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# 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
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

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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.

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3 1 Assessing the impact on intestinal microbiome and clinical outcomes of  
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5 2 antibiotherapy optimization strategies in hematopoietic stem cell transplant  
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7 3 recipients: study protocol for the prospective multi-center OptimBioma study  
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24 10 Trasplante Hematopoyético y Terapia Celular).  
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## 42 **ABSTRACT**

### 43 Introduction

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

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

### 56 Methods and analysis

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



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3 66 peri-transplant time-points. Patients are followed since the pre-conditioning  
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5 67 phase throughout one-year post-transplant and four follow-up visits are  
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7 68 scheduled. Fecal microbiota composition and diversity will be compared  
8  
9 69 between both cohorts along with acute GVHD incidence and severity, severe  
10  
11 70 infections rate, mortality and overall and disease-free survival.  
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#### 15 71 Ethics and dissemination

16  
17  
18 72 The study was approved between 2017 and 2018 by the Ethical Committees of  
19  
20 73 participant centers. Study results will be disseminated through peer-reviewed  
21  
22 74 journals and national and international scientific conferences.  
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26 75 Trial registration number: ClinicalTrials.gov, NCT03727113. Registered on  
27  
28 76 November 1<sup>st</sup>, 2018.  
29  
30

31  
32 77 Keywords: microbiome, microbiota, hematopoietic stem cell transplantation,  
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34 78 graft *versus* host disease, infections, antibiotics.  
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40 80 Word count: 3474  
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## 82 ARTICLE SUMMARY

### 83 Strengths and limitations of the study.

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

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

For peer review only

## 111 INTRODUCTION

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

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

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

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3 135 safe to discontinue empirical antibiotic therapy after resolution of fever when  
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5 136 patients are clinically stable, irrespective of their neutrophils counts, significantly  
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7 137 reducing exposure to antibiotics. On the other hand, the ECIL group (European  
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9 138 Conference of Infections in Leukemia) has proposed<sup>10</sup> specific empirical  
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11 139 antibacterial therapy strategies in hematological patients including HSCT  
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14 140 recipients, in order to optimized antibiotic use. These recommendations are  
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17 141 heterogeneously implemented in the different hematopoietic transplant centers.  
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19 142 This study will investigate if a predefined strategy of optimization of  
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21 143 antimicrobial therapy that includes ECIL recommendations<sup>10-12</sup> and the *How-*  
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23 144 *Long* study<sup>9</sup>, will preserve intestinal microbiota composition and diversity while  
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26 145 reducing the incidence and severity of acute GVHD when compared to a  
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28 146 conventional permissive antibiotic strategy. In addition, severe infections rate,  
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30 147 transplant related mortality and long-term survival will be compared between  
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33 148 both groups.

## 149 **METHODS AND ANALYSIS**

### 150 **Study design**

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

158

## 159 **Study settings**

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

## 166 **Participants**

### 167 *Eligibility criteria*

#### 168 Inclusion Criteria

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

#### 174 Exclusion criteria

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

## 178 **Recruitment process**

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

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3 181 **Intervention**  
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6 182 Centers allocated to the intervention cohort use an antibacterial systematic  
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8 183 approach that includes the following strategies:  
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11 184 1. No routine antibacterial prophylaxis is used.  
12  
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14  
15 185 2. In case of febrile neutropenia:  
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17  
18 186 - Use of escalation / de-escalation strategy for empiric antimicrobial  
19  
20 187 therapy<sup>10</sup>.  
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22  
23 188 - Directed simplification in patients with etiological diagnosis according to  
24  
25 189 *in vitro* susceptibility tests.  
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27  
28  
29 190 - Switch to a narrower-spectrum agent in patients without an etiological  
30  
31 191 diagnosis and clinical stabilization on treatment.  
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33  
34 192 - No broadening the antibacterial spectrum but maintenance of initial  
35  
36 193 antimicrobials therapy in patients with persistent fever if they are clinically  
37  
38 194 stable in the criteria of the physicians in charge of them.  
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40  
41  
42 195 3. Early (in 72 hours) withdrawal of combined treatments, when clinically  
43  
44 196 indicated.  
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47 197 4. Antibacterial therapy withdrawal regardless neutrophils count and  
48  
49 198 expected duration of neutropenia when patient meets all the following  
50  
51 199 criteria (*How-Long* strategy)<sup>9</sup>.  
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54  
55 200 i) Afebrile for  $\geq 72$  h.  
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3 201 ii) Complete resolution of signs, symptoms and alterations in  
4  
5 202 complementary tests secondary to the infection (cough, abdominal  
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7 203 pain, diarrhea, pulmonary infiltrate, etc.) for  $\geq 72$  h.  
8  
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11 204 iii) Normal vital constants (blood pressure, heart rate, respiratory rate  
12  
13 205 and diuresis and, in patients with respiratory involvement, oxygen  
14  
15 206 saturation by pulse oximetry) for  $\geq 72$  h.  
16  
17

18  
19 207 5. Short (7 days) etiological therapy for primary or related to central  
20  
21 208 venous (with catheter removal) no complicated bacteremia, and 14  
22  
23 209 days for *Staphylococcus aureus* non complicated bacteremia if good  
24  
25 210 clinical response and good clinical evolution.  
26  
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29 211 In centers allocated to the control cohort the antimicrobial therapy approach  
30  
31 212 does not include any of the strategies used in the optimization cohort but the  
32  
33 213 following management:  
34  
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36 214 1. Use of antibacterial prophylaxis.  
37

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39 215 2. In case of febrile neutropenia:  
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42 216 - Use of early broad-spectrum antimicrobial therapy without systematic  
43  
44 217 use of escalation / de-escalation strategy.  
45  
46

47 218 - Optional antibiotic simplification in patients with etiological diagnosis  
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49 219 according to *in vitro* susceptibility tests.  
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52 220 - No switching to a narrower-spectrum agent in patients without  
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54 221 etiological diagnosis and clinical response.  
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3 222 - Broadening the spectrum of initial antimicrobials in patients with  
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5 223 persistent fever even without clinical worsening.  
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8 224 3. No early discontinuation of the combined empirical antimicrobial therapy  
9  
10 even in case of clinical response.  
11 225  
12  
13 226 4. No discontinuation of empirical antimicrobial therapy until neutropenia  
14  
15 227 recovery.  
16  
17 228 5. Prolonged etiological treatment for primary or related to central venous  
18  
19 catheter no complicated bacteremia, even in case of early clinical  
20 229  
21 response.  
22 230  
23

### 231 **Schedule of visits and collection of fecal samples**

232 The scheduled visits and assessments are described in table 1.

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

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3 245 alternatively, if the patient did not receive antibiotics or continues receiving  
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5 246 antibiotics at discharge, the day before discharge (or 24 hours in advance).  
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8 247 The stool samples will be collected at different time points (table 1) in Stool  
9  
10 248 Nucleic Acid Collection and Preservation Tubes (Ref. 45660, Norgen Biotek  
11  
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13 249 Corp.).  
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15

16 250 **DNA extraction, prokaryotic 16S ribosomal RNA gene (16S rRNA)**  
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18 251 **sequencing and bioinformatics analysis**  
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21 252 The microbiome studies will be performed at the laboratory of Infectious  
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23 253 Diseases of the Institute of Biomedicine of Seville (IBiS).  
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27 254 DNA will be extracted from faecal samples using the Stool DNA Isolation kit  
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29 255 (Cat. 27600, Norgen Biotek Corp., Ontario, Canadá) according to the  
30  
31 256 manufacturer's protocol. All DNA samples will be stored at  $-20^{\circ}\text{C}$  until further  
32  
33 257 processing. DNA will be quantified by Qubit fluorometry (Invitrogen Qubit 4  
34  
35 258 Fluorometer, ThermoFisher Scientific, Spain) and normalized to  $5\text{ ng}/\mu\text{L}$  with  $10$   
36  
37 259  $\text{mM}$  Tris pH 8.5. Bacterial 16S rRNA amplification and library construction will  
38  
39 260 be performed according to the 16S Metagenomic Sequencing Library  
40  
41 261 Preparation guide from Illumina. Briefly,  $2.5\ \mu\text{L}$  of total DNA per sample will be  
42  
43 262 amplified using primers targeting the 16S rRNA V3 and V4 regions<sup>13-14</sup>. These  
44  
45 263 regions provide ample information for taxonomic classification of microbial  
46  
47 264 communities. Pooled V3-V4 amplicon libraries will be sequenced using the  
48  
49 265 Illumina MiSeq platform with a V3 reagent kit (Illumina, San Diego, California)  
50  
51 266 and paired-end 300-bp reads. Up to 96 libraries could be pooled together for  
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53 267 sequencing. Regarding the bioinformatics analysis of the sequencing data,  
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55 268 machine learning libraries from Scikit-learn<sup>15</sup> will be used to filter out and  
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3 269 discard poor-quality reads. Processed sequences will be subjected to  
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5 270 operational taxonomic unit (OTU) picking against Greengenes (v13.8)<sup>16</sup>, with  
6  
7 271 reads clustered by 97% identity into OTUs using QIIME 2<sup>17</sup>. In-house R scripts  
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10 272 (v3.2.2) will be used to visualize the results.

### 13 273 **Evaluation of results**

16 274 In order to assess the impact of both antimicrobial strategies on intestinal  
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18 275 microbiota diversity it will be characterized as described in the previous section  
19  
20 276 and biological alpha and beta diversity indexes of samples from both cohorts  
21  
22 277 will be compared<sup>18</sup>. Alpha-diversity and beta-diversity refer to diversity within  
23  
24 278 and between samples, respectively. These secondary bioinformatics analyses  
25  
26 279 will be performed with QIIME 2 and included the calculation of the parameters  
27  
28 280 of alpha-diversity: Shannon's diversity index, frequency of observed OTUs,  
29  
30 281 Faith's Phylogenetic Diversity, and Evenness; and the parameters of beta-  
31  
32 282 diversity: Jaccard distance, Bray-Curtis distance, Unweighted UniFrac distance  
33  
34 283 and Unweighted UniFrac distance.

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38  
39 284 In order to assess clinical outcomes (secondary objectives of the study) the  
40  
41 285 following data will be prospectively recorded: incidence and severity of GVHD  
42  
43 286 (evaluated for any degree, degree-II and degree-III/IV up to day 100 post-  
44  
45 287 transplant), transplant related mortality and mortality caused by infection (time  
46  
47 288 frame: Day 0 to Day +30, Day +100 and Day +365 post-transplant), incidence of  
48  
49 289 severe infections (Day 0 to Day +30 post-transplant), and overall and disease  
50  
51 290 free survival (time frame: Day 0 to Day +30, Day +100 and Day +365 post-  
52  
53 291 transplant).

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## 293 **Sample size**

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

304

## 305 **Statistical analysis**

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

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2  
3 317 statistically significant using an alpha error of 5% and LDA log-scores  
4  
5 318 exceeding  $\pm 2.0$  will be visually represented as bar plots. The median values of  
6  
7 319 taxa abundance and the median percentages of taxa presence in both groups  
8  
9  
10 320 will be calculated, and the Manhattan distances will be used for the clustering  
11  
12 321 analysis. The Kruskal-Wallis rank-sum test will be used to identify significant  
13  
14 322 taxa abundance and Fisher's exact test will be used to identify significant taxa  
15  
16  
17 323 presence in the both groups.

18  
19  
20 324 The propensity score will be used to adjust potential confounding effects. To  
21  
22 325 calculate the propensity scores in the logistic model the center factors and key  
23  
24 326 predictive characteristics identified in the baseline comparability analysis will be  
25  
26  
27 327 taken into account. Propensity scores will be used for all adjusted inferential  
28  
29 328 analyzes.

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31  
32 329 Standard descriptive statistical indices will be used according to the nature of  
33  
34 330 each variable. Continuous variables will be analyzed with linear models, binary  
35  
36  
37 331 variables without time factor with logistic models and the time-to-event variables  
38  
39 332 with survival models, all of them incorporating the propensity score as an  
40  
41 333 adjustment factor.

42  
43  
44 334 The survival function of both groups will be described using the Kaplan-Meier  
45  
46 335 method. For the inferential analysis, the stratified log-rank test will be used (with  
47  
48 336 the propensity score value categorized as stratum). Hazard Ratios and its 95%  
49  
50  
51 337 confidence intervals will be estimated using the Cox proportional hazards  
52  
53 338 regression (including the propensity score value).

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57 339 The following strategy will be used for time-dependent variables:  
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3 340 (a) Continuous variables that follow a Gaussian distribution by means of mixed  
4  
5 341 models for repeated measures (mixed longitudinal model for repeated  
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7 342 measurements (MMRM)).  
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11 343 (b) Variables that do not comply with the parametric assumptions will be  
12  
13 344 transformed into ranges and analyzed analogously to those in section a).  
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15

16 345 c) Longitudinal binary data will be analogously analyzed with the marginal  
17  
18 346 models [Generalized Estimation Equation GEE).  
19  
20

21 347 In addition, the following statistical tests will be used when necessary: Fisher's  
22  
23 348 exact test to compare categorical variables between groups, McNemar test or  
24  
25 349 Cochran Q test for the analysis within the groups, dependent or independent t-  
26  
27 350 test for continuous variables when comparing two groups and ANOVA if  
28  
29 351 comparing more than two groups.  
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33 352 Nonparametric methods will be used in case of deviations from the applicability  
34  
35 353 assumptions: according to the data distribution, Mann-Whitney and/or Kruskal-  
36  
37 354 Wallis tests (independent variables), or Wilcoxon or Friedman tests (dependent  
38  
39 355 variables). Correlations will be done with Pearson or Spearman coefficients  
40  
41 356 according to the data distribution. SAS System (Release 9.2) or validated  
42  
43 357 equivalent software will be used. All recruited patients will be included in the  
44  
45 358 main analysis. In addition, a sensitivity analysis will be carried out with those  
46  
47 359 subjects who have complied with the protocol.  
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### 52 53 360 **Patient and public involvement**

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56 361 Neither patients nor public were involved in the development of the study.  
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## 363 **DISCUSSION**

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

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

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3 388 observational study carried out in two groups of centers in which one of the two  
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5 389 antibiotherapy approaches is already implemented turns out to be the safest  
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8 390 and more feasible design. The propensity score matching statistical technique  
9  
10 391 will reduced the possible bias due to confounding variables.

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13 392 Another limitation of the study is that no causal association could be accurately  
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15 393 described because of the observational design. Nevertheless, as an exhaustive  
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17 394 set of prospective clinical data are being recorded for each patient, including  
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19 395 start and stopping date of every antimicrobial used, dates of start and resolution  
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21 396 of main clinical end-points it is likely that meaningful cause-effect relationships  
22  
23 397 might be forwarded.

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28 398 This is the first prospective multicenter study aiming to address the effect of two  
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30 399 antimicrobial therapy strategies on intestinal microbiota and clinical outcomes in  
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32 400 HSCT recipients. The systematic collection of faecal samples at predetermined  
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34 401 peri-transplant time-points and the prospective collection of a wide clinical data  
35  
36 402 set with one year follow up based in pre-scheduled repeated clinical visits will  
37  
38 403 allow to examine changes in the microbiota composition over time and accurate  
39  
40 404 link them to the development of acute GVHD and other clinical outcomes.  
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42  
43 405 Another strength of the study is its design based on daily clinical practice. This  
44  
45 406 will provide valuable data on 'real-life patients' in addition to potential  
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47 407 recommendations on sampling time points and frequency for further studies. In  
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49  
50 408 conclusion, the findings of this study will bring useful insight in the relationship  
51  
52 409 between antibiotherapy use and development of acute graft versus host disease  
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54 410 in HSCT recipients helping to design improved strategies expectedly leading to  
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56 411 better survival, reduced graft versus host disease and improved quality of life.  
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3 412 **Trial status**  
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6 413 At submission the study is running and 140 patients are recruited.  
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9 414 Current approved protocol is version 3.0, dated 29/January/2018.  
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12 415 Date recruitment began at 16 January 2018 (First patient in).  
13  
14

15 416 Approximate date when recruitment will be completed: November 2019.  
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17

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19 417 **Abbreviations**  
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22 418 ASCT: Allogeneic hemopoietic stem cell transplant; CTU: Clinical Trials Unit;  
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24 419 CVC: central venous catheter; ECIL: European Conference of Infections in  
25

26 420 Leukemia; GEE: Generalized Estimation Equation; GVHD: Graft Versus Host  
27

28 421 Disease; ICH: International Council of Harmonisation; ITTP: intention-to-treat  
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30 422 population; MMRM: Mixed longitudinal model for repeated measurements;  
31  
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33 423 MRT: mortality related to transplantation; PP: per-protocol population; RCT:  
34

35 424 Randomized Clinical Trial.  
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38  
39 425 **Author Contributions**  
40  
41

42 426 IE conceived, designed the study and lead the study. IE and CR-F obtained  
43

44 427 funding for the research. SJ-J, GL-H, NR-T, FJM-M, MA-G, MEP-I and CR-F  
45

46 428 collaborated in methodological aspects of the study. SJ-J, NR-T, CR-F, MEP-I  
47

48 429 and CL-R coordinated the study. IE, CS, LV, MK, JMM-B and NR-T were  
49

50 430 responsible for the inclusion, treatment, clinical monitoring and follow-up of the  
51

52 431 patients. CL-R coordinated sample collection. GL-H, MEP-I and YS were  
53

54 432 responsible for the sample management. SJ-J wrote the first draft of the  
55  
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2  
3 433 manuscript. All authors were involved in critically revising the article and  
4  
5 434 approved the final version.  
6  
7

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17  
18

#### 19 439 **Competing interests**

20  
21  
22 440 The authors declared no competing interests  
23  
24

#### 25 441 **Patient consent for Publication**

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27  
28 442 Not required.  
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#### 32 443 **Ethics and dissemination**

33  
34  
35 444 The study was approved between 2017 and 2018 by the five Ethical  
36  
37 445 Committees involved (Comité Coordinador de Ética de la Investigación  
38  
39 446 Biomédica de Andalucía, Comité de Ética de Investigación Clínica de  
40  
41 447 Cantabria, Comité Autonómico de Evaluación de Estudios Posautorización  
42  
43 448 Observacionales, de Seguimiento Prospectivo con medicamentos, Comité de  
44  
45 449 Ética de la investigación con medicamentos del Hospital Univesitario de  
46  
47 450 Salamanca, Dirección General de Inspección y Ordenación de la Consejería de  
48  
49 451 Sanidad de la Comunidad de Madrid). Each substantial protocol amendment  
50  
51 452 will be notified for approval to the relevant ethics committee(s) prior to  
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53 453 implementation. All data collected will be kept strictly confidential and in  
54  
55 454 accordance with all relevant legislation on control and protection of personal  
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3 455 information. Study results will be published in peer-reviewed journals as well as  
4  
5 456 national and international scientific conferences.  
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7

8  
9 457 **Patient and Public Involvement**

10  
11 458 No patient involved.  
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15 459 **Access to data**

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18 460 Data are sustained in an electronic database. Upon request to the  
19  
20 461 corresponding author, the identified participant's data will be made available to  
21  
22 462 researchers whose proposals meet the research criteria. It will be also  
23  
24 463 considered requests for the protocol. To gain access, data requestors must  
25  
26 464 comply to a data access agreement.  
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565 **Table 1. Schedule of enrollment and assessments. *OptimBioma* study.**

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

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

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3 Dear Editor,  
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5 Please find bellow proofs to publish without peer review protocols:  
6

- 7
- 8 • Formal ethical approvals from the five participating sites
  - 9 • Resolution of the granting of the requested funding for the OptimBioma  
10 project issued by the ISCIII (The Instituto de Salud Carlos III (Institute of  
11 Health Carlos III) is the Spanish organization managing the activities of  
12 the Health Research and Development Strategy (AES) under the State  
13 Plan for Scientific and Technical Research and Innovation 2017-2020;  
14 <https://eng.isciii.es/Paginas/Inicio.aspx>)  
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20 Best regards,

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22 Ildfonso Espigado

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24 Corresponding Author  
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# Formal ethical approvals from the five participating sites

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GOBIERNO  
de  
CANTABRIA

CONSEJERÍA DE SANIDAD

Dirección General de Ordenación  
y Atención Sanitaria



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

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18 Expte. N.º: LMCA/ cao  
19 N/Ref.: LMCA/ cao  
20 Asunto: Notificación resolución  
21 Fecha: 20 de diciembre 2017

## NOTIFICACIÓN

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29 Adjunto se remite resolución de la Directora General de Ordenación y Atención  
30 Sanitaria, de fecha 18 de diciembre de 2017, por la que se concede la autorización para la  
31 realización en la Comunidad Autónoma de Cantabria del estudio posautorización  
32 observacional titulado: **“Optimización del tratamiento antibiótico en receptores de**  
33 **alotrasplante hematopoyético: impacto en la microbiota intestinal y en resultados**  
34 **clínicos”,** versión 1.0, de 28 de marzo de 2017 con código de protocolo **FIS-ANT-**  
35 **2017-01,**  
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EL JEFE DE SERVICIO DE  
ORDENACION SANITARIA



Luis M. Cabanzón Alber



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

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Expte. N°: 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 aplicado las disposiciones 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



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**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



E-mail: [comite.etico.husa@saludcastillayleon.es](mailto:comite.etico.husa@saludcastillayleon.es)

## 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,

### CERTIFICA

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:

D<sup>a</sup>. M<sup>a</sup> del Carmen Sánchez García. Asesora Jurídica.

Vicesecretaria:

D<sup>a</sup>. Silvia Jiménez Cabrera. Farmacia Hospitalaria.

Vocales:

D. José Manuel González de Buitrago. Jefe de Servicio de Bioquímica Clínica.

D<sup>a</sup>. Carmen Arias de la Fuente, Técnico Gestor de Ensayos Clínicos.

D<sup>a</sup>. Mercedes López Rico. Especialista en Farmacología Clínica.





**COMPLEJO  
ASISTENCIAL  
UNIVERSITARIO  
DE SALAMANCA**

Paseo de San Vicente, 58-182  
37007 Salamanca

Comité de Ética de la Investigación con medicamentos  
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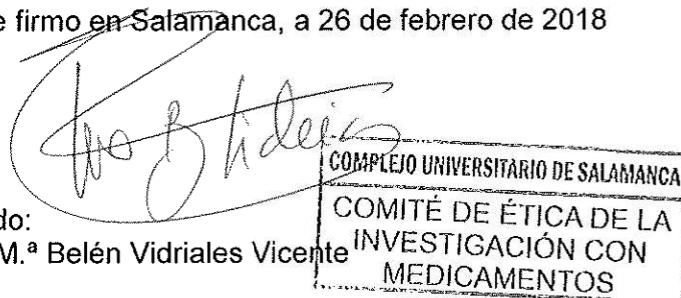
D. Guzmán Franch Arcas. Especialista en Cirugía General y Aparato Digestivo.  
D<sup>a</sup>. Ana Martín García. Especialista en Cardiología  
D<sup>a</sup>. Cristina Hidalgo Calleja. Especialista de Reumatología.  
D<sup>a</sup> Teresa Martín Gómez. Especialista en Oncología  
D<sup>a</sup>. Zulema Ferreras Páez. Especialista Medicina Intensiva UVI – VV  
D<sup>a</sup>. Berta Bote Bonaecha. Especialista en Psiquiatría  
D<sup>a</sup>. 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

Firmado:  
Doña M.<sup>a</sup> Belén Vidriales Vicente





Direcció General de Farmàcia i Productes Sanitaris

NºRef: DGFPS/SGOI/SPFD/CAVIME/ol

**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.

GENERALITAT VALENCIANA  
CONSELLERIA DE SANITAT

Registre General

Data 24 NOV. 2017

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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



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.



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 Sanitat Universal y Salut 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.





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**

**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 noviembre 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)

D<sup>a</sup>. 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



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 microbiótica 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

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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

<b>Código Seguro De Verificación:</b>	0wIMELSV47KhZ1a07Th1wQ==	<b>Fecha</b>	30/11/2017
<b>Normativa</b>	Este documento incorpora firma electrónica reconocida de acuerdo a la Ley 59/2003, de 19 de diciembre, de firma electrónica.		
<b>Firmado Por</b>	Joaquin Alanis Lopez		
<b>Url De Verificación</b>	https://ws058.juntadeandalucia.es/verifirma/code/0wIMELSV47KhZ1a07Th1wQ=	<b>Página</b>	1/1





# JUNTA DE ANDALUCÍA

## 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 microbiótica 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, D<sup>a</sup>. 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 microbiótica 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

<b>Código Seguro De Verificación:</b>	wh6BXW04Kd3y5NRNeQdisw==	<b>Fecha</b>	30/11/2017
<b>Normativa</b>	Este documento incorpora firma electrónica reconocida de acuerdo a la Ley 59/2003, de 19 de diciembre, de firma electrónica.		
<b>Firmado Por</b>	Joaquin Alanis Lopez		
<b>Url De Verificación</b>	https://ws058.juntadeandalucia.es/verifirma/code/wh6BXW04Kd3y5NRNeQdisw= =	<b>Página</b>	1/2



**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 microbiótica 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 microbiótica 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 artículo 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)

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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

<b>Código Seguro De Verificación:</b>	wh6BXW04Kd3y5NRNeQdisw==	<b>Fecha</b>	30/11/2017
<b>Normativa</b>	Este documento incorpora firma electrónica reconocida de acuerdo a la Ley 59/2003, de 19 de diciembre, de firma electrónica.		
<b>Firmado Por</b>	Joaquin Alanis Lopez		
<b>Url De Verificación</b>	https://ws058.juntadeandalucia.es/verifirma/code/wh6BXW04Kd3y5NRNeQdisw=	<b>Página</b>	2/2







## Comunidad de Madrid

Subdirección Gral. de Inspección y Ordenación Farmacéutica  
Área de Control Farmacéutico y Productos Sanitarios  
Ref.: MG/bhz

### NOTIFICACIÓN

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.



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

9  
10 *“2.- (...)A los efectos de esta ley, se entiende por «estudio observacional» el estudio en el que*  
11 *los medicamentos se prescriben de la manera habitual, de acuerdo con las condiciones*  
12 *establecidas en la autorización. La asignación de un paciente a una estrategia terapéutica*  
13 *concreta no estará decidida de antemano por el protocolo de un ensayo, sino que estará*  
14 *determinada por la práctica habitual de la medicina. La decisión de prescribir un medicamento*  
15 *determinado estará claramente dissociada de la decisión de incluir al paciente en el estudio.*  
16 *No se aplicará a los pacientes ninguna intervención, ya sea diagnóstica o de seguimiento,*  
17 *que no sea la habitual de la práctica clínica. Se utilizarán métodos epidemiológicos para el*  
18 *análisis de los datos recogidos.”*

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21 b) La Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal,  
22 define lo que se entiende por datos de carácter personal y regula, entre otros asuntos, las  
23 condiciones de obtención, tratamiento y cesión de datos especialmente protegidos.  
24  
25 c) El capítulo VI del Real Decreto 577/2013, de 26 de julio, por el que se regula la  
26 farmacovigilancia de medicamentos de uso humano establece que:

27  
28 *“-Los estudios posautorización deberán tener como finalidad complementar la información*  
29 *obtenida durante el desarrollo clínico de los medicamentos previo a su autorización. Queda*  
30 *prohibida la planificación, realización o financiación de estudios posautorización con la*  
31 *finalidad de promover la prescripción de los medicamentos.”*

- 32  
33 d) La Orden SAS/3470/2009, de 16 de diciembre por la que se publican las directrices sobre  
34 estudios posautorización de tipo observacional para medicamentos de uso humano  
35  
36 e) La Orden 730/2004 de 30 de junio del Consejero de Sanidad y Consumo establece los  
37 requisitos para la realización de estudios postautorización de tipo observacional con  
38 medicamentos de uso humano en la Comunidad de Madrid.  
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42 **Segundo.-** Evaluada la documentación aportada no se muestran objeciones a su realización en la  
43 Comunidad de Madrid.

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46 **VISTAS** las disposiciones citadas en los Fundamentos Jurídicos esta Dirección General de  
47 Inspección y Ordenación es competente para resolver la solicitud presentada, conforme a las  
48 atribuciones que le reconoce la Ley 19/1998, de 25 de noviembre de Ordenación y Atención  
49 Farmacéutica de la Comunidad de Madrid y el Decreto 195/2015, de 4 de agosto, del Consejo de  
50 Gobierno, por el que se establece la estructura orgánica de la Consejería de Sanidad, modificado por  
51 el Decreto 125/2017, vengo en:  
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58 **RESOLVER**  
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**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 Ezquerro 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  
Huella dig.: d9bd143bedcd2a0e970e191667067166a52b300e

DESTINATARIO:

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



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# 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))



**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
<b>SUBTOTALES</b>	<b>50.000,00</b>	<b>38.500,00</b>	<b>39.000,00</b>	<b>127.500,00</b>
Costes ind. 21,00 %	10.500,00	8.085,00	8.190,00	26.775,00
<b>TOTALES</b>	<b>60.500,00</b>	<b>46.585,00</b>	<b>47.190,00</b>	<b>154.275,00</b>

#### 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.

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3 September 25, 2019  
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5 Adrian Aldcroft  
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7 Editor in Chief  
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10 BMJ Open  
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14 Dear Editor in Chief,  
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18 We are submitting to your consideration the Protocol manuscript entitled “*Assessing the*  
19 *impact on intestinal microbiome and clinical outcomes of antibiotherapy optimization*  
20 *strategies in hematopoietic stem cell transplant recipients: study protocol for the*  
21 *prospective multi-center OptimBioma study*”. The study was approved between 2017  
22 and 2018 by the involved Ethical Committees and has undergone independent peer  
23 review to acquire funding.  
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34 The manuscript summarizes the used methodology in a prospective comparative  
35 observational multicenter study researching the effect of two center-driven antibacterial  
36 strategies, applied on two respective cohorts of hematopoietic stem cell transplant  
37 recipients, on intestinal microbiome composition and acute graft versus host disease rate  
38 and severity. All the two antibiotherapy strategies are described in the manuscript and  
39 named as *optimization* and *standard* strategies. The study is built on systematic  
40 collection of fecal samples at predetermined peri-transplant time-points and on  
41 prospective collection of a wide set of relevant clinical data throughout one year of  
42 follow up by means of scheduled clinical visits. This design will allow examining  
43 changes in the microbiome composition over time in both cohorts of recipients and  
44 linking them to the development of acute graft versus host disease and other relevant  
45 clinical outcomes.  
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3 The findings of this study will bring useful insight into the possible relationship  
4 between antibiotherapy use, intestinal microbiome misbalance and development of  
5 acute graft versus host disease in hematopoietic stem cell transplantation recipients.  
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12 This knowledge will help to design improved antibiotherapy strategies leading to better  
13 survival in this group of patients with frequent poor clinical outcomes.  
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19 This manuscript has been approved by all authors and it has not been published or  
20 submitted for publication elsewhere.  
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26 Thank you very much for your consideration.  
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31 Sincerely,  
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37  
38 Dr. Ildefonso Espigado, PhD.  
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42 **Corresponding author**  
43

44 Postal address: Department of Hematology, University Hospital Virgen del Rocío /  
45 University of Seville / CSIC / Institute of Biomedicine of Seville.  
46  
47

48 Avenida Manuel Siurot s/n - 41013, Seville, Spain  
49

50 Telephone number: +34 636 096 808  
51

52 E-mail: [ildefonso.espigado.sspa@juntadeandalucia.es](mailto:ildefonso.espigado.sspa@juntadeandalucia.es)  
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STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
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

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1	<b>Introduction</b>			
2				
3	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
4				
5				
6		6b	Explanation for choice of comparators	10-12
7				
8	Objectives	7	Specific objectives or hypotheses	8
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial or single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	10-12
11				
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	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
17				
18				
19	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
20				
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
23				
24		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)	_____
25				
26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	_____
27				
28		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_____
29				
30	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
31				
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34	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
35				
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1	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
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4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____
5				

**Methods: Assignment of interventions (for controlled trials)**

Allocation:

10	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	_____
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16	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	_____
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21	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____
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24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____
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**Methods: Data collection, management, and analysis**

33	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	_____
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39		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	_____
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1	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	_____
2				
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5	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
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____
9				
10		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)	_____
11				
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14	<b>Methods: Monitoring</b>			
15				
16	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	_____
17				
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22		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	_____
23				
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25	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	_____
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28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
29				
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32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4,21,22
35				
36				
37	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
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
2				
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____
5				
6				
7	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
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
11				
12				
13	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
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16	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	_____
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20	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
21				
22				
23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	_____
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
27				
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29	<b>Appendices</b>			
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31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____
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34	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	_____
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\*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](https://creativecommons.org/licenses/by/4.0/)" license.

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





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3 1 Assessing the impact on intestinal microbiome and clinical outcomes of  
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5 2 antibiotherapy optimization strategies in hematopoietic stem cell transplant  
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7 3 recipients: study protocol for the prospective multi-center OptimBioma study  
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24 10 Trasplante Hematopoyético y Terapia Celular).  
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3 42 **ABSTRACT**  
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6 43 Introduction  
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9 44 Hematopoietic stem cell transplantation (HSCT) is a lifesaving treatment for a  
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11 45 number of hematologic diseases. Graft versus host disease (GVHD) is its main  
12  
13 46 complication and hampers survival. There is strong evidence that intestinal  
14  
15 47 microbiota diversity of the recipient may increase the risk of GVHD worsening  
16  
17 48 survival. Antibiotic regimens used during the early phase of the transplant may  
18  
19 49 influence clinical outcomes by reducing intestinal microbiota diversity. Present  
20  
21 50 guidelines of *European Conference on Infections in Leukemia* exhort to  
22  
23 51 optimizing antibiotic use in hematologic patients including HSCT recipients.  
24  
25  
26

27  
28 52 The present study aims to investigate if, in HSCT recipients, the optimization of  
29  
30 53 antibacterial use may preserve intestinal microbiota composition reducing the  
31  
32 54 incidence and severity of acute GVHD and improving relevant clinical  
33  
34 55 outcomes.  
35  
36

37  
38 56 Methods and analysis  
39  
40

41  
42 57 This is a prospective longitudinal observational study of two cohorts of HSCT  
43  
44 58 recipients: i) the intervention cohort includes patients treated in centers in which  
45  
46 59 a pre-defined strategy of antibiotherapy optimization is implemented, with the  
47  
48 60 objective of optimizing and reducing antibiotic administration according to  
49  
50 61 clinical criteria and ii) the control cohort includes patients treated in centers in  
51  
52 62 which a classic permissive strategy of antibiotic prophylaxis and treatment is  
53  
54 63 used. Adult patient receiving a first HSCT as a treatment for any hematologic  
55  
56 64 condition are included. Clinical variables are prospectively recorded and up to  
57  
58 65 five fecal samples are collected for microbiota characterization at pre-established  
59  
60

1  
2  
3 66 peri-transplant time-points. Patients are followed since the pre-conditioning  
4  
5 67 phase throughout one-year post-transplant and four follow-up visits are  
6  
7 68 scheduled. Fecal microbiota composition and diversity will be compared  
8  
9 69 between both cohorts along with acute GVHD incidence and severity, severe  
10  
11 70 infections rate, mortality and overall and disease-free survival.  
12  
13  
14

#### 15 71 Ethics and dissemination

16  
17  
18 72 The study was approved between 2017 and 2018 by the Ethical Committees of  
19  
20 73 participant centers. Study results will be disseminated through peer-reviewed  
21  
22 74 journals and national and international scientific conferences.  
23  
24  
25

26 75 Trial registration number: ClinicalTrials.gov, NCT03727113. Registered on  
27  
28 76 November 1<sup>st</sup>, 2018.  
29  
30

31 77 Keywords: microbiome, microbiota, hematopoietic stem cell transplantation,  
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33 78 graft *versus* host disease, infections, antibiotics.  
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40 80 Word count: 3474  
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3 82 **ARTICLE SUMMARY**  
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6 83 Strengths and limitations of the study.  
7  
8

- 9 84 • First-in-class prospective comparative observational multicenter study  
10  
11 addressing the effect of two different (centers-driven) antimicrobial  
12 85  
13 strategies (optimized versus standard antimicrobial use) on intestinal  
14 86  
15 microbiota diversity, acute graft versus host disease and survival in  
16 87  
17 hematopoietic stem cell transplant recipients.  
18 88
- 19 89 • Robust design by systematic collection of fecal samples at  
20  
21 predetermined peri-transplant time-points and prospective collection of a  
22 90  
23 wide relevant clinical data set throughout one-year follow up with  
24 91  
25 scheduled clinical visits.  
26 92
- 27 93 • Limitation: non-randomized design (for security reasons) with propensity  
28  
29 score matching statistical approach to reduce possible bias by  
30 94  
31 confounding variables.  
32 95
- 33 96 • Limitation: no causal mechanistic association could be accurately  
34  
35 concluded although meaningful cause-effect relationships should be  
36 97  
37 advanced.  
38 98
- 39 99 • Results may bring new insights into the relationship between  
40  
41 antibiotherapy use, intestinal microbiome modification and development  
42 100  
43 of acute graft versus host disease in hematopoietic stem cell  
44 101  
45 transplantation recipients, helping to design improved strategies leading  
46 102  
47 to better survival.  
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## 106 INTRODUCTION

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

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

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

1  
2  
3 130 safe to discontinue empirical antibiotic therapy after resolution of fever when  
4  
5 131 patients are clinically stable, irrespective of their neutrophils counts, significantly  
6  
7 132 reducing exposure to antibiotics. On the other hand, the ECIL group (European  
8  
9 133 Conference of Infections in Leukemia) has proposed<sup>10</sup> specific empirical  
10  
11 134 antibacterial therapy strategies in hematological patients including HSCT  
12  
13  
14 135 recipients, in order to optimized antibiotic use. These recommendations are  
15  
16  
17 136 heterogeneously implemented in the different hematopoietic transplant centers.  
18  
19 137 This study will investigate if a predefined strategy of optimization of  
20  
21 138 antimicrobial therapy that includes ECIL recommendations<sup>10-12</sup> and the *How-*  
22  
23 139 *Long* study<sup>9</sup>, will preserve intestinal microbiota composition and diversity while  
24  
25  
26 140 reducing the incidence and severity of acute GVHD when compared to a  
27  
28 141 conventional permissive antibiotic strategy. In addition, severe infections rate,  
29  
30 142 transplant related mortality and long-term survival will be compared between  
31  
32  
33 143 both groups.

## 144 **METHODS AND ANALYSIS**

### 145 **Study design**

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

153

## 154 **Study settings**

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

## 161 **Participants**

### 162 *Eligibility criteria*

#### 163 Inclusion Criteria

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

#### 169 Exclusion criteria

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

## 173 **Recruitment process**

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

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2  
3 176 **Intervention**  
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5  
6 177 Centers allocated to the intervention cohort use an antibacterial systematic  
7  
8 178 approach that includes the following strategies:  
9

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11  
12 179 1. No routine antibacterial prophylaxis is used.  
13

14  
15 180 2. In case of febrile neutropenia:  
16

17  
18 181 - Use of scalation / de-scalation strategy for empiric antimicrobial  
19  
20 182 therapy<sup>10</sup>.  
21

22  
23 183 - Directed simplification in patients with etiological diagnosis according to  
24  
25 184 *in vitro* susceptibility tests.  
26

27  
28  
29 185 - Switch to a narrower-spectrum agent in patients without an etiological  
30  
31 186 diagnosis and clinical stabilization on treatment.  
32

33  
34 187 - No broadening the antibacterial spectrum but maintenance of initial  
35  
36 188 antimicrobials therapy in patients with persistent fever if they are clinically  
37  
38 189 stable in the criteria of the physicians in charge of them.  
39

40  
41  
42 190 3. Early (in 72 hours) withdrawal of combined treatments, when clinically  
43  
44 191 indicated.  
45

46  
47 192 4. Antibacterial therapy withdrawal regardless neutrophils count and  
48  
49 193 expected duration of neutropenia when patient meets all the following  
50  
51 194 criteria (*How-Long* strategy)<sup>9</sup>.  
52

53  
54  
55 195 i) Afebrile for  $\geq 72$  h.  
56  
57  
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3 196 ii) Complete resolution of signs, symptoms and alterations in  
4  
5 197 complementary tests secondary to the infection (cough, abdominal  
6  
7 198 pain, diarrhea, pulmonary infiltrate, etc.) for  $\geq 72$  h.  
9

10  
11 199 iii) Normal vital constants (blood pressure, heart rate, respiratory rate  
12  
13 200 and diuresis and, in patients with respiratory involvement, oxygen  
14  
15 201 saturation by pulse oximetry) for  $\geq 72$  h.  
16

17  
18 202 5. Short (7 days) etiological therapy for primary or related to central  
19  
20 203 venous (with catheter removal) no complicated bacteremia, and 14  
21  
22 204 days for *Staphylococcus aureus* non complicated bacteremia if good  
23  
24 205 clinical response and good clinical evolution.  
25  
26  
27

28  
29 206 In centers allocated to the control cohort the antimicrobial therapy approach  
30  
31 207 does not include any of the strategies used in the optimization cohort but the  
32  
33 208 following management:  
34

35  
36 209 1. Use of antibacterial prophylaxis.  
37

38  
39 210 2. In case of febrile neutropenia:  
40

41  
42 211 - Use of early broad-spectrum antimicrobial therapy without systematic  
43  
44 212 use of escalation / de-escalation strategy.  
45

46  
47 213 - Optional antibiotic simplification in patients with etiological diagnosis  
48  
49 214 according to *in vitro* susceptibility tests.  
50

51  
52 215 - No switching to a narrower-spectrum agent in patients without  
53  
54 216 etiological diagnosis and clinical response.  
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3 217 - Broadening the spectrum of initial antimicrobials in patients with  
4  
5 218 persistent fever even without clinical worsening.  
6  
7  
8  
9 219 3. No early discontinuation of the combined empirical antimicrobial therapy  
10  
11 220 even in case of clinical response.  
12  
13 221 4. No discontinuation of empirical antimicrobial therapy until neutropenia  
14  
15 222 recovery.  
16  
17 223 5. Prolonged etiological treatment for primary or related to central venous  
18  
19 224 catheter no complicated bacteremia, even in case of early clinical  
20  
21 225 response.  
22  
23  
24

#### 25 226 **Schedule of visits and collection of fecal samples**

27  
28 227 The scheduled visits and assessments are described in table 1.

29  
30  
31 228 Follow-up is organized in four planned visits: Visit 1 (seven days pre-transplant),  
32  
33 229 Visit 2 (end of antimicrobial therapy or hospital discharge, whichever occurs  
34  
35 230 first), Visit 3 (100 days post-transplant), and Visit 4 or Final Visit (one-year post-  
36  
37 231 transplant or mortality, whichever occurs first). A minimum of four fecal samples  
38  
39 232 will be collected: Specimen 1, day of starting the conditioning treatment  $\pm$  48  
40  
41 233 hours; Specimen 2, day of transplantation  $\pm$  48 hours; Specimen 3, day +7 post-  
42  
43 234 transplant  $\pm$  24 hours; Specimen 4, when the first episode of fever at any time  
44  
45 235 from the beginning of the conditioning until the end of antimicrobial therapy or  
46  
47 236 hospital discharge, whichever occurs first (this sample is collected at fever  
48  
49 237 onset or within 48 hours, unless the fever starts on the same day that a  
50  
51 238 scheduled fecal sample is already collected); and Specimen 5, day when the  
52  
53 239 antimicrobial therapy is stopped (or within the following 48 hours) or,  
54  
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3 240 alternatively, if the patient did not receive antibiotics or continues receiving  
4  
5 241 antibiotics at discharge, the day before discharge (or 24 hours in advance).  
6  
7

8 242 The stool samples will be collected at different time points (table 1) in Stool  
9  
10 243 Nucleic Acid Collection and Preservation Tubes (Ref. 45660, Norgen Biotek  
11  
12  
13 244 Corp.).  
14  
15

16 245 **DNA extraction, prokaryotic 16S ribosomal RNA gene (16S rRNA)**  
17  
18 246 **sequencing and bioinformatics analysis**  
19

20  
21  
22 247 The microbiome studies will be performed at the laboratory of Infectious  
23  
24 248 Diseases of the Institute of Biomedicine of Seville (IBiS).  
25  
26

27 249 DNA will be extracted from faecal samples using the Stool DNA Isolation kit  
28  
29 250 (Cat. 27600, Norgen Biotek Corp., Ontario, Canadá) according to the  
30  
31 251 manufacturer's protocol. All DNA samples will be stored at  $-20^{\circ}\text{C}$  until further  
32  
33 252 processing. DNA will be quantified by Qubit fluorometry (Invitrogen Qubit 4  
34  
35 253 Fluorometer, ThermoFisher Scientific, Spain) and normalized to  $5\text{ ng}/\mu\text{L}$  with  $10$   
36  
37 254  $\text{mM}$  Tris pH 8.5. Bacterial 16S rRNA amplification and library construction will  
38  
39 255 be performed according to the 16S Metagenomic Sequencing Library  
40  
41 256 Preparation guide from Illumina. Briefly,  $2.5\ \mu\text{L}$  of total DNA per sample will be  
42  
43 257 amplified using primers targeting the 16S rRNA V3 and V4 regions<sup>13-14</sup>. These  
44  
45 258 regions provide ample information for taxonomic classification of microbial  
46  
47 259 communities. Pooled V3-V4 amplicon libraries will be sequenced using the  
48  
49 260 Illumina MiSeq platform with a V3 reagent kit (Illumina, San Diego, California)  
50  
51 261 and paired-end 300-bp reads. Up to 96 libraries could be pooled together for  
52  
53 262 sequencing. Regarding the bioinformatics analysis of the sequencing data,  
54  
55 263 machine learning libraries from Scikit-learn<sup>15</sup> will be used to filter out and  
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2  
3 264 discard poor-quality reads. Processed sequences will be subjected to  
4  
5 265 operational taxonomic unit (OTU) picking against Greengenes (v13.8)<sup>16</sup>, with  
6  
7 266 reads clustered by 97% identity into OTUs using QIIME 2<sup>17</sup>. In-house R scripts  
8  
9 267 (v3.2.2) will be used to visualize the results.  
10  
11  
12

### 13 268 **Evaluation of results**

14  
15  
16 269 In order to assess the impact of both antimicrobial strategies on intestinal  
17  
18 270 microbiota diversity it will be characterized as described in the previous section  
19  
20 271 and biological alpha and beta diversity indexes of samples from both cohorts  
21  
22 272 will be compared<sup>18</sup>. Alpha-diversity and beta-diversity refer to diversity within  
23  
24 273 and between samples, respectively. These secondary bioinformatics analyses  
25  
26 274 will be performed with QIIME 2 and included the calculation of the parameters  
27  
28 275 of alpha-diversity: Shannon's diversity index, frequency of observed OTUs,  
29  
30 276 Faith's Phylogenetic Diversity, and Evenness; and the parameters of beta-  
31  
32 277 diversity: Jaccard distance, Bray-Curtis distance, Unweighted UniFrac distance  
33  
34 278 and Unweighted UniFrac distance.  
35  
36  
37  
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39

40 279 In order to assess clinical outcomes (secondary objectives of the study) the  
41  
42 280 following data will be prospectively recorded: incidence and severity of GVHD  
43  
44 281 (evaluated for any degree, degree-II and degree-III/IV up to day 100 post-  
45  
46 282 transplant), transplant related mortality and mortality caused by infection (time  
47  
48 283 frame: Day 0 to Day +30, Day +100 and Day +365 post-transplant), incidence of  
49  
50 284 severe infections (Day 0 to Day +30 post-transplant), and overall and disease  
51  
52 285 free survival (time frame: Day 0 to Day +30, Day +100 and Day +365 post-  
53  
54 286 transplant).  
55  
56  
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## 288 **Sample size**

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

299

## 300 **Statistical analysis**

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

1  
2  
3 312 statistically significant using an alpha error of 5% and LDA log-scores  
4  
5 313 exceeding  $\pm 2.0$  will be visually represented as bar plots. The median values of  
6  
7 314 taxa abundance and the median percentages of taxa presence in both groups  
8  
9  
10 315 will be calculated, and the Manhattan distances will be used for the clustering  
11  
12 316 analysis. The Kruskal-Wallis rank-sum test will be used to identify significant  
13  
14 317 taxa abundance and Fisher's exact test will be used to identify significant taxa  
15  
16  
17 318 presence in the both groups.

18  
19  
20 319 The propensity score will be used to adjust potential confounding effects. To  
21  
22 320 calculate the propensity scores in the logistic model the center factors and key  
23  
24 321 predictive characteristics identified in the baseline comparability analysis will be  
25  
26  
27 322 taken into account. Propensity scores will be used for all adjusted inferential  
28  
29 323 analyzes.

30  
31  
32 324 Standard descriptive statistical indices will be used according to the nature of  
33  
34 325 each variable. Continuous variables will be analyzed with linear models, binary  
35  
36  
37 326 variables without time factor with logistic models and the time-to-event variables  
38  
39 327 with survival models, all of them incorporating the propensity score as an  
40  
41 328 adjustment factor.

42  
43  
44  
45 329 The survival function of both groups will be described using the Kaplan-Meier  
46  
47 330 method. For the inferential analysis, the stratified log-rank test will be used (with  
48  
49 331 the propensity score value categorized as stratum). Hazard Ratios and its 95%  
50  
51 332 confidence intervals will be estimated using the Cox proportional hazards  
52  
53 333 regression (including the propensity score value).

54  
55  
56  
57 334 The following strategy will be used for time-dependent variables:  
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59  
60

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3 335 (a) Continuous variables that follow a Gaussian distribution by means of mixed  
4  
5 336 models for repeated measures (mixed longitudinal model for repeated  
6  
7 337 measurements (MMRM).  
8  
9

10  
11 338 (b) Variables that do not comply with the parametric assumptions will be  
12  
13 339 transformed into ranges and analyzed analogously to those in section a).  
14  
15

16 340 c) Longitudinal binary data will be analogously analyzed with the marginal  
17  
18 341 models [Generalized Estimation Equation GEE).  
19  
20

21 342 In addition, the following statistical tests will be used when necessary: Fisher's  
22  
23 343 exact test to compare categorical variables between groups, McNemar test or  
24  
25 344 Cochran Q test for the analysis within the groups, dependent or independent t-  
26  
27 345 test for continuous variables when comparing two groups and ANOVA if  
28  
29 346 comparing more than two groups.  
30  
31  
32

33 347 Nonparametric methods will be used in case of deviations from the applicability  
34  
35 348 assumptions: according to the data distribution, Mann-Whitney and/or Kruskal-  
36  
37 349 Wallis tests (independent variables), or Wilcoxon or Friedman tests (dependent  
38  
39 350 variables). Correlations will be done with Pearson or Spearman coefficients  
40  
41 351 according to the data distribution. SAS System (Release 9.2) or validated  
42  
43 352 equivalent software will be used. All recruited patients will be included in the  
44  
45 353 main analysis. In addition, a sensitivity analysis will be carried out with those  
46  
47 354 subjects who have complied with the protocol.  
48  
49  
50  
51

### 52 53 355 **Patient and public involvement**

54  
55

56 356 Neither patients nor public were involved in the development of the study.  
57  
58  
59  
60



## 358 **DISCUSSION**

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

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

1  
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3 383 observational study carried out in two groups of centers in which one of the two  
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5 384 antibiotherapy approaches is already implemented turns out to be the safest  
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7  
8 385 and more feasible design. The propensity score matching statistical technique  
9  
10 386 will reduced the possible bias due to confounding variables.

11  
12  
13 387 Another limitation of the study is that no causal association could be accurately  
14  
15 388 described because of the observational design. Nevertheless, as an exhaustive  
16  
17 389 set of prospective clinical data are being recorded for each patient, including  
18  
19 390 start and stopping date of every antimicrobial used, dates of start and resolution  
20  
21 391 of main clinical end-points it is likely that meaningful cause-effect relationships  
22  
23 392 might be forwarded.

24  
25  
26  
27  
28 393 This is the first prospective multicenter study aiming to address the effect of two  
29  
30 394 antimicrobial therapy strategies on intestinal microbiota and clinical outcomes in  
31  
32 395 HSCT recipients. The systematic collection of faecal samples at predetermined  
33  
34 396 peri-transplant time-points and the prospective collection of a wide clinical data  
35  
36 397 set with one year follow up based in pre-scheduled repeated clinical visits will  
37  
38 398 allow to examine changes in the microbiota composition over time and accurate  
39  
40 399 link them to the development of acute GVHD and other clinical outcomes.

41  
42  
43 400 Another strength of the study is its design based on daily clinical practice. This  
44  
45 401 will provide valuable data on 'real-life patients' in addition to potential  
46  
47 402 recommendations on sampling time points and frequency for further studies. In  
48  
49 403 conclusion, the findings of this study will bring useful insight in the relationship  
50  
51 404 between antibiotherapy use and development of acute graft versus host disease  
52  
53 405 in HSCT recipients helping to design improved strategies expectedly leading to  
54  
55 406 better survival, reduced graft versus host disease and improved quality of life.  
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3 407 **Trial status**  
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5

6 408 At submission the study is running and 140 patients are recruited.  
7

8  
9 409 Current approved protocol is version 3.0, dated 29/January/2018.  
10

11  
12 410 Date recruitment began at 16 January 2018 (First patient in).  
13

14  
15 411 Approximate date when recruitment will be completed: November 2019.  
16  
17

18  
19 412 **Abbreviations**  
20

21  
22 413 ASCT: Allogeneic hemopoietic stem cell transplant; CTU: Clinical Trials Unit;  
23

24 414 CVC: central venous catheter; ECIL: European Conference of Infections in  
25

26 415 Leukemia; GEE: Generalized Estimation Equation; GVHD: Graft Versus Host  
27

28 416 Disease; ICH: International Council of Harmonisation; ITTP: intention-to-treat  
29

30 417 population; MMRM: Mixed longitudinal model for repeated measurements;  
31  
32

33 418 MRT: mortality related to transplantation; PP: per-protocol population; RCT:  
34

35 419 Randomized Clinical Trial.  
36  
37

38  
39 420 **Author Contributions**  
40

41  
42 421 IE conceived, designed the study and lead the study. IE and CR-F obtained  
43

44 422 funding for the research. SJ-J, GL-H, NR-T, FJM-M, MA-G, MEP-I and CR-F  
45

46 423 collaborated in methodological aspects of the study. SJ-J, NR-T, CR-F, MEP-I  
47

48 424 and CL-R coordinated the study. IE, CS, LV-L, MK, JMM-B and NR-T were  
49

50 425 responsible for the inclusion, treatment, clinical monitoring and follow-up of the  
51

52 426 patients. CL-R coordinated sample collection. GL-H, MEP-I and YS were  
53

54 427 responsible for the sample management. SJ-J wrote the first draft of the  
55  
56  
57  
58  
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2  
3 428 manuscript. All authors were involved in critically revising the article and  
4  
5 429 approved the final version.  
6  
7

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15  
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17  
18

### 19 434 **Competing interests**

20  
21  
22 435 The authors declared no competing interests  
23  
24

### 25 436 **Patient consent for Publication**

26  
27  
28 437 Not required.  
29  
30

### 31 438 **Ethics and dissemination**

32  
33  
34  
35 439 The study was approved between 2017 and 2018 by the five Ethical  
36  
37 440 Committees involved (Comité Coordinador de Ética de la Investigación  
38  
39 441 Biomédica de Andalucía, Comité de Ética de Investigación Clínica de  
40  
41 442 Cantabria, Comité Autonómico de Evaluación de Estudios Posautorización  
42  
43 443 Observacionales, de Seguimiento Prospectivo con medicamentos, Comité de  
44  
45 444 Ética de la investigación con medicamentos del Hospital Univesitario de  
46  
47 445 Salamanca, Dirección General de Inspección y Ordenación de la Consejería de  
48  
49 446 Sanidad de la Comunidad de Madrid). Each substantial protocol amendment  
50  
51 447 will be notified for approval to the relevant ethics committee(s) prior to  
52  
53 448 implementation. All data collected will be kept strictly confidential and in  
54  
55 449 accordance with all relevant legislation on control and protection of personal  
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1  
2  
3 450 information. Study results will be published in peer-reviewed journals as well as  
4  
5 451 national and international scientific conferences.  
6  
7

## 8 452 **Patient and Public Involvement**

9  
10  
11 453 No patient involved.  
12  
13

## 14 454 **Access to data**

15  
16  
17 455 Data are sustained in an electronic database. Upon request to the  
18  
19 456 corresponding author, the identified participant's data will be made available to  
20  
21 457 researchers whose proposals meet the research criteria. It will be also  
22  
23 458 considered requests for the protocol. To gain access, data requestors must  
24  
25 459 comply to a data access agreement.  
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30 460

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

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

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

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563



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
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

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1	<b>Introduction</b>			
2				
3	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
4				
5				
6		6b	Explanation for choice of comparators	10-12
7				
8	Objectives	7	Specific objectives or hypotheses	8
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial or single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	10-12
11				
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	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
17				
18				
19	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
20				
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
23				
24		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)	_____
25				
26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	_____
27				
28		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_____
29				
30	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
31				
32				
33				
34	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
35				
36				
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1	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
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____
5				

**Methods: Assignment of interventions (for controlled trials)**

Allocation:

10	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	_____
11				
12				
13				
14				
15				
16	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	_____
17				
18				
19				
20				
21	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____
28				
29				
30				

**Methods: Data collection, management, and analysis**

33	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	_____
34				
35				
36				
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39		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	_____
40				
41				
42				

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1	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	_____
2				
3				
4				
5	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
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____
9				
10		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)	_____
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	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	_____
17				
18				
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21				
22		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	_____
23				
24				
25	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	_____
26				
27				
28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
29				
30				
31				
32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4,21,22
35				
36				
37	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
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____
5				
6				
7	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
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
11				
12				
13	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
14				
15				
16	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	_____
17				
18				
19				
20	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
21				
22				
23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	_____
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____
32				
33				
34	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	_____
35				
36				

\*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.

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

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3 1 Assessing the impact on intestinal microbiome and clinical outcomes of  
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5 2 antibiotherapy optimization strategies in hematopoietic stem cell transplant  
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7 3 recipients: study protocol for the prospective multi-center OptimBioma study  
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3 42 **ABSTRACT**  
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6 43 Introduction  
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9 44 Hematopoietic stem cell transplantation (HSCT) is a lifesaving treatment for a  
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11 45 number of hematologic diseases. Graft versus host disease (GVHD) is its main  
12  
13 46 complication and hampers survival. There is strong evidence that intestinal  
14  
15 47 microbiota diversity of the recipient may increase the risk of GVHD worsening  
16  
17 48 survival. Antibiotic regimens used during the early phase of the transplant may  
18  
19 49 influence clinical outcomes by reducing intestinal microbiota diversity. Present  
20  
21 50 guidelines of *European Conference on Infections in Leukemia* exhort to  
22  
23 51 optimizing antibiotic use in hematologic patients including HSCT recipients.  
24  
25  
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27

28 52 The present study aims to investigate if, in HSCT recipients, the optimization of  
29  
30 53 antibacterial use may preserve intestinal microbiota composition reducing the  
31  
32 54 incidence and severity of acute GVHD and improving relevant clinical  
33  
34 55 outcomes.  
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38 56 Methods and analysis  
39  
40

41 57 This is a prospective longitudinal observational study of two cohorts of HSCT  
42  
43 58 recipients: i) the intervention cohort includes patients treated in centers in which  
44  
45 59 a pre-defined strategy of antibiotherapy optimization is implemented, with the  
46  
47 60 objective of optimizing and reducing antibiotic administration according to  
48  
49 61 clinical criteria and ii) the control cohort includes patients treated in centers in  
50  
51 62 which a classic permissive strategy of antibiotic prophylaxis and treatment is  
52  
53 63 used. Adult patient receiving a first HSCT as a treatment for any hematologic  
54  
55 64 condition are included. Clinical variables are prospectively recorded and up to  
56  
57 65 five fecal samples are collected for microbiota characterization at pre-established  
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3 66 peri-transplant time-points. Patients are followed since the pre-conditioning  
4  
5 67 phase throughout one-year post-transplant and four follow-up visits are  
6  
7 68 scheduled. Fecal microbiota composition and diversity will be compared  
8  
9 69 between both cohorts along with acute GVHD incidence and severity, severe  
10  
11 70 infections rate, mortality and overall and disease-free survival.  
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#### 15 71 Ethics and dissemination

16  
17  
18 72 The study was approved between 2017 and 2018 by the Ethical Committees of  
19  
20 73 participant centers. Study results will be disseminated through peer-reviewed  
21  
22 74 journals and national and international scientific conferences.  
23  
24  
25

26 75 Trial registration number: ClinicalTrials.gov, NCT03727113. Registered on  
27  
28 76 November 1<sup>st</sup>, 2018.  
29  
30

31 77 Keywords: microbiome, microbiota, hematopoietic stem cell transplantation,  
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33 78 graft *versus* host disease, infections, antibiotics.  
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40 80 Word count: 3474  
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3 82 **ARTICLE SUMMARY**  
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6 83 Strengths and limitations of the study.  
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- 9 84 • First-in-class prospective comparative observational multicenter study  
10  
11 addressing the effect of two different (centers-driven) antimicrobial  
12 85  
13 strategies (optimized versus standard antimicrobial use) on intestinal  
14 86  
15 microbiota diversity, acute graft versus host disease and survival in  
16 87  
17 hematopoietic stem cell transplant recipients.  
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19 88
- 20  
21 89 • Robust design by systematic collection of fecal samples at  
22  
23 predetermined peri-transplant time-points and prospective collection of a  
24 90  
25 wide relevant clinical data set throughout one-year follow up with  
26 91  
27 scheduled clinical visits.  
28  
29 92
- 30 93 • Limitation: non-randomized design (for security reasons) with propensity  
31  
32 score matching statistical approach to reduce possible bias by  
33 94  
34 confounding variables.  
35 95  
36
- 37 96 • Limitation: no causal mechanistic association could be accurately  
38  
39 concluded although meaningful cause-effect relationships should be  
40 97  
41 advanced.  
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## 101 INTRODUCTION

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

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

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

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2  
3 125 safe to discontinue empirical antibiotic therapy after resolution of fever when  
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5 126 patients are clinically stable, irrespective of their neutrophils counts, significantly  
6  
7 127 reducing exposure to antibiotics. On the other hand, the ECIL group (European  
8  
9 128 Conference of Infections in Leukemia) has proposed<sup>10</sup> specific empirical  
10  
11 129 antibacterial therapy strategies in hematological patients including HSCT  
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13 130 recipients, in order to optimized antibiotic use. These recommendations are  
14  
15 131 heterogeneously implemented in the different hematopoietic transplant centers.  
16  
17 132 This study will investigate if a predefined strategy of optimization of  
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19 133 antimicrobial therapy that includes ECIL recommendations<sup>10-12</sup> and the *How-*  
20  
21 134 *Long* study<sup>9</sup>, will preserve intestinal microbiota composition and diversity while  
22  
23 135 reducing the incidence and severity of acute GVHD when compared to a  
24  
25 136 conventional permissive antibiotic strategy. In addition, severe infections rate,  
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27 137 transplant related mortality and long-term survival will be compared between  
28  
29 138 both groups.  
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## 36 139 **METHODS AND ANALYSIS**

### 37 38 39 140 **Study design**

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41  
42 141 A prospective longitudinal observational study of two cohorts of HSCT  
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44 142 recipients was established: i) the intervention cohort includes patients treated at  
45  
46 143 centers using an optimized strategy of antibacterial therapy (see *Intervention*  
47  
48 144 section), ii) the control cohort includes patients treated at centers using a  
49  
50 145 classical permissive antibacterial therapy strategy (see *Intervention* section).  
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52 146 Each participating center is allocated in one of the two cohorts according to its  
53  
54 147 clinical practice.  
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## 149 **Study settings**

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

## 156 **Participants**

### 157 *Eligibility criteria*

#### 158 Inclusion Criteria

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

#### 164 Exclusion criteria

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

## 168 **Recruitment process**

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

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3 171 **Intervention**  
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6 172 Centers allocated to the intervention cohort use an antibacterial systematic  
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8 173 approach that includes the following strategies:  
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12 174 1. No routine antibacterial prophylaxis is used.

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15 175 2. In case of febrile neutropenia:

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17  
18 176 - Use of escalation / de-escalation strategy for empiric antimicrobial  
19  
20 177 therapy<sup>10</sup>.

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22  
23 178 - Directed simplification in patients with etiological diagnosis according to  
24  
25 179 *in vitro* susceptibility tests.

26  
27  
28 180 - Switch to a narrower-spectrum agent in patients without an etiological  
29  
30 181 diagnosis and clinical stabilization on treatment.

31  
32  
33 182 - No broadening the antibacterial spectrum but maintenance of initial  
34  
35 183 antimicrobials therapy in patients with persistent fever if they are clinically  
36  
37 184 stable in the criteria of the physicians in charge of them.

38  
39  
40 185 3. Early (in 72 hours) withdrawal of combined treatments, when clinically  
41  
42 186 indicated.

43  
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45 187 4. Antibacterial therapy withdrawal regardless neutrophils count and  
46  
47 188 expected duration of neutropenia when patient meets all the following  
48  
49 189 criteria (*How-Long* strategy)<sup>9</sup>.

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51  
52 190 i) Afebrile for  $\geq 72$  h.  
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3 191 ii) Complete resolution of signs, symptoms and alterations in  
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5 192 complementary tests secondary to the infection (cough, abdominal  
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7 193 pain, diarrhea, pulmonary infiltrate, etc.) for  $\geq 72$  h.  
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11 194 iii) Normal vital constants (blood pressure, heart rate, respiratory rate  
12  
13 195 and diuresis and, in patients with respiratory involvement, oxygen  
14  
15 196 saturation by pulse oximetry) for  $\geq 72$  h.  
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18  
19 197 5. Short (7 days) etiological therapy for primary or related to central  
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21 198 venous (with catheter removal) no complicated bacteremia, and 14  
22  
23 199 days for *Staphylococcus aureus* non complicated bacteremia if good  
24  
25 200 clinical response and good clinical evolution.  
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27

28  
29 201 In centers allocated to the control cohort the antimicrobial therapy approach  
30  
31 202 does not include any of the strategies used in the optimization cohort but the  
32  
33 203 following management:  
34  
35

36 204 1. Use of antibacterial prophylaxis: levofloxacin 500 mg/24h (PO) or  
37  
38 205 ciprofloxacin 500 mg/24h (PO) since the start of conditioning or day 0, until  
39  
40 206 neutrophils count in peripheral blood is  $\geq 0.5 \times 10^9/L$  or empiric antimicrobial  
41  
42 207 therapy is started.  
43  
44

45 208 2. In case of febrile neutropenia:  
46  
47

48  
49 209 - Use of early broad-spectrum antimicrobial therapy without systematic  
50  
51 210 use of escalation / de-escalation strategy.  
52  
53

54 211 - Optional antibiotic simplification in patients with etiological diagnosis  
55  
56 212 according to *in vitro* susceptibility tests.  
57  
58  
59  
60

- 1  
2  
3 213 - No switching to a narrower-spectrum agent in patients without  
4  
5 214 etiological diagnosis and clinical response.  
6  
7  
8 215 - Broadening the spectrum of initial antimicrobials in patients with  
9  
10 216 persistent fever even without clinical worsening.  
11  
12  
13  
14 217 3. No early discontinuation of the combined empirical antimicrobial therapy  
15  
16 218 even in case of clinical response.  
17  
18 219 4. No discontinuation of empirical antimicrobial therapy until neutropenia  
19  
20 220 recovery.  
21  
22  
23 221 5. Prolonged etiological treatment for primary or related to central venous  
24  
25 222 catheter no complicated bacteremia, even in case of early clinical  
26  
27 223 response.

### 224 **Schedule of visits and collection of fecal samples**

225 The scheduled visits and assessments are described in table 1.

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

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3 237 antimicrobial therapy is stopped (or within the following 48 hours) or,  
4  
5 238 alternatively, if the patient did not receive antibiotics or continues receiving  
6  
7 239 antibiotics at discharge, the day before discharge (or 24 hours in advance).  
8  
9

10  
11 240 The stool samples will be collected at different time points (table 1) in Stool  
12  
13 241 Nucleic Acid Collection and Preservation Tubes (Ref. 45660, Norgen Biotek  
14  
15 242 Corp.).  
16  
17

18  
19 243 **DNA extraction, prokaryotic 16S ribosomal RNA gene (16S rRNA)**  
20  
21 244 **sequencing and bioinformatics analysis**  
22

23  
24 245 The microbiome studies will be performed at the laboratory of Infectious  
25  
26 246 Diseases of the Institute of Biomedicine of Seville (IBiS).  
27  
28

29  
30 247 DNA will be extracted from faecal samples using the Stool DNA Isolation kit  
31  
32 248 (Cat. 27600, Norgen Biotek Corp., Ontario, Canadá) according to the  
33  
34 249 manufacturer's protocol. All DNA samples will be stored at  $-20^{\circ}\text{C}$  until further  
35  
36 250 processing. DNA will be quantified by Qubit fluorometry (Invitrogen Qubit 4  
37  
38 251 Fluorometer, ThermoFisher Scientific, Spain) and normalized to  $5\text{ ng}/\mu\text{L}$  with  $10$   
39  
40 252  $\text{mM}$  Tris pH 8.5. Bacterial 16S rRNA amplification and library construction will  
41  
42 253 be performed according to the 16S Metagenomic Sequencing Library  
43  
44 254 Preparation guide from Illumina. Briefly,  $2.5\ \mu\text{l}$  of total DNA per sample will be  
45  
46 255 amplified using primers targeting the 16S rRNA V3 and V4 regions<sup>13-14</sup>. These  
47  
48 256 regions provide ample information for taxonomic classification of microbial  
49  
50 257 communities. Pooled V3-V4 amplicon libraries will be sequenced using the  
51  
52 258 Illumina MiSeq platform with a V3 reagent kit (Illumina, San Diego, California)  
53  
54 259 and paired-end 300-bp reads. Up to 96 libraries could be pooled together for  
55  
56 260 sequencing. Regarding the bioinformatics analysis of the sequencing data,  
57  
58  
59  
60

1  
2  
3 261 machine learning libraries from Scikit-learn<sup>15</sup> will be used to filter out and  
4  
5 262 discard poor-quality reads. Processed sequences will be subjected to  
6  
7 263 operational taxonomic unit (OTU) picking against Greengenes (v13.8)<sup>16</sup>, with  
8  
9 264 reads clustered by 97% identity into OTUs using QIIME 2<sup>17</sup>. In-house R scripts  
10  
11 265 (v3.2.2) will be used to visualize the results.  
12  
13  
14

## 15 266 **Evaluation of results**

16  
17  
18 267 In order to assess the impact of both antimicrobial strategies on intestinal  
19  
20 268 microbiota diversity it will be characterized as described in the previous section  
21  
22 269 and biological alpha and beta diversity indexes of samples from both cohorts  
23  
24 270 will be compared<sup>18</sup>. Alpha-diversity and beta-diversity refer to diversity within  
25  
26 271 and between samples, respectively. These secondary bioinformatics analyses  
27  
28 272 will be performed with QIIME 2 and included the calculation of the parameters  
29  
30 273 of alpha-diversity: Shannon's diversity index, frequency of observed OTUs,  
31  
32 274 Faith's Phylogenetic Diversity, and Evenness; and the parameters of beta-  
33  
34 275 diversity: Jaccard distance, Bray-Curtis distance, Unweighted UniFrac distance  
35  
36 276 and Unweighted UniFrac distance.  
37  
38  
39  
40

41  
42 277 In order to assess clinical outcomes (secondary objectives of the study) the  
43  
44 278 following data will be prospectively recorded: incidence and severity of GVHD  
45  
46 279 (evaluated for any degree, degree-II and degree-III/IV up to day 100 post-  
47  
48 280 transplant), transplant related mortality and mortality caused by infection (time  
49  
50 281 frame: Day 0 to Day +30, Day +100 and Day +365 post-transplant), incidence of  
51  
52 282 severe infections (Day 0 to Day +30 post-transplant), and overall and disease  
53  
54 283 free survival (time frame: Day 0 to Day +30, Day +100 and Day +365 post-  
55  
56 284 transplant).  
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3 285  
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56 286 **Sample size**  
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9 287 Assuming a percentage of patients developing grade two or higher acute graft  
10 288 versus host disease<sup>19</sup> in the control cohort of 42%<sup>5</sup> and 20% in the intervention  
11  
12  
13 289 cohort, a power of 80% and an alpha error of 5%, 90 patients in each study  
14  
15  
16 290 cohort should be enough to detect differences between them. However, 100  
17  
18 291 patients per cohort will be included in order to compensate possible loss of  
19  
20  
21 292 statistical power due to associations between centers and the effect of the  
22  
23 293 optimization strategy versus standard antimicrobial use (unknown at the time of  
24  
25 294 the study design), to increase the statistical power for the secondary objectives  
26  
27  
28 295 (transplant related mortality, infections rate, mortality and survival), along with  
29  
30 296 potential withdrawals<sup>20-22</sup>.

31  
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33 297  
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3536 298 **Statistical analysis**  
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39 299 To determine the impact of both antimicrobial therapy strategies on the  
40  
41 300 intestinal microbiota diversity, in a first step, a multidimensional scaling and a  
42  
43 301 permutational multivariate analysis of variance (PERMANOVA) analyses of both  
44  
45 302 antibiotherapy strategies will be performed using the R statistical package  
46  
47  
48 303 (v3.2.2)<sup>23</sup>. To find out which taxa were most likely to explain the differences  
49  
50 304 between both groups, taxa summaries generated in QIIME 2 will be reformatted  
51  
52  
53 305 for input into LEfSe via the Huttenhower Lab Galaxy Server  
54  
55 306 (<https://huttenhower.sph.harvard.edu/galaxy/root>). This algorithm performs  
56  
57 307 nonparametric statistical testing of whether individual taxa differed between  
58  
59 308 both groups and then differentially ranked the abundant taxa by their linear

14/27

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3 309 discriminate analysis (LDA) log-scores. Differentially abundant taxa that are  
4  
5 310 statistically significant using an alpha error of 5% and LDA log-scores  
6  
7 311 exceeding  $\pm 2.0$  will be visually represented as bar plots. The median values of  
8  
9 312 taxa abundance and the median percentages of taxa presence in both groups  
10  
11 313 will be calculated, and the Manhattan distances will be used for the clustering  
12  
13 314 analysis. The Kruskal-Wallis rank-sum test will be used to identify significant  
14  
15 315 taxa abundance and Fisher's exact test will be used to identify significant taxa  
16  
17 316 presence in the both groups.

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21  
22 317 The propensity score will be used to adjust potential confounding effects. To  
23  
24 318 calculate the propensity scores in the logistic model the center factors and key  
25  
26 319 predictive characteristics identified in the baseline comparability analysis will be  
27  
28 320 taken into account. Propensity scores will be used for all adjusted inferential  
29  
30 321 analyzes.

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33  
34 322 Standard descriptive statistical indices will be used according to the nature of  
35  
36 323 each variable. Continuous variables will be analyzed with linear models, binary  
37  
38 324 variables without time factor with logistic models and the time-to-event variables  
39  
40 325 with survival models, all of them incorporating the propensity score as an  
41  
42 326 adjustment factor.

43  
44  
45  
46  
47 327 The survival function of both groups will be described using the Kaplan-Meier  
48  
49 328 method. For the inferential analysis, the stratified log-rank test will be used (with  
50  
51 329 the propensity score value categorized as stratum). Hazard Ratios and its 95%  
52  
53 330 confidence intervals will be estimated using the Cox proportional hazards  
54  
55 331 regression (including the propensity score value).

56  
57  
58  
59 332 The following strategy will be used for time-dependent variables:  
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1  
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3 333 (a) Continuous variables that follow a Gaussian distribution by means of mixed  
4  
5 334 models for repeated measures (mixed longitudinal model for repeated  
6  
7 335 measurements (MMRM).  
8  
9

10  
11 336 (b) Variables that do not comply with the parametric assumptions will be  
12  
13 337 transformed into ranges and analyzed analogously to those in section a).  
14  
15

16 338 c) Longitudinal binary data will be analogously analyzed with the marginal  
17  
18 339 models [Generalized Estimation Equation GEE).  
19  
20

21 340 In addition, the following statistical tests will be used when necessary: Fisher's  
22  
23 341 exact test to compare categorical variables between groups, McNemar test or  
24  
25 342 Cochran Q test for the analysis within the groups, dependent or independent t-  
26  
27 343 test for continuous variables when comparing two groups and ANOVA if  
28  
29 344 comparing more than two groups.  
30  
31  
32

33 345 Nonparametric methods will be used in case of deviations from the applicability  
34  
35 346 assumptions: according to the data distribution, Mann-Whitney and/or Kruskal-  
36  
37 347 Wallis tests (independent variables), or Wilcoxon or Friedman tests (dependent  
38  
39 348 variables). Correlations will be done with Pearson or Spearman coefficients  
40  
41 349 according to the data distribution. SAS System (Release 9.2) or validated  
42  
43 350 equivalent software will be used. All recruited patients will be included in the  
44  
45 351 main analysis. In addition, a sensitivity analysis will be carried out with those  
46  
47 352 subjects who have complied with the protocol.  
48  
49  
50

### 51 52 53 353 **Patient and public involvement** 54 55

56 354 Neither patients nor public were involved in the development of the study.  
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59  
60



## 356 **DISCUSSION**

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

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

1  
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3 381 observational study carried out in two groups of centers in which one of the two  
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5 382 antibiotherapy approaches is already implemented turns out to be the safest  
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8 383 and more feasible design. The propensity score matching statistical technique  
9  
10 384 will reduced the possible bias due to confounding variables.

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12  
13 385 Another limitation of the study is that no causal association could be accurately  
14  
15 386 described because of the observational design. Nevertheless, as an exhaustive  
16  
17 387 set of prospective clinical data are being recorded for each patient, including  
18  
19 388 start and stopping date of every antimicrobial used, dates of start and resolution  
20  
21 389 of main clinical end-points it is likely that meaningful cause-effect relationships  
22  
23 390 might be forwarded.

24  
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26  
27  
28 391 This is the first prospective multicenter study aiming to address the effect of two  
29  
30 392 antimicrobial therapy strategies on intestinal microbiota and clinical outcomes in  
31  
32 393 HSCT recipients. The systematic collection of faecal samples at predetermined  
33  
34 394 peri-transplant time-points and the prospective collection of a wide clinical data  
35  
36 395 set with one year follow up based in pre-scheduled repeated clinical visits will  
37  
38 396 allow to examine changes in the microbiota composition over time and accurate  
39  
40 397 link them to the development of acute GVHD and other clinical outcomes.  
41  
42  
43 398 Another strength of the study is its design based on daily clinical practice. This  
44  
45 399 will provide valuable data on 'real-life patients' in addition to potential  
46  
47 400 recommendations on sampling time points and frequency for further studies. In  
48  
49 401 conclusion, the findings of this study will bring useful insight in the relationship  
50  
51 402 between antibiotherapy use and development of acute graft versus host disease  
52  
53 403 in HSCT recipients helping to design improved strategies expectedly leading to  
54  
55 404 better survival, reduced graft versus host disease and improved quality of life.  
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3 405 **Trial status**  
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6 406 At submission the study is running and 140 patients are recruited.  
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8  
9 407 Current approved protocol is version 3.0, dated 29/January/2018.  
10

11  
12 408 Date recruitment began at 16 January 2018 (first patient in).  
13

14  
15 409 Approximate date when recruitment will be completed: November 2019.  
16  
17

18  
19 410 **Abbreviations**  
20

21 411 ASCT: Allogeneic hemopoietic stem cell transplant; CTU: Clinical Trials Unit;  
22

23 412 CVC: central venous catheter; ECIL: European Conference of Infections in  
24

25 413 Leukemia; GEE: Generalized Estimation Equation; GVHD: Graft Versus Host  
26

27 414 Disease; ICH: International Council of Harmonisation; ITPP: intention-to-treat  
28

29 415 population; MMRM: Mixed longitudinal model for repeated measurements;  
30

31 416 MRT: mortality related to transplantation; PP: per-protocol population; RCT:  
32

33 417 Randomized Clinical Trial.  
34  
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37  
38

39 418 **Author Contributions**  
40

41 419 IE conceived, designed and lead the study. IE and CR-F obtained funding for  
42

43 420 the research. SJ-J, GL-H, NR-T, FJM-M, MA-G, MEP-I and CR-F collaborated  
44

45 421 in methodological aspects of the study. SJ-J, NR-T, CR-F, MEP-I and CL-R  
46

47 422 coordinated the study. IE, CS, LV-L, MK, JMM-B and NR-T were responsible for  
48

49 423 the inclusion, treatment, clinical monitoring and follow-up of the patients. CL-R  
50

51 424 coordinated sample collection. GL-H, MEP-I and YS were responsible for the  
52

53 425 sample management. SJ-J wrote the first draft of the manuscript. All authors  
54

55 426 were involved in critically revising the article and approved the final version.  
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1  
2  
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11  
12

13  
14 431 **Competing interests**  
15

16  
17 432 The authors declared no competing interests  
18  
19

20 433 **Patient consent for Publication**  
21

22  
23 434 Not required.  
24  
25

26 435 **Ethics and dissemination**  
27

28  
29 436 The study was approved between 2017 and 2018 by the five Ethical  
30  
31 437 Committees involved (Comité Coordinador de Ética de la Investigación  
32  
33 438 Biomédica de Andalucía, Comité de Ética de Investigación Clínica de  
34  
35 439 Cantabria, Comité Autonómico de Evaluación de Estudios Posautorización  
36  
37 440 Observacionales, de Seguimiento Prospectivo con medicamentos, Comité de  
38  
39 441 Ética de la investigación con medicamentos del Hospital Universitario de  
40  
41 442 Salamanca, Dirección General de Inspección y Ordenación de la Consejería de  
42  
43 443 Sanidad de la Comunidad de Madrid). Each substantial protocol amendment  
44  
45 444 will be notified for approval to the relevant ethics committee(s) prior to  
46  
47 445 implementation. All data collected will be kept strictly confidential and in  
48  
49 446 accordance with all relevant legislation on control and protection of personal  
50  
51 447 information. Study results will be published in peer-reviewed journals as well as  
52  
53 448 national and international scientific conferences.  
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3 449 **Patient and Public Involvement**  
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5  
6 450 No patient involved.  
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8  
9 451 **Access to data**  
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11  
12 452 Data are sustained in an electronic database. Upon request to the  
13  
14 453 corresponding author, the identified participant's data will be made available to  
15  
16 454 researchers whose proposals meet the research criteria. It will be also  
17  
18 455 considered requests for the protocol. To gain access, data requestors must  
19  
20 456 comply to a data access agreement.  
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29

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For peer review only

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

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

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

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STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
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

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1	<b>Introduction</b>			
2				
3	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
4				
5				
6		6b	Explanation for choice of comparators	10-12
7				
8	Objectives	7	Specific objectives or hypotheses	8
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial or single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	10-12
11				
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	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
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18				
19	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
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22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-12
23				
24		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)	_____
25				
26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	_____
27				
28		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_____
29				
30	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
31				
32				
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34	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
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1	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
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____
5				

**Methods: Assignment of interventions (for controlled trials)**

Allocation:

10	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	_____
11				
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16	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	_____
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21	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____
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26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____
28				
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**Methods: Data collection, management, and analysis**

33	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	_____
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39		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	_____
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1	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	_____
2				
3				
4				
5	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
6				
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8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____
9				
10		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)	_____
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	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	_____
17				
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22		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	_____
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25	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	_____
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28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
29				
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32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4,21,22
35				
36				
37	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
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____
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7	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
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
11				
12				
13	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
14				
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16	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	_____
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20	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
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	_____
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
27				
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29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____
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33				
34	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	_____
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\*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](https://creativecommons.org/licenses/by/4.0/)" license.

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