. Drug accountability

The pharmacy at the study site will carry out the drug accountability. Batch numbers of medication administered to the patients will be recorded in the patient files and CRF. The central pharmacy at Erasmus MC or at the local site will be responsible for the destruction of medication that is returned pursuant to the ICH/GCP Guidelines, local regulations and the investigator's institutional policies. Clinical supplies will be received by a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only the investigator, a pharmacist or its designated assistant have access. Clinical supplies are dispensed in accordance with the protocol. The investigator is responsible for keeping accurate records of the clinical supplies, the amount dispensed to the ward and returned to the pharmacy by the ward as well as the disposition at the end of the study. The investigator can delegate this task to the hospital pharmacist. A stock of at least 25 vials of 100mg should be in place at the site as long as the study is open for inclusion. If the stock falls below 25 vials, the investigator or the hospital pharmacist will order a new stock of 25 vials, using the order form that will be provided by the Erasmus MC trial pharmacy unit at the time of the first delivery. Ordering more vials is possible after sending a request to the study team (duet.study@erasmusmc.nl)

7. METHODS

Primary hypothesis

For the treatment of IA, combination therapy of voriconazole or isavuconazole or posaconazole with anidulafungin will improve the overall survival compared with voriconazole or isavuconazole or posaconazole monotherapy.

7.1. Study parameters/endpoints

7.1.1. Main study endpoint

Overall survival 42 days after the start of antifungal therapy in the MITT population

7.1.2. Secondary study endpoints

1. Overall aspergillus attributable mortality 12 weeks after the start of antifungal therapy(*).
2. Overall survival 12 weeks after the start of antifungal therapy in the MITT population
3. Overall survival 6 weeks after the start of therapy in the subgroup of patients in the MITT population with a positive serum galactomannan test at baseline.
4. Overall survival 6 weeks after the start of therapy in the subgroup of non-ICU patients who fulfill the EORTC/MSG probable or proven definition (MITT population).
5. Overall survival 6 weeks after the start of therapy in the subgroup of non-ICU patients with an underlying haematological disease (MITT population)
6. Overall survival 6 weeks after the start of therapy in the subgroup of non-ICU patients without an underlying haematological disease (MITT population)
7. Overall survival 6 weeks after the start of therapy in patients that started with triazole monotherapy and in which triazole resistance is detected during follow-up (MITT population)
8. Overall survival 6 weeks after the start of therapy in patients that started with triazole-anidulafungin combination therapy and in which triazole resistance is detected during follow-up (MITT population)
9. In the subgroup of patients with a positive serum galactomannan; Kinetics of serum galactomannan levels with combination versus monotherapy
10. Outcome of patients in which resistance testing was unsuccessful
11. Time to hospital discharge (in the MITT subgroup of patients admitted to the hospital at baseline)
12. Cost-effectivity of azole-anidulafungin combination therapy

(*) Aspergillus attributable mortality is defined according to Vidal G et al. (with some modifications) as one of the following[22]:

When selecting one of the 4 options below, please consider the immediate cause of death as the disease process, injury, or complication immediately preceding death.

IA is considered the cause of death (=IA attributable mortality)

1. IA was considered the cause of death when the immediate cause of death was due to this infection. Examples are neurological complications of an aspergillus infection

that disseminated to the brain, lung bleeding or respiratory insufficiency in a patient with pulmonary aspergillosis *or*

2. IA was judged to have played a major role if death would not have occurred had the patient not had IA, even though another condition was present that also contributed to death. Examples are toxicity, interactions and other side effects of antifungal treatment that played a major role in the cause of death. Another example is a pseudomonas bacteremia in a patient with a cavitating pulmonary aspergillosis in which the lungs are considered the most likely source of the bacteremia.

IA contributed to the death of the patient: Mortality is not considered attributable to IA but rather contributable if IA or treatment of IA was defined as playing a minor role but probably not essential in explaining the patient's death but arguably did play some role in the event. An example is a patient an aspergillus infection as well as severe uncontrolled gastrointestinal GVHD at the time of death

IA did not contribute nor cause the death of the patient

Mortality was classified as not related to IA if there was a clear other cause of death

Unknown

If insufficient data were present about the circumstances of the death of the patient

7.1.3. Pragmatic study design

We have tried to design the study in such a way that the prompt inclusion of patients is facilitated as much as possible.

1. Registration trials of new antifungal drugs typically only include patients that fulfill the strict lung CT radiology criteria (e.g. halo sign) as described in appendix 1. However, in real-life as much as 50% of the patients treated for invasive aspergillosis do not have these typical abnormalities but are treated on the basis of the presence of a host factor, a positive mycological test and a (non-typical) pulmonary infiltrate. The outcome of these patients is similar to those with the typical pulmonary infiltrate.[12] Therefore, we consider the exclusion of these patients undesirable in the context of a pragmatic trial.
2. Typically, patients that have received antifungal therapy for more than 48-96 hours at the time of inclusion are excluded from registration trials. Again, this leads to many patients being excluded from study participation. To avoid this, we will allow that patients at high risk of IA (patents with AML or grade II or higher GVHD) are included at the time the triazole antifungal therapy is initiated by the treating physician even when the mycological criterium is not yet met. These patients will

be followed until day 7 and will leave the study if at that time the mycological tests have turned out to be negative.

3. While the previous randomized study on the use of combination antifungal therapy for IA only included patients with an underlying haematological disease, there is no reason to believe that the hypothesis of improved survival with combination therapy only applies to these patients. Therefore, our study population will not be limited to this subgroup of patients.
4. Our study design has overall mortality as the primary endpoint and no compulsory follow-up CT scans are required.
5. Given the 100% objective primary endpoint we have decided not to use a double-blind design. This will make the study logistics and therefore the inclusion of patients more straightforward. By offering state-of-the-art triazole resistance testing to all patients, the presence of triazole resistance is made very unlikely and therefore the treating physician can be reassured that the treatment given to patients in the control arm is a fully active treatment.

7.1.4. Definitions of the patient populations

ITT: All patients randomized as registered in the IVRS

MITT: All patients randomized as registered in the IVRS, in whom the positive mycological test was available at the time of randomization or became available within 8 days after randomization will be included in the MITT population *unless* resistance to voriconazole was documented by culture or PCR (VIPcheck or Aspergenius, see below). Because Dutch guidelines currently recommend initiating combination antifungal therapy if the presence of an azole resistant A. fumigatus infection cannot be excluded by PCR or culture, Dutch patients in whom the resistance PCR or culture turns out unsuccessful will be excluded from the MITT population as well. The antifungal therapy given to these patients will be decided upon by their treating physician (see also flowdiagram) .

ICU population: Patients already admitted to the ICU at the time of the start of triazole antifungal therapy

7.2. Randomisation, blinding and treatment allocation

This is a phase 3 non-blinded non-placebo controlled pragmatic trial. Randomization will be stratified according to the following 3 risk groups: Acute myeloid leukemia, graft-versus host disease and other.

Randomization and concealed allocation will be performed and guaranteed via randomization using blocks of different length and with the use of a randomization module

in the eCRF (Alea). Subjects will be randomized to a 1:1 allocation into 2 treatment group: monotherapy versus combination therapy.

7.3. Study procedures

The study procedures involve a screening visit and follow-up up to week 24 for overall mortality. Taking the pragmatic design into account, this means that a formal hospital visit is not required for the primary endpoint evaluation after the patient is discharge from the hospital. However, investigators and patients will be encouraged to facilitate the collection of additional data as described in appendix 2 as much as possible to allow for the evaluation of several secondary endpoints in a large study population. Because these data are part of routine medical registration procedures (e.g. use of blood products, hospital days, ICU days, use of medication, blood test results) this will not obstruct the analysis of these secondary endpoints.

All patients will be receiving triazole therapy as prescribed by their treating physician at the time of screening for study participation. Monitoring for treatment related adverse events will be performed as per standard of care during triazole antifungal therapy and typically includes liver enzyme monitoring at least once a week during the first weeks of therapy. Given the overall very good safety profile of anidulafungin and the fact that it can only be administered intravenously at the hospital, no additional safety evaluations are needed.

7.4. Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Patients leaving the study will receive treatment as deemed appropriate by their treating physician

After randomization, the following patients will leave the interventional part of the study, but clinical data will continue to be collected:

1. Patients in whom none of the mycological criteria as described in appendix 1 have become positive within 8 days after randomization. This may be as soon as 48hrs after (e.g. if serum and BAL galactomannan testing and fungal culture result is final 48hrs after randomization).
2. Patients in the Netherlands in whom the resistance tests were unsuccessful (aspergillus culture remained negative and aspergillus PCR demonstrated the presence of A. fumigatus but the resistance PCR is unsuccessful) will receive further treatment according to the choice of the treating physician because guidelines recommend to treat these patients with an azole in combination with a second antifungal drug. If the patient was randomized to the anidulafungin arm, the option will be given to

continue anidulafungin for up to 4 weeks, but these patients will be excluded from the MITT population (see 7.1.4) The 24-week outcome of these patients will be registered. In Belgium, currently no guidelines are in place recommending combination therapy for this patient group. Therefore, this does not apply to Belgian patients. Please note that patients in whom the Aspergenius PCR documents the presence of an aspergillus spp. other than fumigatus will remain in the study and will be included in the MITT population. This specific conclusion will be drawn when the aspergillus spp. PCR is positive with a CT value of 35 or lower but the aspergillus fumigatus PCR is negative as this demonstrates the presence of a non-fumigatus aspergillus infection.

7.5. Replacement of individual subjects after withdrawal

As anidulafungin has limited side-effects, a low percentage of withdrawal can be expected, and patients withdrawn from the study will not be replaced.

7.6. Follow-up of subjects withdrawn from treatment

Patients withdrawn from the study will be followed for overall mortality only

7.7. Premature termination of the study

One interim futility analysis will be performed at the time when the 6-week survival of 50% of the planned sample size of 474 evaluable patients (=237) has become available. If this interim analysis shows that the conditional power after further enrolment of the remaining study population of observing the anticipated 33% reduction in the incidence of the primary endpoint in favor of combination therapy is <10%, the study team (the PI's from Erasmus MC, UZ Leuven and Radboud UMC, the study statistician, a delegate from KCE and from ZONMW and a patient representative) will meet to decide upon further enrollment. This decision will not only take the conditional power into account but also the recruitment speed as well as the relative decrease in mortality observed with the intervention under study (e.g. it may still be useful to continue the study if a 50% rather than a 30% decrease in overall mortality is observed when the conditional power to demonstrate a 30% is <10% as a result of a lower than expected overall absolute mortality in the control arm).

8. SAFETY REPORTING

Background regarding the safety reporting paragraph of this study.
According to the current Dutch SWAB guideline, combination therapy consisting of an azole and an echinocandin (e.g. anidulafungin, the IMP in this study) is one of the

treatment options for patients with IA. Another treatment option recommended in this guideline is azole monotherapy. In Belgium, azole monotherapy is the current standard of care. Combination therapy with voriconazole and anidulafungin has been studied in a preceding phase III study. In this study, the number of AE and SAE in the combination therapy arm was comparable to the azole monotherapy arm. Therefore, in this study 2 treatment options with a well-established safety profile will be compared.

Many if not the majority of the patients in this study will be receiving intensive chemotherapy or suffer from graft-versus-host-disease and its related AEs (e.g. anemia, leuco -or thrombocytopenia, diarrhea, mucositis, nausea, vomiting, alopecia, fever, bacteremia, fatigue, headache, bacterial and viral infections infection.

The study described in this protocol was designed as a pragmatic trial of which the goal is to compare two treatment options in a setting that simulates the treatment of IA in real-life as much as possible. This will the extrapolation of the study results as straightforward as possible.

For the reasons mentioned above, we think that registering all (S)AE will not only be very time consuming because the average number of AE per patient is expected to be very high but will not increase patient safety. We therefore will not register AE but only SAE with the limitations mentioned below.

8.1. Definitions

Serious adverse event (SAE)

A serious adverse event is defined as any untoward medical occurrence or effect that at any dose:

- Results in death
- Is a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- Requires hospitalization or prolongation of an existing hospitalization
- Results in significant or persistent disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (i.e. important adverse events that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the above characteristics/ consequences, including suspected transmission of infectious agents by a medicinal product).

Suspected unexpected serious adverse reaction (SUSAR)

A suspected unexpected serious adverse reaction is defined as all **suspected** Adverse Reactions (AR) which occur in the trial and that are both **unexpected** and **serious**.

Suspected adverse reactions are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected. Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorized medicinal product).

Overdose
This refers to the administration of a quantity of a medicinal product given per administration or cumulatively, which is above the maximum recommended dose according to the authorised product information. Clinical judgement should always be applied.

Misuse
This refers to situations where the medicinal product is intentionally and inappropriately used not in accordance with the terms of the marketing authorisation.

Abuse
This corresponds to the persistent or sporadic, intentional excessive use of a medicinal product, which is accompanied by harmful physical or psychological effects.

Occupational exposure
This refers to the exposure to a medicinal product, as a result of one's professional or non-professional occupation. It does not include the exposure to one of the ingredients during the manufacturing process before the release as finished product.

Medication error
This is an unintended failure in the drug treatment process that leads to or has the potential to lead to harm to the patient.

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC/IRB if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC/IRB, except insofar as suspension would jeopardize the subjects' health. The investigator will take care that all subjects are kept informed.

8.2. Adverse event

8.2.1. Reporting of adverse events

Adverse events that do not fulfill the definition of Serious adverse Event will not be reported.

8.3. Serious Adverse Events

8.3.1. Reporting of serious adverse events

Serious Adverse Events (SAEs) will be reported from the first administration of treatment according to protocol until day 14 days after the last dose of the IMP or until the start of subsequent systemic therapy for the disease under study, if earlier.

Serious adverse events (including death) occurring after day 42 days should also be reported if considered at least possibly related to the investigational medicinal product by the investigator.

SAEs must be reported to HOVON Data Center **within 3 days** after the event was known to the investigator, using the SAE report form provided. This initial report should contain a minimum amount of information regarding the event, associated treatment and patient identification, as described in the detail in the instructions for the SAE report form. Complete detailed information should be provided in a follow-up report within a further 7 business days, if necessary.

The following events do not require to be reported as a serious adverse event:

- Relapse/Progression of the disease under study. However, death or complications as a result of disease progression should be reported as serious adverse events if occurring within 14 days after last dose of study drug.
- Hospitalization for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a serious adverse event.
- Hospitalization for diagnostic investigations (e.g., scans, endoscopy, sampling for laboratory tests, bone marrow sampling) that are not related to an adverse event. Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
- Prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- Hospitalization for a procedure that was planned prior to study participation (i.e. prior to registration or randomization). This should be recorded in the source documents. Prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.

8.3.2. Causality assessment of serious adverse events

The investigator will decide whether the serious adverse event is related to trial medication, i.e. any of the products from the protocol treatment schedule. The decision will be recorded on the serious adverse event report. The assessment of causality is made by the investigator using the following:

Causality term	Assessment criteria*
Certain	¨ Event or laboratory test abnormality, with plausible time relationship to drug intake ¨ Cannot be explained by disease or other drugs ¨ Response to withdrawal plausible (pharmacologically, pathologically) ¨ Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognised pharmacological phenomenon) ¨ Rechallenge satisfactory, if necessary
Probable /Likely	¨ Event or laboratory test abnormality, with reasonable time relationship to drug intake ¨ Unlikely to be attributed to disease or other drugs ¨ Response to withdrawal clinically reasonable ¨ Rechallenge not required
Possible	¨ Event or laboratory test abnormality, with reasonable time relationship to drug intake ¨ Could also be explained by disease or other drugs ¨ Information on drug withdrawal may be lacking or unclear
Unlikely	¨ Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) ¨ Disease or other drugs provide plausible explanations
Conditional / Unclassified	¨ Event or laboratory test abnormality ¨ More data for proper assessment needed, or ¨ Additional data under examination
Unassessable / Unclassifiable	¨ Report suggesting an adverse reaction ¨ Cannot be judged because information is insufficient or contradictory ¨ Data cannot be supplemented or verified

8.3.3. Follow up of serious adverse events

All serious adverse events will be followed clinically until they are resolved or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

Follow up information on SAEs should be reported monthly until recovery or until a stable situation has been reached. The final outcome of the SAE should be reported on a final SAE report.

8.3.4. Processing of serious adverse event reports

HOVON Data Center will forward all SAE reports within 24 hours of receipt to the principal investigator.

The HDC safety desk will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR).

The SmPC will be used as a reference document for expectedness assessment.

Where reporting of SAEs to the ethics committee is required by national laws or regulations or by the procedures of the ethics committee, HOVON Data Center will report those SAEs by means of a six-monthly SAE line listing.

8.4. Reporting Suspected Unexpected Serious Adverse Reactions

The HDC Safety Desk, on behalf of the sponsor, will ensure the reporting of any SUSARs to the ethics committees (EC), the competent authorities (CA) and the investigators in compliance with applicable laws and regulations, and in accordance with any trial specific agreements between the sponsor and a co-sponsor.

Expedited reporting of SUSARs will occur no later than 15 days after HOVON Data Center had first knowledge of the serious adverse event. For fatal or life-threatening cases this will be no later than 7 days for a preliminary report, with another 8 days for a complete report.

The manner of SUSAR reporting will be in compliance with the procedures of the ethics committees and health authorities involved.

8.5. Reporting special situations

Overdose, abuse, misuse, medication error or occupational exposure are special reporting situations and must be reported to HOVON Data Center immediately.

Please inform HOVON Data Center of these events within 3 days hours after the event was known to the investigator by email (hdc@erasmusmc.nl). Note that these special reporting situations in and of themselves are not AEs. If a special reporting situation results in an SAE, an SAE form should be completed and sent to HOVON Data Center (see section 12.3.1).

8.6. Annual safety report

The annual safety report will be combined with the annual progress report (see chapter 12.4).

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;

– a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.7. Follow-up of adverse events

All SAEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported from the first administration of treatment according to protocol until day 14 days after the last dose of the IMP or until the start of subsequent systemic therapy for the disease under study, if earlier.

8.8. Data Safety Monitoring

No data safety monitoring board (DSMB) will be implemented because the investigational product has been very well tolerated in the phase II and III studies and a previous fase III trial did not show any increase in AE or SAE in the anidulafungin voriconazole combination arm compared with the voriconazole monotherapy arm.

9. STATISTICAL ANALYSIS

9.1. Primary endpoint analysis

The primary endpoint is defined as all-cause mortality at 42 days (6 weeks) after randomization. The MITT population is considered the main analysis population.

The relation between randomly allocated treatment and the incidence of the primary endpoint in the MITT population will be described by

- the total number of endpoint events by allocated treatment;
  - a crude, unadjusted odds ratio. Logistic regression analysis will be conducted, with randomly allocated treatment as independent predictor variable and the incidence of the primary endpoint as dependent outcome variable;
  - an adjusted odds ratio. A multivariable logistic regression analysis will be applied. The effect of randomly allocated treatment will be adjusted for age, pulmonary or (also) extrapulmonary disease, ICU admission, baseline serum galactomannan status, post allogeneic stem cell transplantation status, and acute or chronic GVHD for which patients are receiving systemic immunosuppressive therapy.

The adjusted odds ratio will be considered the key primary endpoint analysis

The same analysis will be performed for secondary endpoints 2 to 6. The listed sub-populations will be analyzed using simple and multivariable logistic regression (the adjustment factors - as far as applicable - are listed above). Since these analyses will be considered exploratory only and these endpoints are defined as secondary endpoints, no correction for multiple testing will be performed.

9.2. Secondary endpoints analyses

The 1st secondary endpoint is defined as attributable mortality at 84 days (12 weeks) follow-up, which will be analyzed in the main analysis population. For this analysis, non-attributable mortality will be considered competing risk for attributable mortality

For the analysis of secondary endpoints 2 to 6 see 9.1.

Secondary endpoints 7 and 8 will be analysis using descriptive statistics (6- and 12-week mortality with 95% confidence intervals). If the number of patients in the 2 subgroups described in endpoint 6 and 7 are sufficiently large (□25) we will compare the 6 mortality of both groups using the Fisher-Exact test.

Regarding endpoint 9, several studies have studied the impact of galactomannan kinetics on outcome in patients with IA. Studies have shown a reasonable correlation between galactomannan kinetics in the weeks following the initiation of therapy and outcome[21]. However, several questions remain; What is the optimal timing of the follow-up galactomannan measurement? Is the rate of decline more informative than a simpler binary outcome (decline/no decline or decline with 0.5 OD units)? The statistical analysis plan for this endpoint will be written when the study is completed, and the number of galactomannan positive patients and the number of follow-up plasma samples is known.

Secondary endpoints 10 will be analysis using descriptive statistics (6 and 12-week mortality with 95% confidence intervals).

The 11th secondary endpoint is defined as the total duration of the hospital stay. The relation between randomly allocated treatment and this secondary endpoint will be analyzed using a Mann-Whitney U test.

9.3. Multiplicity

There is only 1 primary endpoint and no interim analysis for efficacy will be performed. Therefore, the primary endpoint will be tested at the α=0.05 level (two-sided test).

9.4. Missing data

Patients that are lost to follow-up after the start of study drugs will be considered treatment failures in the mITT analysis.

9.5. Sample size calculation

In the pivotal trial on voriconazole and anidulafungin combination therapy the mortality in the voriconazole control group was 28% 6 weeks after the start of therapy.[2] This study only included patients with an underlying haematological disease. Furthermore, in a large pragmatic trial with fewer exclusion criteria and in which a small number of ICU patients will be included as well, we expect a somewhat higher mortality of 35%, We consider a 33% lower overall mortality with combination therapy (=from 35% to 23,33%) compared to monotherapy of clinical importance. This leads to a sample size of 237 evaluable patients per group (=included in the mITT population as defined above) to show superiority of combination therapy compared with monotherapy with an alfa of 0.05 and a power of 80%. Follow-up for the primary endpoint is 6 weeks after the start of antifungal therapy.

9.6. Responsibility for data analysis

The coordinating investigator will be responsible for analyzing the study data.

9.7. Monitoring

This trial is part of the HOVON site evaluation visit program. Site evaluation visits will be performed for HOVON trials to review the quality of the site and not specifically the quality of a certain trial. It will enable HOVON to collect quality data and facilitate improvement of the participating sites. Data cleaning or monitoring of the performance of specific trials is not the goal of the site evaluation visits. Site evaluation visits will be performed according to the site evaluation visit plan.

The HOVON site evaluation visit plan applies to sites in the Netherlands and Belgium only. Monitoring of the quality of trial conduct in participating sites from other countries will be organized by the coordinating investigator or co-sponsor. The frequency and content of the site visits in other countries will be at least equal to the specifications of the site evaluation visit plan and are described in a monitoring plan provided by HOVON.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. The sponsor expects that during site visits the relevant investigational staff will be available, the source documentation will be available, and a suitable environment will be provided for review of study-related documents.

9.8. Interim efficacy analysis

An interim efficacy analysis will not be performed. One interim futility analysis will be performed after the inclusion of 50% of the planned sample (see 7.7).

10. COST-EFFECTIVENESS ANALYSIS

The cost-effectivity of combination therapy compared with triazole monotherapy will be analyzed only if a statistical difference (p0.05 or lower) is observed for the primary endpoint or for secondary endpoint 2. The full statistical analysis plan for endpoint 12 is therefore not yet complete but will be developed after the analysis of these endpoints has been completed and in collaboration with prof. dr. C. Uyl-de Groot of the institute of Medical Technology Assessment in Rotterdam (iMTA). The analysis will estimate if combination therapy is cost saving and if this is not the case the cost per quality adjusted life year of combination therapy compared to monotherapy will be calculated. For this, not only quality of life data, the (time to) death but also detailed data on each of the following clinical parameters that are associated with substantial increase in costs will be collected; Duration of hospital stay and number of days admitted to the intensive care unit, blood products and antifungal drugs administered as well as enteral or parenteral nutrition given between baseline and week 12 of follow-up. For the quality of life data collection, the EuroQOL EQ5D-5L questionnaire will be used (including the proxy or telephone version if needed, appendix 5). Because the majority of the patients with IA in our study will consist of patients treated for AML with intensive chemotherapy and of patients that received an allogeneic stem cell transplantation, previously published utility scores from these patient populations will be used to calculate the cost per quality adjusted life years gained (QALY) with combination therapy compared with monotherapy.[19] Apart from quality of life data, we will collect data on loss of income up to 24 weeks after inclusion to allow for an analysis of cost-effectivity from the society perspective. For this purpose, a set of dedicated questionnaires will be used (appendix 5).

The study population will consist of 3 major study populations: Patients receiving chemotherapy for AML, patients with graft-versus-host-disease and other patients. If a significant treatment effect of the intervention is observed in one of these subgroups, an exploratory cost-effectiveness analysis will be done for this subgroup.

11. ETHICAL CONSIDERATIONS

11.1. Regulation statement

The study will be performed in accordance with the protocol, the guidelines of Good Clinical Practice/ICH, which underwrites the principles of the Declaration of Helsinki, as most recently revised by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013.

11.2. Ethical committee approval

The study protocol will be formally submitted to the ethical committee of the Erasmus MC. The study will start after approval from the ethical committee has been obtained. The nature of the study and an outline of those investigative procedures, which might be in excess of their usual care, will be explained to the patients. They will be required to give their written informed consent before entering the study.

11.3. Recruitment and consent

Patients will be recruited at study sites in the Netherlands and Belgium. It is the responsibility of the investigators or the co-investigators to obtain written informed consent from each subject participating in this study, after adequate explanation of the aims, methods, anticipated, and potential hazards of the study.

Besides the specific information regarding the study, the following standard items are covered in the patient information form (Dutch: patiënten informatie formulier):

- Patient's right to withdraw from the clinical study anytime without giving reasons and without any consequences for further medical treatment.
- The information that all study findings will be stored in a computer database and handled confidentially
- Patient names will be kept separate from research data and patients will be identifiable by subject number only.
- Information about the possibility of inspection of relevant parts of the hospital records by regulatory authorities. Inspection will only take place if a confidentiality agreement has been signed.
- The existence of patient insurance policy in case the patient will be harmed by participating in the study (using the study drug)
- All novel clinically relevant information that will become available during the study and is possibly important for the patient will be communicated to him/her by one of the investigators.

The signature of an investigator or co-investigator on the form will attest that the information in the consent form was accurately explained and understood. Thereafter the patient will sign after a period of reflection. If new safety information results in significant changes in the risk/benefit assessment, the consent form will be reviewed and updated after approval by the ethical committee. Then, all subjects (including those already being treated) will be informed of the new information, will be given a copy of the revised form and will be asked to give their consent to continue the study.

11.4. Benefits and risks assessment

Anidulafungin has been registered and used in the Netherlands for the treatment of invasive candida infections for >10 years and is considered a very safe drug. Also, the safety of the combination of anidulafungin and voriconazole that the patients in the intervention group will receive has previously been demonstrated in a large randomization clinical trial.[2] The risks are therefore considered very low.

If the improved mortality with combination therapy that was observed in the study by Marr K et al. is confirmed in our study, the patients in the study as well as future patients may potentially benefit from this combination treatment.

Hepatic metabolism of anidulafungin has not been observed and anidulafungin is not a clinically relevant substrate, inducer, or inhibitor of cytochrome P450 (CYP450) isoenzymes. It is therefore very unlikely that anidulafungin will have significant drug-drug interactions with concomitant medication taken by the patient.

As a result of the underlying disease as well as the chemotherapy, serious adverse events are very frequently observed in this patient population (e.g. bleeding, life threatening infections, death due to progression of the underlying disease). The study will comprise of 4 study visits and as most patients will be hospitalized at the start of therapy few of these will be additional hospital visits on top of the standard of care.

11.5. Compensation for injury

Liability insurance sponsor/investigator

The sponsor has a liability insurance in place for the Dutch study sites in accordance with article 7, subsection 6 of the WMO.

The Belgian coordinating party has a liability insurance in place for the Belgian sites in accordance with Belgian legislation.

Insurance for study participants

The Erasmus MC WMO insurance applies for all patients included in one of the Dutch study sites. The certificate can be found in appendix 4a.
The UZ Gasthuisberg insurance (also called the “no-fault aansprakelijkheidsverzekering”) applies for all patients included in one of the Belgian study sites. The certificate can be found in appendix 4b.

11.6. Incentives

No incentive will be given.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1. Handling and storage of data and documents

Data will be handled confidential and if possible, anonymously. Where it is necessary to be able to trace data to an individual subject, a subject identification code list will be used to link the data to the subject. The code will not be based on the patient initials and birthdate. The key to the code will be safeguarded by the investigator. as the data and human material will be kept for a longer period of time. The handling of personal data will comply with the Belgian and Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, Wbp).

Case record form (CRF)

All data of patients, including results from standard procedures during treatment, collected during the study will be recorded in Case Record Forms. The CRF must be completed fully and legibly. Corrections of possibly erroneous entries must be carried out in such a manner that the initial entry is not rendered illegible. Corrections should be written alongside or above the pertinent place with the date and initials. Correction fluid must not be used.

The investigators are responsible for the quality of the data recorded in the Case Record Forms (CRF). Where the investigators have not been responsible for completing the CRF, an additional signature from the co-investigator overseeing the data entry of the study must be obtained.

In the event that the investigators need to deviate from the protocol, the nature of and reasons for protocol deviation must be recorded in the hospital patient record and in the CRF. In nearly all cases it is desirable that the patient continues the study to allow the most informative intention-to-treat analysis; This does not mean that the treatment to which the patient was randomized needs to be continued. As illustrated by the flow diagram of the study (figure 1) there can be good reasons to change antifungal therapy after randomization. However, also for patients that go off study for the 3 reasons mentioned in figure 1, a limited number of data will be collected in the CRF (e.g. antifungal therapy, survival)

Privacy rules

Patients will be identified in the CRF by their identification code. The investigators will keep a patient identification log, including sufficient information to link the hospital record and CRFs.

The subjects will be informed that the data will be stored electronically, that local regulations for the handling of computerized data will be followed as described in the written patient information / consent form and that identification of individual

patient data will only be possible for the investigators. Furthermore, the subjects will be informed about the possibility of inspections of relevant parts of the hospital records by health authorities. These officials will be identified and have signed a confidentiality agreement. The data are processed and stored using dedicated GCP compliant electronic CRF (ALEA). From this database the data will be transferred to a statistical program for further analysis. Only data, with coded patient identity will be transferred to the statistician for analysis.

Data processing
After a visual plausibility check the CRF data will be entered in the computer and processed using dedicated GCP compliant electronic CRF (ALEA). When all data have been approved by the local investigator, the database will be locked for that site and the data can be transferred from the database to a statistical data file, with conversion in uniform data and formation of a master file for further analysis. The data will also be approved by the investigator and locked after approval at the time when a patient moves from one hospital to another

Data achieving
Patient identification log, hospital records, informed consent forms, case record forms and databases must be kept for at least 15 years after completing the study. If the investigators move or retire, they must nominate someone in writing to be responsible for record keeping. Archived data may be held on microfiche or electronic record, provided that a backup exists, and a hard copy can be obtained from it if required.

12.2. Monitoring and Quality Assurance

Please refer to our monitoring plan.

12.3. Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC/IRB has been given. All amendments will be notified to the METC/IRB that gave a favorable opinion.

A ‘substantial amendment’ is defined as an amendment to the terms of the METC/IRB application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:
- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC/IRB and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC/IRB and the competent authority but will be recorded and filed by the sponsor. Examples of non-substantial amendments are typing errors and administrative changes like changes in names, telephone numbers and other contact details of involved persons mentioned in the submitted study documentation.

12.4. Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC/IRB once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5. End of study report

The sponsor will notify the accredited METC/IRB and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the sponsor will notify the accredited METC/IRB and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC/IRB and the Competent Authority.

12.6. Public disclosure and publication policy

The sponsor is free to publicly disclose and publish all research data. Please refer to the contract between de sponsor and the subsidizing party for arrangements made concerning public disclose and publication of research data.

13. STRUCTURED RISK ANALYSIS

13.1. Potential issues of concern

a. Level of knowledge about mechanism of action

While voriconazole and other azoles inhibit fungal cell membrane synthesis, the echinocandins block production of 1,3-beta-D glucan, a key component of fungal cell walls.

Combination therapy with voriconazole and an echinocandin is an intriguing possibility given the different mechanisms of action of these two agents.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism
See SPCs submitted with this protocol

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?
The purpose of this study is showing efficacy of combination therapy in patients with IA. In a neutropenic rabbit model of invasive pulmonary aspergillosis, the combination of voriconazole and anidulafungin was superior to single agent therapy with respect to mean pulmonary fungal burden and survival, among other measures.[16]

d. Selectivity of the mechanism to target tissue in animals and/or human beings
Voriconazole has been shown to be more selective for fungal cytochrome P-450 enzymes than for various mammalian cytochrome P-450 enzyme systems. Anidulafungin inhibits the synthesis of beta (1,3)-D-glucan, an essential component of the cell wall of many filamentous fungi and yeast. Beta (1,3)-D-glucan is not present in mammalian cells.

e. Analysis of potential effect
The potential positive effects of combination therapy are described in 6.1 and 6.2

f. Pharmacokinetic considerations
Pharmacokinetics of voriconazole/isavuconazole/isavuconazole and anidulafungin are well known and described in the SPCs submitted with this protocol. There are no drug-drug interactions between both drugs.

g. Study population
Immunocompromised patients with a suspected, probable or proven IA as well as ICU patients admitted with influenza diagnosed with IA according to the in -and exclusion criteria in the protocol

h. Interaction with other products
Voriconazole and isavuconazole or posaconazole have no significant drug-drug interactions with anidulafungin. Anidulafungin is not metabolized by the liver and is not renally excreted. Hepatic metabolism of anidulafungin has not been observed and anidulafungin is not a clinically relevant substrate, inducer, or inhibitor of cytochrome P450 (CYP450)

isoenzymes. It is unlikely that anidulafungin will have clinically relevant effects on the metabolism of drugs metabolized by CYP450 isoenzymes.

Anidulafungin undergoes slow chemical degradation at physiologic temperature and pH to a ring-opened peptide that lacks antifungal activity. The in vitro degradation half-life of anidulafungin under physiologic conditions is about 24 hours. In vivo, the ring-opened product is subsequently converted to peptidic degradants and eliminated.

i.Predictability of effect

There are no predictable side-effects of anidulafungin therapy. The effect of combination therapy on the survival in patients with IA is currently not predictable as it has been studied in only 1 randomized clinical trial and this trial was inconclusive.

j. Can effects be managed?

Not applicable

13.2. Synthesis

In conclusion, we think that in this study the potential benefits outweight the risks as the drug that is being used is a registered drug that has been used extensively worldwide, has an overall good safety profile and has no drug-drug interactions.

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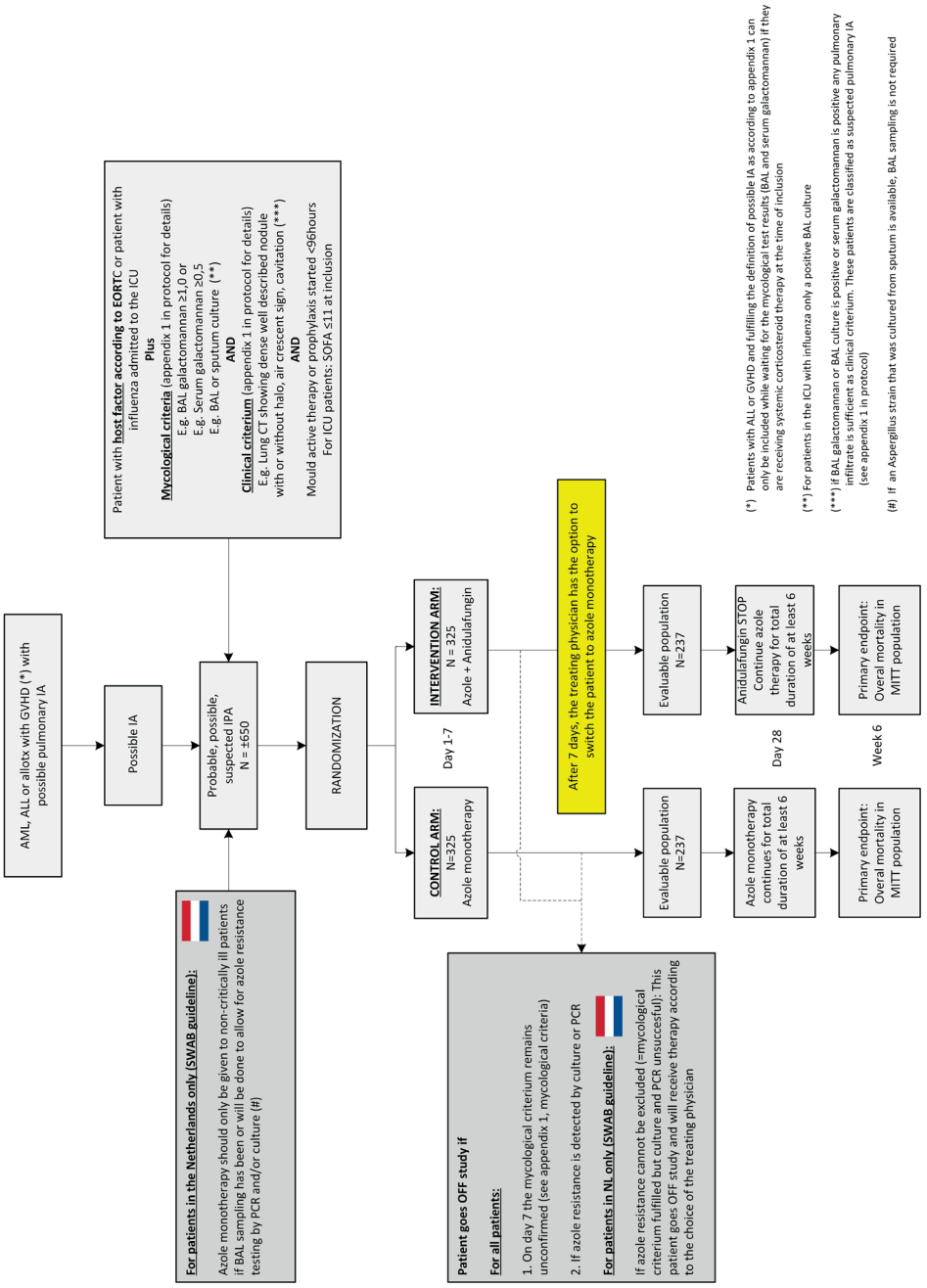

Figure 1. Flow diagram

APPENDIX 1: MODIFIED EORTC/MSG CONSENSUS DEFINITIONS FOR DIAGNOSIS OF PROVEN, PROBABLE, POSSIBLE OR SUSPECTED INVASIVE ASPERGILLOSIS.

Proven invasive aspergillosis

Histopathologic, cytopathologic, or direct microscopic examination of a needle aspiration or biopsy specimen showing hyphal forms with evidence of associated tissue damage (either microscopically or as an infiltrate or lesion by imaging) in combination with a positive aspergillus PCR on the sample

OR

Recovery of a mould by culture from a sample obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL, cranial sinus cavity, and urine.

Probable invasive aspergillosis

Defined by at least:

- One host factor (See below)
  AND
- One clinical criterion (See below)
  AND
- One mycological criterion (See below)

Possible invasive aspergillosis

Defined by at least:

- One host factor (See below)
  AND
- One clinical criterion (See below)

Suspected invasive pulmonary aspergillosis

Please note that the EORTC/MSG classification does not include suspected invasive pulmonary aspergillosis in its classification

Defined by at least:

- One host factor (See below)
  AND
- A pulmonary infiltrate other than a nodule, halo sign, cavity or air-crescent sign
  AND
- One mycological criterion (See below)

Host factors

1. Recent history of neutropenia (<0.5 x 109/L (<500 neutrophils/mm3) for >10 days) temporally related to the onset of fungal disease or ongoing neutropenia; Patients with a newly diagnosed AML can be considered to be neutropenic for at least 10 days and therefore fulfill this criterium also at the time of AML diagnosis.
2. Receipt of an allogeneic stem cell transplant;
3. Prolonged use of corticosteroids (excluding patients with ABPA) at an average minimum dose of 0.3 mg/kg/day prednisone equivalent for >3 weeks;
4. Treatment with other recognized T-cell immune suppressants such as ciclosporin, TNF-α blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days;
5. Inherited severe immunodeficiency (eg, chronic granulomatous disease, severe combined immunodeficiency).

Clinical criteria

Must be consistent with the microbiological findings, if any, and must be temporally related to current episode. Every reasonable attempt should be made to exclude an alternative etiology.

1. Lower respiratory tract fungal disease

The presence of one of the following three signs on CT:

- Dense, well-circumscribed lesion with or without a halo sign;
- Air crescent sign;
- Cavity.
- For patients with a serum galactomannan value of 0.5 or higher or for patients with a BAL galactomannan of 1.0 or higher, the presence of any pulmonary infiltrate is considered sufficient evidence of lower respiratory tract fungal disease

2. Tracheobronchitis:

Tracheobronchial ulceration, nodule, pseudomembrane, plaque or eschar seen on bronchoscopy.

3. Sinonasal infection

Imaging showing sinusitis PLUS at least one of the following:

- Acute localized pain (including pain radiating to the eye);
- Nasal ulcer with black eschar;
- Extension from the paranasal sinus across bony barriers, including into the orbit.

4. CNS infection

At least one of the following:

- Focal lesions on imaging;
- Meningeal enhancement on MRI or CT.

MYCOLOGICAL CRITERIA

1. Cytology, direct microscopy or culture:

- Sputum, BAL and bronchial brush samples demonstrating the presence of fungal elements either by recovery by culture of Aspergillus spp. or detection by cytology or
- direct microscopy of hyphal forms in combination with a positive aspergillus PCR on sputum, BAL or bronchial brush
- Sinus aspirate: recovery by culture of Aspergillus spp. from aspirate *or* the detection of hyphal forms by cytology or microscopy in combination with a positive aspergillus PCR on the aspirate.
- Skin ulcers, draining soft tissue lesions or fissure for which both a positive microscopy (hyphae) and positive Aspergillus culture are required.

2. Detection of galactomannan antigen or DNA of aspergillus defined as one of the following:

- Galactomannan antigen EIA (Platelia):
  1. Serum sample positive for galactomannan (0.5 or higher);
  2. BAL sample positive for galactomannan (1.0 or higher).
- PCR:
  1. Positive Aspergenius spp. PCR on BAL fluid (cycle threshold 38 or lower) in combination with a galactomannan BAL OD value of 0.5-0.9 is considered a positive mycological criterium in this study
  2. Positive Aspergillus spp. PCR on sputum, BAL or bronchial brush sample in combination with hyphal forms detected by cytology or direct microscopy

NOTE: Positive aspergillus PCR results alone will NOT be considered sufficient mycological evidence of invasive fungal disease.

APPENDIX 2: PATIENT VISIT SCHEDULE

	Screening	Baseline (D1)	D2-6	D3	D5	D7	D8-D28	D14	D28	D42	D84	D168	D8-28
Eligibility check(#)	x	x (#)											
Informed consent		x											
Study drug administration(^)		x	x			x	(x)						x
Medical history		x											
Serum sampling(*)		x		x	x	x		x	x				
Patiënt details		x											
Register use of any antifungals		x						x	x	x	x	x	
Register use of TPV or EN after baseline visit										x		x	
Register use of any blood products administered after baseline visit										x		x	
Register hospital and ICU days from baseline to week 24										x		x	
Quality of life questionnaire		x								x	x	x	
Loss of income questionnaire		x									x	x	
Neutropenic status		x				x		x	x	x	x		
Survival status						x		x	x	x	x	x	

Please note that few exceptions notwithstanding, screening and baseline visit will be done on the same day.

Gray columns indicate the days that a hospital visit is required: Although the large majority of the patients will be in the hospital during the first week of therapy some patients will be outpatients. Patients who are outpatients and are included in the study are required to visit the outpatient clinic for study drug administration up until day 7.

Please note that, except for the screening and baseline visit, none of the other visits require an additional patient visit to the hospital as all these data can be collected from the patient files and by contacting the patient or the general practitioner or relatives of the patient.

(^) For the patients randomized in the combination therapy group. The minimum duration of study drug treatment is 7 days. After day 7 and up until day 28 the investigator decides if the treatment needs to be continued for a maximum of 28 days.

(#) For ICU patients the SOFA score should be recalculated if >24hrs pass between screening and baseline and patient excluded if SOFA has increased to >11 points.

(*) This should only be done if the patient is still in the hospital or visiting the outpatient clinic at these study dates. Please note that the day 14 and day 28 sampling can be done between day 11 and 17 and day 25 and 31 respectively (so day 14±3days and day 29±3days). The standard operating procedure regarding serum sampling and storage of serum by the lab is described in appendix 6 in more detail.

TPV= total parenteral nutrition. EN= enteral nutrition.