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Study Protocol Clinical Trial

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Disulfiram repurposing in the combined chemotherapy of Chagas disease

A protocol for phase I/II clinical trial

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Abstract

Background: Chagas disease (CD) has high morbimortality and the available trypanocidal treatment, including benznidazole (BZ), has limited efficacy in chronic patients. Furthermore, BZ causes adverse effects (AE) that prevent treatment completion in up to 30% of patients. The use of repositioned drugs or drug combination may provide an effective trypanocidal treatment. Disulfiram (DF) may enhance BZ activity and decrease BZ related AE. This study aims to assess the safety of a new combination of drugs for CD therapy, assuming BZ as the drug of choice plus DF as repositioned drug.

Methods: This single-centre, open-label, phase I/II clinical trial was designed to evaluate the safety of the combined use of BZ plus DF for CD therapy. Participants are adults with indeterminate form of chronic CD, both sexes, aged from 18 to 70 years old and *Trypanosoma cruzi* polymerase chain reaction-positive. The primary outcome will be the occurrence of serious AE. The secondary outcome will be post-treatment *Trypanosoma cruzi* polymerase chain reaction negativization. Six groups of 9 patients will be sequentially tested. The first group will be allocated to receive BZ 100 mg/d + DF 250 mg/d for 60 days. Upon safety confirmation (<1/3 of participants with serious AE), the combination dose will be gradually increased and dispensed to 5 groups (group II:BZ 200 mg/day+DF 250 mg/d; group III:BZ 300 mg/d + DF 250 mg/d; group IV:BZ 100 mg/d + DF 500 mg/d; group V:BZ 200 mg/d + DF 500 mg/d; group VI: BZ 300 mg/d + DF 500 mg/d) for 60 days in order to determine the maximum tolerated dose.

Discussion: Our hypothesis is that the drug combination will be well tolerated and allow the proposal of phase II trials in larger scale to test the efficacy of the new drug combination in CD. We expect that the studied combination will have less AEs with an efficacy

Trial Status: Protocol version: 18 January 2021, version 5.

The first patient was recruited in February 2020.

The last patient will be recruited in December 2021

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CB, OCM, and MAVS are research fellows of CNPq. CB is CNE researcher from FAPERJ. OCM is JCNE researcher from FAPERJ. The remaining authors report no conflicts of interest.

Data sharing are not applicable to this article as no datasets were generated or analyzed during the current study.

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similar or superior to the current treatment. This will allow the successful treatment of a greater number of patients while decreasing the treatment cost as less patients will need treatment for AEs.

Trial registration: This study was registered on the Brazilian Clinical Trials Database - REBEC (RBR-5n4htp). Registered 7 January 2020. UTN Number: U1111-1246-1293. http://www.ensaiosclinicos.gov.br/rg/RBR-5n4htp/

Abbreviations: AE = adverse events, BZ = benznidazole, CD = Chagas disease, DETC = sodium diethyldithiocarbamate, DF = disulfiram, DNA = deoxyribonucleic acid, EDTA = ethylenediamine tetraacetic acid, GEB = guanidine-EDTA-blood, IC = informed consent, PCR = polymerase chain reaction.

Keywords: benznidazole, Chagas disease, clinical trial, repurposing drug, treatment

1. Introduction

Chagas disease (CD) is a neglected, chronic and potentially fatal disease caused by the *Trypanosoma cruzi (T. cruzi)*. According to World Health Organization, CD affects 8 million people worldwide, mainly in Latin America.[1] CD annual death toll surpasses 10,000 people and over 25 million people are at risk of acquiring the disease.[1] In Brazil, estimates indicate that the number of people infected with *T. cruzi* ranges from 1.9 million to 4.6 million, which is over 2% of the Brazilian population.[2] Moreover, migration movements led to diagnosis of CD in non-endemic countries including autochthonous cases by non-vector transmission, such as blood transfusion, vertical, and organ transplantation.[3]

The only drugs approved for CD treatment in Brazil and by FDA are benznidazole (BZ) and nifurtimox. However, both drugs are associated with possible serious adverse events (AE)[4–5] and up to 30% of the patients treated with BZ abandon treatment due to intolerable AE[6–9] whereas nifurtimox toxicity may be even higher.[10] Moreover, the specific treatment available has limited efficacy in chronic patients[11] and BZ is currently indicated only to patients with acute CD and chronic patients under 50 years old with the indeterminate form of the disease.[2] Thus, new effective and low cost trypanocidal agents are a pressing demand, particularly in countries with tropical climate and low-income populations.

The combination of drugs with different mechanisms of action has already been proposed as a strategy for the treatment of patients with chronic CD.[12] The use of repositioned drugs represents a breakthrough in chemotherapy studies, as it allows a shorter time and lower cost for drug development.[13] In this study, disulfiram (DF-1,1',1",1'"-[disulfanediylbis (carbonothioylnitrilo)]tetraethane), a well-tolerated drug used for the treatment of alcoholism,[14] will be repositioned and used in combination with BZ. Therefore, this study protocol proposes the evaluation of the off-label use of DF associated to BZ in CD treatment. DF has already been tested as a repurposing drug in combination with cisplatin and vinorelbine and was well tolerated and appeared to prolong survival in patients with non-small cell lung cancer.[15] DF was shown to be trypanocidal against *T. cruzi in vitro*.[16–17] In preclinical studies, we demonstrated that DF present synergistic effect with BZ both as a trypanocidal agent *in vitro* and as a protective agent increasing survival of mice infected by *T. cruzi*. Our preclinical data also indicated that the BZ/DF combination presented low systemic toxicity. These data are included in the BZ/DF combination patent deposited at the Brazilian National Institute of Industrial Property (Pat. no. PI09008810).

Thus, the study main objective is to test the safety of a drug combination for CD chronic patients treatment that include BZ as the drug of choice plus DF as repositioned drug. Our hypothesis is that the drug combination will be well tolerated and that *T. cruzi* polymerase chain reaction (PCR) will be largely negative even when using low doses of BZ in combination to DF.

2. Methods/design

2.1. Study setting, aim, and design

It is a single-centre, open-label, randomized, phase I/II clinical trial with 6 parallel arms conducted at Evandro Chagas National Institute of Infectious Diseases, a national reference centre for treatment and research in infectious and tropical diseases in Brazil.

The study main aim is to test the safety of the drug combination BZ/DF for CD chronic patients treatment. We used the SPIRIT reporting guidelines for the preparation of this protocol.[18] The complete checklist is available as a supplemental file, http://links.lww.com/MD2/A198. Trial registration data are depicted in Table 1.

2.2. Eligibility criteria

The inclusion criteria for this study are adults with indeterminate form of human chronic CD, both sexes, aged 18 to 70 years and *T. cruzi* PCR-positive. Two distinct serological tests (enzyme-linked immunosorbent assay, indirect immunofluorescence or chemiluminescence) will be carried out to confirm the diagnosis of CD.

Patients with any of the following conditions will be excluded from the study: previous BZ or DF treatment, contraindications or hypersensitivity; alcoholism; smoking; renal or hepatic impairment; gastrointestinal disorders; pulmonary, epileptic, hematological disorders; pregnancy or lactation; heart disease associated with moderate or severe orovalvar disease, ischemic, congenital or hypertensive heart disease; systemic diseases such as autoimmune disorders, cancer, other infectious diseases such as AIDS; the use of ≥3 regular medications within 2 weeks before starting study treatment; use of any eventual medication within 7 days before starting study treatment; use of any antifungal 60 days before starting study treatment. Individuals who took part in previous intervention studies one year before and who have severe cognitive impairments will also be excluded.

2.3. Withdrawal criteria

The study participant will be discontinued from the study in case of participant request, serious or intolerable AE, or study participant failure to maintain compliance with clinical trial medication or protocol.

Table 1

Trial registration data.

Data category	Information
Primary registry and trial identifying number	ReBEC no. RBR-5n4htp
Date of registration in primary registry	January 7th, 2020
Secondary identifying numbers	UTN no. U1111-1246-1293
Primary sponsor	Oswaldo Cruz Foundation
Secondary sponsors	Fundação Carlos Chagas Filho de Amparo à Pesquisa no Estado do Rio de Janeiro; Conselho Nacional de Desenvolvimento Científico e Tecnológico
Contact for public and scientific queries	Dr Roberto Saraiva, MD, PhD
Public and scientific title	Disulfiram repurposing in the combined chemotherapy of Chagas disease – Phase I/II Clinical Trial
Country of recruitment	Brazil
Health condition	Indeterminate form of chronic Chagas Disease
Intervention	Combined use of benznidazole (BZ) plus disulfiram (DF)
Key inclusion criteria	Adults, both sexes, aged from 18 to 70 years *T. cruzi* PCR-positive
Key exclusion criteria	previous BZ or DF treatment, contraindications or hypersensitivity; pregnancy or lactation; alcoholism; smoking
Study type	Interventional Randomized, open-label Phase I/II
Date of first enrolment	February 3rd, 2020
Recruitment status	Recruiting
Primary outcome	Occurrence of serious AE
Secondary outcome	*T. cruzi* PCR-negative result

AE = adverse events, BZ = benznidazole, DF = disulfiram, PCR = polymerase chain reaction, ReBEC = Brazilian registry of clinical trials, UTN = Universal test number.

2.4. Outcomes

The primary outcome is the occurrence of toxicity or serious AE by the combination of BZ and DF in more than one-third of the participants of a study arm.

Secondary outcome will be *T. cruzi* PCR negativization after treatment in more than 80% of the participants of a study arm.

2.5. Screening

For the screening process (Visit 0 and Visit 1), a computer based random selection of 135 patients with indeterminate form of CD who attend the outpatient clinic at Evandro Chagas National Institute of Infectious Diseases will be done by the non-medical study coordinator. It is required to recruit a greater number of participants during the screening process since only around 40% of patients with indeterminate form of CD will present a positive *T. cruzi* PCR result. During Visit 0 all selected patients should sign the informed consent form to enroll in the study and take part in an interview to verify their eligibility. Subsequently, at Visit 1, the participants will undergo a medical consultation, blood tests, echocardiogram, and electrocardiogram tests (Table 2).

2.6. Intervention and follow-up

After eligibility confirmation, 6 groups of 9 patients will be sequentially tested to verify the safety of treatment. The first group of nine participants will be randomly selected among those with confirmed eligibility and allocated to the first medication treatment level that will start at Visit 2 after medical doctor and

Table 2

Trial activities scheduling.

	Screening		Treatment					Post-treatment			
Timepoint	**Visit 0**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**
Informed consent	X										
Baseline questionnaire	X										
Blood tests		X		X	X	X	X				
T.cruzi PCR		X					X	X	X	X	X
Serological tests		X									
ECHO/ECG		X									
Anthropometry		X	X	X	X	X	X				
Vital status		X	X	X	X	X	X	X	X	X	
Toxicological effects				X	X	X	X				
Nutritional follow-up			X		X		X				
AE assessment				X	X	X	X	X	X		
Drug dispensation			X	X	X	X					
Pharmacokinetics				X	X	X					

AE = adverse events, ECG = electrocardiogram, ECHO = echocardiogram, PCR = polymerase chain reaction.

pharmacist consultations. The pharmacist will be responsible for drug dispensation and directly observed treatment during the assessment visits. The treatment will begin with the lowest dose of BZ for 60 days, corresponding to 100 mg/d associated with 250 mg/d of DF. Upon safety confirmation of the lowest dose of BZ and DF, i.e. after the nine participants have finished 60 days of treatment with less than 3 participants with serious AE, two new groups of 9 participants (group II - 200 mg/d BZ + 250 mg/d DF and group IV - 100 mg/d BZ + 500 mg/d DF) will start treatment to test a higher dose of BZ (group II) or DF (group IV). After confirming the safety of the group II drug combination, another group of nine participants (group III - 300 mg/d BZ + 250 mg/d DF) will start treatment. Similarly, after confirming the safety of the group IV drug combination, another group of nine participants (group V - 200 mg/d BZ + 500 mg/d DF) will start treatment. Finally, after confirming the safety of the group V drug combination, another group of nine participants (group VI - 300 mg/d of BZ + 500 mg/d of DF) will start treatment. If 3 or more participants within any group present serious AE, the treatment will be discontinued and the maximum tolerated dose will be considered those at the previous level (Fig. 1).

At each visit, participants will receive a calendar to register the medication intake over the treatment period until the next visit to the research centre. Each patient will be assessed within one week (Visit 3), three weeks (Visit 4), six weeks (Visit 5), and 60 days (Visit 6) after treatment started at Visit 2. During Visits 3 to 6, blood samples will be collected to verify health status and possible toxicological effects from the BZ/DF combination. In

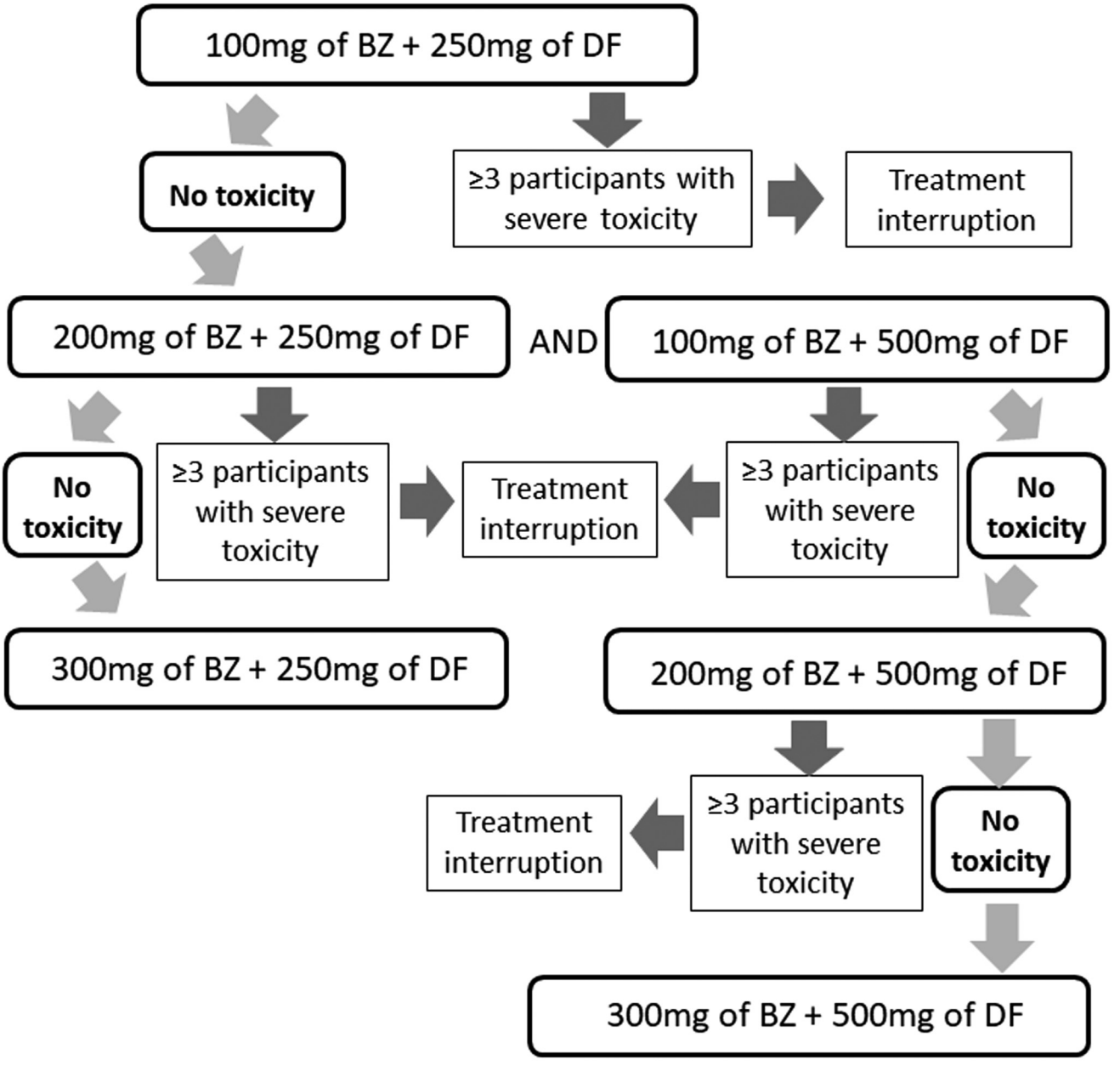

Figure 1. Dose escalation sequence of BZ + DF combination.

addition, blood sampling for *T. cruzi* real time qualitative and quantitative PCR will be performed at Visit 6 (Table 2). Once the treatment is complete, each patient will be evaluated 2 months (Visit 7), 4 months (Visit 8), 6 months (Visit 9) and 12 months (Visit 10) after Visit 6. These visits will also include blood samples collection for *T. cruzi* PCR.

In addition to the scheduled visits, weekly telephone contacts will take place during the first 30 days of the follow-up in order to assess AEs, to check adherence to medication treatment and to confirm the participant next visit. After the first 30 days of follow-up, phone calls will happen 1–2 days before each visit up to the end of the study (Visit 10). As many attempts as necessary to contact the patients will be made.

2.7. Pharmacokinetic study

For the pharmacokinetic analysis of DF and BZ, at Visit 4, ten blood samples will be collected at 00:00 h our (pre-dose), 02:00, 02:30, 03:00, 03:30, 04:00, 04:30, 05:00, 05:30, and 06:00. At each time point, 8 mL of blood will be collected into an ethylenediamine tetraacetic acid (EDTA) K3 vacutainer and centrifuged at 3000 rpm for 10 mins at 4°C. Following centrifugation, the plasma samples will be stored at −70°C until analysis. During Visit 3 and 5, the collected blood samples will be used for baseline values before assisted medication administration. Blood samples will be processed and stored following the same procedures described above.

The quantification of BZ, DF and metabolites (diethyldithiocarbamic acid methyl ester and diethylthiocarbamic acid methyl ester) in human blood will be performed by liquid chromatography coupled with tandem mass spectrometry. Methods will be developed for measuring BZ, and DF and its metabolites, and validation will be performed in accordance with the Brazilian Health Surveillance Agency, resolution n° 27/2012.

2.8. Adverse events

The participant will be informed of all potential AE and will be instructed to report any discomfort perceived all through the study. Any untoward medical occurrence in a participant who has received any of the research-related therapies will be considered an AE. That include abnormal findings on blood tests or vital signs, unfavorable and unintended symptom, or a disease temporally associated or not with therapy. In addition, researchers will evaluate and notify these events during medical appointments and telephone calls.

To evaluate the severity of AEs, the modified classification from Capellà et al[19] will be used. We will consider as mild events those reactions of little importance and of short duration, which may require treatment but do not substantially affect the normal life of the patient. We will classify as moderate all reactions that alter the patient's normal activity, including emergency care services and absenteeism from work or school. We will consider serious AE when threatens the patient's life, requires hospitalization or prolongs hospitalization, results in significant or persistent weakness or disability, or definitive treatment interruption.[8,20]

2.9. Deoxyribonucleic Acid (DNA) extraction and molecular diagnosis

Blood samples for molecular diagnosis will be collected for eligibility assessment (before treatment), at the end of the treatment (Visit 6), and at 2 months (Visit 7), 4 months (Visit 8), 6 months (Visit 9) and 12 months (Visit 10) after the end of the treatment. At each of those visits, 10 mL of peripheral blood will be drawn and from each sample, three DNA extractions will be performed. From each DNA extraction, qualitative real time PCR for *T. cruzi* DNA will be run in duplicate. From every patient's sample with a positive qualitative PCR, one of the positive extractions will be used. In case any of the samples after the end of the treatment test positive, all subsequent samples collected in the remaining visits will not be tested for CD molecular diagnosis as the therapeutic failure will have been already characterized.

Venous blood samples will be collected from each patient in EDTA-K2 vacutainer tubes, immediately mixed with the same volume of 6 M Guanidine-HCl/0.2 M EDTA solution (GEB), heated for 15 minutes in boiling water and stored at 4°C until DNA extraction. Aliquots of 300 μL GEB samples will be processed with the High Pure PCR Template Preparation kit (Roche Diagnostics Corp., Indianapolis, IN) for DNA purification, as described previously.[21]

Multiplex real-time qPCR assays targeting the *T. cruzi* satellite nuclear DNA and the exogenous internal amplification control (plasmid pZErO-2 containing an insert from the *Arabidopsis thaliana* aquaporin gene, 40 amplification cycles) will be performed.[22] The standard curves for absolute quantification will be constructed with 1/10 serial dilutions of total DNA obtained from a negative GEB sample spiked with 10^5 parasite (CL Brener, TcVI) equivalents/mL of blood. The amplifications will be carried out in an ABI Prism 7500 Fast device (Applied Biosystems, Carlsbad, CA).

2.10. Sample size

A total of 135 patents will be recruited for eligibility assessment. The final sample size that will undergo study intervention in case primary outcome does not happen in any study arm will be 54 participants.

2.11. Data collection, management, and analysis

All data will be recorded in specific case report forms and in electronic medical records. All data will be deidentified and included in an electronic password protected database in RedCap. All data entry in the database will be double checked against the data on case report forms. The principal investigator and key personnel designated by him will have access to the final trial dataset.

Descriptive analysis will be performed based on absolute and relative frequency to describe qualitative variables and summary measures (mean, median, quartiles, minimum and maximum) for quantitative variables. The maximum tolerable dose and the minimum effective dose will be described.

Since the regular dose of BZ produces PCR-negative results in 82%[22] to 86.7%[23] of chronic patients, we will consider as the minimum effective dose when over 80% of treated participant shows *T. cruzi* PCR-negative results at all post-treatment visits. We will compare the groups exposed to different doses of BZ (100 mg, 200 mg, and 300 mg) and DF (250 mg and 500 mg) considering the percentage of *T. cruzi* PCR-negative results using McNemar test for paired proportions. The analysis will be run using Stata version 13.0 (StataCorp, College Station, TX). The null hypothesis will be rejected at $P < .05$.

2.12. Trial monitoring

An independent contract research organization company will provide external clinical trial monitoring and will monthly oversee the progress of the clinical trial and ensure that it will be conducted, recorded and reported in accordance with the protocol, good clinical practice and regulatory requirements. A data monitoring committee will not be implemented due to the short-term duration of the intervention, small sample size, non-critical patients' condition, and the fact that the drugs under investigation are well characterized.

2.13. Ethics approval and consent to participate

The study protocol was approved by the institutional ethics committee under number 4.022.927 and will be performed in accordance to the Resolution n° 466/2012 of the Brazilian National Council of Health. Written, informed consent to participate will be obtained from all participants. The consent process will follow the Good Clinical Practice standards and will be conduct by a well-trained team member. All patients will only be ready to sign or fingerprint the informed consent (IC) form after:

(1) reading the form;
(2) receipt of appropriate explanations of the nature and objectives of the study;
(3) understanding of the objectives and procedures to be adopted during the study;
(4) explanation of potential risks and benefits as well as number of visits required until protocol termination;
(5) clarification of all questions or doubts.

In the case of illiterate participants, witnesses who are able to read and sign the IC form must accompany them. All patients will be informed that they may withdraw from the trial at any time without any harm or consequences. The voluntary and confidential nature of participation will also be explained to all participants. The participants will receive reimbursement for any transportation costs they may have to take part in the study. All participants will receive free treatment for any AE they may have during the study. All participants will be informed that their blood specimens will be stored de-identified in a study biorepository for the maximum period of 10 years. After that length of time, all samples will be discarded following local legislation. All participants will also be informed that their samples might be used in ancillary studies and, in that case, the participants will have to give a new signed consent form after approval of the new study by the institutional ethics committee. All participants will also be informed that they may withdraw the authorization for the storage of their blood specimens at any time and in that case their samples will be discarded. Each participant will receive an original signed and dated consent form at the time of initial consent.

Any protocol modification that substantially alters the study plan or the risk to participant's health will be reported to the research ethics committee and will be added to the trial registration. The IC form will include any changes and/or amendments that may occur in the present protocol, and a new consent process will be applied.

The results of the trial will also be communicated to the study participants and those with positive PCR who have received a BZ dose lower than 300 mg/day will be treated with the standard treatment dose.

3. Discussion

The development of new drugs or drug combinations for CD treatment is pursued due to the known AEs associated to the use of BZ or nifurtimox and the limited efficacy in chronic patients.[11] However, until now studies have failed the demonstration of a drug with higher sustained trypanocidal effect than BZ[23–25] or the demonstration of similar trypanocidal effects with lower occurrence of AEs. There is a recent proposal to use lower doses of BZ for CD treatment with the hope of higher treatment adherence and the same trypanocidal effect.[26] Therefore, our proposal to study a new drug combination for CD treatment is highly interesting. However, BZ/DF combination efficacy/safety in humans is not determined yet which brings up the need for phase I to III clinical trials.

The DF trypanocidal mechanism of action may be related to the superoxide dismutase antagonism properties of the DF derivative, sodium diethyldithiocarbamate (DETC). DETC is a superoxide dismutase antagonist and *T. cruzi* superoxide dismutase was shown to function as an important virulence factor, promoting parasite proliferation within cardiomyocytes[27] and macrophages.[28] Moreover, the combination with DF and DETC is remarkably interesting because both compounds inhibit P-glycoprotein[29] which is considered to largely mediate *T. cruzi* drug resistance.[30]

Other potential advantage of the use of DF as a repositioned drug in combination with BZ is that DF and DETC are not only well tolerated,[14] but in animal models DF/DETC was described to be radioprotective,[14] hepatoprotective,[31–34] nephroprotective,[35] neuroprotective[36] or induces neuroprotective action.[37–38] In addition, DF was shown to prevent doxorubicin-induced cardiotoxicity and oxidative stress *in vivo*.[39]

Therefore, the BZ/DF combination may give rise to a safer and more effective agent for CD treatment.

BZ and DF AEs are well known at loose drug regimens.[6–9,14] However, their combined use is new. According to full prescribing information, BZ is contraindicated in patients who have taken DF within the last two weeks because psychotic reactions have been rarely reported in patients concurrently taking DF and other nitroimidazole agents (metronidazole, for example), of which BZ is structurally similar to.[40] In monotherapy, both metronidazole and DF can promote psychosis outbreaks, as described in their package inserts.[41,42] However, most reported cases are not actually psychosis, but toxic delirium.[43] The interaction between DF and metronidazole was described to be linked to elevated dopaminergic activity in the brain.[44] Both substances cross the blood-brain barrier and can increase dopamine by interfering with its catabolism, as DF inhibits dopamine beta-hydroxylase[45] and metronidazole down modulates monoamine oxidase.[46] Therefore, the risk of psychotic AE after co-administration of these two drugs may be expected. On the other hand, no such psychotic effect have ever been reported for patients using BZ monotherapy[6,8,47] and BZ does not show any influence on the enzymes involved in noradrenaline/dopamine synthesis or catabolism processes, in vitro or in mice.[48] Also, according to Food Drug Administration, there was no report of psychotic AE after concomitant use of BZ and DF.[38] Therefore, we do not expect any increase in psychotic AE occurrence with concurrent use of DF and BZ.

Our study is a unicentre phase I/II trial and may be limited to study which arm of the DF/BZ combinations tested has the best trypanocidal effect due to the number of patients to be included in

each arm. However, the main objective of the study is to evaluate the safety of the drug combination. We intend to propose a proof-of-concept, double-blind, randomised phase 2 clinical trial after definition of the safety of the proposed drug combination arms with enough power to study which drug combination has the best trypanocidal effect against full dose BZ monotherapy. The development of a new cheap and better tolerated drug combination to treat CD is very important as a higher number of patients will complete their treatment and benefit from a potential change in the natural course of their disease.

Author contributions

RMS, GPES, ALV, AMSF, JAS, SACPR, and MVS conceived the study. RMS, LFP, LHCS, AMHM, and MFFM designed the study protocol and patients' follow-up. GPES, FMC, PWM, GMSS, RCE, and ALV designed the pharmacokinetic study and adverse events follow-up. NLSG, OCM, and CB designed the PCR technique and follow-up. All authors have contributed for critical revision of the article, read and approved the final manuscript.

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