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RANDOMISED MULTICENTRE CLINICAL TRIAL TO EVALUATE VORICONAZOLE PRE-EMPTIVE GENOTYPING STRATEGY IN PATIENTS WITH RISK OF ASPERGILLOSIS: VORIGENIPHARM STUDY PROTOCOL

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Date of registration in primary registry	18 June 2019
Source(s) of monetary or material support	Institute of Health Carlos III, grants for Health Research Project 2018 of Spain
Primary sponsor	La Paz University Hospital Research Foundation (FIBHULP)
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Public title	Clinical trial to evaluate the effectiveness and efficiency of pre-emptive genotyping before treatment with voriconazole
Scientific title	Randomised multicentre clinical trial to evaluate voriconazole pre-emptive genotyping strategy in patients with risk of aspergillosis: vorigenipharm study protocol
Countries of recruitment	Spain
Health condition(s) or problem(s) studied	Fungal infectious disorders, neutropaenia, chemotherapy-induced neutropaenia, haematopoietic neoplasm, allogeneic peripheral haematopoietic stem cell transplant, autologous peripheral haematopoietic stem cell transplant
Intervention(s)	Pre-emptive genotyping plus post-serum levels vs sole post-serum level adjustment of voriconazole

Key inclusion and exclusion criteria	Ages eligible for study: no limit Sexes eligible for study: both Accepts healthy volunteers: no Inclusion criteria: children who are going to receive an HSCT and those who have diagnosis of acute myeloid leukaemia as well as its relapse. Adults diagnosed with acute leukaemia, with expected prolonged neutropaenia, and those with risk of developing a fungal infection. Exclusion criteria: patients who for any reason should not be included in the study according to the criteria of the research team.
Study type	Interventional Allocation: randomised Intervention model: parallel assignment Masking: single-blind (subject) Primary purpose: prevention Phase IV
Date of first enrolment	January 2020
Target sample size	146
Recruitment status	Not started
Primary outcome(s)	Serum voriconazole concentration within the therapeutic range.
Key secondary outcomes	Therapeutic failure and adverse events. Dose-related adverse events associated with treatment
Ethics Review	Status: Approved. Date of approval: June 6th 2019 and June 18th 2019. Name and contact details of Ethics committee(s): approved by 'La Paz' University Hospital Ethics Committee and the Spanish Drugs and Health Products Agency, respectively.
IPD sharing statement	Plan to share IPD (Yes, No): Yes. Plan description: A copy of the database of data collected during the clinical trial will be attached as an appendix to the publication resulting from this clinical trial. This data will be available at the same time as the results will be published, and will be kept available to everyone without any time limit. Data will be available indefinitely on the publisher's website, as long as it is kept by the Publisher, for anyone who wishes to access the data, for non-commercial purposes.

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ABSTRACT

Introduction: Invasive aspergillosis is the most important cause of morbimortality in patients with haematological diseases. At present, voriconazole is the first-line treatment for invasive fungal disease. The pharmacokinetic interindividual variability of voriconazole depends on genetic factors. CYP450 is involved in 70%–75% of total metabolism of voriconazole, mainly CYP3A4 and CYP2C19, with the remaining 25%–30% of metabolism conducted by monooxygenase flavins. CYP2C19 single nucleotide polymorphisms could explain 50%–55% of variability in voriconazole metabolism.

Materials and Methods: The main objective is to compare efficiency of pre-emptive voriconazole genotyping with routine practice. The primary outcome is serum voriconazole on the fifth day. The secondary outcome is the combined variables of therapeutic failure and adverse events associated with voriconazole. A total of 146 patients at risk of invasive aspergillosis who will potentially receive voriconazole will be recruited, and CYP2C19 will be genotyped. If the patient ultimately receives voriconazole, they will be randomised (1:1 experimental/control). In the experimental arm, patients will receive a dose according to a pharmacogenetic algorithm, including CYP2C19 genotype and clinical and demographic information. In the control arm, patients will receive a dose according to clinical practice guidelines. In addition, a Spanish National Healthcare System (NHS) point-of-view cost-effectiveness evaluation will be performed. Direct cost calculations for each arm will be performed.

Conclusion: This trial will provide information about the viability and cost-effectiveness of the implementation of a pre-emptive voriconazole genotyping strategy in the Spanish NHS.

Ethics and dissemination: A Spanish version of this protocol has been evaluated and approved by the La Paz University Hospital Ethics Committee and the Spanish Agency of Medicines and Medical Devices. Trial results will be submitted for publication in an open peer-reviewed medical speciality-specific publication. Eudra-CT: 2019-000376-41. ClinicalTrials: NCT04238884

STRENGTHS AND LIMITATIONS OF THIS STUDY

(1) The study will be randomised, with objective variables and not influenced by placebo effect (voriconazole serum level).
(2) The effectiveness and safety related to serum voriconazole is well known.
(3) For patients in this study, there is little risk compared with routine clinical practice.
(4) The study is single-blinded.
(5) Our results depend on the frequency of CYP2C19 polymorphism.

INTRODUCTION:

Invasive aspergillosis is the most important cause of morbimortality in patients with haematological diseases. The main risk groups are patients with severe and the prolonged neutropenia induced by chemotherapy drugs, patients with acute leukaemia, and those who receive haematopoietic stem cells transplants. The most recent epidemiologic studies show an incidence of probable or confirmed invasive aspergillosis in patients with high-risk haematological diseases of 6%–11% [1][2]. Mortality due to this complication in patients with acute leukaemia is 27% [1], and up to 40% if we include every malignant haematologic disease [3]. In recent years, great advances in the treatment of this pathology have been made, which have improved the prognosis of haematologic diseases. However, high morbidity and mortality associated with infectious complications continue to be a medical problem. As a result, improving the prognosis for invasive fungal disease has great scientific interest, with aspergillosis being the most prevalent form.

At present, voriconazole is the first-line treatment for invasive fungal disease (1-grade evidence, A-grade recommendation) [4][5]. It is a third-generation triazole antifungal for. Achieving voriconazole serum levels in the therapeutic range during the first treatment week improves the prognosis of fungal infection and the tolerability of the treatment by reducing dose-dependent adverse effects (AEs) [6]. The AE reduction, associated with posology optimization, decreases the amount of withdrawals of this antifungal drug.

Knowledge of the genetic factors that explain a part — very relevant in some cases — of the variability in response to drugs has been exponentially boosted by important advances in the field of genetics made in recent years. Pharmacogenetics and pharmacogenomics have become established as disciplines of great importance in medical knowledge. The therapeutic effect of a drug relies largely on pharmacokinetic and pharmacodynamic processes, which might be simultaneously regulated by other physiological or pathological systems. All these processes involve proteins, enzymes, receptors and transporters, among others, whose expression is genetically coded. Various degrees of activity or function can be presented by these genes, affecting the behaviour of the drug in terms of efficacy and safety. According to this approach, these genetic variations could become markers that help us predict the pharmacological response depending on whether they are present and on their interrelationship with other markers associated with clinical response. In recent years, several groups and organisations have published evidence assessment systems, guidelines, systematic reviews and evaluations for various drugs and pathologies; in some cases, genotyping prior to treatment with various drugs has been shown to be cost-efficient (Plumpton, 2016). The drug regulatory agencies, such as the United States Food and Drug Administration and the European Medicines Agency, include pharmacogenetic information in the summaries of specific drugs. Thus,

we can find basic information about the genotype-phenotype relationship for authors or groups that request the highest methodological rigor (including clinical trials) to recommend a pharmacogenetic test. In addition to this evidence, the individualisation of treatment must be performed through an expert interpretation of the interaction between pharmacogenetics and other aspects (pharmacokinetics, patient demographics, interactions, etc.).

Focusing on voriconazole, its pharmacokinetic interindividual variability depends on genetic factors [15]. CYP450 is involved in 70%–75% of the total metabolism of voriconazole, mainly CYP3A4 and CYP2C19, with the remaining 25%–30% of metabolism conducted by monooxygenase flavins. CYP2C19 single nucleotide polymorphisms could explain 50%–55% of variability in voriconazole metabolism. Some 5%–17% of patients are ultrafast metabolisers (CYP2C19*17/*17), and approximately 25%–33% of patients are fast metabolisers (CYP2C19*1/*17). Both are associated with a high risk of not achieving therapeutic levels for invasive aspergillosis [16][17]. Pharmacogenetics information oriented to dose adjustment is present in European dosing guidelines and in FDA recommendations, in which recommendatons are given according to genotype [15]. This information must be known by every physician in order to reduce adverse effects, improve effectiveness and thus raise patient compliance by reducing morbidity and mortality.

We propose a pragmatic clinical trial to evaluate the effectiveness and efficiency of a pre-emptive genotyping strategy of biomarkers related to the voriconazole response in patients with haematological disease who are at risk of suffering an infection susceptible to treatment with voriconazole.

METHODS AND ANALYSIS

Study design

VORIGENIPHARM is an acronym (VORIconazole PHARMacoGENetIcs) of the clinical trial with code EUDRA-CT: 2019-000376-41, funded by the Spanish Health Research and Development Strategy. It is a phase IV pragmatic, multicentre, randomised, single-blind, parallel arm, centre-stratified clinical trial. A total of 146 patients at risk of invasive aspergillosis who will potentially receive voriconazole will be recruited, and CYP2C19 alleles will be genotyped. The patients who ultimately receive voriconazole will be randomised to receive the dose according to a pharmacogenetic algorithm, including the CYP2C19 genotype and clinical and demographic information, or according to clinical practice (Figure 1).

Study objectives

Primary objective:

Evaluating the effectiveness and efficiency of a pre-emptive genotyping strategy for voriconazole in treatment and prophylaxis of *Aspergillus* fungal infections in patients with haematological diseases.

Secondary objectives:

- Evaluate the effectiveness of a pre-emptive genotyping strategy for voriconazole in achieving adequate therapeutic levels in haematological patients at risk of fungal infection compared with routine clinical practice.
- Evaluate the efficacy and safety of pre-emptive genotyping of voriconazole in haematological patients at risk of fungal infection, comparing it with routine clinical practice.
- Evaluate the efficiency of this pre-emptive genotyping strategy.

Eligibility

The patient will be selected from La Paz University Hospital Paediatrics Hemato-Oncology Department, La Paz University Hospital Haematology Department, La Princesa University Hospital Adult Hematology Department and Gomez-Ulla University Hospital Adult Hematology Department. They will include children and adults who meet the following inclusion criteria:

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.
2) Accepting participation in the study by signing the informed consent (adult patients), or minor patients whose representative/legal guardian has willingly signed the informed consent. In the case of mature minors (12–17 years of age), in addition to the consent signed by the legal guardian, the minor's assent shall be obtained.

The exclusion criteria are as follows:

1) Patients who for any reason should not be included in the study as assessed by the research team.
2) Patients who are not capable of understanding the information form and are unable to sign the informed consent document.

All patients eligible to receive voriconazole will sign an informed consent document for their participation in the clinical trial and for the collection of blood samples destined for genetic studies. They will also be asked for their consent to store an aliquot of their DNA for future studies (i.e., mass sequencing). Patients identified in this phase will be randomised if and when they receive voriconazole.

Randomisation

Patients who meet all inclusion and no exclusion criteria, who have signed the informed consent and will receive voriconazole will be randomised, stratified by centres. The randomisation sequence was created using SAS version 9.4 statistical software (procedure 'PROC PLAN') with a 1:1 allocation. No randomisation seed was specified. The randomisation seed was generated taking the hour of the computer where the program was executed. Randomization will be done centrally through the electronic CRF (MACRO®) in order to conceal the sequence until interventions are assigned.

Masking

The study is single blind, and under no circumstance does the patient know the group to which they have been assigned. The medical researchers are unaware of the randomization scheme. Although there is a risk of unmasking the patient, and the lack of masking for the physician could affect the evaluation variables, we believe that in this clinical trial is permisible since the primary variable is an objective one (voriconazol concentration), and a total masking is not feasible in a study using a pragmatic approach.

Outcomes

Primary outcome

Serum voriconazole levels on the fifth day, which is a subrogated variable strongly related to effectiveness and safety, with high-level evidence. According to the British Society for Medical Mycology, serum levels should be in the range of 1–5.5 μg/mL [6].

Secondary outcomes

- A combined variable of therapeutic failure and AEs.
    - Therapeutic failure is defined as the following:

- Patients with suspected or confirmed invasive aspergillosis: drug change or association because of poor clinical or radiological evolution of the disease.
- Patients who received prophylactic treatment: the necessity of change because of suspected or confirmed invasive fungal disease.

  - Adverse event: Dose-dependent drug AEs, including visual disturbances (e.g., photopsia), skin reactions, neurotoxicity (e.g., confusion and visual hallucinations) and QTc lengthening.
- Costs by AE
- Cost savings by AE
- Quality-adjusted life years.

Study procedures

The study visits and procedures will be performed as shown in Figures 1 and 2.

- Selection visit: After confirming the selection criteria, informing the patient and signing the informed consent document, a blood sample will be taken for genetic analysis. Patients who have not received voriconazole within 3 months of recruitment will not be considered for randomisation, but will be considered for economic evaluation. Patients who, in this 3-month period, receive voriconazole will continue to the randomisation visit.
- Randomisation visit: The patient will be randomised to one of the two branches of the study:
  - Experimental group: based on the genetic study performed and the patient's characteristics (age, weight, indication), the Pharmacogenetics Unit of La Paz University Hospital will indicate the dose to be administered. The dose will be based on the therapeutic individualisation protocol guided by pharmacogenetics, as agreed by all clinical services, and which includes the recommendations of the Clinical Pharmacogenetics Implementation Consortium guide (Moriyana, 2016) and the study by Hicks et al (Hicks, 2014). In the experimental group arm, in cases of patients with rapid, ultrafast or slow metaboliser phenotypes, a determination of serum voriconazole concentrations at 48 hours (+/- 24 hours) may be considered, which will make a dose adjustment possible if required.
  - Control group: No information will be provided and the procedure will be performed according to normal clinical practice, with clinical monitoring by the doctor in charge. In both cases, the request for serum voriconazole levels for subsequent dose adjustment will be recommended, as is standard clinical practice, in accordance with the recommendations of the British Society for Medical Mycology (Ashbee, 2014).

- Primary outcome evaluation visit: Voriconazole concentrations will be measured on no later than the fifth day after the start of treatment (±1 day); if a dose adjustment is necessary, this will be performed according to the criteria of the attending clinician (control group) or the Pharmacogenetic Unit (experimental group); subsequent monitoring (determination of concentrations and dose adjustment) will be performed according to the criteria of the attending clinician in both arms.
- Follow-up visits: While the patient is being treated with voriconazole, follow-up visits will be made according to clinical criteria, and the following information will be recorded:
    - Evaluation of potential voriconazole-related clinical AEs (see evaluation variables). This will be performed by asking the patient questions, performing a physical examination, routine tests (including liver enzymes) and an electrocardiogram.
    - Voriconazole levels, if requested according to medical criteria.
    - The type of consultation will be recorded: outpatient appointment, urgent episode, admission, readmission or phone call.
- End-of-treatment visit: Performed on the last day the patient receives treatment with voriconazole, including the following procedures:
    - A reason for the end of treatment will be indicated (withdrawal due to toxicity or therapeutic failure, or after completion of treatment according to clinical criteria of efficacy)
    - Evaluation of potentially voriconazole-related clinical adverse events, as in follow-up visits.
    - If the patient continues voriconazole treatment beyond 3 months, the end of study visit will be made at that time.
- End-of-study visit: the end-of-study visit will coincide with the end-of-treatment visit, except for those patients who are still undergoing treatment after 3 months. A safety analysis, electrocardiogram, and assessment of adverse events will be performed. Patients who continue treatment beyond this visit will be followed according to routine clinical practice.

Voriconazole genotyping and therapeutic drug monitoring

Genotyping will be performed at the Institute of Medical and Molecular Genetics at La Paz University Hospital using the self-designed SNP-array device (PharmArray v.2.), which makes genotyping of 180 relevant mutations possible for predicting the response to drugs. Among these mutations are the most relevant variants of CYP2C19, a gene that is related to serum levels of voriconazole, with a level of evidence 1A of CPIC: CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893) and CYP2C19*17 (rs12248560).

Serum voriconazole determinations will be tested at the Therapeutic Drug Monitoring Laboratory of the Clinical Pharmacology Service at La Paz University Hospital using an enzyme immunosensing technique on the Abbott ARCHITECT c4000 autoanalyser.

Data collection and outcome measures

A electronic case report form has been designed using MACRO Electronic Data Capture by Elsevier. This system will anonymise patients, and the data will be transferred to a '*.csv' file in order to analyse it with R software (3.5.2 version or newer). To ensure the quality of the data, Data management will be performed by the Spanish Clinical Research Network (SCReN). Data management plan have been approved by the principal investigator and the sponsor. Data collection forms will be included in the final report.

Sample size calculation

In routine clinical practice, 43% of patients have voriconazole serum levels in the therapeutic range on the fifth day of treatment. We expect this to improve to 68%, based on the Hicks article [19]. With a two-tailed type I error of 0.05 and 80% power, it is necessary to recruit 62 patients to receive treatment in both arms. 85 out of every 100 patients recruited are estimated to will receive voriconazole, we will need a total of 146 patients from whom we obtain informed consent. This calculation has been performed with the 'power.prop.test' package, from R version 3.2.0. The necessary time to recruit the whole group is estimated to be 2 years. We estimate 20% of the patients will be selected from the La Princesa University Hospital Adult Haematology Department, and 20% from the Gomez-Ulla University Hospital Adult Haematology Department. The rest of group will be recruited from La Paz University Hospital. Since it is a phase IV clinical trial including patients that are usually followed in the hospital, with pragmatic selection criteria, it is not necessary to plan any strategy for achieving adequate participant enrolment.

Statistical analysis

Frequency results will be expressed in absolute terms, as percentages and confidence intervals. Continuous variables will be expressed as mean (SD) and median (range) according to Kolmogorov Smirnov's test of normality.

For the main dichotomous variable (level of voriconazole within the therapeutic range) a generalised logistic model will be used. After that, "elastic net" techniques will be employed with the purpose of selecting variables so that they are not as rigid as in Lasso's case. Variables with some degree of correlation between themselves will be accepted in the model, as in Ridge regression, aiming at the best possible predictive model. Finally, more advanced methods of statistical learning will be used to give the variables consistency by the method described above. Predictive ability will be calculated

using receiver operating characteristic curves both at a specific time and over the course of the patient's follow-up.

A survival analysis will be performed for the secondary variables to test the Cox proportional hazards model and the Weibull model, analysing the time until the first event, using Kaplan Meier curves. A penalised Cox regression will be fitted to determine whether there are differences from the start of the target drug to the appearance of the event (secondary variable) between the control group and the experimental group, and finally, if possible the Weibull model will be used. A cross-validation approach will be used, dividing the sample into k subgroups. Later, a subgroup analysis will be performed taking into consideration the recruiting centre, calculating the *a posteriori* analysis power of every objective, given that this can be a source of hypothesis generation for future research lines.

The analysis of the primary variable of efficacy will be made on an intention-to-treat (ITT) basis. It will include all the patients who are randomized, whether they have received the study treatment or not. The safety analyses will be held in the safety population, which includes patients who receive at least one dose of the drug during the study. No interim analysis or stopping rules will be carry out in this clinical trial.

A bilateral significance level of 0.05 and bilateral 95% confidence intervals will be assumed. The statistical software R (R Core Team [2014]) will be used. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Economic evaluation

A Spanish National Healthcare Service (NHS) point-of-view cost-effectiveness evaluation will be performed. Direct cost calculations for each arm will be performed.

Direct costs will be calculated for both alternatives by registering healthcare resources used during the follow-up and the unit cost per patient, as well as the costs incurred by the centres to implement the intervention.

The use of resources and their unit costs, such as doctor's visits, medications and any interventions performed on those patients in the hospital during follow-up shall be recorded. Costs for each patient for each intervention group will be totalled. This calculation will allow us to compare the average cost per event avoided between the intervention group and the control group. The results will be expressed in cost per event and avoided events. As far as possible, the utility of each intervention should be calculated.

A univariate and a probabilistic sensitivity analysis will be performed to assess the uncertainty about the model and the unit costs, if they are obtained from a source other than the clinical centres.

Data monitoring

Coordination, management, monitoring, data management and statistical analysis of the study will be performed by the Spanish Clinical Research Network (SCReN).

Auditing

During the progress of the study, audit visits can be conducted at the participating centres. The investigator will allow direct access to the source data/documents for monitoring, auditing, review by the ethical research committee and inspection by the Health Authorities

Funding

This clinical trial has been funded by the Instituto de Salud Carlos III (ISCIII), Minister of Innovation and Science of Spain in a competitive and public grant (Research Projects 2018, Spanish Health Research and Development Strategy). Project code: PI18/01322.

Conflicts of Interest

The Funders and Sponsor not interfere in the selection processes of the patients, analysis of the data and/or publication of the results, or any other process that might come into play with the results of the study. Funding will be independent of the results of the study. The Principal Investigator has ultimate authority over any of these activities.

Patient and Public Involvement

The development of the research question and outcome measures were based on the oncologists' experience treating this profile of patients the desire to optimize voriconazol treatment. Patients and patient advisers were not involved in the design, recruitment or conduct of this study. The patients or their families will be notified of the study results in writing and verbally, and we will invite them to help us develop our dissemination strategy.

Access to data.

This section is included in the data sharing plan.

Disemination policy

Outputs from this study will include journal publications, conference presentations and community reporting. Outputs will not identify participants.

Ethical considerations

The researchers will adhere strictly to the provisions of this protocol and will complete the case report forms. The study will be performed according to the recommendations for clinical studies and the evaluation of drugs in humans, as contained in the Declaration of Helsinki (revised in successive world assemblies) and in the current Spanish and European legislation on clinical studies and patient data confidentiality. The study will follow the principles of Good Clinical Practice. This study has been approved by the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain) and by the Spanish Agency of Medication and Health Products, and has been registered in Eudra CT (Eudra CT: 2019-000376-41) and ClinicalTrials.gov (NCT04238884).

This clinical trial has been classified by the Spanish Agency for Medicines and Healthcare Products as a "low-intervention clinical trial". The additional diagnostic or monitoring procedures do not pose more than minimal additional risk or burden to the safety of the subjects compared to normal clinical.

No additional use of the system for compensation shall be required from the sponsor for low-intervention clinical trials. If any possible damage that could be suffered by a subject, resulting from the use of the investigational medicinal product in accordance with the protocol of that specific clinical trial, it is covered by the applicable compensation system already in place.

All protocol amendments will be evaluated by the Ethics Committee and the Spanish Agency of Medication and Health Products, following the principles of Good Clinical Practice and national legislation.

DISCUSSION

Some important barriers have been detected to implementation of pharmacogenetics in usual clinical practice. The reasons detected in the literature include the difficulty of obtaining high-level evidence on genetic markers' efficacy, effectivity and efficiency, and a lack of consensus. Another problem is the absence of pharmacogenetic techniques' formation and its interpretation. We also have to consider the financial, logistic and legal limitations. Finally, there is no specific implementation strategy in clinical practice. There have been two proposed models for the implementation of pharmacogenetics biomarkers in usual clinical practice:

- Case-to-case model: The decision is made individually and is based on the need for using a drug whose effectiveness or safety is modified by specific genetics variations. The limitations are the costs and the latency between sample extraction and the results obtained.
- Pre-emptive genotyping model: This alternative assesses the patient's genetic information at the very beginning, even before starting treatment. Currently, the development of arrays permits detection of a significant number of mutations in which therapeutic impact is probable or definite. Other authors have developed a similar strategy [10][11], for instance St. Jude's Hospital [12], as well as our group [13]. Rasmussen-Torvik's group has proposed the

eMERGE-PGx project, in which pre-emptive pharmacogenotyping is integrated with clinical history [14].

In the case of voriconazole, several studies have linked CYP2C19 polymorphism to the response. In addition, some studies have shown that genotyping CYP2C19 is effective. The cost-efficiency of pre-treatment genotyping in some drugs has been already demonstrated in some articles [7]; however, there is no evidence of voriconazole efficiency specifically in the Spanish NHS, or even whether voriconazole-related cytochrome genotyping is efficient in other countries.

This pragmatic clinical trial is proposed by our group to evaluate effectiveness and efficiency of pre-emptive biomarker genotyping and its interpretation by a Pharmacogenetic Unit for patients with haematological diseases and a risk of fungal infection, who are susceptible to receiving voriconazole treatment. If our hypothesis is proven, this strategy will help us improve the prevention and treatment of this infectious complication. This trial will provide information about the viability of the translation of this strategy to routine clinical practice. In addition, our pre-emptive pharmacogenetic strategy, combined with therapeutic drug monitoring, has the potential to improve efficacy and safety, with a high level of evidence [6], lowering the likelihood of AE occurrences.

AUTHOR CONTRIBUTIONS

All authors conceptualized and contributed to the study design and reviewed and revised this article. AMB supervised the study design and takes responsibility for the paper as a whole.

All authors attest to meeting the four ICMJE.org authorship criteria: (1) Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND (2) Drafting the work or revising it critically for important intellectual content; AND (3) Final approval of the version to be published; AND (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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VISIT	Visit of selection	Visit of randomisation	Primary outcome evaluation (visit)	Follow-up visit	End-of-treatment visit	End-of-study visit
Day of the study	**Day 0**	**Maximum 3 months after selection**	**5th day of treatment (+/- 1 day)**	**Weekly until end of treatment**	**Last day of treatment**	**2 weeks later after ending treatment(1)**
Selection criteria meeting	X					
Informed Consent Signing	X					
Genetic sample	X					
Randomisation		X				
Starting treatment		X				
Voriconazole serum levels			X	X (2)		
Lab analysis (3)		X	X	X	X	X
Electrocardigram		X	X	X	X	X
AE(4) evaluation		X	X	X	X	X

(1) Patients who after 3 months continue being treated, End-of-study visit will be done on the last day of third month.
(2) According to physician criteria
(3) Analysis including liver enzimes.
(4) AE (Adverse Events)

Table1. Study visits planning and study procedures.

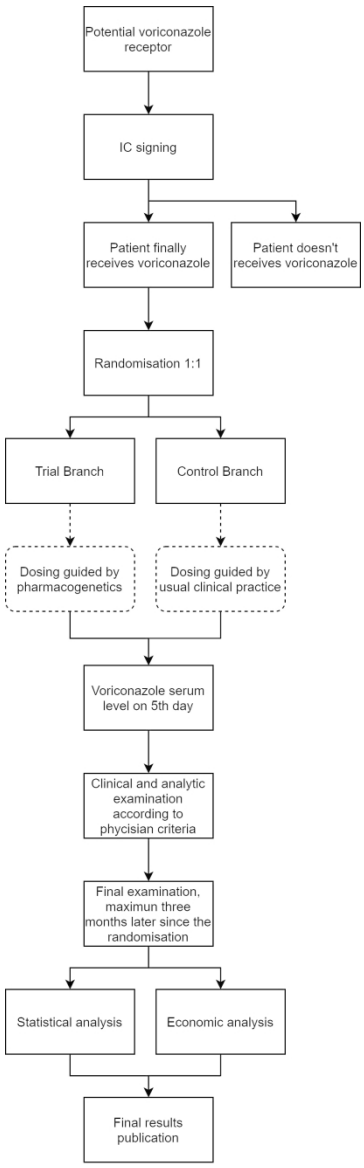

Figure 1. Study Flowchart

Figure 1. Study flowchart

APPENDIX 1: Trial Investigation Group

- Trial coordination: Alberto M. Borobia, MD, PhD; Antonio J. Carcas, MD, PhD.
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    - Pediatric Onco-Haematology Department: David Bueno, MD, PhD.
    - Haematology Department: Ana Lopez, MD, PhD; Teresa de Soto, MD
  - La Princesa University Hospital:
    - Haematology Department: Rafael de la Camara, MD, PD.
    - Clinical Pharmacology Department: Francisco Abad Santos, MD, PhD; Gina Mejia, MD, PhD.
  - Gómez Ulla Hospital:
    - Infectious Diseases. Internal Medicine Department: Miriam Estébanez, MD, PhD.
    - Haematology Department: María José Otero, MD, PhD.
- Pharmacoeconomic analyses:
  - Francisco de Vitoria University - Health Technologies Assessment Department: Cristina Antón, MD, PhD.
- Study monitoring: Foundation for Biomedical Research, University Hospital La Paz: Central Unit for Clinical Research and Clinical Trials. SCReN.

SPIRIT checklist.

Included	Section/item	Item No	Description	Pg.
Administrative information				
☑	Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
☑	Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
☑		2b	All items from the World Health Organization Trial Registration Data Set	1-2
☑	Protocol version	3	Date and version identifier	1
☑	Funding	4	Sources and types of financial, material, and other support	14
☑	Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	16, 21
☑		5b	Name and contact information for the trial sponsor	1
☑		5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
☑		5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction				
☑	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6, 7
☑		6b	Explanation for choice of comparators	6, 7
☑	Objectives	7	Specific objectives or hypotheses	8

☑	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes				**Pg.**
☑	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8, 12
☑	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
☑	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10, 11
☑		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11
☑		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11, 12
☑		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7, 12, 16
☑	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9, 10
☑	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10,11,20

☑	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
☑	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	12
Methods: Assignment of interventions (for controlled trials)				
	Allocation:			
☑	Sequence generation	16a	Method of generating the allocation sequence (eg, computer- generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
☑	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
☑	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9, 10, 11, 21
☑	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
☑		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9

Methods: Data collection, management, and analysis				
☑	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12

☑		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	12
☑	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
☑	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12,13
☑		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12,13
☑		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	12,13

Methods: Monitoring				
☑	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14
☑		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12, 13
☑	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
☑	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14

Ethics and dissemination	

☑	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15
☑	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	15
☑	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
☑		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9
☑	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
☑	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
☑	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	14
☑	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
☑	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2,14
☑		31b	Authorship eligibility guidelines and any intended use of professional writers	16
☑		31c	Plans, if any, for granting public access to the full protocol, participant- level dataset, and statistical code	2, 14

Appendices	

☑	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	*
☑	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	9, *

COMMENTS:

* Some parts of the documentation will be attached to the submission, such as the model consent form and other related documentation.

BMJ Open

RANDOMISED MULTICENTRE CLINICAL TRIAL TO EVALUATE VORICONAZOLE PRE-EMPTIVE GENOTYPING STRATEGY IN PATIENTS WITH RISK OF ASPERGILLOSIS: VORIGENIPHARM STUDY PROTOCOL

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Primary Subject Heading:	Genetics and genomics
Secondary Subject Heading:	Infectious diseases
Keywords:	CLINICAL PHARMACOLOGY, INFECTIOUS DISEASES, Clinical trials < THERAPEUTICS

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TITLE: RANDOMISED MULTICENTRE CLINICAL TRIAL TO EVALUATE VORICONAZOLE PRE-EMPTIVE GENOTYPING STRATEGY IN PATIENTS WITH RISK OF ASPERGILLOSIS: VORIGENIPHARM STUDY PROTOCOL

Data category	Information
Primary registry and trial identification number	EU Clinical Trials Register https://www.clinicaltrialsregister.eu/ctr-search/search 2019-000376-41 ClinicalTrials.Gov https://clinicaltrials.gov/ct2/show/NCT04238884 NCT04238884
Date of registration in primary registry	18 June 2019
Source(s) of monetary or material support	Institute of Health Carlos III, grants for Health Research Project 2018 of Spain
Primary sponsor	La Paz University Hospital Research Foundation (FIBHULP)
Contact for public queries	Alberto M. Borobia Perez, MD, PhD, La Paz University Hospital, Clinical Pharmacology Department, +34917277558, alberto.borobia@salud.madrid.org
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Public title	Clinical trial to evaluate the effectiveness and efficiency of pre-emptive genotyping before treatment with voriconazole
Scientific title	Randomised multicentre clinical trial to evaluate voriconazole pre-emptive genotyping strategy in patients with risk of aspergillosis: vorigenipharm study protocol
Countries of recruitment	Spain
Health condition(s) or problem(s) studied	Fungal infectious disorders, neutropaenia, chemotherapy-induced neutropaenia, haematopoietic neoplasm, allogeneic peripheral haematopoietic stem cell transplant, autologous peripheral haematopoietic stem cell transplant
Intervention(s)	Pre-emptive genotyping plus post-serum levels vs sole post-serum level adjustment of voriconazole

Key inclusion and exclusion criteria	Ages eligible for study: no limit Sexes eligible for study: both Accepts healthy volunteers: no Inclusion criteria: children who are going to receive an HSCT and those who have diagnosis of acute myeloid leukaemia as well as its relapse. Adults diagnosed with acute leukaemia, with expected prolonged neutropaenia, and those with risk of developing a fungal infection. Exclusion criteria: patients who for any reason should not be included in the study according to the criteria of the research team.
Study type	Interventional Allocation: randomised Intervention model: parallel assignment Masking: single-blind (subject) Primary purpose: prevention Phase IV
Date of first enrolment	January 2020
Target sample size	146
Recruitment status	Not started
Primary outcome(s)	Serum voriconazole concentration within the therapeutic range.
Key secondary outcomes	Therapeutic failure and adverse events. Dose-related adverse events associated with treatment
Ethics Review	Status: Approved. Date of approval: June 6th 2019 and June 18th 2019. Name and contact details of Ethics committee(s): approved by 'La Paz' University Hospital Ethics Committee and the Spanish Drugs and Health Products Agency, respectively.
IPD sharing statement	Plan to share IPD (Yes, No): Yes. Plan description: A copy of the database of data collected during the clinical trial will be attached as an appendix to the publication resulting from this clinical trial. This data will be available at the same time as the results will be published, and will be kept available to everyone without any time limit. Data will be available indefinitely on the publisher's website, as long as it is kept by the Publisher, for anyone who wishes to access the data, for non-commercial purposes.

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WORD COUNTS
Main text: 4020 words
Abstract: 297 words

ABSTRACT

Introduction: Invasive aspergillosis is the most important cause of morbidity and mortality in patients with haematological diseases. At present, voriconazole is the first-line treatment for invasive fungal disease. The pharmacokinetic interindividual variability of voriconazole depends on genetic factors. CYP450 is involved in 70%–75% of total metabolism of voriconazole, mainly CYP3A4 and CYP2C19, with the remaining 25%–30% of metabolism conducted by monooxygenase flavins. CYP2C19 single nucleotide polymorphisms could explain 50%–55% of variability in voriconazole metabolism.

Materials and Methods: The main objective is to compare efficiency of pre-emptive voriconazole genotyping with routine practice. The primary outcome is serum voriconazole on the fifth day within the therapeutic range. The secondary outcome is the combined variables of therapeutic failure and adverse events within 90 days of first administration, associated with voriconazole. A total of 146 patients at risk of invasive aspergillosis who will potentially receive voriconazole will be recruited, and CYP2C19 will be genotyped. If the patient ultimately receives voriconazole, they will be randomised (1:1 experimental/control). In the experimental arm, patients will receive a dose according to a pharmacogenetic algorithm, including CYP2C19 genotype and clinical and demographic information. In the control arm, patients will receive a dose according to clinical practice guidelines. In addition, a Spanish National Healthcare System (NHS) point-of-view cost-effectiveness evaluation will be performed. Direct cost calculations for each arm will be performed.

Conclusion: This trial will provide information about the viability and cost-effectiveness of the implementation of a pre-emptive voriconazole genotyping strategy in the Spanish NHS.

Ethics and dissemination: A Spanish version of this protocol has been evaluated and approved by the La Paz University Hospital Ethics Committee and the Spanish Agency of Medicines and Medical Devices. Trial results will be submitted for publication in an open peer-reviewed medical speciality-specific publication. Eudra-CT: 2019-000376-41. ClinicalTrials: NCT04238884

STRENGTHS AND LIMITATIONS OF THIS STUDY

(1) The study will be randomised, with objective variables and not influenced by placebo effect (voriconazole serum level).
(2) The effectiveness and safety related to serum voriconazole is well known.
(3) For patients in this study, there is little risk compared with routine clinical practice.
(4) The study is single-blinded.
(5) Our results depend on the frequency of CYP2C19 polymorphism.

INTRODUCTION:

Invasive aspergillosis is the most important cause of morbidity and mortality in patients with haematological diseases. The most recent epidemiologic studies show an incidence of probable or proven invasive aspergillosis in patients with high-risk haematological diseases of 6%–11% [1][2]. Mortality due to this complication in patients with acute leukaemia is 27% [1], and up to 40% if we include every malignant haematologic disease [3]. In recent years, great advances in the treatment of this pathology have been made, which have improved the prognosis of haematologic diseases. However, high morbidity and mortality associated with infectious complications continue to be a medical problem. As a result, improving the prognosis for invasive fungal disease has great scientific interest, with aspergillosis being the most prevalent form.

At present, voriconazole is the first-line treatment for invasive fungal disease (1-grade evidence, A-grade recommendation) [4][5]. It is a third-generation triazole antifungal with broad-spectrum activity. Achieving voriconazole serum levels in the therapeutic range during the first treatment week improves the prognosis of fungal infection and the tolerability of the treatment by reducing dose-dependent adverse effects (AEs) [6]. The AE reduction, associated with posology optimization, decreases the amount of withdrawals of this antifungal drug.

Genetic variations could become markers that help us predict the pharmacological response depending on whether they are present and on their interrelationship with other markers associated with clinical response. In recent years, several groups and organisations have published evidence assessment systems, guidelines, systematic reviews and evaluations for various drugs and pathologies; in some cases, genotyping prior to treatment with various drugs has been shown to be cost-efficient [7].

Focusing on voriconazole, its pharmacokinetic interindividual variability depends on genetic factors [8]. CYP450 is involved in 70%–75% of the total metabolism of voriconazole, mainly CYP3A4 and CYP2C19, with the remaining 25%–30% of metabolism conducted by monooxygenase flavins. CYP2C19 single nucleotide polymorphisms could explain 50%–55% of variability in voriconazole metabolism. Some 5%–17% of patients are ultrafast metabolisers (CYP2C19*17/*17), and approximately 25%–33% of patients are fast metabolisers (CYP2C19*1/*17). Both are associated with a high risk of not achieving therapeutic levels for invasive aspergillosis [9][10]. Pharmacogenetics information oriented to dose adjustment is present in European dosing guidelines and in FDA recommendations, in which recommendatons are given according to genotype [8]. This information must be known by every physician in order to reduce adverse effects, improve effectiveness and thus raise patient compliance by reducing morbidity and mortality.

We propose a pragmatic clinical trial to evaluate the effectiveness and efficiency of a pre-emptive genotyping strategy of biomarkers related to the voriconazole response in patients with haematological disease who are at risk of suffering an infection susceptible to treatment with voriconazole.

METHODS AND ANALYSIS

Study design

VORIGENIPHARM is an acronym (VORIconazole PHARMacoGENetIcs) of the clinical trial with code EUDRA-CT: 2019-000376-41, funded by the Spanish Health Research and Development Strategy. It is a phase IV pragmatic, multicentre, randomised, single-blind, parallel arm, centre-stratified clinical trial. A total of 146 patients at risk of invasive aspergillosis who will potentially receive voriconazole will be recruited, and CYP2C19 alleles will be genotyped. The patients who ultimately receive voriconazole will be randomised to receive the dose according to a pharmacogenetic algorithm, including the CYP2C19 genotype and clinical and demographic information, or according to clinical practice (Figure 1).

Study objectives

General objective:

Evaluating the effectiveness and efficiency of a pre-emptive genotyping strategy for voriconazole in treatment and prophylaxis of *Aspergillus* fungal infections in patients with haematological diseases.

Primary objective:

- Evaluate the effectiveness of a pre-emptive genotyping strategy for voriconazole in achieving adequate therapeutic levels in haematological patients at risk of fungal infection compared with routine clinical practice.

Secondary objectives:

- Evaluate the safety of pre-emptive genotyping of voriconazole in haematological patients at risk of fungal infection, comparing it with routine clinical practice.
- 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.

Eligibility

The patient will be selected from La Paz University Hospital Paediatrics Hemato-Oncology Department, La Paz University Hospital Haematology Department, La Princesa University Hospital Adult Hematology Department and Gomez-Ulla University Hospital Adult Hematology Department. They will include children and adults who meet the following inclusion criteria:

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.
2) Accepting participation in the study by signing the informed consent (adult patients), or minor patients whose representative/legal guardian has willingly signed the informed consent. In the case of mature minors (12–17 years of age), in addition to the consent signed by the legal guardian, the minor's assent shall be obtained.

The exclusion criteria are as follows:

1) Patients who for any reason should not be included in the study as assessed by the research team.
2) Patients who are not capable of understanding the information form and are unable to sign the informed consent document.

All patients eligible to receive voriconazole will sign an informed consent document for their participation in the clinical trial and for the collection of blood samples destined for genetic studies. They will also be asked for their consent to store an aliquot of their DNA for future studies (i.e., mass

sequencing). Patients identified in this phase will be randomised if and when they receive voriconazole.

Randomisation

Patients who meet all inclusion and no exclusion criteria, who have signed the informed consent and will receive voriconazole will be randomised, stratified by centres. The randomisation sequence was created using SAS version 9.4 statistical software (procedure 'PROC PLAN') with a 1:1 allocation. No randomisation seed was specified. The randomisation seed was generated taking the hour of the computer where the program was executed. Randomization will be done centrally through the electronic CRF (MACRO®) in order to conceal the sequence until interventions are assigned.

Masking

The study is single blind, and under no circumstance does the patient know the group to which they have been assigned. The medical researchers are unaware of the randomization scheme. Although there is a risk of unmasking the patient, and the lack of masking for the physician could affect the evaluation variables, we believe that in this clinical trial is permisible since the primary variable is an objective one (voriconazol concentration), and a total masking is not feasible in a study using a pragmatic approach.

Outcomes

Primary outcome

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 [11].

Secondary outcomes

- A combined variable of therapeutic failure and AEs occurring within 90 days of first voriconazole administration.
    - Therapeutic failure is defined following EORTC consensus [12]:
        - Patients with probable or proven invasive aspergillosis: drug change or association because of poor clinical or radiological evolution of the disease.

        - Patients who received prophylactic treatment: the necessity of change because of probable or proven invasive fungal disease.
    - Adverse event: Dose-dependent drug AEs, including visual disturbances (e.g., photopsia), skin reactions, neurotoxicity (e.g., confusion and visual hallucinations) and QTc lengthening, requiring voriconazole withdrawal.
- Costs by AE
- Cost savings by AE
- Quality-adjusted life years.

Study procedures

The study visits and procedures will be performed as shown in Table 1.

- Selection visit: After confirming the selection criteria, informing the patient and signing the informed consent document, a blood sample will be taken for genetic analysis. Patients who have not received voriconazole within 3 months of recruitment will not be considered for randomisation, but will be considered for economic evaluation. Patients who, in this 3-month period, receive voriconazole will continue to the randomisation visit.
- Randomisation visit: The patient will be randomised to one of the two branches of the study:
    - Experimental group: based on the genetic study performed and the patient's characteristics (age, weight, indication), the Pharmacogenetics Unit of La Paz University Hospital will indicate the dose to be administered. The dose will be based on the therapeutic individualisation protocol guided by pharmacogenetics, as agreed by all clinical services, and which includes the recommendations of the Clinical Pharmacogenetics Implementation Consortium guide (Moriyana, 2016) and the study by Hicks et al (Hicks, 2014). In the experimental group arm, in cases of patients with rapid, ultrafast or slow metaboliser phenotypes, a determination of serum voriconazole concentrations at 48 hours (+/- 24 hours) may be considered, which will make a dose adjustment possible if required.
    - Control group: No information will be provided and the procedure will be performed according to normal clinical practice, with clinical monitoring by the doctor in charge. In both cases, the request for serum voriconazole levels for subsequent dose adjustment will be recommended, as is standard clinical practice, in accordance with the recommendations of the British Society for Medical Mycology (Ashbee, 2014).

- Primary outcome evaluation visit: Voriconazole concentrations will be measured on no later than the fifth day after the start of treatment (±1 day); if a dose adjustment is necessary, this

will be performed according to the criteria of the attending clinician (control group) or the Pharmacogenetic Unit (experimental group); subsequent monitoring (determination of concentrations and dose adjustment) will be performed according to the criteria of the attending clinician in both arms.

- Follow-up visits: While the patient is being treated with voriconazole, follow-up visits will be made according to clinical criteria, and the following information will be recorded:
    - Evaluation of potential voriconazole-related clinical AEs (see evaluation variables). This will be performed by asking the patient questions, performing a physical examination, routine tests (including liver enzymes) and an electrocardiogram.
    - Voriconazole levels, if requested according to medical criteria.
    - The type of consultation will be recorded: outpatient appointment, urgent episode, admission, readmission or phone call.
- End-of-treatment visit: Performed on the last day the patient receives treatment with voriconazole, including the following procedures:
    - A reason for the end of treatment will be indicated (withdrawal due to toxicity or therapeutic failure, or after completion of treatment according to clinical criteria of efficacy)
    - Evaluation of potentially voriconazole-related clinical adverse events, as in follow-up visits.
    - If the patient continues voriconazole treatment beyond 3 months, the end of study visit will be made at that time.
- End-of-study visit: the end-of-study visit will coincide with the end-of-treatment visit, except for those patients who are still undergoing treatment after 3 months. A safety analysis, electrocardiogram, and assessment of adverse events will be performed. Patients who continue treatment beyond this visit will be followed according to routine clinical practice.

Voriconazole genotyping and therapeutic drug monitoring

Genotyping will be performed at the Institute of Medical and Molecular Genetics at La Paz University Hospital using the self-designed SNP-array device (PharmArray v.2.), which makes genotyping of 180 relevant mutations possible for predicting the response to drugs. Among these mutations are the most relevant variants of CYP2C19, a gene that is related to serum levels of voriconazole, with a level of evidence 1A of CPIC: CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893) and CYP2C19*17 (rs12248560).

Serum voriconazole determinations will be tested at the Therapeutic Drug Monitoring Laboratory of the Clinical Pharmacology Service at La Paz University Hospital using an enzyme immunosensing technique on the Abbott ARCHITECT c4000 autoanalyser.

Data collection and outcome measures

A electronic case report form has been designed using MACRO Electronic Data Capture by Elsevier. This system will anonymise patients, and the data will be transferred to a '*.csv' file in order to analyse it with R software (3.5.2 version or newer). To ensure the quality of the data, Data management will be performed by the Spanish Clinical Research Network (SCReN). Data management plan have been approved by the principal investigator and the sponsor. Data collection forms will be included in the final report.

Sample size calculation

In routine clinical practice, 43% of patients have voriconazole serum levels in the therapeutic range on the fifth day of treatment. We expect this to improve to 68%, based on the Hicks article [9]. With a two-tailed type I error of 0.05 and 80% power, it is necessary to recruit 62 patients to receive treatment in both arms. 85 out of every 100 patients recruited are estimated to will receive voriconazole, we will need a total of 146 patients from whom we obtain informed consent. This calculation has been performed with the 'power.prop.test' package, from R version 3.2.0. The necessary time to recruit the whole group is estimated to be 2 years. We estimate 20% of the patients will be selected from the La Princesa University Hospital Adult Haematology Department, and 20% from the Gomez-Ulla University Hospital Adult Haematology Department. The rest of group will be recruited from La Paz University Hospital. Since it is a phase IV clinical trial including patients that are usually followed in the hospital, with pragmatic selection criteria, it is not necessary to plan any strategy for achieving adequate participant enrolment.

Statistical analysis

Frequency results will be expressed in absolute terms, as percentages and confidence intervals. Continuous variables will be expressed as mean (SD) and median (range) according to Kolmogorov Smirnov's test of normality.

For the main dichotomous variable (level of voriconazole within the therapeutic range) a generalised logistic model will be used. After that, "elastic net" techniques will be employed with the purpose of selecting variables so that they are not as rigid as in Lasso's case. Variables with some degree of correlation between themselves will be accepted in the model, as in Ridge regression, aiming at the best possible predictive model. Finally, more advanced methods of statistical learning will be used to give the variables consistency by the method described above. Predictive ability will be calculated

using receiver operating characteristic curves both at a specific time and over the course of the patient's follow-up.

A survival analysis will be performed for the secondary variables to test the Cox proportional hazards model and the Weibull model, analysing the time until the first event, using Kaplan Meier curves. A penalised Cox regression will be fitted to determine whether there are differences from the start of the target drug to the appearance of the event (secondary variable) between the control group and the experimental group, and finally, if possible the Weibull model will be used. A cross-validation approach will be used, dividing the sample into k subgroups. Later, a subgroup analysis will be performed taking into consideration the recruiting centre, calculating the *a posteriori* analysis power of every objective, given that this can be a source of hypothesis generation for future research lines.

The analysis of the primary variable of efficacy will be made on an intention-to-treat (ITT) basis. It will include all the patients who are randomized, whether they have received the study treatment or not. The safety analyses will be held in the safety population, which includes patients who receive at least one dose of the drug during the study. No interim analysis or stopping rules will be carry out in this clinical trial.

A bilateral significance level of 0.05 and bilateral 95% confidence intervals will be assumed. The statistical software R (R Core Team [2014]) will be used. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Economic evaluation

A Spanish National Healthcare Service (NHS) point-of-view cost-effectiveness evaluation will be performed. Direct cost calculations for each arm will be performed.

Direct costs will be calculated for both alternatives by registering healthcare resources used during the follow-up and the unit cost per patient, as well as the costs incurred by the centres to implement the intervention.

The use of resources and their unit costs, such as doctor's visits, medications and any interventions performed on those patients in the hospital during follow-up shall be recorded. Costs for each patient for each intervention group will be totalled. This calculation will allow us to compare the average cost per event avoided between the intervention group and the control group. The results will be expressed in cost per event and avoided events. As far as possible, the utility of each intervention should be calculated.

A univariate and a probabilistic sensitivity analysis will be performed to assess the uncertainty about the model and the unit costs, if they are obtained from a source other than the clinical centres.

Data monitoring

Coordination, management, monitoring, data management and statistical analysis of the study will be performed by the Spanish Clinical Research Network (SCReN).

Auditing

During the progress of the study, audit visits can be conducted at the participating centres. The investigator will allow direct access to the source data/documents for monitoring, auditing, review by the ethical research committee and inspection by the Health Authorities

Conflicts of Interest

The Funders and Sponsor not interfere in the selection processes of the patients, analysis of the data and/or publication of the results, or any other process that might come into play with the results of the study. Funding will be independent of the results of the study. The Principal Investigator has ultimate authority over any of these activities.

Patient and Public Involvement

The development of the research question and outcome measures were based on the oncologists' experience treating this profile of patients the desire to optimize voriconazol treatment. Patients and patient advisers were not involved in the design, recruitment or conduct of this study. The patients or their families will be notified of the study results in writing and verbally, and we will invite them to help us develop our dissemination strategy.

Access to data.

This section is included in the data sharing plan.

Disemination policy

Outputs from this study will include journal publications, conference presentations and community reporting. Outputs will not identify participants.

Ethical considerations

The researchers will adhere strictly to the provisions of this protocol and will complete the case report forms. The study will be performed according to the recommendations for clinical studies and the evaluation of drugs in humans, as contained in the Declaration of Helsinki (revised in successive world assemblies) and in the current Spanish and European legislation on clinical studies and patient

data confidentiality. The study will follow the principles of Good Clinical Practice. This study has been approved by the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain) and by the Spanish Agency of Medication and Health Products, and has been registered in Eudra CT (Eudra CT: 2019-000376-41) and ClinicalTrials.gov (NCT04238884).

This clinical trial has been classified by the Spanish Agency for Medicines and Healthcare Products as a "low-intervention clinical trial". The additional diagnostic or monitoring procedures do not pose more than minimal additional risk or burden to the safety of the subjects compared to normal clinical.

No additional use of the system for compensation shall be required from the sponsor for low-intervention clinical trials. If any possible damage that could be suffered by a subject, resulting from the use of the investigational medicinal product in accordance with the protocol of that specific clinical trial, it is covered by the applicable compensation system already in place.

All protocol amendments will be evaluated by the Ethics Committee and the Spanish Agency of Medication and Health Products, following the principles of Good Clinical Practice and national legislation.

DISCUSSION

Some important barriers have been detected to implementation of pharmacogenetics in usual clinical practice. The reasons detected in the literature include the difficulty of obtaining high-level evidence on genetic markers' efficacy, effectivity and efficiency, and a lack of consensus. Another problem is the absence of pharmacogenetic techniques' formation and its interpretation. We also have to consider the financial, logistic and legal limitations. Finally, there is no specific implementation strategy in clinical practice. There have been two proposed models for the implementation of pharmacogenetics biomarkers in usual clinical practice:

- Case-to-case model: The decision is made individually and is based on the need for using a drug whose effectiveness or safety is modified by specific genetics variations. The limitations are the costs and the latency between sample extraction and the results obtained.
- Pre-emptive genotyping model: This alternative assesses the patient's genetic information at the very beginning, even before starting treatment. Currently, the development of arrays permits detection of a significant number of mutations in which therapeutic impact is probable or definite. Other authors have developed a similar strategy [11][12], for instance St. Jude's Hospital [13], as well as our group [14]. Rasmussen-Torvik's group has proposed the eMERGE-PGx project, in which pre-emptive pharmacogenotyping is integrated with clinical history [15].

In the case of voriconazole, several studies have linked CYP2C19 polymorphism to the response. In addition, some studies have shown that genotyping CYP2C19 is effective. The cost-efficiency of pre-treatment genotyping in some drugs has been already demonstrated in some articles [7]; however, there is no evidence of voriconazole efficiency specifically in the Spanish NHS, or even whether voriconazole-related cytochrome genotyping is efficient in other countries [16].

This pragmatic clinical trial is proposed by our group to evaluate effectiveness and efficiency of pre-emptive biomarker genotyping and its interpretation by a Pharmacogenetic Unit for patients with haematological diseases and a risk of fungal infection, who are susceptible to receiving voriconazole treatment. If our hypothesis is proven, this strategy will help us improve the prevention and treatment of this infectious complication. This trial will provide information about the viability of the translation of this strategy to routine clinical practice. In addition, our pre-emptive pharmacogenetic strategy, combined with therapeutic drug monitoring, has the potential to improve efficacy and safety, with a high level of evidence [6], lowering the likelihood of AE occurrences.

Author contributions

AMB, AJC and JFI conceived the study. JMV, IG, FAS, CA, GM, ER, JFI, AJC and AMB designed the study. DB, RC, ME, AL and MJO are responsible of the data collecting and management. JMV and IG drafted this manuscript. AMB, AJC and JFI critically revised the manuscript. JMV and IG contributed equally. The final version of the manuscript was reviewed and approved by all authors.

Competing interests

None declared.

Funding statement

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VISIT	Visit of selection	Visit of randomisation	Primary outcome evaluation (visit)	Follow-up visit	End-of-treatment visit	End-of-study visit
Day of the study	**Day 0**	**Maximum 3 months after selection**	**5th day of treatment (+/- 1 day)**	**Weekly until end of treatment**	**Last day of treatment**	**2 weeks later after ending treatment(1)**
Selection criteria meeting	X					
Informed Consent Signing	X					
Genetic sample	X					
Randomisation		X				
Starting treatment		X				
Voriconazole serum levels			X	X (2)		
Lab analysis (3)		X	X	X	X	X
Electrocardigram		X	X	X	X	X
AE(4) evaluation		X	X	X	X	X

(1) Patients who after 3 months continue being treated, End-of-study visit will be done on the last day of third month.
(2) According to physician criteria
(3) Analysis including liver enzimes.
(4) AE (Adverse Events)

Table1. Study visits planning and study procedures.

Figure legend

Figure 1. Study Flowchart.
Proposed reporting of the flow of trial participants.

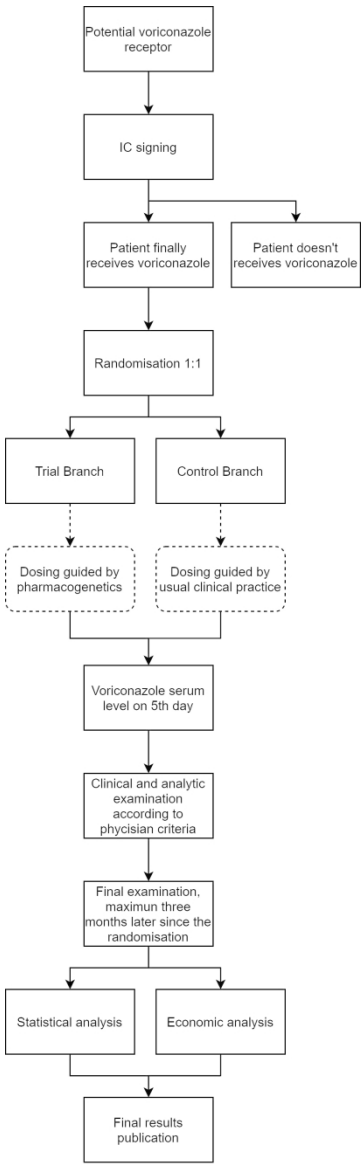

Figure 1. Study Flowchart

Figure 1. Study flowchart

APPENDIX 1: Trial Investigation Group

- Trial coordination: Alberto M. Borobia, MD, PhD; Antonio J. Carcas, MD, PhD.
- Clinical Researches:
  - La Paz University Hospital:
    - Clinical Pharmacology Department: Jaime Monserrat-Villatoro, MD; Irene Garcia, MD; Elena Ramírez, MD, PhD; Jesús Frías Iniesta, MD, PhD.
    - Pediatric Onco-Haematology Department: David Bueno, MD, PhD.
    - Haematology Department: Ana Lopez, MD, PhD; Teresa de Soto, MD
  - La Princesa University Hospital:
    - Haematology Department: Rafael de la Camara, MD, PD.
    - Clinical Pharmacology Department: Francisco Abad Santos, MD, PhD; Gina Mejia, MD, PhD.
  - Gómez Ulla Hospital:
    - Infectious Diseases. Internal Medicine Department: Miriam Estébanez, MD, PhD.
    - Haematology Department: María José Otero, MD, PhD.
- Pharmacoeconomic analyses:
  - Francisco de Vitoria University - Health Technologies Assessment Department: Cristina Antón, MD, PhD.
- Study monitoring: Foundation for Biomedical Research, University Hospital La Paz: Central Unit for Clinical Research and Clinical Trials. SCReN.

SPIRIT checklist.

Included	Section/item	Item No	Description	Pg.
Administrative information				
☑	Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
☑	Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
☑		2b	All items from the World Health Organization Trial Registration Data Set	1-2
☑	Protocol version	3	Date and version identifier	1
☑	Funding	4	Sources and types of financial, material, and other support	14
☑	Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	16, 21
☑		5b	Name and contact information for the trial sponsor	1
☑		5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
☑		5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction				
☑	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6, 7
☑		6b	Explanation for choice of comparators	6, 7
☑	Objectives	7	Specific objectives or hypotheses	8

☑	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes				**Pg.**
☑	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8, 12
☑	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
☑	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10, 11
☑		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11
☑		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11, 12
☑		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7, 12, 16
☑	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9, 10
☑	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10,11,20

☑	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
☑	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	12
Methods: Assignment of interventions (for controlled trials)				
	Allocation:			
☑	Sequence generation	16a	Method of generating the allocation sequence (eg, computer- generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
☑	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
☑	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9, 10, 11, 21
☑	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
☑		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9

Methods: Data collection, management, and analysis				
☑	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12

☑		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	12
☑	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
☑	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12,13
☑		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12,13
☑		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	12,13

Methods: Monitoring				
☑	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14
☑		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12, 13
☑	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
☑	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14

Ethics and dissemination	

☑	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15
☑	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	15
☑	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
☑		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9
☑	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
☑	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
☑	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	14
☑	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
☑	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2,14
☑		31b	Authorship eligibility guidelines and any intended use of professional writers	16
☑		31c	Plans, if any, for granting public access to the full protocol, participant- level dataset, and statistical code	2, 14

Appendices	

☑	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	*
☑	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	9, *

COMMENTS:

* Some parts of the documentation will be attached to the submission, such as the model consent form and other related documentation.

BMJ Open

RANDOMISED MULTICENTRE CLINICAL TRIAL TO EVALUATE VORICONAZOLE PRE-EMPTIVE GENOTYPING STRATEGY IN PATIENTS WITH RISK OF ASPERGILLOSIS: VORIGENIPHARM STUDY PROTOCOL

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Primary Subject Heading:	Genetics and genomics
Secondary Subject Heading:	Infectious diseases
Keywords:	CLINICAL PHARMACOLOGY, INFECTIOUS DISEASES, Clinical trials < THERAPEUTICS

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TITLE: RANDOMISED MULTICENTRE CLINICAL TRIAL TO EVALUATE VORICONAZOLE PRE-EMPTIVE GENOTYPING STRATEGY IN PATIENTS WITH RISK OF ASPERGILLOSIS: VORIGENIPHARM STUDY PROTOCOL

Data category	Information
Primary registry and trial identification number	EU Clinical Trials Register https://www.clinicaltrialsregister.eu/ctr-search/search 2019-000376-41 ClinicalTrials.Gov https://clinicaltrials.gov/ct2/show/NCT04238884 NCT04238884
Date of registration in primary registry	18 June 2019
Source(s) of monetary or material support	Institute of Health Carlos III, grants for Health Research Project 2018 of Spain
Primary sponsor	La Paz University Hospital Research Foundation (FIBHULP)
Contact for public queries	Alberto M. Borobia Perez, MD, PhD, La Paz University Hospital, Clinical Pharmacology Department, +34917277558, alberto.borobia@salud.madrid.org
Contact for scientific queries	Alberto M. Borobia Perez, MD, PhD, La Paz University Hospital, Clinical Pharmacology Department, +34917277558, alberto.borobia@salud.madrid.org
Public title	Clinical trial to evaluate the effectiveness and efficiency of pre-emptive genotyping before treatment with voriconazole
Scientific title	Randomised multicentre clinical trial to evaluate voriconazole pre-emptive genotyping strategy in patients with risk of aspergillosis: vorigenipharm study protocol
Countries of recruitment	Spain
Health condition(s) or problem(s) studied	Fungal infectious disorders, neutropaenia, chemotherapy-induced neutropaenia, haematopoietic neoplasm, allogeneic peripheral haematopoietic stem cell transplant, autologous peripheral haematopoietic stem cell transplant
Intervention(s)	Pre-emptive genotyping plus post-serum levels vs sole post-serum level adjustment of voriconazole

Key inclusion and exclusion criteria	Ages eligible for study: no limit Sexes eligible for study: both Accepts healthy volunteers: no Inclusion criteria: children who are going to receive an HSCT and those who have diagnosis of acute myeloid leukaemia as well as its relapse. Adults diagnosed with acute leukaemia, with expected prolonged neutropaenia, and those with risk of developing a fungal infection. Exclusion criteria: patients who for any reason should not be included in the study according to the criteria of the research team.
Study type	Interventional Allocation: randomised Intervention model: parallel assignment Masking: single-blind (subject) Primary purpose: prevention Phase IV
Date of first enrolment	January 2020
Target sample size	146
Recruitment status	Not started
Primary outcome(s)	Serum voriconazole concentration within the therapeutic range.
Key secondary outcomes	Therapeutic failure and adverse events. Dose-related adverse events associated with treatment
Ethics Review	Status: Approved. Date of approval: June 6th 2019 and June 18th 2019. Name and contact details of Ethics committee(s): approved by 'La Paz' University Hospital Ethics Committee and the Spanish Drugs and Health Products Agency, respectively.
IPD sharing statement	Plan to share IPD (Yes, No): Yes. Plan description: A copy of the database of data collected during the clinical trial will be attached as an appendix to the publication resulting from this clinical trial. This data will be available at the same time as the results will be published, and will be kept available to everyone without any time limit. Data will be available indefinitely on the publisher's website, as long as it is kept by the Publisher, for anyone who wishes to access the data, for non-commercial purposes.

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ABSTRACT

Introduction: Invasive aspergillosis is the most important cause of morbidity and mortality in patients with haematological diseases. At present, voriconazole is the first-line treatment for invasive fungal disease. The pharmacokinetic interindividual variability of voriconazole depends on genetic factors. CYP450 is involved in 70%–75% of total metabolism of voriconazole, mainly CYP3A4 and CYP2C19, with the remaining 25%–30% of metabolism conducted by monooxygenase flavins. CYP2C19 single nucleotide polymorphisms could explain 50%–55% of variability in voriconazole metabolism.

Materials and Methods: The main objective is to compare efficiency of pre-emptive voriconazole genotyping with routine practice. The primary outcome is serum voriconazole on the fifth day within the therapeutic range. The secondary outcome is the combined variables of therapeutic failure and adverse events within 90 days of first administration, associated with voriconazole. A total of 146 patients at risk of invasive aspergillosis who will potentially receive voriconazole will be recruited, and CYP2C19 will be genotyped. If the patient ultimately receives voriconazole, they will be randomised (1:1 experimental/control). In the experimental arm, patients will receive a dose according to a pharmacogenetic algorithm, including CYP2C19 genotype and clinical and demographic information. In the control arm, patients will receive a dose according to clinical practice guidelines. In addition, a Spanish National Healthcare System (NHS) point-of-view cost-effectiveness evaluation will be performed. Direct cost calculations for each arm will be performed.

Conclusion: This trial will provide information about the viability and cost-effectiveness of the implementation of a pre-emptive voriconazole genotyping strategy in the Spanish NHS.

Ethics and dissemination: A Spanish version of this protocol has been evaluated and approved by the La Paz University Hospital Ethics Committee and the Spanish Agency of Medicines and Medical Devices. Trial results will be submitted for publication in an open peer-reviewed medical speciality-specific publication. Eudra-CT: 2019-000376-41. ClinicalTrials: NCT04238884

STRENGTHS AND LIMITATIONS OF THIS STUDY

(1) The study will be randomised, with objective variables and not influenced by placebo effect (voriconazole serum level).
(2) The effectiveness and safety related to serum voriconazole is well known.
(3) For patients in this study, there is little risk compared with routine clinical practice.
(4) The study is single-blinded.
(5) Our results depend on the frequency of CYP2C19 polymorphism.

INTRODUCTION:

Invasive aspergillosis is the most important cause of morbidity and mortality in patients with haematological diseases. The most recent epidemiologic studies show an incidence of probable or proven invasive aspergillosis in patients with high-risk haematological diseases of 6%–11% [1][2]. Mortality due to this complication in patients with acute leukaemia is 27% [1], and up to 40% if we include every malignant haematologic disease [3]. In recent years, great advances in the treatment of this pathology have been made, which have improved the prognosis of haematologic diseases. However, high morbidity and mortality associated with infectious complications continue to be a medical problem. As a result, improving the prognosis for invasive fungal disease has great scientific interest, with aspergillosis being the most prevalent form.

At present, voriconazole is the first-line treatment for invasive fungal disease (1-grade evidence, A-grade recommendation) [4][5]. It is a third-generation triazole antifungal with broad-spectrum activity. Achieving voriconazole serum levels in the therapeutic range during the first treatment week improves the prognosis of fungal infection and the tolerability of the treatment by reducing dose-dependent adverse effects (AEs) [6]. The AE reduction, associated with posology optimization, decreases the amount of withdrawals of this antifungal drug.

Genetic variations could become markers that help us predict the pharmacological response depending on whether they are present and on their interrelationship with other markers associated with clinical response. In recent years, several groups and organisations have published evidence assessment systems, guidelines, systematic reviews and evaluations for various drugs and pathologies; in some cases, genotyping prior to treatment with various drugs has been shown to be cost-efficient [7].

Focusing on voriconazole, its pharmacokinetic interindividual variability depends on genetic factors [8]. CYP450 is involved in 70%–75% of the total metabolism of voriconazole, mainly CYP3A4 and CYP2C19, with the remaining 25%–30% of metabolism conducted by monooxygenase flavins. CYP2C19 single nucleotide polymorphisms could explain 50%–55% of variability in voriconazole metabolism. Some 5%–17% of patients are ultrafast metabolisers (CYP2C19*17/*17), and approximately 25%–33% of patients are fast metabolisers (CYP2C19*1/*17). Both are associated with a high risk of not achieving therapeutic levels for invasive aspergillosis [9][10]. Pharmacogenetics information oriented to dose adjustment is present in European dosing guidelines and in FDA recommendations, in which recommendatons are given according to genotype [8]. This information must be known by every physician in order to reduce adverse effects, improve effectiveness and thus raise patient compliance by reducing morbidity and mortality.

We propose a pragmatic clinical trial to evaluate the effectiveness and efficiency of a pre-emptive genotyping strategy of biomarkers related to the voriconazole response in patients with haematological disease who are at risk of suffering an infection susceptible to treatment with voriconazole.

METHODS AND ANALYSIS

Study design

VORIGENIPHARM is an acronym (VORIconazole PHARMacoGENetIcs) of the clinical trial with code EUDRA-CT: 2019-000376-41, funded by the Spanish Health Research and Development Strategy. It is a phase IV pragmatic, multicentre, randomised, single-blind, parallel arm, centre-stratified clinical trial. A total of 146 patients at risk of invasive aspergillosis who will potentially receive voriconazole will be recruited, and CYP2C19 alleles will be genotyped. The patients who ultimately receive voriconazole will be randomised to receive the dose according to a pharmacogenetic algorithm, including the CYP2C19 genotype and clinical and demographic information, or according to clinical practice (Figure 1).

Study objectives

General objective:

Evaluating the effectiveness and efficiency of a pre-emptive genotyping strategy for voriconazole in treatment and prophylaxis of *Aspergillus* fungal infections in patients with haematological diseases.

Primary objective:

- Evaluate the effectiveness of a pre-emptive genotyping strategy for voriconazole in achieving adequate therapeutic levels in haematological patients at risk of fungal infection compared with routine clinical practice.

Secondary objectives:

- Evaluate the safety of pre-emptive genotyping of voriconazole in haematological patients at risk of fungal infection, comparing it with routine clinical practice.
- 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.

Eligibility

The patient will be selected from La Paz University Hospital Paediatrics Hemato-Oncology Department, La Paz University Hospital Haematology Department, La Princesa University Hospital Adult Hematology Department and Gomez-Ulla University Hospital Adult Hematology Department. They will include children and adults who meet the following inclusion criteria:

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.
2) Accepting participation in the study by signing the informed consent (adult patients), or minor patients whose representative/legal guardian has willingly signed the informed consent. In the case of mature minors (12–17 years of age), in addition to the consent signed by the legal guardian, the minor's assent shall be obtained.

The exclusion criteria are as follows:

1) Patients who for any reason should not be included in the study as assessed by the research team.
2) Patients who are not capable of understanding the information form and are unable to sign the informed consent document.

All patients eligible to receive voriconazole will sign an informed consent document for their participation in the clinical trial and for the collection of blood samples destined for genetic studies. They will also be asked for their consent to store an aliquot of their DNA for future studies (i.e., mass

sequencing). Patients identified in this phase will be randomised if and when they receive voriconazole.

Randomisation

Patients who meet all inclusion and no exclusion criteria, who have signed the informed consent and will receive voriconazole will be randomised, stratified by centres. The randomisation sequence was created using SAS version 9.4 statistical software (procedure 'PROC PLAN') with a 1:1 allocation. No randomisation seed was specified. The randomisation seed was generated taking the hour of the computer where the program was executed. Randomization will be done centrally through the electronic CRF (MACRO®) in order to conceal the sequence until interventions are assigned.

Masking

The study is single blind, and under no circumstance does the patient know the group to which they have been assigned. The medical researchers are unaware of the randomization scheme. Although there is a risk of unmasking the patient, and the lack of masking for the physician could affect the evaluation variables, we believe that in this clinical trial is permisible since the primary variable is an objective one (voriconazol concentration), and a total masking is not feasible in a study using a pragmatic approach.

Outcomes

Primary outcome

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 [11].

Secondary outcomes

- A combined variable of therapeutic failure and AEs occurring within 90 days of first voriconazole administration.
    - Therapeutic failure is defined following EORTC consensus [12]:
        - Patients with probable or proven invasive aspergillosis: drug change or association because of poor clinical or radiological evolution of the disease.

        - Patients who received prophylactic treatment: the necessity of change because of probable or proven invasive fungal disease.
    - Adverse event: Dose-dependent drug AEs, including visual disturbances (e.g., photopsia), skin reactions, neurotoxicity (e.g., confusion and visual hallucinations) and QTc lengthening, requiring voriconazole withdrawal.
- Costs by AE
- Cost savings by AE
- Quality-adjusted life years.

Study procedures

The study visits and procedures will be performed as shown in Table 1.

- Selection visit: After confirming the selection criteria, informing the patient and signing the informed consent document, a blood sample will be taken for genetic analysis. Patients who have not received voriconazole within 3 months of recruitment will not be considered for randomisation, but will be considered for economic evaluation. Patients who, in this 3-month period, receive voriconazole will continue to the randomisation visit.
- Randomisation visit: The patient will be randomised to one of the two branches of the study:
    - Experimental group: based on the genetic study performed and the patient's characteristics (age, weight, indication), the Pharmacogenetics Unit of La Paz University Hospital will indicate the dose to be administered. The dose will be based on the therapeutic individualisation protocol guided by pharmacogenetics, as agreed by all clinical services, and which includes the recommendations of the Clinical Pharmacogenetics Implementation Consortium guide (Moriyana, 2016) and the study by Hicks et al (Hicks, 2014). In the experimental group arm, in cases of patients with rapid, ultrafast or slow metaboliser phenotypes, a determination of serum voriconazole concentrations at 48 hours (+/- 24 hours) may be considered, which will make a dose adjustment possible if required.
    - Control group: No information will be provided and the procedure will be performed according to normal clinical practice, with clinical monitoring by the doctor in charge. In both cases, the request for serum voriconazole levels for subsequent dose adjustment will be recommended, as is standard clinical practice, in accordance with the recommendations of the British Society for Medical Mycology (Ashbee, 2014).

- Primary outcome evaluation visit: Voriconazole concentrations will be measured on no later than the fifth day after the start of treatment (±1 day); if a dose adjustment is necessary, this

will be performed according to the criteria of the attending clinician (control group) or the Pharmacogenetic Unit (experimental group); subsequent monitoring (determination of concentrations and dose adjustment) will be performed according to the criteria of the attending clinician in both arms.

- Follow-up visits: While the patient is being treated with voriconazole, follow-up visits will be made according to clinical criteria, and the following information will be recorded:
    - Evaluation of potential voriconazole-related clinical AEs (see evaluation variables). This will be performed by asking the patient questions, performing a physical examination, routine tests (including liver enzymes) and an electrocardiogram.
    - Voriconazole levels, if requested according to medical criteria.
    - The type of consultation will be recorded: outpatient appointment, urgent episode, admission, readmission or phone call.
- End-of-treatment visit: Performed on the last day the patient receives treatment with voriconazole, including the following procedures:
    - A reason for the end of treatment will be indicated (withdrawal due to toxicity or therapeutic failure, or after completion of treatment according to clinical criteria of efficacy)
    - Evaluation of potentially voriconazole-related clinical adverse events, as in follow-up visits.
    - If the patient continues voriconazole treatment beyond 3 months, the end of study visit will be made at that time.
- End-of-study visit: the end-of-study visit will coincide with the end-of-treatment visit, except for those patients who are still undergoing treatment after 3 months. A safety analysis, electrocardiogram, and assessment of adverse events will be performed. Patients who continue treatment beyond this visit will be followed according to routine clinical practice.

Voriconazole genotyping and therapeutic drug monitoring

Genotyping will be performed at the Institute of Medical and Molecular Genetics at La Paz University Hospital using the self-designed SNP-array device (PharmArray v.2.), which makes genotyping of 180 relevant mutations possible for predicting the response to drugs. Among these mutations are the most relevant variants of CYP2C19, a gene that is related to serum levels of voriconazole, with a level of evidence 1A of CPIC: CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893) and CYP2C19*17 (rs12248560).

Serum voriconazole determinations will be tested at the Therapeutic Drug Monitoring Laboratory of the Clinical Pharmacology Service at La Paz University Hospital using an enzyme immunosensing technique on the Abbott ARCHITECT c4000 autoanalyser.

Data collection and outcome measures

A electronic case report form (eCRF) has been designed using MACRO Electronic Data Capture by Elsevier. This eCRF will included all the variables described and also concomitant medication and other clinical and demographic condition that could interfere with voriconazol This system will anonymise patients, and the data will be transferred to a '*.csv' file in order to analyse it with R software (3.5.2 version or newer). To ensure the quality of the data, Data management will be performed by the Spanish Clinical Research Network (SCReN). Data management plan have been approved by the principal investigator and the sponsor. Data collection forms will be included in the final report.

Sample size calculation

In routine clinical practice, 43% of patients have voriconazole serum levels in the therapeutic range on the fifth day of treatment. We expect this to improve to 68%, based on the Hicks article [9]. With a two-tailed type I error of 0.05 and 80% power, it is necessary to recruit 62 patients to receive treatment in both arms. 85 out of every 100 patients recruited are estimated to will receive voriconazole, we will need a total of 146 patients from whom we obtain informed consent. This calculation has been performed with the 'power.prop.test' package, from R version 3.2.0. The necessary time to recruit the whole group is estimated to be 2 years. We estimate 20% of the patients will be selected from the La Princesa University Hospital Adult Haematology Department, and 20% from the Gomez-Ulla University Hospital Adult Haematology Department. The rest of group will be recruited from La Paz University Hospital. Since it is a phase IV clinical trial including patients that are usually followed in the hospital, with pragmatic selection criteria, it is not necessary to plan any strategy for achieving adequate participant enrolment.

Statistical analysis

Frequency results will be expressed in absolute terms, as percentages and confidence intervals. Continuous variables will be expressed as mean (SD) and median (range) according to Kolmogorov Smirnov's test of normality.

For the main dichotomous variable (level of voriconazole within the therapeutic range) a generalised logistic model will be used. After that, "elastic net" techniques will be employed with the purpose of selecting variables so that they are not as rigid as in Lasso's case. Variables with some degree of correlation between themselves will be accepted in the model, as in Ridge regression, aiming at the

best possible predictive model. Finally, more advanced methods of statistical learning will be used to give the variables consistency by the method described above. Predictive ability will be calculated using receiver operating characteristic curves both at a specific time and over the course of the patient's follow-up.

A survival analysis will be performed for the secondary variables to test the Cox proportional hazards model and the Weibull model, analysing the time until the first event, using Kaplan Meier curves. A penalised Cox regression will be fitted to determine whether there are differences from the start of the target drug to the appearance of the event (secondary variable) between the control group and the experimental group, and finally, if possible the Weibull model will be used. A cross-validation approach will be used, dividing the sample into k subgroups. Later, a subgroup analysis will be performed taking into consideration the recruiting centre, calculating the *a posteriori* analysis power of every objective, given that this can be a source of hypothesis generation for future research lines.

The analysis of the primary variable of efficacy will be made on an intention-to-treat (ITT) basis. It will include all the patients who are randomized, whether they have received the study treatment or not. The safety analyses will be held in the safety population, which includes patients who receive at least one dose of the drug during the study. No interim analysis or stopping rules will be carry out in this clinical trial.

A bilateral significance level of 0.05 and bilateral 95% confidence intervals will be assumed. The statistical software R (R Core Team [2014]) will be used. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Economic evaluation

A Spanish National Healthcare Service (NHS) point-of-view cost-effectiveness evaluation will be performed. Direct cost calculations for each arm will be performed.

Direct costs will be calculated for both alternatives by registering healthcare resources used during the follow-up and the unit cost per patient, as well as the costs incurred by the centres to implement the intervention.

The use of resources and their unit costs, such as doctor's visits, medications and any interventions performed on those patients in the hospital during follow-up shall be recorded. Costs for each patient for each intervention group will be totalled. This calculation will allow us to compare the average cost per event avoided between the intervention group and the control group. The results will be expressed in cost per event and avoided events. As far as possible, the utility of each intervention should be calculated.

A univariate and a probabilistic sensitivity analysis will be performed to assess the uncertainty about the model and the unit costs, if they are obtained from a source other than the clinical centres.

Data monitoring

Coordination, management, monitoring, data management and statistical analysis of the study will be performed by the Spanish Clinical Research Network (SCReN).

Auditing

During the progress of the study, audit visits can be conducted at the participating centres. The investigator will allow direct access to the source data/documents for monitoring, auditing, review by the ethical research committee and inspection by the Health Authorities

Conflicts of Interest

The Funders and Sponsor not interfere in the selection processes of the patients, analysis of the data and/or publication of the results, or any other process that might come into play with the results of the study. Funding will be independent of the results of the study. The Principal Investigator has ultimate authority over any of these activities.

Patient and Public Involvement

The development of the research question and outcome measures were based on the oncologists' experience treating this profile of patients the desire to optimize voriconazol treatment. Patients and patient advisers were not involved in the design, recruitment or conduct of this study. The patients or their families will be notified of the study results in writing and verbally, and we will invite them to help us develop our dissemination strategy.

Access to data.

This section is included in the data sharing plan.

Disemination policy

Outputs from this study will include journal publications, conference presentations and community reporting. Outputs will not identify participants.

Ethical considerations

The researchers will adhere strictly to the provisions of this protocol and will complete the case report forms. The study will be performed according to the recommendations for clinical studies and the

evaluation of drugs in humans, as contained in the Declaration of Helsinki (revised in successive world assemblies) and in the current Spanish and European legislation on clinical studies and patient data confidentiality. The study will follow the principles of Good Clinical Practice. This study has been approved by the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain) and by the Spanish Agency of Medication and Health Products, and has been registered in Eudra CT (Eudra CT: 2019-000376-41) and ClinicalTrials.gov (NCT04238884).

This clinical trial has been classified by the Spanish Agency for Medicines and Healthcare Products as a "low-intervention clinical trial". The additional diagnostic or monitoring procedures do not pose more than minimal additional risk or burden to the safety of the subjects compared to normal clinical.

No additional use of the system for compensation shall be required from the sponsor for low-intervention clinical trials. If any possible damage that could be suffered by a subject, resulting from the use of the investigational medicinal product in accordance with the protocol of that specific clinical trial, it is covered by the applicable compensation system already in place.

All protocol amendments will be evaluated by the Ethics Committee and the Spanish Agency of Medication and Health Products, following the principles of Good Clinical Practice and national legislation.

DISCUSSION

Some important barriers have been detected to implementation of pharmacogenetics in usual clinical practice. The reasons detected in the literature include the difficulty of obtaining high-level evidence on genetic markers' efficacy, effectivity and efficiency, and a lack of consensus. Another problem is the absence of pharmacogenetic techniques' formation and its interpretation. We also have to consider the financial, logistic and legal limitations. Finally, there is no specific implementation strategy in clinical practice. There have been two proposed models for the implementation of pharmacogenetics biomarkers in usual clinical practice:

- Case-to-case model: The decision is made individually and is based on the need for using a drug whose effectiveness or safety is modified by specific genetics variations. The limitations are the costs and the latency between sample extraction and the results obtained.
- Pre-emptive genotyping model: This alternative assesses the patient's genetic information at the very beginning, even before starting treatment. Currently, the development of arrays permits detection of a significant number of mutations in which therapeutic impact is probable or definite. Other authors have developed a similar strategy [11][12], for instance St. Jude's Hospital [13], as well as our group [14]. Rasmussen-Torvik's group has proposed the eMERGE-PGx project, in which pre-emptive pharmacogenotyping is integrated with clinical history [15].

In the case of voriconazole, several studies have linked CYP2C19 polymorphism to the response. In addition, some studies have shown that genotyping CYP2C19 is effective. The cost-efficiency of pre-treatment genotyping in some drugs has been already demonstrated in some articles [7]; however, there is no evidence of voriconazole efficiency specifically in the Spanish NHS, or even whether voriconazole-related cytochrome genotyping is efficient in other countries [16].

This pragmatic clinical trial is proposed by our group to evaluate effectiveness and efficiency of pre-emptive biomarker genotyping and its interpretation by a Pharmacogenetic Unit for patients with haematological diseases and a risk of fungal infection, who are susceptible to receiving voriconazole treatment. If our hypothesis is proven, this strategy will help us improve the prevention and treatment of this infectious complication. This trial will provide information about the viability of the translation of this strategy to routine clinical practice. In addition, our pre-emptive pharmacogenetic strategy, combined with therapeutic drug monitoring, has the potential to improve efficacy and safety, with a high level of evidence [6], lowering the likelihood of AE occurrences.

Author contributions

AMB, AJC and JFI conceived the study. JMV, IG, FAS, CA, GM, ER, JFI, AJC and AMB designed the study. DB, RC, ME, AL and MJO are responsible of the data collecting and management. JMV and IG drafted this manuscript. AMB, AJC and JFI critically revised the manuscript. JMV and IG contributed equally. The final version of the manuscript was reviewed and approved by all authors.

Competing interests

None declared.

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VISIT	Visit of selection	Visit of randomisation	Primary outcome evaluation (visit)	Follow-up visit	End-of-treatment visit	End-of-study visit
Day of the study	**Day 0**	**Maximum 3 months after selection**	**5th day of treatment (+/- 1 day)**	**Weekly until end of treatment**	**Last day of treatment**	**2 weeks later after ending treatment(1)**
Selection criteria meeting	X					
Informed Consent Signing	X					
Genetic sample	X					
Randomisation		X				
Starting treatment		X				
Voriconazole serum levels			X	X (2)		
Lab analysis (3)		X	X	X	X	X
Electrocardigram		X	X	X	X	X
AE(4) evaluation		X	X	X	X	X

(1) Patients who after 3 months continue being treated, End-of-study visit will be done on the last day of third month.
(2) According to physician criteria
(3) Analysis including liver enzimes.
(4) AE (Adverse Events)

Table1. Study visits planning and study procedures.

Figure legend

Figure 1. Study Flowchart.
Proposed reporting of the flow of trial participants.

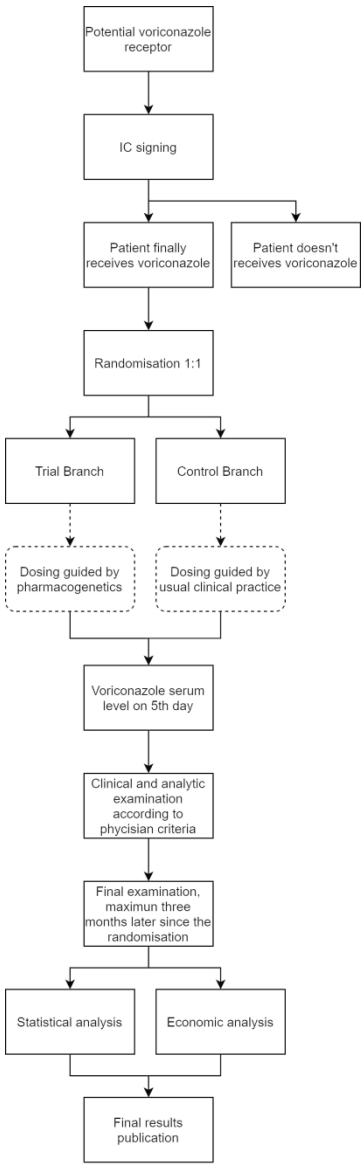

Figure 1. Study Flowchart

Figure 1. Study flowchart

SPIRIT checklist.

Included	Section/item	Item No	Description	Pg.
Administrative information				
☑	Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
☑	Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
☑		2b	All items from the World Health Organization Trial Registration Data Set	1-2
☑	Protocol version	3	Date and version identifier	1
☑	Funding	4	Sources and types of financial, material, and other support	14
☑	Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	16, 21
☑		5b	Name and contact information for the trial sponsor	1
☑		5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
☑		5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction				
☑	Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6, 7
☑		6b	Explanation for choice of comparators	6, 7
☑	Objectives	7	Specific objectives or hypotheses	8

☑	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes				**Pg.**
☑	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8, 12
☑	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
☑	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10, 11
☑		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11
☑		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11, 12
☑		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7, 12, 16
☑	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9, 10
☑	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10,11,20

☑	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
☑	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	12
Methods: Assignment of interventions (for controlled trials)				
	Allocation:			
☑	Sequence generation	16a	Method of generating the allocation sequence (eg, computer- generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
☑	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
☑	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9, 10, 11, 21
☑	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
☑		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9

Methods: Data collection, management, and analysis				
☑	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12

☑		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	12
☑	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
☑	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12,13
☑		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12,13
☑		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	12,13

Methods: Monitoring				
☑	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14
☑		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12, 13
☑	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
☑	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14

Ethics and dissemination	

☑	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	15
☑	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	15
☑	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
☑		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9
☑	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
☑	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
☑	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	14
☑	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
☑	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2,14
☑		31b	Authorship eligibility guidelines and any intended use of professional writers	16
☑		31c	Plans, if any, for granting public access to the full protocol, participant- level dataset, and statistical code	2, 14

Appendices	

☑	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	*
☑	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	9, *

COMMENTS:

* Some parts of the documentation will be attached to the submission, such as the model consent form and other related documentation.