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Assessing the impact on intestinal microbiome and clinical outcomes of antibiotherapy optimization strategies in hematopoietic stem cell transplant recipients: study protocol for the prospective multi-center OptimBioma study

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Keywords:	microbiome, microbiota, hematopoietic stem cell transplantation, graft versus host disease, infections, antibiotics

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Note from the Editors: Instructions for reviewers of study protocols

Since launching in 2011, BMJ Open has published study protocols for planned or ongoing research studies. If data collection is complete, we will not consider the manuscript.

Publishing study protocols enables researchers and funding bodies to stay up to date in their fields by providing exposure to research activity that may not otherwise be widely publicised. This can help prevent unnecessary duplication of work and will hopefully enable collaboration. Publishing protocols in full also makes available more information than is currently required by trial registries and increases transparency, making it easier for others (editors, reviewers and readers) to see and understand any deviations from the protocol that occur during the conduct of the study.

The scientific integrity and the credibility of the study data depend substantially on the study design and methodology, which is why the study protocol requires a thorough peer-review.

BMJ Open will consider for publication protocols for any study design, including observational studies and systematic reviews.

Some things to keep in mind when reviewing the study protocol:

- Protocol papers should report planned or ongoing studies. The dates of the study should be included in the manuscript.
- Unfortunately we are unable to customize the reviewer report form for study protocols. As such, some of the items (i.e., those pertaining to results) on the form should be scored as Not Applicable (N/A).
- While some baseline data can be presented, there should be no results or conclusions present in the study protocol.
- For studies that are ongoing, it is generally the case that very few changes can be made to the methodology. As such, requests for revisions are generally clarifications for the rationale or details relating to the methods. If there is a major flaw in the study that would prevent a sound interpretation of the data, we would expect the study protocol to be rejected.

Assessing the impact on intestinal microbiome and clinical outcomes of antibiotherapy optimization strategies in hematopoietic stem cell transplant recipients: study protocol for the prospective multi-center OptimBioma study

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ABSTRACT

Introduction

Hematopoietic stem cell transplantation (HSCT) is a lifesaving treatment for a number of hematologic diseases. Graft versus host disease (GVHD) is its main complication and hampers survival. There is strong evidence that intestinal microbiota diversity of the recipient may increase the risk of GVHD worsening survival. Antibiotic regimens used during the early phase of the transplant may influence clinical outcomes by reducing intestinal microbiota diversity. Present guidelines of *European Conference on Infections in Leukemia* exhort to optimizing antibiotic use in hematologic patients including HSCT recipients.

The present study aims to investigate if, in HSCT recipients, the optimization of antibacterial use may preserve intestinal microbiota composition reducing the incidence and severity of acute GVHD and improving relevant clinical outcomes.

Methods and analysis

This is a prospective longitudinal observational study of two cohorts of HSCT recipients: i) the intervention cohort includes patients treated in centers in which a pre-defined strategy of antibiotherapy optimization is implemented, with the objective of optimizing and reducing antibiotic administration according to clinical criteria and ii) the control cohort includes patients treated in centers in which a classic permissive strategy of antibiotic prophylaxis and treatment is used. Adult patient receiving a first HSCT as a treatment for any hematologic condition are included. Clinical variables are prospectively recorded and up to five fecal samples are collected for microbiota characterization at pre-stablished

peri-transplant time-points. Patients are followed since the pre-conditioning phase throughout one-year post-transplant and four follow-up visits are scheduled. Fecal microbiota composition and diversity will be compared between both cohorts along with acute GVHD incidence and severity, severe infections rate, mortality and overall and disease-free survival.

Ethics and dissemination

The study was approved between 2017 and 2018 by the Ethical Committees of participant centers. Study results will be disseminated through peer-reviewed journals and national and international scientific conferences.

Trial registration number: ClinicalTrials.gov, NCT03727113. Registered on November 1st, 2018.

Keywords: microbiome, microbiota, hematopoietic stem cell transplantation, graft *versus* host disease, infections, antibiotics.

Word count: 3474

ARTICLE SUMMARY

Strengths and limitations of the study.

- This is the first prospective comparative observational multicenter study addressing the effect of two centers-driven antimicrobial strategy cohorts of hematopoietic stem cell transplant recipients (optimized *versus* standard antimicrobial use) on intestinal microbiota diversity and acute graft *versus* host disease.
- The study is built on systematic collection of fecal samples at predetermined peri-transplant time-points and prospective collection of a wide relevant clinical data set throughout one-year follow up with scheduled clinical visits. This design will allow to examine changes in the microbiota diversity pre-transplant over time and to analyze its possible association with the development of acute GVHD, severe infections, and survival.
- One limitation is that the study is non-randomized. Nevertheless, propensity score matching statistical approach will be used in order to reduce possible bias by confounding variables.
- Because of the observational design, other limitation is that no causal mechanistic association could be accurately concluded. Even though, the analysis of exhaustive set of prospectively recorded clinical data and systematic collection of faecal samples obtained for each patient will allow for meaningful cause-effect relationships being predicted.
- In conclusion, the findings of this study may bring useful new insights into the relationship between antibiotherapy use and development of acute graft *versus* host disease in hematopoietic stem cell transplantation

(HSCT) recipients and may help to design improved strategies leading to better survival.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a lifesaving treatment for many severe hematological disorders. However, the deep immunosuppression associated to the procedure results in high risk of infectious complications prompting the administration of antimicrobial prophylaxis and therapy. Antibiotics fight off pathogenic bacteria but at the same time they may damage intestinal commensal bacteria leading to changes in intestinal microbiota composition and reduced diversity[1]. There is evidence that loss of intestinal microbiota diversity during HSCT may cause an increased risk of acute graft-versus-host disease (GVHD) worsening of short and long-term clinical outcomes.

Pioneering preclinical studies suggested that selective intestinal decontamination with antibiotics could reduce the incidence of GVHD[2 3] leading to the clinical use of pre-transplant antibacterial prophylaxis. These expectations were not subsequently confirmed in the clinical setting and this practice is not currently performed in many transplant centers. However, recent studies suggest that changes in intestinal microbiota composition may play an important role in the development of GVHD and in clinical outcomes of HSCT[1 4 5].

Therefore, antibacterial therapy strategies currently used in HSCT clinical practice should be re-evaluated in order to avoid as much as possible intestinal microbiota misbalance[6-8]. Our group has recently demonstrated in an academic prospective multicenter randomized clinical trial (*How-Long* study)[9], that in hematological patients (including HSCT recipients) with febrile neutropenia it is

safe to discontinue empirical antibiotic therapy after resolution of fever when patients are clinically stable, irrespective of their neutrophils counts, significantly reducing exposure to antibiotics. On the other hand, the ECIL group (European Conference of Infections in Leukemia) has proposed[10] specific empirical antibacterial therapy strategies in hematological patients including HSCT recipients, in order to optimized antibiotic use. These recommendations are heterogeneously implemented in the different hematopoietic transplant centers. This study will investigate if a predefined strategy of optimization of antimicrobial therapy that includes ECIL recommendations[10-12] and the *How-Long* study[9], will preserve intestinal microbiota composition and diversity while reducing the incidence and severity of acute GVHD when compared to a conventional permissive antibiotic strategy. In addition, severe infections rate, transplant related mortality and long-term survival will be compared between both groups.

METHODS AND ANALYSIS

Study design

A prospective longitudinal observational study of two cohorts of HSCT recipients was established: i) the intervention cohort includes patients treated at centers using an optimized strategy of antibacterial therapy (see *Intervention* section), ii) the control cohort includes patients treated at centers using a classical permissive antibacterial therapy strategy (see *Intervention* section). Each participating center is allocated in one of the two cohorts according to its clinical practice.

Study settings

Multicenter study conducted at five academic hospitals in Spain, two allocated to the intervention cohort [Virgen del Rocío University Hospital (Seville) and Marqués de Valdecilla University Hospital (Santander)] and three to the control cohort [Valencia Clinic Hospital (Valencia), Salamanca University Hospital (Salamanca), Gregorio Marañón University Hospital (Madrid)]. A four years study period is estimated (2017 – 2020).

Participants

Eligibility criteria

Inclusion Criteria

- Adult patients admitted to receive their first allogeneic hematopoietic transplant as a treatment for any hematological disease.
- Patients who have signed the study informed consent to participate.
- Patients who have received a previous autologous transplant are not excluded.

Exclusion criteria

- Non-compliance of the patient to sign the informed consent.
- Patients who have initiated the conditioning regimen previously to entering the study will not be included.

Recruitment process

Patients who meet the eligibility criteria and sign the informed consent will be recruited by investigators of the hematology services on the participating sites.

Intervention

Centers allocated to the intervention cohort use an antibacterial systematic approach that includes the following strategies:

1. No routine antibacterial prophylaxis is used.

2. In case of febrile neutropenia:

   - Use of scalation / de-scalation strategy for empiric antimicrobial therapy[10].

   - Directed simplification in patients with etiological diagnosis according to *in vitro* susceptibility tests.

   - Switch to a narrower-spectrum agent in patients without an etiological diagnosis and clinical stabilization on treatment.

   - No broadening the antibacterial spectrum but maintenance of initial antimicrobials therapy in patients with persistent fever if they are clinically stable in the criteria of the physicians in charge of theam.

3. Early (in 72 hours) withdrawal of combined treatments, when clinically indicated.

4. Antibacterial therapy withdrawal regardless neutrophils count and expected duration of neutropenia when patient meets all the following criteria (*How-Long* strategy)[9].

   i) Afebrile for ≥ 72 h.

ii) Complete resolution of signs, symptoms and alterations in complementary tests secondary to the infection (cough, abdominal pain, diarrhea, pulmonary infiltrate, etc.) for ≥ 72 h.

iii) Normal vital constants (blood pressure, heart rate, respiratory rate and diuresis and, in patients with respiratory involvement, oxygen saturation by pulse oximetry) for ≥72 h.

5. Short (7 days) etiological therapy for primary or related to central venous (with catheter removal) no complicated bacteremia, and 14 days for *Staphylococcus aureus* non complicated bacteremia if good clinical response and good clinical evolution.

In centers allocated to the control cohort the antimicrobial therapy approach does not include any of the strategies used in the optimization cohort but the following management:

1. Use of antibacterial prophylaxis.
2. In case of febrile neutropenia:

- Use of early broad-spectrum antimicrobial therapy without systematic use of escalation / de-escalation strategy.

- Optional antibiotic simplification in patients with etiological diagnosis according to *in vitro* susceptibility tests.

- No switching to a narrower-spectrum agent in patients without etiological diagnosis and clinical response.

- Broadening the spectrum of initial antimicrobials in patients with persistent fever even without clinical worsening.

3. No early discontinuation of the combined empirical antimicrobial therapy even in case of clinical response.
4. No discontinuation of empirical antimicrobial therapy until neutropenia recovery.
5. Prolonged etiological treatment for primary or related to central venous catheter no complicated bacteremia, even in case of early clinical response.

Schedule of visits and collection of fecal samples

The scheduled visits and assessments are described in table 1.

Follow-up is organized in four planned visits: Visit 1 (seven days pre-transplant), Visit 2 (end of antimicrobial therapy or hospital discharge, whichever occurs first), Visit 3 (100 days post-transplant), and Visit 4 or Final Visit (one-year post-transplant or mortality, whichever occurs first). A minimum of four fecal samples will be collected: Specimen 1, day of starting the conditioning treatment ± 48 hours; Specimen 2, day of transplantation ± 48 hours; Specimen 3, day +7 post-transplant ± 24 hours; Specimen 4, when the first episode of fever at any time from the beginning of the conditioning until the end of antimicrobial therapy or hospital discharge, whichever occurs first (this sample is collected at fever onset or within 48 hours, unless the fever starts on the same day that a scheduled fecal sample is already collected); and Specimen 5, day when the antimicrobial therapy is stopped (or within the following 48 hours) or,

alternatively, if the patient did not receive antibiotics or continues receiving antibiotics at discharge, the day before discharge (or 24 hours in advance).

The stool samples will be collected at different time points (table 1) in Stool Nucleic Acid Collection and Preservation Tubes (Ref. 45660, Norgen Biotek Corp.).

DNA extraction, prokaryotic 16S ribosomal RNA gene (16S rRNA) sequencing and bioinformatics analysis

The microbiome studies will be performed at the laboratory of Infectious Diseases of the Institute of Biomedicine of Seville (IBiS).

DNA will be extracted from faecal samples using the Stool DNA Isolation kit (Cat. 27600, Norgen Biotek Corp., Ontario, Canadá) according to the manufacturer's protocol. All DNA samples will be stored at −20 °C until further processing. DNA will be quantified by Qubit fluorometry (Invitrogen Qubit 4 Fluorometer, ThermoFisher Scientific, Spain) and normalized to 5 ng/μL with 10 mM Tris pH 8.5. Bacterial 16S rRNA amplification and library construction will be performed according to the 16S Metagenomic Sequencing Library Preparation guide from Illumina. Briefly, 2.5 μl of total DNA per sample will be amplified using primers targeting the 16S rRNA V3 and V4 regions[13-14]. These regions provide ample information for taxonomic classification of microbial communities. Pooled V3-V4 amplicon libraries will be sequenced using the Illumina MiSeq platform with a V3 reagent kit (Illumina, San Diego, California) and paired-end 300-bp reads. Up to 96 libraries could be pooled together for sequencing. Regarding the bioinformatics analysis of the sequencing data, machine learning libraries from Scikit-learn[15] will be used to filter out and

discard poor-quality reads. Processed sequences will be subjected to operational taxonomic unit (OTU) picking against Greengenes (v13.8)[16], with reads clustered by 97% identity into OTUs using QIIME 2[17]. In-house R scripts (v3.2.2) will be used to visualize the results.

Evaluation of results

In order to assess the impact of both antimicrobial strategies on intestinal microbiota diversity it will be characterized as described in the previous section and biological alpha and beta diversity indexes of samples from both cohorts will be compared[18]. Alpha-diversity and beta-diversity refer to diversity within and between samples, respectively. These secondary bioinformatics analyses will be performed with QIIME 2 and included the calculation of the parameters of alpha-diversity: Shannon's diversity index, frequency of observed OTUs, Faith's Phylogenetic Diversity, and Evenness; and the parameters of beta-diversity: Jaccard distance, Bray-Curtis distance, Unweighted UniFrac distance and Unweighted UniFrac distance.

In order to asses clinical outcomes (secondary objectives of the study) the following data will be prospectively recorded: incidence and severity of GVHD (evaluated for any degree, degree-II and degree-III/IV up to day 100 post-transplant), transplant related mortality and mortality caused by infection (time frame: Day 0 to Day +30, Day +100 and Day +365 post-transplant), incidence of severe infections (Day 0 to Day +30 post-transplant), and overall and disease free survival (time frame: Day 0 to Day +30, Day +100 and Day +365 post-transplant).

Sample size

Assuming a percentage of patients developing grade two or higher acute graft versus host disease[19] in the control cohort of 42%[5] and 20% in the intervention cohort, a power of 80% and an alpha error of 5%, 90 patients in each study cohort should be enough to detect differences between them. However, 100 patients per cohort will be included in order to compensate possible loss of statistical power due to associations between centers and the effect of the optimization strategy versus standard antimicrobial use (unknown at the time of the study design), to increase the statistical power for the secondary objectives (transplant related mortality, infections rate, mortality and survival), along with potential withdrawals[20-22].

Statistical analysis

To determine the impact of both antimicrobial therapy strategies on the intestinal microbiota diversity, in a first step, a multidimensional scaling and a permutational multivariate analysis of variance (PERMANOVA) analyses of both antibiotherapy strategies will be performed using the R statistical package (v3.2.2)[23]. To find out which taxa were most likely to explain the differences between both groups, taxa summaries generated in QIIME 2 will be reformatted for input into LEfSe via the Huttenhower Lab Galaxy Server (https://huttenhower.sph.harvard.edu/galaxy/root). This algorithm performs nonparametric statistical testing of whether individual taxa differed between both groups and then differentially ranked the abundant taxa by their linear discriminate analysis (LDA) log-scores. Differentially abundant taxa that are

statistically significant using an alpha error of 5% and LDA log-scores exceeding ±2.0 will be visually represented as bar plots. The median values of taxa abundance and the median percentages of taxa presence in both groups will be calculated, and the Manhattan distances will be used for the clustering analysis. The Kruskal-Wallis rank-sum test will be used to identify significant taxa abundance and Fisher's exact test will be used to identify significant taxa presence in the both groups.

The propensity score will be used to adjust potential confounding effects. To calculate the propensity scores in the logistic model the center factors and key predictive characteristics identified in the baseline comparability analysis will be taken into account. Propensity scores will be used for all adjusted inferential analyzes.

Standard descriptive statistical indices will be used according to the nature of each variable. Continuous variables will be analyzed with linear models, binary variables without time factor with logistic models and the time-to-event variables with survival models, all of them incorporating the propensity score as an adjustment factor.

The survival function of both groups will be described using the Kaplan-Meyer method. For the inferential analysis, the stratified log-rank test will be used (with the propensity score value categorized as stratum). Hazard Ratios and its 95% confidence intervals will be estimated using the Cox proportional hazards regression (including the propensity score value).

The following strategy will be used for time-dependent variables:

(a) Continuous variables that follow a Gaussian distribution by means of mixed models for repeated measures (mixed longitudinal model for repeated measurements (MMRM).

(b) Variables that do not comply with the parametric assumptions will be transformed into ranges and analyzed analogously to those in section a).

c) Longitudinal binary data will be analogously analyzed with the marginal models [Generalized Estimation Equation GEE).

In addition, the following statistical tests will be used when necessary: Fisher's exact test to compare categorical variables between groups, McNemar test or Cochran Q test for the analysis within the groups, dependent or independent t-test for continuous variables when comparing two groups and ANOVA if comparing more than two groups.

Nonparametric methods will be used in case of deviations from the applicability assumptions: according to the data distribution, Mann-Whitney and/or Kruskall-Wallis tests (independent variables), or Wilcoxon or Friedman tests (dependent variables). Correlations will be done with Pearson or Spearman coefficients according to the data distribution. SAS System (Release 9.2) or validated equivalent software will be used. All recruited patients will be included in the main analysis. In addition, a sensitivity analysis will be carried out with those subjects who have complied with the protocol.

Patient and public involvement

Neither patients nor public were involved in the development of the study.

DISCUSSION

The aim of this study is to prospectively investigate if an antimicrobial therapy in HSCT recipients has lesser impact on the intestinal microbiota composition and diversity than a non-restrictive standard antimicrobial therapy approach and if it correlates with a decrease incidence and severity of acute GVHD leading to improved clinical outcomes as reduced transplant related mortality, severe infections rate and improved survival. If this hypothesis proved to be certain, current antibacterial strategies in HSCT setting may need to be fully reviewed in order to avoid decrease the intestinal microbiota diversity. A prospective longitudinal observational study of two cohorts of patients will be used to address these objectives. The intervention cohort includes patients treated at two centers in which the antimicrobial therapy approach is optimized according to clinical criteria (as specified at intervention paragraph) and the control cohort includes three centers in which the classic management of antimicrobial therapy treatment is used (also specified at intervention paragraph).

One limitation of the study is a non-randomized design. Randomized controlled trials (RCT) are widely considered the design of choice for the assessment of effectiveness of healthcare intervention as the randomization process makes the comparison groups equal with respect to both known and unknown prognostic factors at baseline[24]. Nevertheless, RCT design is not applicable in this study. The implementation of a whole antibacterial therapy strategy in this frail setting of patients requires that it is solidly grounded in the daily practice of the clinical team, in order to be safe. The randomization scenario would implicate the use of unfamiliar antibacterial strategies by the clinical teams. This would be unsafe for patients and then ethically inadmissible. Therefore, an

observational study carried out in two groups of centers in which one of the two antibiotherapy approaches is already implemented turns out to be the safest and more feasible design. The propensity score matching statistical technique will reduced the possible bias due to confounding variables.

Another limitation of the study is that no causal association could be accurately described because of the observational design. Nevertheless, as an exhaustive set of prospective clinical data are being recorded for each patient, including start and stopping date of every antimicrobial used, dates of start and resolution of main clinical end-points it is likely that meaningful cause-effect relationships might be forwarded.

This is the first prospective multicenter study aiming to address the effect of two antimicrobial therapy strategies on intestinal microbiota and clinical outcomes in HSCT recipients. The systematic collection of faecal samples at predetermined peri-transplant time-points and the prospective collection of a wide clinical data set with one year follow up based in pre-scheduled repeated clinical visits will allow to examine changes in the microbiota composition over time and accurate link them to the development of acute GVHD and other clinical outcomes. Another strength of the study is its design based on daily clinical practice. This will provide valuable data on 'real-life patients' in addition to potential recommendations on sampling time points and frequency for further studies. In conclusion, the findings of this study will bring useful insight in the relationship between antibiotherapy use and development of acute graft versus host disease in HSCT recipients helping to design improved strategies expectedly leading to better survival, reduced graft versus host disease and improved quality of life.

Trial status

At submission the study is running and 140 patients are recruited.

Current approved protocol is version 3.0, dated 29/January/2018.

Date recruitment began at 16 January 2018 (First patient in).

Approximate date when recruitment will be completed: November 2019.

Abbreviations

ASCT: Allogeneic hemopoietic stem cell transplant; CTU: Clinical Trials Unit; CVC: central venous catheter; ECIL: European Conference of Infections in Leukemia; GEE: Generalized Estimation Equation; GVHD: Graft Versus Host Disease; ICH: International Council of Harmonisation; ITPP: intention-to-treat population; MMRM: Mixed longitudinal model for repeated measurements; MRT: mortality related to transplantation; PP: per-protocol population; RCT: Randomized Clinical Trial.

Author Contributions

IE conceived, designed the study and lead the study. IE and CR-F obtained funding for the research. SJ-J, GL-H, NR-T, FJM-M, MA-G, MEP-I and CR-F collaborated in methodological aspects of the study. SJ-J, NR-T, CR-F, MEP-I and CL-R coordinated the study. IE, CS, LV, MK, JMM-B and NR-T were responsible for the inclusion, treatment, clinical monitoring and follow-up of the patients. CL-R coordinated sample collection. GL-H, MEP-I and YS were responsible for the sample management. SJ-J wrote the first draft of the

manuscript. All authors were involved in critically revising the article and approved the final version.

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Competing interests

The authors declared no competing interests

Patient consent for Publication

Not required.

Ethics and dissemination

The study was approved between 2017 and 2018 by the five Ethical Committees involved (Comité Coordinador de Ética de la Investigación Biomédica de Andalucía, Comité de Ética de Investigación Clínica de Cantabria, Comité Autonómico de Evaluación de Estudios Posautorización Observacionales, de Seguimiento Prospectivo con medicamentos, Comité de Ética de la investigación con medicamentos del Hospital Univesitario de Salamanca, Dirección General de Inspección y Ordenación de la Consejería de Sanidad de la Comunidad de Madrid). Each substantial protocol amendment will be notified for approval to the relevant ethics committee(s) prior to implementation. All data collected will be kept strictly confidential and in accordance with all relevant legislation on control and protection of personal

information. Study results will be published in peer-reviewed journals as well as national and international scientific conferences.

Patient and Public Involvement

No patient involved.

Access to data

Data are sustained in an electronic database. Upon request to the corresponding author, the identified participant's data will be made available to researchers whose proposals meet the research criteria. It will be also considered requests for the protocol. To gain access, data requestors must comply to a data access agreement.

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Table 1. Schedule of enrollment and assessments. *OptimBioma study*.

	VISIT 1 (7 days Pre-transplant ± 48h)	**Day of transplant (day 0)** (±48h)	**7 days post-transplant (day +7)** (± 24h)	**Day of Fever onset (if fever occurs)** (+ 48h)	**VISIT 2** (End anti-biotherapy or discharge)*	**VISIT 3** (100 days post-transplant) Day +100	**VISIT 4** (1 year post-transplant or *exitus letalis* ± 1 week)
Inclusion/Exclusion criterion	**X**						
Signature of informed consent	**X**						
Clinical Data Collection	**X**				**X**	**X**	**X**
Fecal Sample Collection	**Specimen 1**	**Specimen 2**	**Specimen 3**	**Specimen 4**	**Specimen 5***		

* End of antibiotic therapy or discharge, whichever occurs first.

Dear Editor,

Please find bellow proofs to publish without peer review protocols:

- Formal ethical approvals from the five participating sites
- Resolution of the granting of the requested funding for the OptimBioma project issued by the ISCIII (The Instituto de Salud Carlos III (Institute of Health Carlos III) is the Spanish organization managing the activities of the Health Research and Development Strategy (AES) under the State Plan for Scientific and Technical Research and Innovation 2017-2020; https://eng.isciii.es/Paginas/Inicio.aspx)

Best regards,

Ildefonso Espigado

Corresponding Author

Formal ethical approvals from the five participating sites

GOBIERNO de CANTABRIA

CONSEJERÍA DE SANIDAD

Dirección General de Ordenación y Atención Sanitaria

Llébana Cantabria 2017 Año Jubilar

GOBIERNO de CANTABRIA CONSEJERÍA DE SANIDAD REGISTRO AUXILIAR DE LA CONSEJERÍA DE SANIDAD
22 DIC. 2017
Hora: 15:02
Nº DE REGISTRO E 12938

Clara María Rossso Fernández
Unidad de Investigación Clínica y EECC
Hospital Universitario Virgen del Rocío
Hospital General planta baja
Avda. Manuel Siurot, s/n
41013-Sevilla

Expte. Nº: EP A-SP. 14/17
N/Ref.: LMCA/ cao
Asunto: Notificación resolución
Fecha: 20 de diciembre 2017

NOTIFICACIÓN

Adjunto se remite resolución de la Directora General de Ordenación y Atención Sanitaria, de fecha 18 de diciembre de 2017, por la que se concede la autorización para la realización en la Comunidad Autónoma de Cantabria del estudio posautorización observacional titulado: **"Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: impacto en la microbiota intestinal y en resultados clínicos"**, versión 1.0, de 28 de marzo de 2017 con código de protocolo **FIS-ANT-2017-01**,

EL JEFE DE SERVICIO DE ORDENACION SANITARIA

Luis M. Cabanzón Alber

DIRECCIÓN GENERAL DE ORDENACIÓN Y ATENCIÓN SANITARIA Consejería de Sanidad

SECCIÓN DE ORDENACIÓN FARMACÉUTICA
C/ Federico Vial, 13
39009 SANTANDER
TEL.: 942-207701/ 207702
FAX: 942 208239

GOBIERNO de CANTABRIA
CONSEJERÍA DE SANIDAD
Dirección General de Ordenación y Atención Sanitaria

Llébana Cantabria 2017 Año Jubilar

Expte. Nº: EPA-SP 14/17

RESOLUCIÓN

Visto el expediente de solicitud de autorización para la realización del estudio posautorización de tipo observacional titulado: "Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: impacto en la microbiota intestinal y en resultados clínicos", versión 1.0, de 28 de marzo de 2017, código de protocolo FIS-ANT-2017-01, formulada por Doña Clara María Rosso Fernández, responsable de la Unidad de Investigación Clínica y EECC-HUVR, con domicilio en Avda. Manuel Siurot, s/n, 41013-Sevilla, en representación de FISEVI: Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla, con domicilio en Hospital Universitario Virgen del Rocío. Avda. Manuel Siurot, s/n., Edif. de Laboratorios 6º planta., 41013-Sevilla, promotor del estudio y teniendo en cuenta los siguientes

ANTECEDENTES DE HECHO

PRIMERO.- Con fecha 6 de noviembre de 2017, se presenta en esta Consejería de Sanidad, solicitud de autorización para la realización en la Comunidad Autónoma de Cantabria del estudio posautorización de tipo observacional titulado: "Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: impacto en la microbiota intestinal y en resultados clínicos", versión 1.0, de 28 de marzo de 2017, código de protocolo FIS-ANT-2017-01, formulada por Doña Clara María Rosso Fernández, responsable de la Unidad de Investigación Clínica y EECC-HUVR, con domicilio en Avda. Manuel Siurot, s/n, 41013-Sevilla, en representación de FISEVI: Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla, con domicilio en Hospital Universitario Virgen del Rocío. Avda. Manuel Siurot, s/n., Edif. de Laboratorios 6º planta., 41013-Sevilla, promotor del estudio.

SEGUNDO.- Con fecha 20 de noviembre de 2017, se requiere a Clara María Rosso Fernández, responsable de la Unidad de Investigación Clínica y EECC-HUVR, documentación adicional en relación con su solicitud, que es recibida en esta Consejería de Sanidad, el día 12 de diciembre de 2017

TERCERO.- Con fecha 18 de diciembre de 2017, tras solicitar la evaluación del estudio al Servicio Cántabro de Salud y al Centro de Farmacovigilancia de Cantabria, la Sección de Ordenación Farmacéutica emite informe considerando que procede la realización del mismo en la Comunidad Autónoma de Cantabria

FUNDAMENTOS DE DERECHO

I.- Es competente para dictar la presente resolución la Directora General de Ordenación y Atención Sanitaria, de acuerdo con lo establecido en el artículo 5.5 h) del Decreto 24/2002, de 7 de marzo, de Estructura Orgánica de la Consejería de Sanidad,

Consumo y Servicios Sociales, según la redacción dada por el Decreto 60/2007, de 24 de mayo, de modificación parcial de las estructuras orgánicas y de las relaciones de puestos de trabajo de la Consejería de Sanidad y Servicios Sociales y de la Dirección Gerencia del Servicio Cántabro de Salud.

II.- Evaluada la solicitud, de acuerdo con lo dispuesto en el Real Decreto 577/2013, de 26 de julio, por el que se regula la farmacovigilancia de medicamentos de uso humano así como la Orden SAS/3470/2009, de 16 de diciembre, por la que se publican las directrices sobre estudios posautorización de tipo observacional para medicamentos de uso humano, se concluye que el estudio cumple los requisitos mínimos establecidos en las citadas normas y por lo tanto, se considera procedente la realización del estudio en la Comunidad Autónoma de Cantabria.

III.- En la tramitación del expediente se han observado las prescripciones establecidas en la Ley 39/2015, de 1 de octubre, del procedimiento administrativo común de las administraciones públicas

Vistos los antecedentes de hecho concurrentes y los fundamentos de derecho de aplicación, por la presente

RESUELVO

Conceder la autorización para la realización del estudio posautorización de tipo observacional titulado: "Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: impacto en la microbiota intestinal y en resultados clínicos", versión 1.0, de 28 de marzo de 2017, código de protocolo FIS-ANT-2017-01, en el Hospital Universitario Marqués de Valdecilla por la siguiente investigadora:

- Doña Lucrecia Yañez del Servicio de Hematología

de acuerdo con la solicitud formulada por Doña Clara María Rosso Fernández, responsable de la Unidad de Investigación Clínica y EECC-HUVR, con domicilio en Avda. Manuel Siurot, s/n, 41013-Sevilla, en representación de FISEVI: Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla, con domicilio en Hospital Universitario Virgen del Rocío. Avda. Manuel Siurot, s/n., Edif. de Laboratorios 6º planta., 41013-Sevilla, promotor del estudio.

Contra la presente resolución, que no agota la vía administrativa, se podrá interponer recurso de alzada en el plazo de un mes, contado desde el día siguiente al de su notificación, ante la Consejera de Sanidad, de acuerdo con lo dispuesto en el artículo 128 de la Ley 6/2002, de 10 de diciembre, de Régimen Jurídico del Gobierno y de la Administración de la Comunidad Autónoma de Cantabria.

Santander, 18 de diciembre de 2017

LA DIRECTORA GENERAL DE ORDENACIÓN
Y ATENCIÓN SANITARIA

María Antonia Mora González

COMPLEJO ASISTENCIAL UNIVERSITARIO DE SALAMANCA
Paseo de San Vicente, 58-182
37007 Salamanca
Comité de Ética de la Investigación con medicamentos
Teléfono: 923 29 11 00 – Ext. 55 515

Sacyl
SALUD DE CASTILLA Y LEÓN
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INFORME DEL COMITE DE ÉTICA DE LA INVESTIGACION CON MEDICAMENTOS

Doña María Belén Vidriales Vicente, Secretaria Técnica del Comité de Ética de la Investigación con medicamentos del Hospital Universitario de Salamanca,

C E R T I F I C A

Que este Comité ha evaluado la propuesta del promotor Fundación Pública Andaluza para la Gestión de la Investigación en Salud en Sevilla (FISEVI), para que se realice el Estudio Observacional, código de protocolo OptimBioma (FIS-ANT-2017-01), Versión 3.0 de 29 enero de 2018 y Hoja de información al paciente y Consentimiento informado, Versión 3.0 de 29 enero de 2018 (Ref. CEIC: E.O.17/549), titulado:

"Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: impacto en la microbiota intestinal y en resultados clínicos".

Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.

La capacidad del investigador y los medios disponibles son apropiados para llevar a cabo el estudio.

Son adecuados tanto el procedimiento para obtener el consentimiento informado como la compensación prevista para los sujetos por daños que pudieran derivarse de su participación en el estudio.

El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.

Y que este Comité acepta que dicho estudio sea realizado por la Dra. Lourdes Vázquez López.

La composición del CEIC del Hospital Universitario de Salamanca que evaluó este Estudio es la siguiente:

Vicepresidente:
Dª. Mª del Carmen Sánchez García. Asesora Jurídica.
Vicesecretaria:
Dª. Silvia Jiménez Cabrera. Farmacia Hospitalaria.

Vocales:
D. José Manuel González de Buitrago. Jefe de Servicio de Bioquímica Clínica.
Dª. Carmen Arias de la Fuente, Técnico Gestor de Ensayos Clínicos.
Dª. Mercedes López Rico. Especialista en Farmacología Clínica.

COMPLEJO ASISTENCIAL UNIVERSITARIO D SALAMANCA
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SANIDAD DE CASTILLA Y LEÓN

E-mail: comite.etico.husa@saludcastillayleon.es

D. Guzmán Franch Arcas. Especialista en Cirugía General y Aparato Digestivo.
Dª. Ana Martín García. Especialista en Cardiología
Dª. Cristina Hidalgo Calleja. Especialista de Reumatología.
Dª Teresa Martín Gómez. Especialista en Oncología
Dª. Zulema Ferreras Páez. Especialista Medicina Intensiva UVI – VV
Dª. Berta Bote Bonaechea. Especialista en Psiquiatría
Dª. Carmen Velayos Castelo, Profesora Titular de Ética y Filosofía Política de la Facultad de Filosofía de la USAL

En dicha reunión se cumplieron los requisitos establecidos en la legislación vigente – Orden SAS 3470/2009, 16 de diciembre – para que la decisión del citado CEIC sea válida.

El CEIC del Hospital Universitario de Salamanca, tanto en su composición como en sus PNT, cumple con las normas de BPC.

.

Lo que firmo en Salamanca, a 26 de febrero de 2018

COMPLEJO UNIVERSITARIO DE SALAMANCA
COMITÉ DE ÉTICA DE LA INVESTIGACIÓN CON MEDICAMENTOS

Firmado:
Doña M.ª Belén Vidriales Vicente

GENERALITAT VALENCIANA | TOTS A UNA veu
CONSELLERIA DE SANITAT UNIVERSAL I SALUT PUBLICA

Direcció General de Farmàcia i Productes Sanitaris

NºRef: DGFPS/SGOI/SPFD/CAVIME/ol

GENERALITAT VALENCIANA
CONSELLERIA DE SANITAT
Registre General
Data 24 NOV. 2017
EIXIDA 50191

FUNDACIÓN PÚBLICA ANDALUZA PARA LA GESTIÓN DE LA INVESTIGACIÓN EN SEVILLA
A/A D. ª Clara Rosso Fernández
Hospital Universitario Virgen del Rocío
Edificio Laboratorio, 6ª planta
Avda. / Manuel Siurot s/n
41013 Sevilla.

Adjunto remito resolución de autorización de la Dirección General de Farmacia y Productos Sanitarios para la realización del estudio titulado *Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: impacto de la microbiota intestinal y en resultados clínicos.* Código: *FIS-ANT-2017-01.*

Valencia, 20 de noviembre de 2017
EL SUBDIRECTOR GENERAL DE
OPTIMIZACIÓN E INTEGRACIÓN

José Manuel Ventura Cerdá

GENERALITAT VALENCIANA | TOTS A UNA veu
CONSELLERIA DE SANITAT UNIVERSAL I SALUT PÚBLICA
Direcció General de Farmàcia i Productes Sanitaris

RESOLUCIÓN DE AUTORIZACIÓN DE ESTUDIO POSAUTORIZACIÓN OBSERVACIONAL PROSPECTIVO CON MEDICAMENTOS

DESTINATARIO: FUNDACIÓN PÚBLICA ANDALUZA PARA LA GESTIÓN DE LA INVESTIGACIÓN EN SEVILLA (FISEVI).

Vista la solicitud formulada por FISEVI, con domicilio a efectos de notificación Hospital Universitario Virgen del Rocío – Edificio Laboratorio, 6ª planta. Avda./ Manuel Siurot s/n. 41013 Sevilla.

ANTECEDENTES

PRIMERO- Con fecha de entrada en la Conselleria de Sanidad Universal y Salud Pública 25 de octubre de 2017, FISEVI solicita la autorización para la realización del estudio posautorización observacional titulado *Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: impacto de la microbiota intestinal y en resultados clínicos.* Código: *FIS-ANT-2017-01.*

SEGUNDO- El Comité Autonómico de Estudios Postautorización Observacionales Prospectivos de Medicamentos de la Comunitat Valenciana (CAEPO) evalúa el citado estudio y emite informe favorable a la Directora General de Farmacia y Productos Sanitarios por no contravenir las directrices establecidas en el artículo 24 del RD. 577/2013, de 26 de julio, del Ministerio de Sanidad, Servicios Sociales e Igualdad por el que se regula la farmacovigilancia de medicamentos de uso humano y el artículo 8 de la Resolución de 16 de julio de 2009, de la Conselleria de Sanitat, de regulación de los procedimientos, documentación y plazos a observar en la presentación y modificaciones en procesos relacionados con los ensayos clínicos y estudios post-autorización observacionales de medicamentos y productos sanitarios en la Comunitat Valenciana.

GENERALITAT VALENCIANA | TOTS A UNA veu
CONSELLERIA DE SANITAT UNIVERSAL I SALUT PÚBLICA

Direcció General de Farmàcia i Productes Sanitaris

FUNDAMENTOS DE DERECHO

1º- La Dirección General de Farmacia y Productos Sanitarios es competente para autorizar la realización de los estudios postautorización de acuerdo a lo dispuesto en el Decreto 37/2017, de 10 de marzo, del Consell, por el que se aprueba el Reglamento Orgánico y Funcional de la Conselleria de Sanidad Universal y Salud Pública.

2º- RD. 577/2013, de 26 de julio, del Ministerio de Sanidad, Servicios Sociales e Igualdad por el que se regula la farmacovigilancia de medicamentos de uso humano, establece en su artículo 24 que los estudios postautorización deberán tener como finalidad complementar la información obtenida durante el desarrollo clínico de los medicamentos previo a su autorización y queda prohibida la planificación realización o financiación de estudios postautorización con la finalidad de promover la prescripción de los medicamentos.

3º- La Orden SAS/3470/2009, de 16 de diciembre, por la que se publican las directrices sobre estudios postautorización de tipo observacional para medicamentos de uso humano.

4º- Decreto 73/2009, de 5 de junio, del Consell, por el que se regula la gestión de ensayos clínicos y estudios postautorización observacionales con medicamentos y productos sanitarios.

5º- Resolución de 16 de julio de 2009, de la Conselleria de Sanitat, de regulación de los procedimientos, documentación y plazos a observar en la presentación y modificaciones en procesos relacionados con los ensayos clínicos y estudios post-autorización observacionales de medicamentos y productos sanitarios en la Comunitat Valenciana.

GENERALITAT VALENCIANA | TOTS A UNA veu

CONSELLERIA DE SANITAT UNIVERSAL I SALUT PÚBLICA

Direcció General de Farmàcia i Productes Sanitaris

De conformidad con ello, la Dirección General de Farmacia y Productos Sanitarios

RESUELVE

1º- **AUTORIZAR** la realización del estudio:

Título: Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: impacto de la microbiota intestinal y en resultados clínicos. Protocolo versión 1.0 de 28 de marzo de 2017. HIP/CI versión 1.0 de 28 de marzo de 2017

Código: FIS-ANT-2017-01.

en el centro sanitario propuesto en la solicitud:

CENTROS	INVESTIGADORES
Hospital Clínico Valencia	Dr. Carlos Solano Vercet

2º- La realización del estudio está condicionada a que el Gerente del Departamento dé el visto bueno para su realización y firme el correspondiente contrato con el promotor.

3º- El promotor deberá comunicar a esta Dirección General la fecha efectiva de comienzo del estudio en el centro. Asimismo, enviará el informe de seguimiento anual y deberá comunicar cualquier incidencia relevante de forma inmediata. Tras la finalización de la recopilación de los datos, presentará el informe final del estudio antes de doce meses.

Contra esta Resolución, que no pone fin a la vía administrativa, podrá interponerse Recurso de Alzada ante la Secretaría Autonómica de Salud Pública y del Sistema Sanitario Público en el plazo de un mes a contar desde el día siguiente al de la recepción de la presente notificación.

Valencia 20 de noviembre de 2017

LA DIRECTORA GENERAL DE FARMACIA Y PRODUCTOS SANITARIOS

Patricia Lacruz Gimeno

Misser Mascó, 31 · 46010 València · Tel. 961 928 000 · Fax 961 928 799
www.san.gva.es

JUNTA DE ANDALUCIA

CONSEJERÍA DE SALUD
Dirección General de Investigación y Gestión del Conocimiento
Comité Coordinador de Ética de la Investigación Biomédica de Andalucía

Fecha: 29 de noviembe de 2017
Protocolo:FIS-ANT-2017-01
Promotor: FUNDACIÓN PÚBLICA ANDALUZA GESTIÓN DE LA INVESTIGACIÓN EN SALUD DE SEVILLA (FISEVI)
Asunto: Comunicación de Resolución de estudio postautorización (EPA-SP)

Dª. Clara María Rosso Fernández
HOSPITAL UNIV. VIRGEN DEL ROCIO
Unidad de Investigación Clínica y EE.CC
Hospital General Planta Baja
Avda. Manuel Siurot, s/n
41013 - SEVILLA

SALIDA
JUNTA DE ANDALUCIA
CONSEJERIA DE SALUD
30 NOV. 2017
Registro General 17
24308 Sevilla

Adjunto se remite Resolución de fecha 29 de noviembre de 2017, del Presidente del Comité Coordinador de Ética de la Investigación Biomédica de Andalucía, del estudio titulado*: "Optimización del tratamiento antibiótico en receptores de alotrasplante hemotopoyético: impacto en la microbiotica intestinal y en resultados clínicos*", por la que se autoriza la realización de dicho estudio en los centros sanitarios de Andalucía, previa firma de contrato, o en su caso visto bueno de la Dirección Gerencia de cada centro.

EI SECRETARIO DEL COMITÉ COORDINADOR DE ÉTICA DE LA INVESTIGACIÓN BIOMÉDICA DE ANDALUCÍA.

Fdo.: Joaquín Alanís López

Avda. de la Innovación, s/n. Edificio Arena 1. Apdo. Correos 17.111. 41080 Sevilla
Teléf. 95 500 63 00. Fax 95 500 63 31

Código Seguro De Verificación:	0wIMELSV47KhZlaO7ThlwQ==	Fecha	30/11/2017
Normativa	Este documento incorpora firma electrónica reconocida de acuerdo a la Ley 59/2003, de 19 de diciembre, de firma electrónica.		
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JUNTA DE ANDALUCIA

CONSEJERÍA DE SALUD
Dirección General de Investigación y Gestión del Conocimiento
Comité Coordinador de Ética de la Investigación Biomédica de Andalucía

RESOLUCIÓN

Visto el procedimiento de autorización administrativa para la realización del estudio posautorización con medicamentos código de protocolo FIS-ANT-2017-01 titulado: *"Optimización del tratamiento antibiótico en receptores de alotrasplante hemotopoyético: impacto en la microbiotica intestinal y en resultados clínicos"*, Protocolo versión 1.0 de 28 de marzo de 2017, HIP/ y CI versión 1.0 de 28 de marzo de 2017, se constata lo siguiente:

HECHOS

PRIMERO.- Con fecha 25 de septiembre de 2017, Dª. Clara María Rosso Fernández, en nombre y representación del promotor, solicita la autorización administrativa para la realización del estudio anteriormente mencionado, cuyo promotor es FUNDACIÓN PÚBLICA ANDALUZA GESTIÓN DE LA INVESTIGACIÓN EN SALUD DE SEVILLA (FISEVI).

SEGUNDO.- Que el Comité Coordinador de Ética de la Investigación Biomédica de Andalucía en su reunión del 24 de octubre de 2017, ha evaluado el mencionado estudio solicitando ACLARACIONES MAYORES al protocolo.

TERCERO.- Que el promotor remite las respuesta a dichas aclaraciones con fecha 16 de noviembre de 2017, valorándose las mismas por el Comité Coordinador de Ética de la Investigación Biomédica de Andalucía en su reunión de fecha 28 de noviembre de 2017 (Acta 10/17) considerándolas adecuadas y emitiendo el correspondiente informe FAVORABLE.

FUNDAMENTOS JURÍDICOS

PRIMERO.- Este Comité Coordinador de Ética de la Investigación Biomédica de Andalucía es competente para la emisión de la presente Resolución en virtud de las competencias atribuidas en el artículo 7.3d) del Decreto 439/2012, de 14 de diciembre, por el que se regulan los órganos de ética asistencial y de la investigación biomédica de Andalucía, BOJA núm. 251 de 27 de diciembre de 2010.

SEGUNDO.- De conformidad con lo establecido en el artículo 24 del Real Decreto 577/2013, de 26 de julio, por el que se regula la farmacovigilancia de medicamentos de uso humano, BOE núm. 179, de 27 de julio de 2013, el estudio *"Optimización del tratamiento antibiótico en receptores de alotrasplante hemotopoyético: impacto en la microbiotica intestinal y en resultados clínicos"*, cumple con la finalidad de completar la información obtenida durante el desarrollo clínico de los medicamentos previo a su autorización.

Avda. de la Innovación, s/n. Edificio Arena 1. Apdo. Correos 17.111. 41080 Sevilla
Teléf. 95 500 63 00. Fax 95 500 63 31

JUNTA DE ANDALUCIA

CONSEJERÍA DE SALUD
Dirección General de Investigación y Gestión del Conocimiento
Comité Coordinador de Ética de la Investigación Biomédica de Andalucía

TERCERO.- Consta en el procedimiento tramitado al efecto que el estudio *"Optimización del tratamiento antibiótico en receptores de alotrasplante hemotopoyético: impacto en la microbiotica intestinal y en resultados clínicos"*, respeta las directrices publicadas en la Orden SAS 3470/2009, de 16 de diciembre, por la que se publican las directrices sobre estudios posautorización de tipo observacional para medicamentos de uso humano, BOE núm. 310, de 25 de diciembre de 2009.

CUARTO.- Conforme a lo establecido en el artículo 8. de la Orden SAS 3470/2009, de 16 de diciembre, por la que se publican las directrices sobre estudios posautorización de tipo observacional para medicamentos de uso humano, BOE núm. 310, de 25 de diciembre de 2009, el promotor deberá:

- Formalizar el correspondiente contrato, si procede, con los centros antes de iniciar el estudio. En cualquier caso se deberá contar con la valoración de la correspondiente pertinencia de la Dirección Gerencia de los centros.
- Comunicar al Comité Coordinador de Ética de la Investigación Biomédica de Andalucía la fecha efectiva del inicio del estudio en cada uno de los centros y, anualmente, deberá presentar un informe de seguimiento.
- Comunicar cualquier cambio metodológico o incidencia que afecte al estudio autorizado.

VISTOS la normativa citada y demás de general y pertinente aplicación,

RESUELVO

Primero.- AUTORIZAR la realización del estudio *"Optimización del tratamiento antibiótico en receptores de alotrasplante hemotopoyético: impacto en la microbiotica intestinal y en resultados clínicos"*, Protocolo versión 1.0 de 28 de marzo de 2017, HIP/ y CI versión 1.0 de 28 de marzo de 2017, en los centros sanitarios de Andalucía, sometido a las condiciones enumeradas en el fundamento jurídico cuarto de la presente Resolución.

Contra la presente resolución, que no agota la vía administrativa, podrá interponerse recurso de alzada ante el titular de la Dirección General de Investigación y Gestión del Conocimiento, en el plazo de un mes a contar desde el día siguiente a la notificación de la presente resolución, de acuerdo con el articulo 121 y 122 de la Ley 39/2015, de 1 de octubre, del Procedimiento Administrativo Común de las Administraciones Públicas.

Sevilla, 29 de noviembre de 2017
El Presidente del Comité Coordinador de Ética
de la Investigación Biomédica de Andalucía
(P.A. El Secretario del Comité Coordinador
de Ética de la Investigación Biomédica de Andalucía)

Fdo.: Joaquín Alanís López

Avda. de la Innovación, s/n. Edificio Arena 1. Apdo. Correos 17.111. 41080 Sevilla
Teléf. 95 500 63 00. Fax 95 500 63 31

Código Seguro De Verificación:	wh6BXWO4Kd3y5NRNeQdisw==	Fecha	30/11/2017
Normativa	Este documento incorpora firma electrónica reconocida de acuerdo a la Ley 59/2003, de 19 de diciembre, de firma electrónica.		
Firmado Por	Joaquin Alanis Lopez		
Url De Verificación	https://ws058.juntadeandalucia.es/verifirma/code/wh6BXWO4Kd3y5NRNeQdisw==	Página	2/2

Dirección General de Inspección y Ordenación
CONSEJERÍA DE SANIDAD
Comunidad de Madrid
Subdirección Gral. de Inspección y Ordenación Farmacéutica
Área de Control Farmacéutico y Productos Sanitarios
Ref.: MG/bhz

N O T I F I C A C I Ó N

Con fecha 19 de mayo de 2018 el Ilmo. Sr. Director General de Inspección y Ordenación, ha dictado la **RESOLUCIÓN** que se transcribe:

VISTA la solicitud formulada por la Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla (FISEVI), relativa a la autorización del estudio:: "**Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: Impacto en la microbiota intestinal y en resultados clínicos". Código OptimBioma**. Versión 3.0 de 29 de enero de 2018. HIP y CI versión 3.0 de 29/01/2018. **Código FIS-ANT-2017-01**.

A) ANTECEDENTES DE HECHO

Primero.- El promotor del estudio ha presentado la solicitud cumpliendo los requisitos mencionados en el artículo 5 de la *ORDEN 730/2004, de 30 de junio, del Consejero de Sanidad y Consumo, por la que se establecen los requisitos para la realización de estudios postautorización de tipo observacional con medicamentos de uso humano en la Comunidad de Madrid.* Entre estos requisitos se presentó el informe favorable del el Comité de Ética de la Investigación con medicamentos del Hospital Universitario de Salamanca de 26/02/2018, para la realización del estudio referenciado.

Segundo.- Los técnicos del Área de Control Farmacéutico y Productos Sanitarios emitieron informe de fecha 17 de mayo de 2018.

B) FUNDAMENTOS JURÍDICOS

Primero.- En la tramitación del presente expediente se han observado las disposiciones legalmente aplicables que recogen los requisitos y el marco jurídico para realizar los estudios post-autorización con interés científico, estableciendo una clara separación entre éstos y aquellos cuyos fines son puramente promocionales.

a) El art. 58 del Real Decreto Legislativo 1/2015, de 24 de julio, por el que se aprueba el texto refundido de la Ley de garantías y uso racional de los medicamentos y productos, establece en su punto 2:

"2.- (…)A los efectos de esta ley, se entiende por «estudio observacional» el estudio en el que los medicamentos se prescriben de la manera habitual, de acuerdo con las condiciones establecidas en la autorización. La asignación de un paciente a una estrategia terapéutica concreta no estará decidida de antemano por el protocolo de un ensayo, sino que estará determinada por la práctica habitual de la medicina. La decisión de prescribir un medicamento determinado estará claramente disociada de la decisión de incluir al paciente en el estudio. No se aplicará a los pacientes ninguna intervención, ya sea diagnóstica o de seguimiento, que no sea la habitual de la práctica clínica. Se utilizarán métodos epidemiológicos para el análisis de los datos recogidos."

b) La Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal, define lo que se entiende por datos de carácter personal y regula, entre otros asuntos, las condiciones de obtención, tratamiento y cesión de datos especialmente protegidos.

c) El capitulo VI del Real Decreto 577/2013, de 26 de julio, por el que se regula la farmacovigilancia de medicamentos de uso humano establece que:

"-Los estudios posautorización deberán tener como finalidad complementar la información obtenida durante el desarrollo clínico de los medicamentos previo a su autorización. Queda prohibida la planificación, realización o financiación de estudios posautorización con la finalidad de promover la prescripción de los medicamentos."

d) La Orden SAS/3470/2009, de 16 de diciembre por la que se publican las directrices sobre estudios posautorización de tipo observacional para medicamentos de uso humano

e) La Orden 730/2004 de 30 de junio del Consejero de Sanidad y Consumo establece los requisitos para la realización de estudios postautorización de tipo observacional con medicamentos de uso humano en la Comunidad de Madrid.

Segundo.- Evaluada la documentación aportada no se muestran objeciones a su realización en la Comunidad de Madrid.

VISTAS las disposiciones citadas en los Fundamentos Jurídicos esta Dirección General de Inspección y Ordenación es competente para resolver la solicitud presentada, conforme a las atribuciones que le reconoce la Ley 19/1998, de 25 de noviembre de Ordenación y Atención Farmacéutica de la Comunidad de Madrid y el Decreto 195/2015, de 4 de agosto, del Consejo de Gobierno, por el que se establece la estructura orgánica de la Consejería de Sanidad, modificado por el Decreto 125/2017, vengo en:

R E S O L V E R

AUTORIZAR la solicitud formulada por la Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla (FISEVI), relativa a la autorización del estudio:: "**Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: Impacto en la microbiota intestinal y en resultados clínicos". Código OptimBioma**. Versión 3.0 de 29 de enero de 2018. HIP y CI versión 3.0 de 29/01/2018. **Código FIS-ANT-2017-01**.

La presente Resolución, que no pone fin a la vía administrativa, podrá ser recurrida en alzada en el plazo de un mes a partir del día siguiente al de su notificación, ante la Viceconsejería de Sanidad de conformidad con lo establecido en los artículos 121 y 122 de la Ley 39/2015 de 1 de octubre, de Procedimiento Administrativo Común de las Administraciones Públicas, y el artículo 44.2d) de la Ley 1/1983, de 13 de diciembre, de Gobierno y Administración de la Comunidad de Madrid, todo ello sin perjuicio de interponer cualquier otro recurso que estime oportuno.

En Madrid, diecinueve de mayo de dos mil dieciocho. Firmado: Adolfo Ezquerra Canalejo, Director General de Inspección y Ordenación**."**

La anterior Resolución se notifica conforme a lo previsto en el artículo 40 de la Ley 39/2015, de 1 de octubre, del Procedimiento Administrativo Común de las Administraciones Públicas.

En Madrid,
LA SUBDIRECTORA GENERAL DE INSPECCIÓN Y ORDENACIÓN FARMACÉUTICA

Firmado digitalmente por MARÍA JESÚS GUILLÓ IZQUIERDO
Organización: COMUNIDAD DE MADRID
Fecha: 2018.05.28 15:33:58 CEST
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DESTINATARIO:

Fundación Pública Andaluza para la Gestión de
la Investigación en Salud de Sevilla (FISEVI)
Avda. Manuel Siurot s/n
41013 Sevilla

Resolution of the granting of the requested funding for the OptimBioma project issued by the ISCIII (The Instituto de Salud Carlos III (Institute of Health Carlos III)

GOBIERNO DE ESPAÑA · MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD · Instituto de Salud Carlos III

RESULTADO EXPEDIENTE - PI16/02010

Centro Solicitante: FUNDACION PUBLICA ANDALUZA PARA LA GESTION DE LA INVESTIGACION EN SALUD EN SEVILLA

Centro Realizador: INSTITUTO DE BIOMEDICINA DE SEVILLA - IBIS

Título: Optimización del tratamiento antibiótico en receptores de alotrasplante hematopoyético: impacto en la microbiota intestinal y en resultados clínicos

RESOLUCIÓN PROVISIONAL DE CONCESIÓN

Ayuda susceptible de ser cofinanciada por el FEDER"

Estado de Resolución Provisional de Concesión : CONCEDIDO

PRESUPUESTO CONCEDIDO PROVISIONAL				
	1ª ANUALIDAD	**2ª ANUALIDAD**	**3ª ANUALIDAD**	**TOTAL**
BIENES/SRV	50.000,00	20.000,00	20.000,00	**90.000,00**
PERSONAL	0,00	18.000,00	18.000,00	**36.000,00**
VIAJES	0,00	500,00	1.000,00	**1.500,00**
SUBTOTALES	**50.000,00**	**38.500,00**	**39.000,00**	**127.500,00**
Costes ind. 21,00 %	10.500,00	8.085,00	8.190,00	26.775,00
TOTALES	**60.500,00**	**46.585,00**	**47.190,00**	**154.275,00**

PERSONAL CONCEDIDO PROVISIONAL CON CARGO AL PROYECTO

Personal con Cargo	Concedido Provisional
Personal facultativo	1
Personal de enfermería	0

EQUIPO DE INVESTIGACIÓN

Nombre	Apellido 1	Apellido 2	Tipo	Ded.
FRANCISCO JOSE	MARQUEZ	MALAVER	Colaborador	COMPARTIDA
JOSE	GONZALEZ	CAMPOS	Colaborador	COMPARTIDA
MARIA ISABEL	MONTERO	CUADRADO	Colaborador	COMPARTIDA
CLARA MARIA	ROSSO	FERNANDEZ	Colaborador	COMPARTIDA
ILDEFONSO	ESPIGADO	TOCINO	IP	UNICA
JOSE FRANCISCO	FALANTES	GONZALEZ	Colaborador	COMPARTIDA
JOSE	MOLINA	GIL-BERMEJO	Colaborador	COMPARTIDA
GEMA	LABRADOR	HERRERA	Colaborador	COMPARTIDA
VIRGINIA	ESCAMILLA	GOMEZ	Colaborador	COMPARTIDA
Mª CARMEN	LIMON	RAMOS	Colaborador	COMPARTIDA
SILVIA	VERDESOTO	COZZARELLI	Colaborador	COMPARTIDA

RESOLUCIÓN PROVISIONAL DE CONCESIÓN

El Órgano instructor, visto el informe emitido por la Comisión de Selección, art. 9 de la Convocatoria AES 2016, regulada mediante la Resolución del Instituto de Salud Carlos III, de 28 de marzo de 2016 (extracto publicado en el BOE 01/04/2016 – código BDNS 302836), en su reunión de 22 de septiembre de 2015 y, de acuerdo con las disponibilidades presupuestarias, propone la financiación de la ayuda solicitada para la realización de su proyecto en los términos económicos indicados anteriormente. El presupuesto solicitado se ha modificado en el proceso de evaluación de acuerdo con la valoración económica de los objetivos propuestos y las limitaciones presupuestarias.

September 25, 2019

Adrian Aldcroft

Editor in Chief

BMJ Open

Dear Editor in Chief,

We are submitting to your consideration the Protocol manuscript entitled *"Assessing the impact on intestinal microbiome and clinical outcomes of antibiotherapy optimization strategies in hematopoietic stem cell transplant recipients: study protocol for the prospective multi-center OptimBioma study"*. The study was approved between 2017 and 2018 by the involved Ethical Committees and has undergone independent peer review to acquire funding.

The manuscript summarizes the used methodology in a prospective comparative observational multicenter study researching the effect of two center-driven antibacterial strategies, applied on two respective cohorts of hematopoietic stem cell transplant recipients, on intestinal microbiome composition and acute graft versus host disease rate and severity. All the two antibiotherapy strategies are described in the manuscript and named as *optimization* and *standard* strategies. The study is built on systematic collection of fecal samples at predetermined peri-transplant time-points and on prospective collection of a wide set of relevant clinical data throughout one year of follow up by means of scheduled clinical visits. This design will allow examining changes in the microbiome composition over time in both cohorts of recipients and linking them to the development of acute graft versus host disease and other relevant clinical outcomes.

The findings of this study will bring useful insight into the possible relationship between antibiotherapy use, intestinal microbiome misbalance and development of acute graft versus host disease in hematopoietic stem cell transplantation recipients.

This knowledge will help to design improved antibiotherapy strategies leading to better survival in this group of patients with frequent poor clinical outcomes.

This manuscript has been approved by all authors and it has not been published or submitted for publication elsewhere.

Thank you very much for your consideration.

Sincerely,

Dr. Ildefonso Espigado, PhD.

Corresponding author

Postal address: Department of Hematology, University Hospital Virgen del Rocío / University of Seville / CSIC / Institute of Biomedicine of Seville.

Avenida Manuel Siurot s/n - 41013, Seville, Spain

Telephone number: +34 636 096 808

E-mail: ildefonso.espigado.sspa@juntadeandalucia.es

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
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	4
	2b	All items from the World Health Organization Trial Registration Data Set	_____
Protocol version	3	Date and version identifier	20
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,20,21
	5b	Name and contact information for the trial sponsor	_____
	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	_____
	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)	20,21

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	7,8
	6b	Explanation for choice of comparators	10-12
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)	10-12
Methods: Participants, interventions, and outcomes			
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	9
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)	9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
	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)	_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_____
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	14-17
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)	12,13,Table

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	15
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____

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	_____
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	_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____

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	_____
	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	_____

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	_____
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	15-17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____
	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)	_____
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	_____
	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	_____
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	_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4,21,22
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)	21,22

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____
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	21,22
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
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	22
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	_____
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	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
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	_____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Assessing the impact on intestinal microbiome and clinical outcomes of antibiotherapy optimization strategies in hematopoietic stem cell transplant recipients: study protocol for the prospective multi-center OptimBioma study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-034570.R1
Article Type:	Protocol
Date Submitted by the Author:	11-Feb-2020

Complete List of Authors:	Jiménez-Jorge, Silvia; Clinical Trial Unit, University Hospital Virgen del Rocío/ University of Seville / CSIC / Institute of Biomedicine of Seville Labrador-Herrera, Gema; Clinical Unit of Infectious Diseases, Microbiology, and Preventive Medicine, University Hospital Virgen del Rocío / University of Seville / CSIC / Institute of Biomedicine of Seville Rosso-Fernández, Clara; Clinical Trial Unit, University Hospital Virgen del Rocío/ University of Seville / CSIC / Institute of Biomedicine of Seville; Clinical Pharmacology Department, University Hospital Virgen del Rocío Rodríguez-Torres, Nancy; Department of Hematology, University Hospital Virgen del Rocío / University of Seville / CSIC / Institute of Biomedicine of Seville Pachón-Ibáñez, María Eugenia ; Clinical Unit of Infectious Diseases, Microbiology, and Preventive Medicine, University Hospital Virgen del Rocío / University of Seville / CSIC / Institute of Biomedicine of Seville; Department of Medicine, School of Medicine; University of Seville Smani, Younes; Clinical Unit of Infectious Diseases, Microbiology, and Preventive Medicine, University Hospital Virgen del Rocío / University of Seville / CSIC / Institute of Biomedicine of Seville Márquez-Malaver, Francisco ; Department of Hematology, University Hospital Virgen del Rocío / University of Seville / CSIC / Institute of Biomedicine of Seville Limón Ramos, Carmen; Department of Hematology, University Hospital Virgen del Rocío / University of Seville / CSIC / Institute of Biomedicine of Seville Solano, Carlos; Department of Hematology, Hospital Clínico Universitario, Institute for Research INCLIVA; Department of Medicine, School of Medicine, University of Valencia Vázquez-López, Lourdes; Department of Hematology, University Hospital of Salamanca Kwon, Mi; Department of Hematology, Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón Mora Barrios, Joan; Department of Hematology, University Hospital Marqués de Valdecilla Aguilar-Guisado, Manuela; Clinical Unit of Infectious Diseases, Microbiology, and Preventive Medicine, University Hospital Virgen del Rocío / University of Seville / CSIC / Institute of Biomedicine of Seville Espigado, Ildefonso; Department of Hematology, University Hospital Virgen del Rocío / University of Seville / CSIC / Institute of Biomedicine

	of Seville
Primary Subject Heading:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Evidence based practice, Infectious diseases
Keywords:	microbiome, microbiota, hematopoietic stem cell transplantation, graft versus host disease, infections, antibiotics

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Assessing the impact on intestinal microbiome and clinical outcomes of antibiotherapy optimization strategies in hematopoietic stem cell transplant recipients: study protocol for the prospective multi-center OptimBioma study

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ABSTRACT

Introduction

Hematopoietic stem cell transplantation (HSCT) is a lifesaving treatment for a number of hematologic diseases. Graft versus host disease (GVHD) is its main complication and hampers survival. There is strong evidence that intestinal microbiota diversity of the recipient may increase the risk of GVHD worsening survival. Antibiotic regimens used during the early phase of the transplant may influence clinical outcomes by reducing intestinal microbiota diversity. Present guidelines of *European Conference on Infections in Leukemia* exhort to optimizing antibiotic use in hematologic patients including HSCT recipients.

The present study aims to investigate if, in HSCT recipients, the optimization of antibacterial use may preserve intestinal microbiota composition reducing the incidence and severity of acute GVHD and improving relevant clinical outcomes.

Methods and analysis

This is a prospective longitudinal observational study of two cohorts of HSCT recipients: i) the intervention cohort includes patients treated in centers in which a pre-defined strategy of antibiotherapy optimization is implemented, with the objective of optimizing and reducing antibiotic administration according to clinical criteria and ii) the control cohort includes patients treated in centers in which a classic permissive strategy of antibiotic prophylaxis and treatment is used. Adult patient receiving a first HSCT as a treatment for any hematologic condition are included. Clinical variables are prospectively recorded and up to five fecal samples are collected for microbiota characterization at pre-stablished

peri-transplant time-points. Patients are followed since the pre-conditioning phase throughout one-year post-transplant and four follow-up visits are scheduled. Fecal microbiota composition and diversity will be compared between both cohorts along with acute GVHD incidence and severity, severe infections rate, mortality and overall and disease-free survival.

Ethics and dissemination

The study was approved between 2017 and 2018 by the Ethical Committees of participant centers. Study results will be disseminated through peer-reviewed journals and national and international scientific conferences.

Trial registration number: ClinicalTrials.gov, NCT03727113. Registered on November 1st, 2018.

Keywords: microbiome, microbiota, hematopoietic stem cell transplantation, graft *versus* host disease, infections, antibiotics.

Word count: 3474

ARTICLE SUMMARY

Strengths and limitations of the study.

- First-in-class prospective comparative observational multicenter study addressing the effect of two different (centers-driven) antimicrobial strategies (optimized versus standard antimicrobial use) on intestinal microbiota diversity, acute graft versus host disease and survival in hematopoietic stem cell transplant recipients.
- Robust design by systematic collection of fecal samples at predetermined peri-transplant time-points and prospective collection of a wide relevant clinical data set throughout one-year follow up with scheduled clinical visits.
- Limitation: non-randomized design (for security reasons) with propensity score matching statistical approach to reduce possible bias by confounding variables.
- Limitation: no causal mechanistic association could be accurately concluded although meaningful cause-effect relationships should be advanced.
- Results may bring new insights into the relationship between antibiotherapy use, intestinal microbiome modification and development of acute graft versus host disease in hematopoietic stem cell transplantation recipients, helping to design improved strategies leading to better survival.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a lifesaving treatment for many severe hematological disorders. However, the deep immunosuppression associated to the procedure results in high risk of infectious complications prompting the administration of antimicrobial prophylaxis and therapy. Antibiotics fight off pathogenic bacteria but at the same time they may damage intestinal commensal bacteria leading to changes in intestinal microbiota composition and reduced diversity[1]. There is evidence that loss of intestinal microbiota diversity during HSCT may cause an increased risk of acute graft-versus-host disease (GVHD) worsening of short and long-term clinical outcomes.

Pioneering preclinical studies suggested that selective intestinal decontamination with antibiotics could reduce the incidence of GVHD[2 3] leading to the clinical use of pre-transplant antibacterial prophylaxis. These expectations were not subsequently confirmed in the clinical setting and this practice is not currently performed in many transplant centers. However, recent studies suggest that changes in intestinal microbiota composition may play an important role in the development of GVHD and in clinical outcomes of HSCT[1 4 5].

Therefore, antibacterial therapy strategies currently used in HSCT clinical practice should be re-evaluated in order to avoid as much as possible intestinal microbiota misbalance[6-8]. Our group has recently demonstrated in an academic prospective multicenter randomized clinical trial (*How-Long* study)[9], that in hematological patients (including HSCT recipients) with febrile neutropenia it is

safe to discontinue empirical antibiotic therapy after resolution of fever when patients are clinically stable, irrespective of their neutrophils counts, significantly reducing exposure to antibiotics. On the other hand, the ECIL group (European Conference of Infections in Leukemia) has proposed[10] specific empirical antibacterial therapy strategies in hematological patients including HSCT recipients, in order to optimized antibiotic use. These recommendations are heterogeneously implemented in the different hematopoietic transplant centers. This study will investigate if a predefined strategy of optimization of antimicrobial therapy that includes ECIL recommendations[10-12] and the *How-Long* study[9], will preserve intestinal microbiota composition and diversity while reducing the incidence and severity of acute GVHD when compared to a conventional permissive antibiotic strategy. In addition, severe infections rate, transplant related mortality and long-term survival will be compared between both groups.

METHODS AND ANALYSIS

Study design

A prospective longitudinal observational study of two cohorts of HSCT recipients was established: i) the intervention cohort includes patients treated at centers using an optimized strategy of antibacterial therapy (see *Intervention* section), ii) the control cohort includes patients treated at centers using a classical permissive antibacterial therapy strategy (see *Intervention* section). Each participating center is allocated in one of the two cohorts according to its clinical practice.

Study settings

Multicenter study conducted at five academic hospitals in Spain, two allocated to the intervention cohort [Virgen del Rocío University Hospital (Seville) and Marqués de Valdecilla University Hospital (Santander)] and three to the control cohort [Valencia Clinic Hospital (Valencia), Salamanca University Hospital (Salamanca), Gregorio Marañón University Hospital (Madrid)]. A four years study period is estimated (2017 – 2020).

Participants

Eligibility criteria

Inclusion Criteria

- Adult patients admitted to receive their first allogeneic hematopoietic transplant as a treatment for any hematological disease.
- Patients who have signed the study informed consent to participate.
- Patients who have received a previous autologous transplant are not excluded.

Exclusion criteria

- Non-compliance of the patient to sign the informed consent.
- Patients who have initiated the conditioning regimen previously to entering the study will not be included.

Recruitment process

Patients who meet the eligibility criteria and sign the informed consent will be recruited by investigators of the hematology services on the participating sites.

Intervention

Centers allocated to the intervention cohort use an antibacterial systematic approach that includes the following strategies:

1. No routine antibacterial prophylaxis is used.

2. In case of febrile neutropenia:

   - Use of scalation / de-scalation strategy for empiric antimicrobial therapy[10].

   - Directed simplification in patients with etiological diagnosis according to *in vitro* susceptibility tests.

   - Switch to a narrower-spectrum agent in patients without an etiological diagnosis and clinical stabilization on treatment.

   - No broadening the antibacterial spectrum but maintenance of initial antimicrobials therapy in patients with persistent fever if they are clinically stable in the criteria of the physicians in charge of theam.

3. Early (in 72 hours) withdrawal of combined treatments, when clinically indicated.

4. Antibacterial therapy withdrawal regardless neutrophils count and expected duration of neutropenia when patient meets all the following criteria (*How-Long* strategy)[9].

   i) Afebrile for ≥ 72 h.

ii) Complete resolution of signs, symptoms and alterations in complementary tests secondary to the infection (cough, abdominal pain, diarrhea, pulmonary infiltrate, etc.) for ≥ 72 h.

iii) Normal vital constants (blood pressure, heart rate, respiratory rate and diuresis and, in patients with respiratory involvement, oxygen saturation by pulse oximetry) for ≥72 h.

5. Short (7 days) etiological therapy for primary or related to central venous (with catheter removal) no complicated bacteremia, and 14 days for *Staphylococcus aureus* non complicated bacteremia if good clinical response and good clinical evolution.

In centers allocated to the control cohort the antimicrobial therapy approach does not include any of the strategies used in the optimization cohort but the following management:

1. Use of antibacterial prophylaxis.
2. In case of febrile neutropenia:

- Use of early broad-spectrum antimicrobial therapy without systematic use of escalation / de-escalation strategy.

- Optional antibiotic simplification in patients with etiological diagnosis according to *in vitro* susceptibility tests.

- No switching to a narrower-spectrum agent in patients without etiological diagnosis and clinical response.

- Broadening the spectrum of initial antimicrobials in patients with persistent fever even without clinical worsening.

3. No early discontinuation of the combined empirical antimicrobial therapy even in case of clinical response.
4. No discontinuation of empirical antimicrobial therapy until neutropenia recovery.
5. Prolonged etiological treatment for primary or related to central venous catheter no complicated bacteremia, even in case of early clinical response.

Schedule of visits and collection of fecal samples

The scheduled visits and assessments are described in table 1.

Follow-up is organized in four planned visits: Visit 1 (seven days pre-transplant), Visit 2 (end of antimicrobial therapy or hospital discharge, whichever occurs first), Visit 3 (100 days post-transplant), and Visit 4 or Final Visit (one-year post-transplant or mortality, whichever occurs first). A minimum of four fecal samples will be collected: Specimen 1, day of starting the conditioning treatment ± 48 hours; Specimen 2, day of transplantation ± 48 hours; Specimen 3, day +7 post-transplant ± 24 hours; Specimen 4, when the first episode of fever at any time from the beginning of the conditioning until the end of antimicrobial therapy or hospital discharge, whichever occurs first (this sample is collected at fever onset or within 48 hours, unless the fever starts on the same day that a scheduled fecal sample is already collected); and Specimen 5, day when the antimicrobial therapy is stopped (or within the following 48 hours) or,

alternatively, if the patient did not receive antibiotics or continues receiving antibiotics at discharge, the day before discharge (or 24 hours in advance).

The stool samples will be collected at different time points (table 1) in Stool Nucleic Acid Collection and Preservation Tubes (Ref. 45660, Norgen Biotek Corp.).

DNA extraction, prokaryotic 16S ribosomal RNA gene (16S rRNA) sequencing and bioinformatics analysis

The microbiome studies will be performed at the laboratory of Infectious Diseases of the Institute of Biomedicine of Seville (IBiS).

DNA will be extracted from faecal samples using the Stool DNA Isolation kit (Cat. 27600, Norgen Biotek Corp., Ontario, Canadá) according to the manufacturer's protocol. All DNA samples will be stored at −20 °C until further processing. DNA will be quantified by Qubit fluorometry (Invitrogen Qubit 4 Fluorometer, ThermoFisher Scientific, Spain) and normalized to 5 ng/μL with 10 mM Tris pH 8.5. Bacterial 16S rRNA amplification and library construction will be performed according to the 16S Metagenomic Sequencing Library Preparation guide from Illumina. Briefly, 2.5 μl of total DNA per sample will be amplified using primers targeting the 16S rRNA V3 and V4 regions[13-14]. These regions provide ample information for taxonomic classification of microbial communities. Pooled V3-V4 amplicon libraries will be sequenced using the Illumina MiSeq platform with a V3 reagent kit (Illumina, San Diego, California) and paired-end 300-bp reads. Up to 96 libraries could be pooled together for sequencing. Regarding the bioinformatics analysis of the sequencing data, machine learning libraries from Scikit-learn[15] will be used to filter out and

discard poor-quality reads. Processed sequences will be subjected to operational taxonomic unit (OTU) picking against Greengenes (v13.8)[16], with reads clustered by 97% identity into OTUs using QIIME 2[17]. In-house R scripts (v3.2.2) will be used to visualize the results.

Evaluation of results

In order to assess the impact of both antimicrobial strategies on intestinal microbiota diversity it will be characterized as described in the previous section and biological alpha and beta diversity indexes of samples from both cohorts will be compared[18]. Alpha-diversity and beta-diversity refer to diversity within and between samples, respectively. These secondary bioinformatics analyses will be performed with QIIME 2 and included the calculation of the parameters of alpha-diversity: Shannon's diversity index, frequency of observed OTUs, Faith's Phylogenetic Diversity, and Evenness; and the parameters of beta-diversity: Jaccard distance, Bray-Curtis distance, Unweighted UniFrac distance and Unweighted UniFrac distance.

In order to asses clinical outcomes (secondary objectives of the study) the following data will be prospectively recorded: incidence and severity of GVHD (evaluated for any degree, degree-II and degree-III/IV up to day 100 post-transplant), transplant related mortality and mortality caused by infection (time frame: Day 0 to Day +30, Day +100 and Day +365 post-transplant), incidence of severe infections (Day 0 to Day +30 post-transplant), and overall and disease free survival (time frame: Day 0 to Day +30, Day +100 and Day +365 post-transplant).

Sample size

Assuming a percentage of patients developing grade two or higher acute graft versus host disease[19] in the control cohort of 42%[5] and 20% in the intervention cohort, a power of 80% and an alpha error of 5%, 90 patients in each study cohort should be enough to detect differences between them. However, 100 patients per cohort will be included in order to compensate possible loss of statistical power due to associations between centers and the effect of the optimization strategy versus standard antimicrobial use (unknown at the time of the study design), to increase the statistical power for the secondary objectives (transplant related mortality, infections rate, mortality and survival), along with potential withdrawals[20-22].

Statistical analysis

To determine the impact of both antimicrobial therapy strategies on the intestinal microbiota diversity, in a first step, a multidimensional scaling and a permutational multivariate analysis of variance (PERMANOVA) analyses of both antibiotherapy strategies will be performed using the R statistical package (v3.2.2)[23]. To find out which taxa were most likely to explain the differences between both groups, taxa summaries generated in QIIME 2 will be reformatted for input into LEfSe via the Huttenhower Lab Galaxy Server (https://huttenhower.sph.harvard.edu/galaxy/root). This algorithm performs nonparametric statistical testing of whether individual taxa differed between both groups and then differentially ranked the abundant taxa by their linear discriminate analysis (LDA) log-scores. Differentially abundant taxa that are

statistically significant using an alpha error of 5% and LDA log-scores exceeding ±2.0 will be visually represented as bar plots. The median values of taxa abundance and the median percentages of taxa presence in both groups will be calculated, and the Manhattan distances will be used for the clustering analysis. The Kruskal-Wallis rank-sum test will be used to identify significant taxa abundance and Fisher's exact test will be used to identify significant taxa presence in the both groups.

The propensity score will be used to adjust potential confounding effects. To calculate the propensity scores in the logistic model the center factors and key predictive characteristics identified in the baseline comparability analysis will be taken into account. Propensity scores will be used for all adjusted inferential analyzes.

Standard descriptive statistical indices will be used according to the nature of each variable. Continuous variables will be analyzed with linear models, binary variables without time factor with logistic models and the time-to-event variables with survival models, all of them incorporating the propensity score as an adjustment factor.

The survival function of both groups will be described using the Kaplan-Meyer method. For the inferential analysis, the stratified log-rank test will be used (with the propensity score value categorized as stratum). Hazard Ratios and its 95% confidence intervals will be estimated using the Cox proportional hazards regression (including the propensity score value).

The following strategy will be used for time-dependent variables:

(a) Continuous variables that follow a Gaussian distribution by means of mixed models for repeated measures (mixed longitudinal model for repeated measurements (MMRM).

(b) Variables that do not comply with the parametric assumptions will be transformed into ranges and analyzed analogously to those in section a).

c) Longitudinal binary data will be analogously analyzed with the marginal models [Generalized Estimation Equation GEE).

In addition, the following statistical tests will be used when necessary: Fisher's exact test to compare categorical variables between groups, McNemar test or Cochran Q test for the analysis within the groups, dependent or independent t-test for continuous variables when comparing two groups and ANOVA if comparing more than two groups.

Nonparametric methods will be used in case of deviations from the applicability assumptions: according to the data distribution, Mann-Whitney and/or Kruskall-Wallis tests (independent variables), or Wilcoxon or Friedman tests (dependent variables). Correlations will be done with Pearson or Spearman coefficients according to the data distribution. SAS System (Release 9.2) or validated equivalent software will be used. All recruited patients will be included in the main analysis. In addition, a sensitivity analysis will be carried out with those subjects who have complied with the protocol.

Patient and public involvement

Neither patients nor public were involved in the development of the study.

DISCUSSION

The aim of this study is to prospectively investigate if an antimicrobial therapy in HSCT recipients has lesser impact on the intestinal microbiota composition and diversity than a non-restrictive standard antimicrobial therapy approach and if it correlates with a decrease incidence and severity of acute GVHD leading to improved clinical outcomes as reduced transplant related mortality, severe infections rate and improved survival. If this hypothesis proved to be certain, current antibacterial strategies in HSCT setting may need to be fully reviewed in order to avoid decrease the intestinal microbiota diversity. A prospective longitudinal observational study of two cohorts of patients will be used to address these objectives. The intervention cohort includes patients treated at two centers in which the antimicrobial therapy approach is optimized according to clinical criteria (as specified at intervention paragraph) and the control cohort includes three centers in which the classic management of antimicrobial therapy treatment is used (also specified at intervention paragraph).

One limitation of the study is a non-randomized design. Randomized controlled trials (RCT) are widely considered the design of choice for the assessment of effectiveness of healthcare intervention as the randomization process makes the comparison groups equal with respect to both known and unknown prognostic factors at baseline[24]. Nevertheless, RCT design is not applicable in this study. The implementation of a whole antibacterial therapy strategy in this frail setting of patients requires that it is solidly grounded in the daily practice of the clinical team, in order to be safe. The randomization scenario would implicate the use of unfamiliar antibacterial strategies by the clinical teams. This would be unsafe for patients and then ethically inadmissible. Therefore, an

observational study carried out in two groups of centers in which one of the two antibiotherapy approaches is already implemented turns out to be the safest and more feasible design. The propensity score matching statistical technique will reduced the possible bias due to confounding variables.

Another limitation of the study is that no causal association could be accurately described because of the observational design. Nevertheless, as an exhaustive set of prospective clinical data are being recorded for each patient, including start and stopping date of every antimicrobial used, dates of start and resolution of main clinical end-points it is likely that meaningful cause-effect relationships might be forwarded.

This is the first prospective multicenter study aiming to address the effect of two antimicrobial therapy strategies on intestinal microbiota and clinical outcomes in HSCT recipients. The systematic collection of faecal samples at predetermined peri-transplant time-points and the prospective collection of a wide clinical data set with one year follow up based in pre-scheduled repeated clinical visits will allow to examine changes in the microbiota composition over time and accurate link them to the development of acute GVHD and other clinical outcomes. Another strength of the study is its design based on daily clinical practice. This will provide valuable data on 'real-life patients' in addition to potential recommendations on sampling time points and frequency for further studies. In conclusion, the findings of this study will bring useful insight in the relationship between antibiotherapy use and development of acute graft versus host disease in HSCT recipients helping to design improved strategies expectedly leading to better survival, reduced graft versus host disease and improved quality of life.

Trial status

At submission the study is running and 140 patients are recruited.

Current approved protocol is version 3.0, dated 29/January/2018.

Date recruitment began at 16 January 2018 (First patient in).

Approximate date when recruitment will be completed: November 2019.

Abbreviations

ASCT: Allogeneic hemopoietic stem cell transplant; CTU: Clinical Trials Unit; CVC: central venous catheter; ECIL: European Conference of Infections in Leukemia; GEE: Generalized Estimation Equation; GVHD: Graft Versus Host Disease; ICH: International Council of Harmonisation; ITPP: intention-to-treat population; MMRM: Mixed longitudinal model for repeated measurements; MRT: mortality related to transplantation; PP: per-protocol population; RCT: Randomized Clinical Trial.

Author Contributions

IE conceived, designed the study and lead the study. IE and CR-F obtained funding for the research. SJ-J, GL-H, NR-T, FJM-M, MA-G, MEP-I and CR-F collaborated in methodological aspects of the study. SJ-J, NR-T, CR-F, MEP-I and CL-R coordinated the study. IE, CS, LV-L, MK, JMM-B and NR-T were responsible for the inclusion, treatment, clinical monitoring and follow-up of the patients. CL-R coordinated sample collection. GL-H, MEP-I and YS were responsible for the sample management. SJ-J wrote the first draft of the

manuscript. All authors were involved in critically revising the article and approved the final version.

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Competing interests

The authors declared no competing interests

Patient consent for Publication

Not required.

Ethics and dissemination

The study was approved between 2017 and 2018 by the five Ethical Committees involved (Comité Coordinador de Ética de la Investigación Biomédica de Andalucía, Comité de Ética de Investigación Clínica de Cantabria, Comité Autonómico de Evaluación de Estudios Posautorización Observacionales, de Seguimiento Prospectivo con medicamentos, Comité de Ética de la investigación con medicamentos del Hospital Univesitario de Salamanca, Dirección General de Inspección y Ordenación de la Consejería de Sanidad de la Comunidad de Madrid). Each substantial protocol amendment will be notified for approval to the relevant ethics committee(s) prior to implementation. All data collected will be kept strictly confidential and in accordance with all relevant legislation on control and protection of personal

information. Study results will be published in peer-reviewed journals as well as national and international scientific conferences.

Patient and Public Involvement

No patient involved.

Access to data

Data are sustained in an electronic database. Upon request to the corresponding author, the identified participant's data will be made available to researchers whose proposals meet the research criteria. It will be also considered requests for the protocol. To gain access, data requestors must comply to a data access agreement.

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Table 1. Schedule of enrollment and assessments. *OptimBioma study*.

	VISIT 1 (7 days Pre-transplant ± 48h)	Day of transplant (day 0) (±48h)	7 days post-transplant (day +7) (± 24h)	Day of Fever onset (if fever occurs) (+ 48h)	VISIT 2 (End anti-biotherapy or discharge)*	VISIT 3 (100 days post-transplant) Day +100	VISIT 4 (1 year post-transplant or *exitus letalis* ± 1 week)
Inclusion/Exclusion criterion	**X**						
Signature of informed consent	**X**						
Clinical Data Collection	**X**				**X**	**X**	**X**
Fecal Sample Collection	**Specimen 1**	**Specimen 2**	**Specimen 3**	**Specimen 4**	**Specimen 5***		

* End of antibiotic therapy or discharge, whichever occurs first.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
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	4
	2b	All items from the World Health Organization Trial Registration Data Set	_____
Protocol version	3	Date and version identifier	20
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,20,21
	5b	Name and contact information for the trial sponsor	_____
	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	_____
	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)	20,21

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	7,8
	6b	Explanation for choice of comparators	10-12
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)	10-12
Methods: Participants, interventions, and outcomes			
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	9
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)	9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
	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)	_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_____
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	14-17
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)	12,13,Table

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	15
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____
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	_____
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	_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____
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	_____
	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	_____

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	_____
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	15-17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____
	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)	_____
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	_____
	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	_____
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	_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4,21,22
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)	21,22

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____
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	21,22
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
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	22
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	_____
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	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
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	_____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Assessing the impact on intestinal microbiome and clinical outcomes of antibiotherapy optimization strategies in hematopoietic stem cell transplant recipients: study protocol for the prospective multi-center OptimBioma study

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	of Seville
Primary Subject Heading:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Evidence based practice, Infectious diseases
Keywords:	microbiome, microbiota, hematopoietic stem cell transplantation, graft versus host disease, infections, antibiotics

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Assessing the impact on intestinal microbiome and clinical outcomes of antibiotherapy optimization strategies in hematopoietic stem cell transplant recipients: study protocol for the prospective multi-center OptimBioma study

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ABSTRACT

Introduction

Hematopoietic stem cell transplantation (HSCT) is a lifesaving treatment for a number of hematologic diseases. Graft versus host disease (GVHD) is its main complication and hampers survival. There is strong evidence that intestinal microbiota diversity of the recipient may increase the risk of GVHD worsening survival. Antibiotic regimens used during the early phase of the transplant may influence clinical outcomes by reducing intestinal microbiota diversity. Present guidelines of *European Conference on Infections in Leukemia* exhort to optimizing antibiotic use in hematologic patients including HSCT recipients.

The present study aims to investigate if, in HSCT recipients, the optimization of antibacterial use may preserve intestinal microbiota composition reducing the incidence and severity of acute GVHD and improving relevant clinical outcomes.

Methods and analysis

This is a prospective longitudinal observational study of two cohorts of HSCT recipients: i) the intervention cohort includes patients treated in centers in which a pre-defined strategy of antibiotherapy optimization is implemented, with the objective of optimizing and reducing antibiotic administration according to clinical criteria and ii) the control cohort includes patients treated in centers in which a classic permissive strategy of antibiotic prophylaxis and treatment is used. Adult patient receiving a first HSCT as a treatment for any hematologic condition are included. Clinical variables are prospectively recorded and up to five fecal samples are collected for microbiota characterization at pre-stablished

peri-transplant time-points. Patients are followed since the pre-conditioning phase throughout one-year post-transplant and four follow-up visits are scheduled. Fecal microbiota composition and diversity will be compared between both cohorts along with acute GVHD incidence and severity, severe infections rate, mortality and overall and disease-free survival.

Ethics and dissemination

The study was approved between 2017 and 2018 by the Ethical Committees of participant centers. Study results will be disseminated through peer-reviewed journals and national and international scientific conferences.

Trial registration number: ClinicalTrials.gov, NCT03727113. Registered on November 1st, 2018.

Keywords: microbiome, microbiota, hematopoietic stem cell transplantation, graft *versus* host disease, infections, antibiotics.

Word count: 3474

ARTICLE SUMMARY

Strengths and limitations of the study.

- First-in-class prospective comparative observational multicenter study addressing the effect of two different (centers-driven) antimicrobial strategies (optimized versus standard antimicrobial use) on intestinal microbiota diversity, acute graft versus host disease and survival in hematopoietic stem cell transplant recipients.
- Robust design by systematic collection of fecal samples at predetermined peri-transplant time-points and prospective collection of a wide relevant clinical data set throughout one-year follow up with scheduled clinical visits.
- Limitation: non-randomized design (for security reasons) with propensity score matching statistical approach to reduce possible bias by confounding variables.
- Limitation: no causal mechanistic association could be accurately concluded although meaningful cause-effect relationships should be advanced.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a lifesaving treatment for many severe hematological disorders. However, the deep immunosuppression associated to the procedure results in high risk of infectious complications prompting the administration of antimicrobial prophylaxis and therapy. Antibiotics fight off pathogenic bacteria but at the same time they may damage intestinal commensal bacteria leading to changes in intestinal microbiota composition and reduced diversity[1]. There is evidence that loss of intestinal microbiota diversity during HSCT may cause an increased risk of acute graft-versus-host disease (GVHD) worsening of short and long-term clinical outcomes.

Pioneering preclinical studies suggested that selective intestinal decontamination with antibiotics could reduce the incidence of GVHD[2 3] leading to the clinical use of pre-transplant antibacterial prophylaxis. These expectations were not subsequently confirmed in the clinical setting and this practice is not currently performed in many transplant centers. However, recent studies suggest that changes in intestinal microbiota composition may play an important role in the development of GVHD and in clinical outcomes of HSCT[1 4 5].

Therefore, antibacterial therapy strategies currently used in HSCT clinical practice should be re-evaluated in order to avoid as much as possible intestinal microbiota misbalance[6-8]. Our group has recently demonstrated in an academic prospective multicenter randomized clinical trial (*How-Long* study)[9], that in hematological patients (including HSCT recipients) with febrile neutropenia it is

safe to discontinue empirical antibiotic therapy after resolution of fever when patients are clinically stable, irrespective of their neutrophils counts, significantly reducing exposure to antibiotics. On the other hand, the ECIL group (European Conference of Infections in Leukemia) has proposed[10] specific empirical antibacterial therapy strategies in hematological patients including HSCT recipients, in order to optimized antibiotic use. These recommendations are heterogeneously implemented in the different hematopoietic transplant centers. This study will investigate if a predefined strategy of optimization of antimicrobial therapy that includes ECIL recommendations[10-12] and the *How-Long* study[9], will preserve intestinal microbiota composition and diversity while reducing the incidence and severity of acute GVHD when compared to a conventional permissive antibiotic strategy. In addition, severe infections rate, transplant related mortality and long-term survival will be compared between both groups.

METHODS AND ANALYSIS

Study design

A prospective longitudinal observational study of two cohorts of HSCT recipients was established: i) the intervention cohort includes patients treated at centers using an optimized strategy of antibacterial therapy (see *Intervention* section), ii) the control cohort includes patients treated at centers using a classical permissive antibacterial therapy strategy (see *Intervention* section). Each participating center is allocated in one of the two cohorts according to its clinical practice.

Study settings

Multicenter study conducted at five academic hospitals in Spain, two allocated to the intervention cohort [Virgen del Rocío University Hospital (Seville) and Marqués de Valdecilla University Hospital (Santander)] and three to the control cohort [Valencia Clinic Hospital (Valencia), Salamanca University Hospital (Salamanca), Gregorio Marañón University Hospital (Madrid)]. A four years study period is estimated (2017 – 2020).

Participants

Eligibility criteria

Inclusion Criteria

- Adult patients admitted to receive their first allogeneic hematopoietic transplant as a treatment for any hematological disease.
- Patients who have signed the study informed consent to participate.
- Patients who have received a previous autologous transplant are not excluded.

Exclusion criteria

- Non-compliance of the patient to sign the informed consent.
- Patients who have initiated the conditioning regimen previously to entering the study will not be included.

Recruitment process

Patients who meet the eligibility criteria and sign the informed consent will be recruited by investigators of the hematology services on the participating sites.

Intervention

Centers allocated to the intervention cohort use an antibacterial systematic approach that includes the following strategies:

1. No routine antibacterial prophylaxis is used.

2. In case of febrile neutropenia:

   - Use of scalation / de-scalation strategy for empiric antimicrobial therapy[10].

   - Directed simplification in patients with etiological diagnosis according to *in vitro* susceptibility tests.

   - Switch to a narrower-spectrum agent in patients without an etiological diagnosis and clinical stabilization on treatment.

   - No broadening the antibacterial spectrum but maintenance of initial antimicrobials therapy in patients with persistent fever if they are clinically stable in the criteria of the physicians in charge of theam.

3. Early (in 72 hours) withdrawal of combined treatments, when clinically indicated.

4. Antibacterial therapy withdrawal regardless neutrophils count and expected duration of neutropenia when patient meets all the following criteria (*How-Long* strategy)[9].

   i) Afebrile for ≥ 72 h.

ii) Complete resolution of signs, symptoms and alterations in complementary tests secondary to the infection (cough, abdominal pain, diarrhea, pulmonary infiltrate, etc.) for ≥ 72 h.

iii) Normal vital constants (blood pressure, heart rate, respiratory rate and diuresis and, in patients with respiratory involvement, oxygen saturation by pulse oximetry) for ≥72 h.

5. Short (7 days) etiological therapy for primary or related to central venous (with catheter removal) no complicated bacteremia, and 14 days for *Staphylococcus aureus* non complicated bacteremia if good clinical response and good clinical evolution.

In centers allocated to the control cohort the antimicrobial therapy approach does not include any of the strategies used in the optimization cohort but the following management:

1. Use of antibacterial prophylaxis: levofloxacine 500 mg/24h (PO) or ciprofloxacine 500 mg/24h (PO) since the start of conditioning or day 0, until neutrophils count in peripheral blood is ≥0.5x10E9/L or empiric antimicrobial therapy is started.

2. In case of febrile neutropenia:

- Use of early broad-spectrum antimicrobial therapy without systematic use of escalation / de-escalation strategy.

- Optional antibiotic simplification in patients with etiological diagnosis according to *in vitro* susceptibility tests.

- No switching to a narrower-spectrum agent in patients without etiological diagnosis and clinical response.

- Broadening the spectrum of initial antimicrobials in patients with persistent fever even without clinical worsening.

3. No early discontinuation of the combined empirical antimicrobial therapy even in case of clinical response.
4. No discontinuation of empirical antimicrobial therapy until neutropenia recovery.
5. Prolonged etiological treatment for primary or related to central venous catheter no complicated bacteremia, even in case of early clinical response.

Schedule of visits and collection of fecal samples

The scheduled visits and assessments are described in table 1.

Follow-up is organized in four planned visits: Visit 1 (seven days pre-transplant), Visit 2 (end of antimicrobial therapy or hospital discharge, whichever occurs first), Visit 3 (100 days post-transplant), and Visit 4 or Final Visit (one-year post-transplant or mortality, whichever occurs first). A minimum of four fecal samples will be collected: Specimen 1, day of starting the conditioning treatment ± 48 hours; Specimen 2, day of transplantation ± 48 hours; Specimen 3, day +7 post-transplant ± 24 hours; Specimen 4, when the first episode of fever at any time from the beginning of the conditioning until the end of antimicrobial therapy or hospital discharge, whichever occurs first (this sample is collected at fever onset or within 48 hours, unless the fever starts on the same day that a scheduled fecal sample is already collected); and Specimen 5, day when the

antimicrobial therapy is stopped (or within the following 48 hours) or, alternatively, if the patient did not receive antibiotics or continues receiving antibiotics at discharge, the day before discharge (or 24 hours in advance).

The stool samples will be collected at different time points (table 1) in Stool Nucleic Acid Collection and Preservation Tubes (Ref. 45660, Norgen Biotek Corp.).

DNA extraction, prokaryotic 16S ribosomal RNA gene (16S rRNA) sequencing and bioinformatics analysis

The microbiome studies will be performed at the laboratory of Infectious Diseases of the Institute of Biomedicine of Seville (IBiS).

DNA will be extracted from faecal samples using the Stool DNA Isolation kit (Cat. 27600, Norgen Biotek Corp., Ontario, Canadá) according to the manufacturer's protocol. All DNA samples will be stored at −20 °C until further processing. DNA will be quantified by Qubit fluorometry (Invitrogen Qubit 4 Fluorometer, ThermoFisher Scientific, Spain) and normalized to 5 ng/μL with 10 mM Tris pH 8.5. Bacterial 16S rRNA amplification and library construction will be performed according to the 16S Metagenomic Sequencing Library Preparation guide from Illumina. Briefly, 2.5 μl of total DNA per sample will be amplified using primers targeting the 16S rRNA V3 and V4 regions[13-14]. These regions provide ample information for taxonomic classification of microbial communities. Pooled V3-V4 amplicon libraries will be sequenced using the Illumina MiSeq platform with a V3 reagent kit (Illumina, San Diego, California) and paired-end 300-bp reads. Up to 96 libraries could be pooled together for sequencing. Regarding the bioinformatics analysis of the sequencing data,

machine learning libraries from Scikit-learn[15] will be used to filter out and discard poor-quality reads. Processed sequences will be subjected to operational taxonomic unit (OTU) picking against Greengenes (v13.8)[16], with reads clustered by 97% identity into OTUs using QIIME 2[17]. In-house R scripts (v3.2.2) will be used to visualize the results.

Evaluation of results

In order to assess the impact of both antimicrobial strategies on intestinal microbiota diversity it will be characterized as described in the previous section and biological alpha and beta diversity indexes of samples from both cohorts will be compared[18]. Alpha-diversity and beta-diversity refer to diversity within and between samples, respectively. These secondary bioinformatics analyses will be performed with QIIME 2 and included the calculation of the parameters of alpha-diversity: Shannon's diversity index, frequency of observed OTUs, Faith's Phylogenetic Diversity, and Evenness; and the parameters of beta-diversity: Jaccard distance, Bray-Curtis distance, Unweighted UniFrac distance and Unweighted UniFrac distance.

In order to asses clinical outcomes (secondary objectives of the study) the following data will be prospectively recorded: incidence and severity of GVHD (evaluated for any degree, degree-II and degree-III/IV up to day 100 post-transplant), transplant related mortality and mortality caused by infection (time frame: Day 0 to Day +30, Day +100 and Day +365 post-transplant), incidence of severe infections (Day 0 to Day +30 post-transplant), and overall and disease free survival (time frame: Day 0 to Day +30, Day +100 and Day +365 post-transplant).

Sample size

Assuming a percentage of patients developing grade two or higher acute graft versus host disease[19] in the control cohort of 42%[5] and 20% in the intervention cohort, a power of 80% and an alpha error of 5%, 90 patients in each study cohort should be enough to detect differences between them. However, 100 patients per cohort will be included in order to compensate possible loss of statistical power due to associations between centers and the effect of the optimization strategy versus standard antimicrobial use (unknown at the time of the study design), to increase the statistical power for the secondary objectives (transplant related mortality, infections rate, mortality and survival), along with potential withdrawals[20-22].

Statistical analysis

To determine the impact of both antimicrobial therapy strategies on the intestinal microbiota diversity, in a first step, a multidimensional scaling and a permutational multivariate analysis of variance (PERMANOVA) analyses of both antibiotherapy strategies will be performed using the R statistical package (v3.2.2)[23]. To find out which taxa were most likely to explain the differences between both groups, taxa summaries generated in QIIME 2 will be reformatted for input into LEfSe via the Huttenhower Lab Galaxy Server (https://huttenhower.sph.harvard.edu/galaxy/root). This algorithm performs nonparametric statistical testing of whether individual taxa differed between both groups and then differentially ranked the abundant taxa by their linear

discriminate analysis (LDA) log-scores. Differentially abundant taxa that are statistically significant using an alpha error of 5% and LDA log-scores exceeding ±2.0 will be visually represented as bar plots. The median values of taxa abundance and the median percentages of taxa presence in both groups will be calculated, and the Manhattan distances will be used for the clustering analysis. The Kruskal-Wallis rank-sum test will be used to identify significant taxa abundance and Fisher's exact test will be used to identify significant taxa presence in the both groups.

The propensity score will be used to adjust potential confounding effects. To calculate the propensity scores in the logistic model the center factors and key predictive characteristics identified in the baseline comparability analysis will be taken into account. Propensity scores will be used for all adjusted inferential analyzes.

Standard descriptive statistical indices will be used according to the nature of each variable. Continuous variables will be analyzed with linear models, binary variables without time factor with logistic models and the time-to-event variables with survival models, all of them incorporating the propensity score as an adjustment factor.

The survival function of both groups will be described using the Kaplan-Meyer method. For the inferential analysis, the stratified log-rank test will be used (with the propensity score value categorized as stratum). Hazard Ratios and its 95% confidence intervals will be estimated using the Cox proportional hazards regression (including the propensity score value).

The following strategy will be used for time-dependent variables:

(a) Continuous variables that follow a Gaussian distribution by means of mixed models for repeated measures (mixed longitudinal model for repeated measurements (MMRM).

(b) Variables that do not comply with the parametric assumptions will be transformed into ranges and analyzed analogously to those in section a).

c) Longitudinal binary data will be analogously analyzed with the marginal models [Generalized Estimation Equation GEE).

In addition, the following statistical tests will be used when necessary: Fisher's exact test to compare categorical variables between groups, McNemar test or Cochran Q test for the analysis within the groups, dependent or independent t-test for continuous variables when comparing two groups and ANOVA if comparing more than two groups.

Nonparametric methods will be used in case of deviations from the applicability assumptions: according to the data distribution, Mann-Whitney and/or Kruskall-Wallis tests (independent variables), or Wilcoxon or Friedman tests (dependent variables). Correlations will be done with Pearson or Spearman coefficients according to the data distribution. SAS System (Release 9.2) or validated equivalent software will be used. All recruited patients will be included in the main analysis. In addition, a sensitivity analysis will be carried out with those subjects who have complied with the protocol.

Patient and public involvement

Neither patients nor public were involved in the development of the study.

DISCUSSION

The aim of this study is to prospectively investigate if an antimicrobial therapy in HSCT recipients has lesser impact on the intestinal microbiota composition and diversity than a non-restrictive standard antimicrobial therapy approach and if it correlates with a decrease incidence and severity of acute GVHD leading to improved clinical outcomes as reduced transplant related mortality, severe infections rate and improved survival. If this hypothesis proved to be certain, current antibacterial strategies in HSCT setting may need to be fully reviewed in order to avoid decrease the intestinal microbiota diversity. A prospective longitudinal observational study of two cohorts of patients will be used to address these objectives. The intervention cohort includes patients treated at two centers in which the antimicrobial therapy approach is optimized according to clinical criteria (as specified at intervention paragraph) and the control cohort includes three centers in which the classic management of antimicrobial therapy treatment is used (also specified at intervention paragraph).

One limitation of the study is a non-randomized design. Randomized controlled trials (RCT) are widely considered the design of choice for the assessment of effectiveness of healthcare intervention as the randomization process makes the comparison groups equal with respect to both known and unknown prognostic factors at baseline[24]. Nevertheless, RCT design is not applicable in this study. The implementation of a whole antibacterial therapy strategy in this frail setting of patients requires that it is solidly grounded in the daily practice of the clinical team, in order to be safe. The randomization scenario would implicate the use of unfamiliar antibacterial strategies by the clinical teams. This would be unsafe for patients and then ethically inadmissible. Therefore, an

observational study carried out in two groups of centers in which one of the two antibiotherapy approaches is already implemented turns out to be the safest and more feasible design. The propensity score matching statistical technique will reduced the possible bias due to confounding variables.

Another limitation of the study is that no causal association could be accurately described because of the observational design. Nevertheless, as an exhaustive set of prospective clinical data are being recorded for each patient, including start and stopping date of every antimicrobial used, dates of start and resolution of main clinical end-points it is likely that meaningful cause-effect relationships might be forwarded.

This is the first prospective multicenter study aiming to address the effect of two antimicrobial therapy strategies on intestinal microbiota and clinical outcomes in HSCT recipients. The systematic collection of faecal samples at predetermined peri-transplant time-points and the prospective collection of a wide clinical data set with one year follow up based in pre-scheduled repeated clinical visits will allow to examine changes in the microbiota composition over time and accurate link them to the development of acute GVHD and other clinical outcomes. Another strength of the study is its design based on daily clinical practice. This will provide valuable data on 'real-life patients' in addition to potential recommendations on sampling time points and frequency for further studies. In conclusion, the findings of this study will bring useful insight in the relationship between antibiotherapy use and development of acute graft versus host disease in HSCT recipients helping to design improved strategies expectedly leading to better survival, reduced graft versus host disease and improved quality of life.

Trial status

At submission the study is running and 140 patients are recruited.

Current approved protocol is version 3.0, dated 29/January/2018.

Date recruitment began at 16 January 2018 (first patient in).

Approximate date when recruitment will be completed: November 2019.

Abbreviations

ASCT: Allogeneic hemopoietic stem cell transplant; CTU: Clinical Trials Unit; CVC: central venous catheter; ECIL: European Conference of Infections in Leukemia; GEE: Generalized Estimation Equation; GVHD: Graft Versus Host Disease; ICH: International Council of Harmonisation; ITPP: intention-to-treat population; MMRM: Mixed longitudinal model for repeated measurements; MRT: mortality related to transplantation; PP: per-protocol population; RCT: Randomized Clinical Trial.

Author Contributions

IE conceived, designed and lead the study. IE and CR-F obtained funding for the research. SJ-J, GL-H, NR-T, FJM-M, MA-G, MEP-I and CR-F collaborated in methodological aspects of the study. SJ-J, NR-T, CR-F, MEP-I and CL-R coordinated the study. IE, CS, LV-L, MK, JMM-B and NR-T were responsible for the inclusion, treatment, clinical monitoring and follow-up of the patients. CL-R coordinated sample collection. GL-H, MEP-I and YS were responsible for the sample management. SJ-J wrote the first draft of the manuscript. All authors were involved in critically revising the article and approved the final version.

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Competing interests

The authors declared no competing interests

Patient consent for Publication

Not required.

Ethics and dissemination

The study was approved between 2017 and 2018 by the five Ethical Committees involved (Comité Coordinador de Ética de la Investigación Biomédica de Andalucía, Comité de Ética de Investigación Clínica de Cantabria, Comité Autonómico de Evaluación de Estudios Posautorización Observacionales, de Seguimiento Prospectivo con medicamentos, Comité de Ética de la investigación con medicamentos del Hospital Univesitario de Salamanca, Dirección General de Inspección y Ordenación de la Consejería de Sanidad de la Comunidad de Madrid). Each substantial protocol amendment will be notified for approval to the relevant ethics committee(s) prior to implementation. All data collected will be kept strictly confidential and in accordance with all relevant legislation on control and protection of personal information. Study results will be published in peer-reviewed journals as well as national and international scientific conferences.

Patient and Public Involvement

No patient involved.

Access to data

Data are sustained in an electronic database. Upon request to the corresponding author, the identified participant's data will be made available to researchers whose proposals meet the research criteria. It will be also considered requests for the protocol. To gain access, data requestors must comply to a data access agreement.

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Table 1. Schedule of enrollment and assessments. *OptimBioma study*.

	VISIT 1 (7 days Pre-transplant ± 48h)	Day of transplant (day 0) (±48h)	7 days post-transplant (day +7) (± 24h)	Day of Fever onset (if fever occurs) (+ 48h)	VISIT 2 (End anti-biotherapy or discharge)*	VISIT 3 (100 days post-transplant) Day +100	VISIT 4 (1 year post-transplant or *exitus letalis* ± 1 week)
Inclusion/Exclusion criterion	**X**						
Signature of informed consent	**X**						
Clinical Data Collection	**X**				**X**	**X**	**X**
Fecal Sample Collection	**Specimen 1**	**Specimen 2**	**Specimen 3**	**Specimen 4**	**Specimen 5***		

* End of antibiotic therapy or discharge, whichever occurs first.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
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	4
	2b	All items from the World Health Organization Trial Registration Data Set	_____
Protocol version	3	Date and version identifier	20
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,20,21
	5b	Name and contact information for the trial sponsor	_____
	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	_____
	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)	20,21

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	7,8
	6b	Explanation for choice of comparators	10-12
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)	10-12
Methods: Participants, interventions, and outcomes			
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	9
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)	9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
	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)	_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_____
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	14-17
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)	12,13,Table

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	15
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____
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	_____
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	_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____
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	_____
	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	_____

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	_____
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	15-17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____
	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)	_____
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	_____
	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	_____
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	_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4,21,22
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)	21,22

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____
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	21,22
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
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	22
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	_____
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	22
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
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	_____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.