PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	RANDOMISED MULTICENTRE CLINICAL TRIAL TO EVALUATE VORICONAZOLE PRE-EMPTIVE GENOTYPING STRATEGY IN PATIENTS WITH RISK OF ASPERGILLOSIS: VORIGENIPHARM STUDY PROTOCOL
AUTHORS	Monserrat Villatoro, Jaime; García García, Irene; Bueno, David; de la Cámara, Rafael; Estébanez, Miriam; López de la Guía, Ana; Abad-Santos, Francisco; Antón, Cristina; Mejía, Gina; Otero, María José; Ramírez García, Elena; Frías Iniesta, Jesús; Carcas, Antonio; Borobia, Alberto

VERSION 1 - REVIEW

REVIEWER	Matthew P. Cheng Brigham and Women's Hospital, USA
REVIEW RETURNED	01-Mar-2020

GENERAL COMMENTS	Monserrat-Villatoro et al. propose a randomized controlled trial of comparing a pharmacogenetic algorithm to the standard of care for voriconazole administration for patients with IA. I believe this trial will contribute to the current body of literature not only for the management of patients with IA, but also towards emerging data regarding the importance of precision and individualized medicine. The proposed methodology is generally appropriate, but I believe that clarifications and revisions to the methods paper may help the investigators and would certainly add clarity to the readers. Major comments: P5L19 (and throughout the text): The primary outcome is not clearly defined. A serum voriconazole level is vague and would technically be 100% in both arms if it is ordered. Are the authors referring to a voriconazole level within the therapeutic range? Are they hoping for a more precise window within that range? P5L20 (and throughout the text): The secondary outcomes could be better defined as well. During what time frame is this composite endpoint being assessed? Day 42? 84? End of treatment? Other? Furthermore, therapeutic failure should be clarified. Of note, I am surprised that the authors did not use, and did not cite, the EORTC consensus definitions on defining responses to therapy (Segal BH, Herbrecht R, Stevens DA, et al. Defining responses to therapy and study outcomes in clinical trials of invasive fungal diseases:

	Mycoses Study Group and European Organization for Research and Treatment of Cancer consensus criteria. Clin. Infect. Dis. 2008; 47:674–683.) P6L10: Standard terminology for the definitions of IA are possible, probable, and proven. The authors should consider referencing standard terminology throughout the manuscript (https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciz1008/5645434). P8L20: The objectives cannot evaluate both effectiveness and efficacy; "efficacy can be defined as the performance of an intervention under ideal and controlled circumstances, whereas effectiveness refers to its performance under 'real-world' conditions": https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3912314/ P8L23: How is efficiency defined? P8L49: The eligibility criteria are vague. The investigators suggest that patients would be considered if "the clinician in charge considers might be at risk of developing fungal infection". Instead, I would recommend the investigators utilize robust eligibility criteria, based on hematological disease, treatment, and/or laboratory parameters. I recognize the need and the benefits of a pragmatic trial, yet the authors should clearly define the eligible population to ensure the that their methodology is reproducible. P9L60: The definition of therapeutic failure for patients on prophylactic treatment is confusing on several levels. First, if the patient is already on voriconazole for prophylaxis, how can the outcome be to measure voriconazole levels after the start of treatment? Second, what does suspected or confirmed invasive fungal disease mean? I would strongly recommend the authors rely on the EORTC consensus definitions for invasive fungal disease. P12L24: I find the sample size calculation to be overly optimistic. The authors expect that 85% of recruited patients will require voriconazole, which seems extraordinarily high. Furthermore, the authors do not account for early terminations (before day 5) and patients who will be lost to follow-up. I am concerned that 146 patients will be insufficient to reach the primary endpoint, but will defer to a statistical expert. Other: - How will treatment response be measured? Will routine GM or imaging studies be performed? Will treatment response be individualized per site or standardized throughout? - Should the authors consider stratifying their results if patients received PO or IV formulations of voriconazole? At least, a discussion regarding this point should be mentioned. Minor comments: L5: While I recognize what the authors mean by morbimortality, and it is certainly an understandable term, I do not believe it is an actual word. Would suggest spelling out morbidity and mortality throughout the manuscript. L14: The authors should clarify which "great advances" have been made in the treatment of IA in recent years, as the standard of care treatment remains voriconazole, which has been used since the

	early 2000s. L25: The sentence is incomplete as it ends with "for". L36: The fact that the investigators are unaware of the randomization scheme is not important if they are not blinded to treatment allocation.

REVIEWER	Maud Pichon Hôpital Victor Dupouy, Argenteuil, France
REVIEW RETURNED	17-Mar-2020

GENERAL COMMENTS	The survey is well written and the questions are relevant in a context of health politics and health economics. A few comments: - some parameters could be taken into account in the data collection, particularly the administration of drugs that could interfere with voriconazole even if this impact remains debated On the form, there are some typos in the bibliography with a duplicate: reference 15 and 19.

REVIEWER	Peter Herbison University of Otago New Zealand
REVIEW RETURNED	26-May-2020

GENERAL COMMENTS	My first impression of this paper is that the introduction is too long and is unnecessarily complicated. It could do with being reduced in size considerably. But on the whole the rest of the paper is much better. I do have an issue with the statistical analysis section. The purpose of an RCT is to estimate the difference between treatments, but the analysis section looks like they are trying to create a prognostic model, rather than looking at the difference between treatments and seeing if there were confounders. The power of the study is based on differences the authors expect to happen. These often turn out to be smaller when the study is done. Have to authors considered trying to estimate a minimally clinically important difference (MCID) and basing the power on this?

VERSION 1 – AUTHOR RESPONSE

Reviewer #1

Major comments:
P5L19 (and throughout the text): The primary outcome is not clearly defined. A serum voriconazole level is vague and would technically be 100% in both arms if it is ordered. Are the authors referring to a voriconazole level within the therapeutic range? Are they hoping for a more precise window within that range?

Yes, the reviewer is right. We have modified the description of the primary outcome in the abstract and in the methodology section. The primary outcome is serum voriconazole levels in the range of 1–5.5 µg/mL on the fifth day, according to the British Society for Medical Mycology, which is a subrogated variable strongly related to effectiveness and safety, with high-level evidence [6].

P5L20 (and throughout the text): The secondary outcomes could be better defined as well. During what time frame is this composite endpoint being assessed? Day 42? 84? End of treatment? Other? We agree with the reviewer and we have clarified it in the text. The secondary outcome is a combined variable of therapeutic failure and AEs occurring within 90 days of first voriconazole administration. Furthermore, therapeutic failure should be clarified. Of note, I am surprised that the authors did not use, and did not cite, the EORTC consensus definitions on defining responses to therapy (Segal BH, Herbrecht R, Stevens DA, et al. Defining responses to therapy and study outcomes in clinical trials of invasive fungal diseases: Mycoses Study Group and European Organization for Research and Treatment of Cancer consensus criteria. Clin. Infect. Dis. 2008; 47:674–683.)
The reviewer is right. We have reviewed the article suggested and we have modified the definitions in the manuscript, and also added the article in the bibliography.

P6L10: Standard terminology for the definitions of IA are possible, probable, and proven. The authors should consider referencing standard terminology throughout the manuscript (https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciz1008/5645434).
We agree with the reviewer and we have modified it.

P8L20: The objectives cannot evaluate both effectiveness and efficacy; "efficacy can be defined as the performance of an intervention under ideal and controlled circumstances, whereas effectiveness refers to its performance under 'real-world' conditions":
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3912314/
Yes, the reviewer is right. We would like to evaluate the effectiveness. Modified in the article.
P8L23: How is efficiency defined?
We agree with the reviewer that the definition is not clear enough. We have chanted it in order to clarify it: “Evaluate the efficiency of this pre-emptive genotyping strategy. By mean of a cost-effectiveness analysis. Cost includes the mean total direct cost per patient, also including the cost of adverse events. The effectiveness will be measured as a combined variable of therapeutic failure described in the outcomes section”

P8L49: The eligibility criteria are vague. The investigators suggest that patients would be considered if "the clinician in charge considers might be at risk of developing fungal infection". Instead, I would recommend the investigators utilize robust eligibility criteria, based on hematological disease, treatment, and/or laboratory parameters. I recognize the need and the benefits of a pragmatic trial, yet the authors should clearly define the eligible population to ensure that their methodology is reproducible.
As mentioned by the reviewer, we set the selection criteria from a very pragmatic perspective and taking in consideration that the main objective is to obtain a serum concentration of voriconazole within the defined therapeutic range. This range has been defined through its relationship with efficacy and safety data and therefore is considered a good surrogate marker. The patients to be genotyped are patients with high probability of future voriconazole precribing (prophylaxis or treatment). As stated, these patients are recruited from Paediatric Hemato-Oncology and Oncology Hematology Departments. Therefore the diagnosis to be included are already described in “Eligibility” section:
1) Risk of developing invasive aspergillosis who are potentially eligible for treatment or prophylaxis with voriconazole:
a. Paediatric population: children undergoing haematopoietic stem cell transplantation, with acute myeloid leukaemia, as well as their relapses.

b. Adult population: patients diagnosed with acute leukaemia and those with long-term expected neutropaenia secondary to haematological disease and/or undergoing specific treatment (e.g., aplastic anaemia and variants, myelodysplastic syndrome, solid organ or bone marrow transplantation), and patients who the clinician in charge considers might be at risk of developing fungal infection.
We think that these criteria are clear enough to be recognized by any reader treating these disease.
P9L60: The definition of therapeutic failure for patients on prophylactic treatment is confusing on several levels. First, if the patient is already on voriconazole for prophylaxis, how can the outcome be to measure voriconazole levels after the start of treatment? Second, what does suspected or confirmed invasive fungal disease mean? I would strongly recommend the authors rely on the EORTC consensus definitions for invasive fungal disease.
The reviewer is right, and we have modified the definitions of invasive fungal disease. On the other hand, the voriconazole levels are an indirect marker of effectiveness and safety, also in prophylaxis, so that we are going to measure the levels in every patient who have been administered voriconazole, no matter what the indication of treatment or prophylaxis.
P12L24: I find the sample size calculation to be overly optimistic. The authors expect that 85% of recruited patients will require voriconazole, which seems extraordinarily high. Furthermore, the authors do not account for early terminations (before day 5) and patients who will be lost to follow-up. I am concerned that 146 patients will be insufficient to reach the primary endpoint, but will defer to a statistical expert.
We have considered the reviewer's advice, but we have based our calculations in previous data and clinical experience of our hospital (not yet published). The calculations have been carried out by an expert in statistics.
Other:
- How will treatment response be measured? Will routine GM or imaging studies be performed? Will treatment response be individualized per site or standardized throughout?
The effectiveness of treatment will be measured by a surrogate variable (serum voriconazole levels), which has already been shown to be associated with clinical response to therapy. In addition, as it is a pragmatic clinical trial, physician will perform the usual procedures in the routine practice (imaging studies for example) and this procedures will be taken into account in the economic analysis.
On the other hand, as the reviewer suggests, the response to treatment will be measured stratified by centre, and also globally. We did not include it because we reached the word limit.
- Should the authors consider stratifying their results if patients received PO or IV formulations of voriconazole? At least, a discussion regarding this point should be mentioned.
We consider the reviewer's suggestion, but since the response will be measured by voriconazole serum level, it will not be necessary to consider the formulation, because the adjustment will be made to the dose in any of them.
Minor comments:
L5: While I recognize what the authors mean by morbimortality, and it is certainly an understandable term, I do not believe it is an actual word. Would suggest spelling out morbidity and mortality throughout the manuscript.
The reviewer is right. We have modified it.
L14: The authors should clarify which "great advances" have been made in the treatment of IA in recent years, as the standard of care treatment remains voriconazole, which has been used since the early 2000s.
We have considered the reviewer's advice, but the major advances do not concern invasive fungal disease, but the treatment of haematological disease.
L25: The sentence is incomplete as it ends with "for".
The reviewer is right. We have modified it.
L36: The fact that the investigators are unaware of the randomization scheme is not important if they are not blinded to treatment allocation.

We have considered the reviewer's advice, but we think it is important that researchers do not know the sequence of randomization, so that they cannot use it to assign patients to the treatment arms they prefer, altering the order of inclusion of patients.

Reviewer #2
A few comments:
- some parameters could be taken into account in the data collection, particularly the administration of drugs that could interfere with voriconazole even if this impact remains debated
We agree with the reviewer that is an important issue to collect all potential factors (including drugs) that could interfere with voriconazol. In the eCRF of the trial we have included concomitant medication and other clinical and demographic condition. We have included this information in the manuscript.
On the form, there are some typos in the bibliography with a duplicate: reference 15 and 19.
The reviewer is right. We have corrected it.

Reviewer #3
My first impression of this paper is that the introduction is too long and is unnecessarily complicated. It could do with being reduced in size considerably.
Thank you for your comment and suggestion. We have reduced it.
But on the whole the rest of the paper is much better. I do have an issue with the statistical analysis section. The purpose of an RCT is to estimate the difference between treatments, but the analysis section looks like they are trying to create a prognostic model, rather than looking at the difference between treatments and seeing if there were confounders.
We understand the reviewer concerns, but in this case, the main objective of the RCT is to evaluate the effectiveness of a therapeutic strategy that includes pre-emptive genotyping and therapeutic drug monitoring. To evaluate this strategy we have selected patients that potentially will receive voriconazole. Our objective is not to create a prognosis model.
The power of the study is based on differences the authors expect to happen. These often turn out to be smaller when the study is done. Have to authors considered trying to estimate a minimally clinically important difference (MCID) and basing the power on this?
It is a good appreciation. We will consider this suggestion to calculate the power.

VERSION 2 – REVIEW

REVIEWER	Matthew P. Cheng McGill University Health Centre, Montreal, Canada
REVIEW RETURNED	27-Jun-2020

GENERAL COMMENTS	I appreciate the efforts the authors made to address the issues in the previous version of their manuscript, which has strengthened it considerably.

REVIEWER	Peter Herbison University of Otago New Zealand
REVIEW RETURNED	03-Jul-2020

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.