BENLATINO Trial: Double blind, phase III randomized, multicenter, safety and efficacy non-inferiority trial to evaluate two short benznidazole regimens for the treatment of adults in the chronic phase of Chagas disease in its indeterminate and mild cardiac forms in Bolivia and Colombia.

Study code	BENLATINO Trial
Short title	Short Benznidazole (BZN) regimen for chronic phase Chagas disease patients
Investigational product and comparator	• Group A (B300/2) - BZN (Abarax, tablet 100mg), 300 mg - 2 weeks: BZN 300 mg, divided in three daily doses, for 2 weeks. • Group B (B300/4) - BZN (Abarax, tablet 100mg), 300 mg - 4 weeks: BZN 300 mg, divided in three daily doses, for 4 weeks. • Group C (B300/8) - BZN (Abarax, tablet 100mg), BZN 300 mg, divided in three daily doses for 8 weeks.
Project	CUIDA Chagas – Communities United for Innovation, Development and Care for Chagas disease – 'Towards elimination of congenital transmission of Chagas disease in Latin America'
Date of protocol	Febuary 14th 2023
Protocol version	4.0
Protocol registry number	ERC.0003625
Funder	Unitaid and Brazilian Ministry of Health
Sponsor	FIOCRUZ (Oswaldo Cruz Foundation)
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Glossary

AE	Adverse events
AESI	Adverse Event of Special Interest
ALT	Alanine aminotransferase
AR	Adverse reactions
AST	Aspartate aminotransferase
BENDITA	Benznidazole New Doses Improved. Treatment & Therapeutic Associations
BETTY	Short-course Benznidazole treatment to reduce *Trypanosoma cruzi* parasitic load in women of reproductive age
BZN	Benznidazole
CBC	Complete blood count
CD	Chagas disease
COVID-19	Corona virus disease 2019
CRF	Case report form
CS	Civil Society
CSO	Civil Society Organizations
CTCAE	National Cancer Institute's Common Terminology Criteria for Adverse Events
CUIDA Chagas	Communities United for Innovation, Development and Care for Chagas disease
DNA	Deoxyribonucleic acid
eCRF	Electronic case report form
EKG	Electrocardiogram
ELEA	Laboratorio ELEA-Phoenix
EOT	End of treatment
EQ-5D-3L	Euroqol 5 dimensions 3 levels
ERC	Ethics Review Committee
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
ICER	Incremental cost-effectiveness ratios
ICH	International committee for Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
ISPOR	International Society for Pharmacoeconomics and Outcomes Research, Inc.
INR	International normalized ratio
ITT	Intention to Treat
LAFEPE	Laboratório Farmacêutico do Estado de Pernambuco S/A
MULTIBENZ	Efficacy and safety assessment of different dosage of benznidazole for the treatment of Chagas disease in chronic phase in adults
NFX	Nifurtimox
NTD	Neglected tropical disease
PCR	Polymerase Chain Reaction
PI	Principal Investigator

QALY	Quality-adjusted life-year
QOL	Quality of life
SAE	Serious adverse events
TESEO	New Therapies and Biomarkers for Chagas Infection Clinical trial
ULN	Upper limits of normal
USD	United States dollars
WBC	White blood cells
WHO	World Health Organization
WHOQOL BREF	World Health Organization instrument to evaluate quality of life

Expanded Title: Double blind, phase III randomized, multicenter, safety and efficacy non-inferiority trial to evaluate two short benznidazole regimens for the treatment of adults in the chronic phase of Chagas disease in its indeterminate and mild cardiac forms in ~~Brazil~~Bolivia and Colombia.

Short Title: Short benznidazole regimen for chronic phase Chagas disease patients

Name: BENLATINO Trial

Abstract:

Chagas disease is a major cause of heart disease, morbidity, and premature loss of life in the Americas. Eliminating the *Trypanosoma cruzi* parasite using antitrypanosomal drugs has shown to produce cure in children, halt future congenital transmission, and reduce morbidity from the disease. However, the current treatment regimens are lengthy (60 days) and entail frequent side effects, causing about 20% of patients to drop out of treatment, and discouraging others from starting. Recent research found that a reduced treatment of benznidazole still has adequate efficacy with few side effects.
In this international, multicenter, double-blind, phase III, placebo-controlled study, 672 participants will be randomly assigned to receive the standard-dose of benznidazole (300 mg daily for 8 weeks) or the short experimental regimens (benznidazole 300 mg daily for the first 2 weeks plus placebo for the last 6 weeks or benznidazole 300 mg daily for the first 4 weeks plus placebo for the last 4 weeks). Efficacy will be assessed considering a non-inferiority design and through the detection of parasite deoxyribonucleic acid (DNA) through molecular biology (Polymerase Chain Reaction - PCR). Meanwhile, safety will be evaluated through a superiority design, with the aim to find the new regimen as effective as the standard one, but superior in terms of safety. An intention-to-treat analysis will be performed, and statistical significance will be set at 0.025 for the non-inferiority outcome (positive PCR) and 0.05 for the superiority outcome.
The study population will be adult participants, 18 years or older, with chronic Chagas disease in its indeterminate or mild cardiac forms, with a positive diagnosis from two serological assays. The trial will be conducted in four sites: two in Bolivia, and two in Colombia. The primary endpoint will be parasitological response determined as sustained negative qualitative PCR at 24 months after treatment. The proportion of participants with positive qualitative PCR will be measured at 1, 4, 6, 8, 12, 18, and 24 months from end of treatment . The frequency of adverse events leading to treatment discontinuation will be compared. An economic evaluation will be conducted assessing the direct and indirect costs, including procedures associated with the management of adverse events.

Key words
Chagas disease, treatment, short regimen, benznidazole.

1. Rationale and background information

Chagas disease (CD), a neglected tropical disease (NTD) also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). *T. cruzi* parasites are mainly transmitted through contact with feces/urine of infected blood-sucking triatomine ('kissing') bugs. Other forms of transmission include consumption of contaminated food, transmission from an infected mother to her newborn during pregnancy or childbirth, blood or blood product transfusion from infected donors, organ transplants using organs from infected donors, and laboratory accidents. (1) CD has an acute phase, which can last up to a few weeks or months and is usually mild or asymptomatic, and a chronic phase. An estimated 30-40% of infected and untreated people will develop severe and sometimes life-threatening medical problems over the course of their lives, including cardiac alterations, digestive manifestations, and neurological or mixed alterations, which may require specific treatment. If untreated, infection is lifelong. (2)

CD is mainly found in endemic areas of 21 continental Latin American countries, with approximately 65 million people at risk of contracting the disease. An estimated 6 to 7 million people worldwide are infected with the *T. cruzi* parasite, of which the large majority resides in Latin America. Every year, over 10,000 CD-related deaths are reported. (3–5) Symptomatic CD imposes a substantial financial burden on healthcare systems and societies (6) with an estimated United States dollar (USD) 690 million in healthcare costs and USD 8 billion in annual economic losses, the CD economic burden equals or exceeds that caused by other prominent infectious diseases, such as Zika virus disease (USD 3.7 billion). (7,8) However, despite the high morbidity and mortality of CD and the significant associated economic burden, only 7% of people with CD have been diagnosed and only about 1% receive etiological treatment. (9) Timely identification and treatment of CD has important benefits, including prevention of future congenital transmission in treated mothers, serological cure in infants and children, and the potential reduction of progression to advanced forms of the disease in adults. (10–12,14,15) However, once the disease has progressed to an advanced phase with severe cardiac or digestive disease, etiological treatment does not appear to have clinical benefits. (16) This supports the need for improved diagnostics and early access to safe and effective treatment. (17)

The current treatment for CD has significant limitations and is restricted to two nitro-heterocyclic drugs, nifurtimox (NFX) and benznidazole (BZN). Both include long treatment durations as well as safety and tolerability concerns. BZN, a nitroimidazole introduced by Roche in 1971, is marketed by Laboratório Farmacêutico do Estado de Pernambuco S/A (LAFEPE) - Brazil and Laboratory ELEA-Phoenix (ELEA) – Argentina. It is supplied in tablet strengths of 12.5, 50 and 100 mg, administered twice a day at a dose of 5 mg/kg body weight/day for adults and 5-10 mg/kg body weight/day for children for 60 days. (18)
Current treatment regimens have been derived from old studies and with very limited comparisons. (19) Data from recently concluded trials suggest opportunities for optimization of existing treatment regimens of BZN as described below.

2. Chagas disease treatment

Acute phase of the disease

Treatment during the acute phase of CD is highly effective. Cure rates between 65-80% have been documented, reaching almost 100% in cases of congenital transmission that are treated during the first years of life. Antiparasitic treatment should therefore be provided as soon as possible following detection of acute *T. cruzi* infection. (20)

Chronic phase of the disease

Sero-negativization has been considered the gold standard for cure for decades. Although complete sero-negativization takes five years in at least 70% of children treated, this rate only reaches about 30% in adult patients after roughly 20 years of follow-up. Besides the long period needed to demonstrate cure, this measurement also depends on the time of infection, age when patients were treated, length of follow-up and region where the patient was infected. (21)

In cases of chronic infection, cure rates between 60–93%, using serology, are achieved in children aged up to 13 years and 2–40% in adults with late chronic disease. (18) Although the evidence of treating patients in the chronic phase could be seen as controversial due to the marginal effects found after treating patients with moderate-to-severe cardiomyopathy, several observational studies have demonstrated that etiological treatment could prevent progression of cardiomyopathy. In addition, treatment can clear parasitemia in approximately 80% of treated patients with chronic indeterminate disease and may eventually lead to serological cure in a smaller subset (<40%) after years or decades, as well as interrupt vertical transmission. (10,11) Despite the uncertain rates of cure in the chronic phase, current recommendations advocate treatment for patients as long as they do not have severe heart disease. (22) This consensus is based mainly on the inferior long-term clinical progression observed in patients treated with benznidazole after an average follow-up of about 10 years, in the prevention of chagasic cardiomyopathy, as well as in the prevention of congenital transmission of children born to infected and treated women. (10,23)

2.1 Treatment toxicity

The main drawback of BZN and NFX is their high adverse event ratio. The frequency of adverse effects with NFX ranges from 43.0 to 97.5%. with the most common being anorexia, weight loss, neurological disorders (irritability, insomnia, disorientation, mood changes, paresthesia, and peripheral neuropathy), digestive manifestations such as nausea and vomiting, and, occasionally, fever and rash. Treatment is discontinued in 14.5–75.0% of cases. (24) For BZN, the most common adverse effects involve hypersensitivity, mainly in the form of skin rash (29–50%), digestive intolerance (5–15%), and general symptoms such as anorexia, asthenia, headache, and sleeping disorders (40%). Neuropathy and depression of bone marrow are considered rare. Treatment is discontinued in 9–29% of cases, even though most reactions are reversible, and severe cases occur in less than 1%. BZN is generally preferred over NFX because of its better tolerability profile. (18)

Since the major limitation of nitroderivative-based therapy is its toxicity, which hampers its efficacy and adherence rates, different approaches have been designed to improve tolerance, mainly based on overall dose reduction. (25)

2.2 Benznidazole dosage

The current BZN recommended dose of 5 mg/kg per day for 60 days, or 300 mg daily, is the result of studies carried out at the end of the 1970s, which were empirical and had small sample sizes. (26)
Based on the toxicity profile and treatment interruption rates of up to 29%, new regimens with a dose reduction have been proposed. This hypothesis has driven the realization of three randomized clinical trials, which are currently being assessed, to determine the efficacy and safety of different BZN regimens in adults with chronic phase CD. The BENDITA (27) and MULTIBENZ (NCT03191162) studies evaluated a treatment course of two weeks, while the BETTY study (NCT03672487) is evaluating a treatment course of 30 days.
Primary efficacy endpoint is the parasitological response measured through PCR at the end of treatment and maintained until 12 months, as is common practice in all the recent randomized clinical trials.
The BENDITA trial was a randomized, double-blind, phase II, placebo-controlled trial conducted in Bolivia between 2016 and 2018. (27) Two-hundred and ten participants at chronic phase CD were randomized in seven groups, each group receiving BZN in shorter regimens and in combination with fosravuconazole: BZN 300 mg daily for 8 weeks, 4 weeks, or 2 weeks, BZN 150 mg daily for 4 weeks, BZN 150 mg daily for 4 weeks plus fosravuconazole, BZN 300 mg once per week for 8 weeks plus fosravuconazole, or placebo, with 30 participants in each group and a 12-month follow-up period. The intention-to-treat primary efficacy analysis showed that 89.3% had sustained clearance at 6 months on parasitemia on BZN 300mg 8 weeks and 4 weeks; 82.8% on BZN 300mg 2 weeks; 83.3% on BZN 150mg 4 weeks; 85.2% on BZN 150mg 4 weeks in combination with fosravuconazole and 82.8% on BZN 300mg weekly in combination with fosravuconazole, compared with 3.3% for placebo. Safety results suggested high adherence to treatment in all groups. Six participants (20%) discontinued treatment of BZN 300mg 8 weeks; 1 (3.3%) in 4 weeks; none in 2 weeks; 1 (3.3%) in BZN 150mg 4 weeks; 3 (10%) in BZN 150mg 4 weeks in combination with fosravuconazole and 4 (13.3%) in BZN 300mg weekly in combination with fosravuconazole. Most adverse events (AE) were mild to moderate, with only 6 participants presenting with serious adverse events (SAE). No SAEs were reported in BZN 300mg 2 weeks and 150mg 4 weeks. There are no differences between 4 weeks and 2 weeks arms regarding parasitological response or safety.

These results suggest that a shorter treatment with BZN effectively sustained parasitological clearance, was well tolerated, and has the potential to be a new therapeutic regimen for participants with chronic CD. Therefore, the current study proposes to evaluate a shortened therapeutic regimen with a larger number of participants.

Recently, the MULTIBENZ clinical trial, a phase IIb non-inferiority, randomized, blind, multicenter and international clinical trial, designed to evaluate the efficacy and safety of three different BZN regimens for the treatment of chronic phase CD, was completed. The study was conducted in four different countries: Argentina, Brazil, Colombia and Spain. (33)

The main objective of the MULTIBENZ clinical trial was to evaluate the efficacy of different BZN regimens at 12 months post-initiation of treatment in patients with CD in the chronic phase. The primary efficacy outcome was defined as the proportion of patients with suppression of sustained parasitic load in peripheral blood measured by polymerase chain reaction (PCR) during the first 12 months of follow-up after randomization.

Patients were randomly assigned to receive BZN 150 mg/day for 60 days, 400 mg/day for 15 days, or the

standard regimen (300mg/day for 60 days). The total number of patients who were estimated to be included in the study was 240, 60 participants per country and 80 per treatment arm.

The preliminary efficacy analysis of the study (not yet published) did not demonstrate statistically significant differences between the therapeutic regimens evaluated in Bolivia, Argentina, and Colombia. However, patients treated for 15 days had 10% of treatment failure compared with 5% of those receiving the standard regimen (although they did not reach statistical significance).
Therefore, taking into account the results of the MULTIBENZ and BENDITA studies, where different therapeutic regimens have been evaluated, we consider that both the options of Benznidazole 300 mg per day for 2 or 4 weeks, are equally valid to be evaluated in a phase III clinical trial compared to the standard dose of Benznidazole of 300 mg per day for 8 weeks.

3. Objectives and Endpoints

Objectives	Endpoints
Primary	
• To compare the efficacy of BZN in two reduced treatment schemes with a fixed oral dose of 300mg/day for 2 weeks or 300mg/day for 4 weeks compared to the standard treatment of 300mg/day for 8 weeks in the treatment of chronic CD, in its indeterminate or mild cardiac forms.	• Proportion of participants with sustained negative PCR at 24 months of follow-up after treatment.
Secondary	
• To evaluate the safety profile of a fixed oral dose of 300mg/day for 2 weeks or 300mg/day for 4 weeks compared to the standard treatment of 300mg/day for 8 weeks in the treatment of chronic CD, in its indeterminate or mild cardiac forms.	• Incidence of AE leading to treatment discontinuation.
• To evaluate parasitic kinetics by detecting parasitic DNA measured by qualitative PCR in peripheral blood in 1, 4, 6, 8, 12, 18 and 24 months after the end of treatment in participants with chronic CD, in its indeterminate or mild cardiac forms, treated with each of the different therapeutic regimens.	• Proportion of participants with positive PCR at the different time points: 1, 4, 6, 8, 12, 18 and 24 months after the end of treatment.

Exploratory objectives	
• To evaluate the incidence and severity of clinically relevant events attributed to CD such as hospitalization, death, or progression of cardiac disease.	• Incidence of clinically relevant events: number of hospitalizations related to CD or deaths. • Proportion of participants which presented progression to chronic cardiac form. • Proportion of patients with any new alteration on electrocardiogram (EKG) in comparison to the baseline. • Proportion of patients with any new alteration on echocardiogram (worsening of systolic function or change in the pattern of diffuse contractility deficit to segmental) in comparison to the baseline.
• To measure the quality of life (QoL) of participants with chronic CD in its indeterminate or mild cardiac forms, treated with each of the three therapeutic regimens.	• Longitudinal changes on QoL scores (WHOQoL-BREF and EQ5-D).
• To assess the cost-effectiveness of three regimens, a fixed oral dose of 300mg/day for 2 weeks or 300mg/day for 4 weeks compared to the standard treatment of 300mg/day for 8 weeks in the treatment of chronic CD.	• Number of monetary units used per-patient per each treatment arm. • Cost per quality-adjusted life-year (QALY) gained. • Incremental cost-effectiveness ratios (obtained by dividing the incremental cost by the incremental health benefit).

3.1 Assessment of Efficacy

Efficacy of study treatment will be assessed by comparing the parasitological clearance rate, determined by sustained parasitological clearance until 24 months of follow-up after treatment. Secondary assessments of PCR will be conducted at 1, 4, 6, 8, 12 and 18 months

As this is a double-blind study, this will not interfere with the schedule of events, since all participants will have all required samples planned at each visit. In addition, a qualitative and quantitative efficacy assessment will be performed, correlating incidence of and time to parasitological clearance with changes in *T. cruzi* serology.

The study manual of operations will provide full details regarding procedures for blood samples (tube types and number, aliquots volume), handling, storage, and shipment of the samples for laboratory evaluation. Quality assurance systems will be in place and described. Training of personnel in each of the activities described above will take place before initiation of this clinical trial. All parasitological and other

laboratory assessments will be performed blinded to treatment allocation.
The PCR samples will all be evaluated by trained specialists in a central laboratory in each country, using harmonized protocols, and similar techniques. Quality control will be carried out in the two countries by the same team of molecular biology specialists.

3.2 Assessment of Safety

Safety and tolerability of treatment will be assessed through routine monitoring of AE. During each study visit, the participants will be asked about current AE or any events observed during the period before the visit. Evaluations of hematology and blood chemistry values, regular measurement of vital signs and physical examinations will be done at each scheduled visit during the 8 weeks of treatment. Specific criteria for treatment discontinuation are described in sections 6.9 and 8.13. In addition, participants will be advised to return to the clinic at any time during the follow-up period should they present with any medical occurrence, as to allow for AE assessments during unscheduled visits. All safety assessments will be performed by a study team member, who is blinded to the treatment allocation. AEs will be recorded in the appropriate section of the case report form (CRF).

4. Study Design

This clinical trial is part of a larger project called CUIDA Chagas: Communities United for Innovation, Development and Care for Chagas disease - 'Towards elimination of congenital transmission of Chagas disease in Latin America'. This project includes three different research elements: (i) implementation research that will be conducted in four countries, targeting 234,000 women of childbearing age, their infants and children, and their household contacts, (ii) a validation study where a rapid diagnostic test-based algorithm will be validated for use in the field, and (iii) the proposed clinical trial.

4.1 Study overview

Double-blind, non-inferior, phase III, randomized, comparative with three-parallel groups, multicenter, international clinical trial. The trial will be conducted in four study sites (two in Bolivia and two in Colombia).

Each participant will be blindly assigned with a treatment number according to the randomization schedule. The trial drug label will only indicate the trial number, trial site and participant treatment numbers (according to local regulations).

Primary efficacy analyses will be performed when all participants complete the 24 months follow-up visit. Sponsor and study sites staff, including principal investigators (PI), will remain blinded to treatment allocation. A preliminary analysis will be conducted at 12 months. According to this analysis, the study may be interrupted if:

- At that time point, the experimental arms (B300/2 or B300/4) show superiority in terms of treatment failure compared to the control arm (B300/8).

An independent data and safety monitoring board (DSMB) will be convened for this study with expertise in CD, clinical trials as well as biostatistics. The purpose of the DSMB is to analyze the preliminary data at 12 months, to help in the decision of interrupting any arm.
The decision for interim analysis will be made in a blinded manner (e.g., based on pooled number of events). The interim monitoring plan will define monitoring bounds to maintain the two-sided type I error rate at the desired 5% (97.5% or higher probability of superiority over the control group).

5. Study Population

Adult patients will be >18 with weight between 50 kg and 95 kg with serological tests confirming the diagnosis of *T. cruzi* infection. The following eligibility criteria are designed to select patients whose inclusion in the protocol is considered appropriate. All relevant medical and non-medical conditions should be considered when deciding whether a patient may be included. The eligibility criteria cannot be waived by any researcher. Any questions about a patient's eligibility should be discussed with Fiocruz's medical coordinator, prior to the inclusion of the patient.

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Eligibility criteria

5.1.1 Inclusion Criteria

- Adults aged ≥ 18 years;[1]
- CD diagnosis through the positivity of two serological tests that use different antigens (recombinant and native antigens, according to World Health Organization (WHO) recommendations) (28).
- Informed consent form read and signed by the participant.
- Weight ≥ 50 kg to ≤ 95 kg.
- Ability to carry out all tests and visits to the protocol site and have a fixed address.
- Women of childbearing age should have a negative urine or serum pregnancy test at baseline.

[1] It is considered that neither the dynamics of parasitemia, nor the therapeutic response and safety profile, demonstrate differences in the chronic phase adult population up to 60 years of age. Considering that there is no contraindication for administering antiparasitic treatment beyond 60 years of age, and that the objective is to see the antiparasitic effect and the safety profile, recruiting patients older than 60, which will allow a greater probability of recruiting patients in the shortest possible time, optimizing studytimes, financial resources, and the opportunity to have the results available.

A contraceptive method should be used.[2]

Note that although PCR will be determined at baseline, this will not be considered as an inclusion criterion.

5.1.2 Exclusion Criteria

- Currently pregnant, breastfeeding[3] or expressing gestational desire for the next 2 months.
- Previously received treatment with BZN or NFX (either completely or incompletely);
- Any concomitant use or documented history of using allopurinol or antifungals (ketoconazole, itraconazole and posaconazole);
- History of hypersensitivity, allergic or SAE to any "nitroimidazole", and/or its components;
- Acute or chronic health problems that, in the informed opinion of the investigator, may interfere with the evaluation of the efficacy and/or safety of the drug. Examples are acute infections, Human Immunodeficiency Virus (HIV) infections, liver disease with liver failure and kidney disease requiring support treatment;
- Signs and/or symptoms of severe cardiac form of CD[4];
- History of cardiomyopathy, heart failure or severe ventricular arrhythmia of any etiology;
- Alcoholic participants or those with a history of alcohol abuse (considered as intake of >4 drinks on any single day AND >14 drinks per week for men and >3 drinks on any single day AND >7 drinks per week for women);
- Have basic laboratory parameters outside the normal range or parameters that are considered clinically relevant by the physician responsible for the participant;
- Participation in another clinical trial over the past 12 months.

Pregnancy will be considered an exclusion criterion. There is a strong recommendation for not treating pregnant women due to the possibility of teratogenic effects (experiments performed in animal models). There are inconsistent data regarding chronic Chagas disease during pregnancy; some studies showed increased risk of pregnancy loss, prematurity, and neonatal mortality while other studies did not. Since pregnancy data are inconsistent and chronic Chagas disease is generally not life-threatening, use of this

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2 A subject will be considered fertile if, in the opinion of the researcher, she is biologically capable of having children and is sexually active. Women who are not fertile must meet at least one of the following criteria: have undergone a documented bilateral hysterectomy and/or oophorectomy; Have clinically confirmed ovarian insufficiency; have reached the postmenopausal state, defined as: cessation of regular menstruation for at least 12 consecutive months without alternative pathological or physiological cause. All women of childbearing age should be advised of the risk of teratogenesis and should use barrier contraceptives during the 60-day period of treatment (may additionally maintain other preferred contraceptive methods that are eventually in use).

3 The amount of this drug excreted into milk is much lower than the amount used to treat infants. Therefore, the use of this drug innursing mothers who receive an indication of treating Chagas disease might be considered acceptable due to low drug levels in breastmilk and safety when given directly to infants. However, in those cases a close follow up of the newborns is recommended to detect and treat any adverse events. For the purpose of the clinical trial, we consider excluding these women since will add a complexity and a responsibility that could not be covered by the study.

[4] Cardiac involvement will be assessed through clinical interview, physical examination, and electrocardiogram (EKG). Patients withabnormal EKGs (other rhythms than sinus rhythm, right or left bundle branch block, hemiblocks, ventricular extrasystoles, any heart conduction disorders etc.) will undergo an echocardiogram.

drug is not recommended due to risk of embryofetal toxicity.

5.2 Screening Failures

Screening failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study treatment. A minimal set of screening failure information is required to ensure transparent reporting of screening failure participants, in order to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal obligatory information includes demography, screening failure details, eligibility criteria, and any SAE. Individuals who do not meet the criteria for participation in this study (screening failure) can be rescreened if there is a change in their eligibility.

5.3 Recruitment

The clinical trial is embedded in the CUIDA Chagas project. This project includes three separate, but interconnected studies, namely: implementation research, this clinical trial and an RDT algorithm validation study. The implementation study includes a large number of activities, aimed at generating demand among the target population. This will have an effect on the number of new Chagas patients that will be identified and who will feed the pre-existing patient referral circuits.
Due to the nature of the implementation research and the importance of developing and maintaining adequate control actions related to congenital CD, strategies for the recruitment of the study's target population should be widely discussed with health professionals, (health) managers, civil society and patient representation. This discussion will allow for the implementation of feasible and sustainable strategies in different contexts. The different priority populations suitable for the clinical trial will be recruited as follows:

- Women of Childbearing Age: carrying out information and educational campaigns in the community and at the health unit; invitation to women who attend the primary health care units for other reasons (family planning, vaccination, clinical care, prenatal care, etc.); conduct counseling and testing in primary health facilities.
- Household contacts: advising women with *T. cruzi* infection on surveillance of contacts; home visit for active search.

The centers chosen to participate in the study are already considered reference centers for the treatment of Chagas disease and have established track records of high-quality clinical research integrated into clinical care settings. Each center has its own patient referral flows framed within each health system (patients referred from primary care, specialized centers, etc.). This presupposes an added advantage since there are natural patient referral flows (recruitment projections have been made taking into account such data). In this case, doctors who treat the patients will offer them the possibility of participating in the clinical trial as long as they meet all the inclusion criteria and none of the exclusion criteria.

The main steps should take place as follows:

- The Principal Investigator (PI) or his representative will identify among the people seen at the health facility those who meet the inclusion criteria for the study.
- The PI or its representative will obtain further information to confirm the eligibility of individuals (completion of inclusion criteria and absence of exclusion criteria).
- If the individual is eligible, the PI or his designee will obtain his informed consent, as detailed in the SOP "Informed consent process" and in the section 16.

Therefore, considering the baseline recruitment potential of the chosen centers, as well as the context of the entire CUIDA Chagas project, we do not consider necessary the creation of materials specifically designed for the recruitment of patients for the clinical trial.

Sites recruiting patients will be:

- *Colombia*
  - Instituto nacional de Salud, Bogotá
  - Fundación Cardio infantil, Bogotá.

- *Bolivia*
  - Hospital Básico de Villa Montes, departamento de Tarija.
  - Hospital Dr. Marcos C. Rojas Zurita, de Padilla, departamento de Chuquisaca.

6. Treatments

6.1 Doses and treatment regimens

Subjects will be randomly assigned into one of the following balanced groups:

- **Group A (B300/2)** - BZN (Abarax®, tablet 100mg), 300 mg - 2 weeks: BZN 300 mg, divided in three daily doses, for 2 weeks matching with placebo tablet administered in three daily doses for 6 more weeks.
- **Group B (B300/4)** - BZN (Abarax®, tablet 100mg), 300 mg - 4 weeks: BZN 300 mg, divided in three daily doses, for 4 weeks matching with placebo tablet administered in three daily doses for 4 more weeks.
- **Group C (B300/8)** - BZN (Abarax®, tablet 100mg), BZN 300 mg, divided in three daily doses for 8 weeks.

BZN will be administered as 100mg tablets in blister packaging. Fixed doses of 300 mg will be used during the clinical trial. Participants will be advised that treatment must be taken in three divided doses, accompanied by a meal. During the visits throughout the treatment period, the subject will receive enough medication until the next scheduled visit. Participants are required to bring all remaining study drugs to the end of treatment (EOT) visit in order to check compliance with prescribed treatment and ensure drug accountability. As per recommendations in the BZN package insert, subjects will be advised to abstain from alcohol during treatment.

Placebo tablets will be provided by the same producer of Abarax® and will have the same size, format and include the same excipients as Benznidazol tablets formulation for oral administration. Quality control for both active treatment and placebo will be in charge of the manufacturer, Laboratorio ELEA.

6.2 Rescue treatment

Patients with CD included in this study will be in the chronic phase, and no rescue is envisaged during the treatment period or until the end of the study. All participants in the experimental arm that remain PCR positive at the end of the study will be treated with BZN 5mg/Kg/day, divided in three daily doses for 60 days. In addition, all participants with positive PCR in the standard dose arm will be offered BZN at the cited doses at the end of the study.

6.3 Drugs labelling, packaging

Detailed information on study medication secondary packaging and labelling will be included in the study manual of operations. Minimum information to be included on the study medication label is:

- Name of Sponsor, name and contact details of the PI;
- Study number;
- Name of the drug, dose;
- Pharmaceutical dosage form, route of administration, quantity of dosage units;
- Directions for use;
- "For clinical trial use only";
- Batch number and treatment number;
- Expiry date and storage conditions.

6.4 Accountability and compliance

BZN/placebo clinical trial kits will be shipped to the referral Clinical Trial Site in each country that will act as the study medication's depot and will distribute the assigned kits to the participating centers during the trial.
Study specific forms will be used for drug accountability. Adequate records on the study medication's receipt, use, return, loss, or other disposition will be documented and maintained by the study site investigators or delegated staff authorized by the study PI. Study monitors will also be in charge of checking for drug accountability during the regular monitoring visits to the study sites. All study treatments must be kept in a locked room at the pharmacy or other designated locations (such as the PI's office) where restricted access by the pharmacist or authorized study personnel and appropriate storage conditions can be ensured.
BZN/placebo clinical trial kits that will be provided for this study will not be used for other purposes other than this protocol. Under no circumstances may the investigator or site staff supply the study treatment

to other investigators or healthcare services or allow the medication to be used in ways other than as directed by this protocol. Participants will be instructed to bring the clinical trial kits during each visit to the study center for review and drug accountability check.

6.5 Storage

BZN does not need to be shipped or stored under refrigeration. However, if local temperatures rise above 30° C, medication should be kept in a room with air conditioning. Site storage conditions and temperature should be monitored by site personnel for adherence to product specifications. Records should be available.

6.6 Treatment assignment

This is a double-blind trial in which neither the participant nor the investigators will know the duration of treatment that has been assigned to each participant. At the moment of the inclusion a consecutive numeric code will be generated. That number will be linked with a randomization code that will correspond to the treatment kit number. That number will never reveal the therapeutic branch designation. Each center will have a randomization list with the inclusion number and the kit number, and a randomization closed envelope which links the kit number with the treatment. To avoid bias in the allocation of treatment groups, randomization will be centralized, and investigators will not have access to the sequence of allocation. Participants will be randomized 1:1:1 to each of the treatment arms and balanced by country.

6.7 Blinding and procedures for unblinding

The process of masking will be done as followed: closed numbered boxes will be made available per participant. Inside, there will be three numbered boxes that will correspond to tablets with an active ingredient 100mg and/or placebo: box 1 for the first two weeks and box 2 for the following two weeks of the treatment and box 3 for the following 4 weeks. All the tablets will be the same, both in shape, color, and taste. Inside each box, cards will be placed where the sequence of the drug intake will be graphically reflected.

Code breaking for any participant should only be done in case of emergencies, eg a threat to the participants life, and may only be done by the investigator or by an authorized person. The Sponsor and trial monitors should be informed promptly of the need for unmasking, ideally before it is done. Notification will be ensured through the use of a specific form, which will include the participant number, the date of opening of the blind, the reason and the signature of both the person responsible for the decision and the PI.

Each center will have randomization envelopes that should only be opened for breaking the codes. Likewise, an SAE form must also be filled out if the reason for breaking the code meets the previously defined criteria.

The possibility that there are situations in which unmasking does not necessarily justify withdrawal of the participant from the trial is contemplated. In such cases, participants should continue with the visits and respective evaluations as planned.

6.8 Concomitant medication

Participants may receive concomitant therapy for medical occurrences during the study. Medications that are considered necessary for the participant's welfare may be prescribed at the discretion of the investigator. However, specific drugs that elevate liver enzymes, are immune-suppressants or are known to have activity against *T. cruzi* should be avoided during the treatment phase of the study. It is requested that women of childbearing age do not initiate contraceptive drugs (pills, injection, implant, or patch) during the 60 days of study drug administration, as these may also be associated with increased liver enzymes (contraception will be avoided with barrier contraceptives). However, contraceptives can be maintained in those women who were already using them prior to the clinical trial. Although it is not a formal exclusion criterion, it is recommended not to take herbal medicines, food supplements and energetic drinks during the treatment period. In case of doubt patients should be advice to ask for permission to its physician. The PI should be contacted in case of any questions regarding concomitant medications.

All concomitant medications taken by the participant during the study, from the date of signature of the informed consent until the last study follow-up visit, will be recorded in the appropriate section of the CRF.

6.9 Adverse reactions to investigational product

In the case of mild or moderate AE, temporary suspension of the administration of BZN will be considered, while severe reactions will lead to an evaluation of permanent treatment suspension.

6.9.1 Hypersensitivity reactions

In the event of a hypersensitivity related AE depending on the severity, the following symptomatic treatment regimen will be followed, at the investigator's discretion:

- Mild AE: cetirizine 10 mg every 24 hours orally (or other similar antihistamine drug).
- Moderate AE: cetirizine 10mg every 12 hours or +/- prednisone 0.5 mg/kg every 24 hours orally.
- Serious AE: cetirizine 10 mg every 8 hours or +/- prednisone 1mg/kg mg every 24 hours orally. Indication of hospitalization will be assessed.

6.9.2 Hepatotoxicity

In the event of hepatotoxicity, a control of liver function (aminotransferase, bilirubin, alkaline phosphatase, gamma glutamate transferase among others), complete blood count (CBC) and narrow coagulation will be carried out (apart from the studies necessary to rule out other possible sources of hepatotoxicity).
If a participant experiences elevation in serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) exceeding three times upper limits of normal (ULN) but less than 8 times ULN, treatment can be continued if the participant is asymptomatic and does not manifest a total bilirubin elevation >2x ULN. If a participant develops signs/symptoms of liver injury (fatigue, nausea, vomiting, right upper quadrant pain/tenderness, dark urine, fever, rash, jaundice, and/or eosinophilia (<5%)), they will be instructed to immediately contact the study site for evaluation. Adequate care will be provided by the investigator, with support from a specialist (hepatologist) as needed. Discontinuation of treatment should be considered if participant presents such symptoms/signs and associated liver transaminase increase >3x ULN. If the values are 5 times above the normal range for liver enzymes, the medication will be discontinued, and the participant's admission will be assessed.

6.9.3 Neutropenia

Hematological assessments will be performed as previously described. If Absolute Neutrophil Counts ≤ 1000 cells/µl are documented, treatment should be discontinued, and CBC will be repeated as soon as possible after the initial abnormality is identified. After that, the participant should be followed every 48 hours (2 days), or the closest possible interval, until the neutrophil counts increase above 1000 cells/mm or stabilize. The event should be immediately reported as an SAE.
In case Absolute Neutrophil Count is found to be < 500 cells/µl, the treatment should be immediately interrupted, and a control hematological assessment is required within one day. Participants will be instructed to avoid exposure to people with respiratory tract infections and to respect general hygiene rules. Depending on profile (decreasing phase, stabilization phase, resolution phase), controls will be required every one to three days until normalization. The event should be immediately reported as an SAE.

In case Absolute Neutrophil Count is found to be < 100 cells/µl, or below 1000 with associated fever (even of mild intensity) or infectious origin symptoms, the participant should immediately be hospitalized and reported as an SAE.

7. Laboratory assessment

Blood samples for hemogram and biochemistry (5mL each) will be collected in the same day at screening, at days 14 (allowable window of + 2 days), and 56 (allowable window of + 7 days).
Blood samples for serology will be collected at screening visit and at month 24 after EOT.
Blood samples for PCR will be collected at screening visit and at 1, 4, 6, 8, 12, 18 and 24 months after EOT (allowable window of + 10 days. Blood samples collected for PCR shall be immediately added to a tube containing one volume (5mL) of a solution of Guanidine/ClH 6M EDTA 0.2M pH 8,0 Buffer (GEB). Samples

with guanidine buffer can remain at room temperature. Trained nurses will be responsible for the blood collection, sample processing and storage.
The PCR technique that will be used is the one described by Duffy et al. (29) and optimized by the Molecular Biology Institute of Paraná (IBMP). That procedure allows the amplification of a *T.cruzi* satellite DNA region and a linearized recombinant plasmid used as an internal amplification control. The PCR will be carried out in duplicate from each of three extractions. At least one amplification of the six performed with an amplification cycle (Ct) of *T.cruzi* below 40 and a correct value of internal amplification control will be interpreted as positive. To assess the homogeneity of the results obtained by the different laboratories, a harmonization panel will be constituted and sent to the laboratories.

Back-up samples for serology and PCR will be stored in each laboratory as for quality and warranty purposes.

The study manual of operations will provide full details regarding procedures for sampling (tube types and amount, aliquots volume), handling, storage, and shipment of the samples for laboratory evaluation. Quality assurance systems will be in place and described. Training of personnel in each of the activities described above will take place before initiation of this clinical trial. All parasitological and other laboratory assessments will be performed blinded to treatment allocation.

7.1 Governance of biological specimens

The study will be performed in two different countries, Bolivia and Colombia. After consulting the regulatory agencies of each country, it has been confirmed that there is no minimum time required for sample storage. The storage period for human biological material in a Biorepository must be in accordance with the corresponding research schedule with a maximum for up to ten years. By consensus, we have decided to store samples for up to 5 years after completion of the study.
Samples will be stored at national reference laboratories previously identified in each country:

- Colombia: Instituto Nacional de Salud, Ac. 26 #5120, Bogotá, Colômbia.
    - Responsible for sample custody: Dra. Marcela Mercado and Dr. Mario Olivera.

- Bolivia: Instituto Nacional de Laboratorios de Salud (INLASA). Av. René Zavaleta, La Paz, Bolivia.
    - Responsible for sample custody: Dr. Justo Chungara

The objective of storing samples is part of good clinical practice commitments, in case we need to repeat or confirm any result. If another research needs to use stored samples, it will be submitted for approval by the Research Ethics Committee and patients will be asked to reconsent. Although the final responsibility over samples lies with the sponsor, Oswaldo Cruz Foundation (Fiocruz), a democratic mechanism for decision-making will be established, considering all the opinions of the country Principal Investigators.
In the case that samples need to be destroyed, a certified enterprise will be contracted.

8. Scheduled Visits

As described previously, study assessments will be done at Screening, Day 0 (baseline), at 2 weeks (allowable window of ±2 days), 4 weeks (allowable window of ±2 days), and 8 weeks (allowable window of ±7 days). Afterwards, assessments will be done every 6 months (allowable window of ±14 days) until the end of study (24 months).
Patients will have a total of 5 visits between the inclusion and the treatment period, each visit lasting approximately 2 hours. Afterwards, there will be 7 additional visits which will be shorter, lasting approximately 1 hour each. The per protocol participation will take 26 months of follow up.

8.1 Screening visit

The centers chosen to participate in the study are already considered reference centers for the treatment of Chagas disease. Each center has its own patient referral flows framed within each health system. The doctors who treat the patients themselves who will offer them the possibility of participating in the clinical trial as long as they meet all the inclusion criteria and none of the exclusion criteria.

During screening, the following assessments will be done to evaluate participant eligibility for the study:

- Signature after reading the Informed Consent Form;
- Complete medical history with an emphasis on CD;
- Demographic data, history of medications and toxic habits;
- Physical examination, body weight/height and vital signs;
- CD serology: serum sample will be documented at screening for conventional CD serology. Participant must have at least 2 positive tests to be eligible;
- Real-time PCR collected for quantitative assessment for all participants (result is not required for randomization);
- Clinical safety laboratory evaluations (whole blood and serum sample): hemoglobin, total white blood cells (WBC) count, differential WBC count, numerical platelet count, ALT, AST, urea, creatinine;
- Serum pregnancy test: women of reproductive age will undergo a serum pregnancy test at screening. The test must be negative for the woman to be considered eligible for this study;
- EKG: will be obtained at screening. Participants with abnormal EKGs will undergo an echocardiogram.

8.2 Baseline visit

At baseline (Day 0) the following assessments will be performed before the first dose of medication is administered, to obtain baseline safety data and data for further comparative analyses and multiple parameter correlations:

- Physical examination (including weight);
- Vital signs: axillary temperature, blood pressure and pulse rate;
- WHOQOL Bref, EQ-5D-3L and cost-effectiveness questionnaires will be distributed and will be completed by the participant (See Annexes 1, 2 and 3, respectively).

Concomitant medications at baseline will be recorded in the appropriate CRF.

The participant's eligibility criteria evaluation will be performed at day 0, or when the investigator has access to all results necessary for the participant's inclusion/exclusion evaluation. After participant eligibility is confirmed, randomization will occur, and the participant will receive the first kit of medication at the clinic.

8.3 Treatment visit

Three regular visits will be required during the treatment period, at 2, 4 and 8 weeks after the start of treatment. During each visit, the participant will be asked about the appearance of AE. If the researcher considers that such signs and symptoms are related to the drug (BZN), all measures that the researcher deems necessary will be carried out. Each case will be assessed individually and depending on severity and causality, treatment may be withdrawn definitively, suspended momentarily or concomitant medication may be administered.

- Visit at 2 weeks (+/- 2 days): Directed anamnesis and physical examination. Analytical extraction (hematology, biochemistry. Evaluation of AE and changes in concomitant medication. At this visit, the 1st kit of medications (BZN or placebo) will be collected, and the 2nd kit will be distributed to the participant.
- Visit at 4 weeks (+/- 2 days): Directed anamnesis and physical examination. Evaluation of AE and changes in concomitant medication. At this visit, the 2nd kit of medications (BZN or placebo) will be collected, and the 3rd kit will be distributed to the participant.
- Visit at 8 weeks (+/- 7 days): Directed anamnesis and physical examination. Extraction of laboratory tests (hematology, biochemistry. Evaluation of AE and changes in concomitant medication. In this visit the 3rd kit of medications (BZN or placebo) will be collected.

EQ-5D-3L and cost-effectiveness questionnaires will be distributed to and completed by the participant during each visit.

8.4 Adverse event management

During the treatment phase, participants should seek medical consultation in the event of any AE. The participant then will undergo directed anamnesis and physical examination. The clinical need for analytical extraction will be considered (hematology and biochemistry). AE will be evaluated, as well as potential changes in concomitant medication.
The criteria for withdrawal of the medication will be decided by the doctor treating the participant, considering the severity, intensity and extent of the AE. In addition, a participant may decide to suspend medication at any time during the treatment or upon the appearance of any AE.

If an AE occurs and the opinion of the investigator advises the interruption of treatment, the medication will be suspended, and symptomatic treatment will be instituted following the scheme defined in section

6.9 or at medical discretion. The participant may present different AE during the treatment phase that require treatment interruption. The duration of the study medication interruptions will depend on each AE and will be done at the discretion of the doctor treating the participant. If the drug suspension exceeds 15 days, the study medication will be permanently withdrawn from the participant. If finally, it is decided to definitively withdraw the drug due to an AE, the participant will be recalled 14 days later and will be questioned again about the possibility of late AE. Should the participant not be able to make a visit, a phone call will be accepted, which must be recorded in the source document.

8.5 Follow up visit

During the follow-up phase, visits will be made every six months, regardless of the assigned study branch. At each visit, the following will be carried out: directed anamnesis, physical examination, EKG and determination of the parasitic DNA by PCR. Depending on evolutionary electrocardiographic changes and availability, an echocardiogram may also be requested. The times of visits will be in month 1, 4, 6, 8, 12 and 18 after the end of treatment (with a window of +/- 10 days).
EQ-5D-3L and cost-effectiveness questionnaires will be distributed to and completed by the participant during each visit.

8.6 End of study visit

At the end of the follow-up of each participant, coinciding with month 24 (+/- 15 days) after the end of treatment, a directed anamnesis, physical examination, EKG, determination of the parasite DNA by PCR and serology will be performed and changes in concomitant medication will be recorded. Depending on evolutionary electrocardiographic changes and availability, an echocardiogram may also be requested. WHOQOL BREF and EQ-5D-3L questionnaires will be distributed to and completed by the participant.

9. Pharmacovigilance

9.1 Adverse event definition

AE is defined as any unexpected medical occurrence in a participant or clinical trial participant after the administration of a pharmaceutical product and which does not necessarily have a causal relationship with the treatment. AE include worsening (in severity and frequency) of pre-existing conditions ("Medical history") before the first Investigational Medicinal Product (IMP) administration and abnormalities of procedures (e.g. EKG) or laboratory results which are assessed as "clinically significant".

<u>Laboratory / procedure abnormalities considered as AE</u>

Laboratory / procedure abnormalities (or worsening in severity or frequency of pre-existing abnormalities)

assessed as “clinically significant” must be reported as an AE only if they meet at least one of the following conditions:

- The abnormality suggests a disease and/or organ toxicity AND this abnormality was not present at the screening visit or is assessed as having evolved since the screening visit;
- The abnormality results in discontinuation of the study drug;
- The abnormality requires medical intervention or concomitant therapy.

When reporting an abnormal laboratory/procedure result, a clinical diagnosis should be recorded rather than the abnormal value itself (for example, “anemia” rather than “decreased red blood cell count”).

What is not an AE?

- Medical conditions present at the initial study visit that do not worsen in severity or frequency during the study are NOT considered as AE;
- Symptoms, exacerbation or worsening of the studied disease will NOT to be considered as AE nor captured on the AE page of the CRF if consistent with the anticipated natural progression of the disease (overall and for this given participant);
- Lack of efficacy of the investigational product is NOT considered as AE.

The investigator or appropriate site personnel will examine any participant experiencing an AE as soon as possible. The investigator will do whatever is medically necessary for the safety and well-being of the participant.
The participant will remain under observation as long as a participant is receiving trial drug, and for two months following the last day of drug administration, or longer if medically indicated in the opinion of the investigator. All AEs observed or reported following administration of IMP will be followed until resolved or until medically stable. All AE identified will be assessed for seriousness, severity, and causality and recorded in the appropriate AE section of the CRF using standard medical terminology to avoid the use of vague, ambiguous, or colloquial expressions.

9.2 Serious adverse event

An SAE is any unexpected medical occurrence that:

- Results in death;
- Is life-threatening, i.e in this context refers to an AE in which the participant was at risk of death at the time of the AE; it does not refer to an AE that hypothetically might have caused death if more severe;
- Results in persistent or significant disability or incapacity, i.e. the AE resulted in a substantial disruption of the participant’s ability to conduct normal activities;
- Requires in-patient hospitalization or prolongation of existing hospitalization (note: this clinical trial does not have any period of scheduled hospitalization), i.e. the AE requires at least an overnight admission or prolongs a hospitalization beyond the expected length of stay. Hospital admissions for surgery planned before study entry, for social reasons, for any elective surgery (i.e. plastic surgery) or for normal disease management (including treatment adjustment) are not to

be considered as SAE according to this criterion (i.e. if the protocol or the standard management of the disease under study requires planned hospitalization)*;*

- Congenital anomaly/birth defect, i.e. an AE outcome in a child or fetus of a participant exposed to the IMP (or marketed medicinal product (Note: only to be added for marketed drug)) before conception or during pregnancy;
- Important medical event, i.e. is medically significant. Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious events/reactions, such as important medical events that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the participant or might require intervention to prevent one of the other outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

9.3 Adverse event of special interest

An AE (serious or non-serious) of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the Sponsor may be appropriate. Such an event may require further investigation to characterize and understand it. For this study, no predefined AE of special interest is listed since the investigational drug is a well-known treatment.

9.4 Eliciting Adverse Event information

The investigator is required to report all directly observed AE and all AE spontaneously reported by the trial participant, using concise medical terminology. In addition, during each trial visit, the participant will be interviewed using a checklist and will undergo a physical exam for AE evaluation.

9.5 Adverse Event reporting period

The AE reporting period for this trial begins upon administration of the first dose of trial medication at Day 1 for non-serious events; or upon participant enrolment in the trial (after signature of informed consent) for serious events. This period finishes at the end of patient participation in the trial. All AE that occurs during the AE reporting period specified in the protocol must be reported in the CRF, when considered treatment related. In addition, any SAE that occurs after the AE reporting period, and which is assessed by the investigator as related to the investigational medication should also be reported as an AE.

9.6 Adverse Event reporting requirements

Information on AE must be evaluated by a physician. Each AE is to be classified by the investigator as

serious or non-serious. This classification will determine the reporting procedure for the event.
All SAE are to be reported immediately (within 24 hours of awareness of the SAE by the investigator) to the study PI, using the SAE report form. This includes a description of the event, onset date and type, duration, severity, relationship to trial drug, outcome, measures taken, and all other relevant clinical and laboratory data. The initial report is to be followed by the submission of additional information (follow-up SAE form) as it becomes available. Any follow-up reports should be submitted as soon as possible, but within 5 working days.
SAE should also be reported on the clinical trial AE CRF. It should be noted that the SAE form is not the same as the AE section of the CRF. Although the same data are collected, the two forms must be completed in a consistent manner, and the same medical terminology should be used. Any other additional reporting requirement will be addressed in the trial manual.
Non-serious AE are to be reported in the CRF. In the CRF, a given AE will be recorded only once per participant, and the severity recorded should be the maximum level reached. If several distinct episodes of the same condition occur, their number will be recorded in the CRF. In addition to immediately reporting SAEs to the Sponsor, investigators are responsible for reporting SAEs occurring at their site to their Independent Ethics Committee (IEC), and any periodic safety reporting, following the local requirements of their institution.

9.7 Grading of Adverse Event severity

Severity is a clinical determination of the intensity of an AE. The severity for an AE should be graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE, version 5.0)[5], defining the System Organ Class (using preferred terms defined by MedDRA 13.1). In case of AEs that are not described in the CTCAE v5.0, the investigator will use the terminology MILD, MODERATE, or SEVERE to describe the maximum severity of the AE as follows:

- **MILD** Does not interfere with the patient's usual functions;
- **MODERATE** Interferes to some extent with the patient's usual functions;
- **SEVERE** Interferes significantly with the patient's usual functions.

This information on AE grading will be entered in the AE section of the CRF. It is to be noted the distinction between severity and seriousness of AE. An SAE is not necessarily a serious event.

9.8 Adverse Event causality assessment

For both serious and non-serious AE, the investigator is required to assess the potential relationship between the AE and the trial drug, i.e. to determine whether there exists a reasonable possibility that the trial drug caused or contributed to the AE.

[5] https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf

To help investigators with the decision binary tree in the evaluation of causality, investigators will be asked to consider the following before reaching a decision:

- Medical history;
- Lack of efficacy / worsening of existing condition;
- Trial medications;
- Other medications (concomitant or previous);
- Withdrawal of trial medication, especially following trial discontinuation / end of trial medication;
- Erroneous treatment with trial medication (or concomitant);
- Protocol related procedure.

The relationship of an AE to investigational treatment is assessed and determined by the investigator after careful consideration of the event in terms of biological plausibility, possible unrelated causes, any pre-existing medical conditions or concomitant medications, temporal relationship between administration of investigational treatment and the onset (or worsening) of the event and known patterns of response to trial medications in general. The causality assessment of an AE to the investigational medicinal product will be rated as follows:

- *Not related:* There is no reasonable possibility of causal relationship;
- *Related*: There is at least a reasonable possibility of a causal relationship between an AE and an IMP. This means that there are facts (evidence) or arguments to suggest a causal relationship.

The decision to suspend and resume treatment or to permanently interrupt treatment due to an AE will be left to the trial clinician in charge. The clinician should take into consideration the Assessment of Safety defined for this protocol and the rules for permanently interrupting trial treatment.

9.9 Exposure *in utero*

In this trial, women of childbearing age must have a negative serum pregnancy test at screening, should not be breastfeeding, and should use a double method of contraception to avoid pregnancy during exposure to the study drugs and up to three months after completion of the treatment. Women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device with a hormonal component are required to use an additional barrier method of contraception for the period specified. To assure that requirement, health professional should explain the teratogenic risk and future implications for the fetus and family as a whole in case of getting pregnant within the treatment period. In the event that the participant does not use any contraceptive method, barrier contraceptives will be provided by the research team. If any trial participant becomes or is found to be pregnant while receiving any of the study treatment drugs (or during study post-treatment follow-up period +3 months), the medication must be interrupted, and the investigator must submit the event on a 'Clinical Study Pregnancy Report Form' together with the first day of the last menstruation period and the expected date of birth. This must be done irrespective of the occurrence of an AE. The information submitted should include the anticipated date of delivery. The investigator will follow the participant until completion of the pregnancy or until pregnancy termination (i.e., induced/spontaneous abortion). The

investigator will provide pregnancy outcome information in a 'Clinical Study Pregnancy Outcome Report Form'.

Note: A pregnancy is not an SAE. Any unfavorable outcome meeting a seriousness criterion, i.e., in the case of unfavorable pregnancy outcome (miscarriage, stillbirth), birth defects, or congenital abnormalities, the incident will be reported using the SAE form (in addition to the Pregnancy Outcome Form).

9.10 Adverse event follow-up

All AE should be followed until they are resolved, until the investigator assesses them as chronic or stable, or until the participant participation in the trial ends (i.e., until a final report is completed for that participant).
In addition, all SAE and non-serious events assessed by the investigator as possibly related to the investigational drug, must be followed even after the participant participation in the trial is over. Such events should be followed until they are resolved or until the investigator assesses them as "chronic" or "stable."

9.11 Withdrawal criteria

A participant should be withdrawn from the trial treatment if, in the opinion of the investigator, it is medically necessary, or if it is the wish of the participant. If a participant does not return for a scheduled visit, every effort should be made to contact the participant.
In any circumstance, every effort should be made to document participant outcome.
If the participant withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data, except for safety data, which should be collected if possible and in accordance with participant consent.
If a participant withdraws from the trial, the reason must be noted on the CRF.
If a participant is withdrawn from the trial because of a treatment limiting AE, thorough efforts should be made to clearly document the outcome of AE.

9.12 Rules in case of treatment suspension or temporarily interruption

Treatment discontinuation does not automatically imply withdrawal from the trial. The duration of study medication interruptions will depend on each AE and is determined at the discretion of the investigator. The sum of the interruptions may not exceed 15 days. If the event exceeds 15 days, the study medication would be permanently withdrawn from the participant. Treatment can be resumed, according to the assessment of the trial investigator in charge of the participant, observing the instructions for safety assessment and follow-up as per section 6.9. These participants should continue with trial visits and assessments as planned, but the reasons for treatment discontinuation must be recorded in the

appropriate source documentation and CRF.

9.13 Rules for permanently interrupting trial treatment

The Investigator will permanently interrupt the treatment based on the following:

- ALT or AST >5x upper limits of normal (ULN) for more than two weeks;
- ALT or AST >3x ULN and Total bilirubin 2x ULN or International Normalized Ratio (INR) >1.5, known as Hy's law criteria, to be reported as a SAE;
- ALT or AST >3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%);
- AE or any other condition which, in the investigator's opinion, would put the participant at undue risk by continuing the trial treatment;
- Major protocol deviation incompatible with the continuation/participation on the trial;
- Any condition that the investigators consider medically necessary to interrupt the treatment, such as:
  - Significant leukopenia (<500 cells/mm^3);
  - Severe gastrointestinal symptoms;
  - Severe allergic dermopathy;
  - Peripheral sensitive neuropathy.
- Pregnancy.

Regardless of the reasons for trial treatment interruption, the investigator will make all necessary arrangements to ensure that the participant receives the appropriate treatment for the relevant medical condition. Rescue treatment will be offered for these participants, if appropriate, upon trial completion.

9.14 Lost to Follow-up

A participant will be considered lost to follow-up if they are unable to be contacted by the study team. The following actions must be taken if a participant fails to comply with required study procedures:

- The clinical trial team must attempt to contact the participant as soon as possible and counsel the participant on the importance of maintaining the assigned procedure schedule and ascertain whether the participant wishes to and/or should continue in the study or not.
- Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

10. Statistical Considerations

10.1 Sample Size Determination

A total sample size of 672 subjects (224 per trial arm, and 336 per country) will be necessary to achieve 90% power to detect a non-inferiority margin difference of 15%, assuming that the standard regimen failure rate is 10% and the intervention regimen is 12%, for a one-sided Z test at a significance level of 0.025. This total sample size has been increased in anticipation in 40% to assure a minimum 60% of participants with active infection (positive PCR) and further by 10% to account for potential losses to follow-up, in order to maintain the specified power and inferiority/superiority margins. This means that individuals removed from the study for the reasons mentioned will not be replaced. This sample size will allow the study to also demonstrate a superiority margin of difference of 10% with 80% power regarding the safety of the experimental arm. Considering the percentage of treatment interruption for conventional treatment of 20%, and a reduction of 50% in the experimental arm, power of 90% and α=0.05, 197 patients will be needed in each arm.

10.2 Populations for Analyses

For analysis purposes, the following populations are defined and will be described in greater detail in the trial Statistical Analysis Plan:

Population	Description
Intention to Treat (ITT)	All enrolled participants who are randomized into the study.
Per Protocol	All patients completing the study without major protocol deviations. Patients with a positive PCR result prior to time of exclusion, will be included and considered as treatment failure.
Safety population	All subjects who received at least one dose of study medication.

10.3 Statistical Analyses

Categorical variables will be presented as frequencies and percentages and continuous variables as median (interquartile range) or mean (standard deviation) according to the data distribution. Normality of distribution will be checked through histograms and the Kolmogorov-Smirnov test.
The chi-squared test or Fisher exact test will be performed to compare the frequencies across categorical variables and the Mann-Whitney test or Student t-test will be used to compare the continuous variables. Intention-to-treat analysis will be performed to evaluate the efficacy and safety that includes all participants randomized to the study groups. The primary analysis will compare proportion of patients with sustained negative PCR at 24 months in their randomly assigned treatment groups (intention-to-treat analysis). Moreover, the proportion of participants with adverse drug events will be also compared. Comparison analyses between groups will be performed using generalized linear models with a log-link

and binomial distribution. Longitudinal differences on quality-of-life scores between groups will be fitted using linear mixed models.
A per protocol analysis will also be performed. Those participants who do not comply with treatment or permanently interrupt or modify the proposed treatment will be excluded from the analysis unless they had a positive PCR prior to that time-point, in which case they will be included in the analysis and considered as a treatment failure.

For PCR analyses, means and bilateral 95% confidence intervals will be presented by group and visit. In addition, time to sustained parasite clearance until 6, 12, 18 and 24 months of follow-up visits will be assessed using Kaplan–Meier survival analysis with log rank test for significance. Repeated measures analysis will also be performed.
Statistical significance will be set at 0.025 for the non-inferiority outcome (positive PCR) and 0.05 for the superiority outcome (percentage of adverse drug reactions and QOL).

The interim analysis for early stopping will be based on superiority and conducted when all participants have reached 12 months of follow up since the end of treatment. An independent data and safety monitoring board (DSMB) will be convened for this study with expertise in CD, clinical trials as well as biostatistics. The purpose of the DSMB is to analyze the preliminary data at 12 months to help in the decision of interrupting any arm.
A control event rate is expected to be 10% of treatment failure (at least one positive PCR during follow up period) and a 20% of treatment withdrawn due to any AE. Control arm showing posterior probability of superiority of 97.5% or higher will be considered superior. This will be calculated for the experimental intervention versus the control group based on the observed data at the time of interim assessment with a superiority p-value stopping boundary of 0.010.
The decision for interim analysis will be made in a blinded manner (e.g., based on pooled number of events).
All analyses will be performed using Stata 13.0 for Windows.

10.4 Justification of Non-Inferiority Margin

A non-inferiority margin of 15% has been defined for our study. This means that a maximum acceptable difference in treatment failure rate of 15% with the comparator is considered the difference that would still result in a significant proportion of participants treated appropriately. Historical data on therapeutic failure in most randomized controlled trials is around 20% where the effectiveness rates were measured by parasitic DNA detection on peripheral blood. In adults the failure rate ranges between 10 and 40%. (30,31) If the placebo is considered, it would still be much more advantageous, since the failure rates range between 85 and 95%. (16,32)
For these reasons the study prefers to be conservative in choosing the non-inferiority margin, as the consequence of choosing a margin greater than the actual effect of the active control treatment in the study can be a false conclusion that a new regime is effective, and therefore have an undesirable result for public health. As the intention is treating participants with CD as a public health strategy for reducing the transmission of congenital transmission, reducing parasitemia, through an increase in adherence, a higher rate of compliance in addition to a reduction in costs programmatic level is intended.

10.5 Participant Disposition

At the end of the study the following will be described:

- Number of participants screened;
- Number of participants assessed for eligibility excluded from the study, including reasons for exclusion;
- Number of participants who have been randomized in the study, as per original allocation (ITT set);
- Number of participants randomized into each study arm;
- Number and percentage of participants who have received at least one dose of study medication(s) (safety population – All Treated Set), per study arm;
- Number and percentage of participants who have received a full course of treatment and have completed EOT assessments (treatment completers), per study arm;
- Number and percentage of participants who have completed the study per study arm (study completers);
- Number and percentage of participants lost to follow-up and reasons of loss, total and per study arm;
- Number and percentage of participants who have withdrawn from the study, per study arm and reasons for withdrawal;
- Number and percentage of participants excluded from analysis per study arm and reasons for exclusion;
- Number and percentage of participants with at least one protocol violation and nature of protocol violation (major, minor).

10.6 Baseline

The following baseline characteristics of the study population will be described:

- Age distribution;
- Gender distribution;
- Nationality and country(ies) of residence;
- Proportion of participants with positive PCR;
- Antibody titers assessed by conventional and selected non-conventional serology, per type of assay;
- EKG abnormalities will be described per treatment arm as the proportion of participants per type of EKG finding, clinical relevance and changes over time.

10.7 Treatment Compliance

This is a double blinded study and compliance will be considered according to treatment course. During

the study, treatment compliance will be monitored through follow- up visits, and all information will be registered in the appropriate section of the CRF. Treatment compliance will be presented in the study report, describing the number and percentage of participants who have and have not completed a full course of treatment per study arm, their intake of meals, and the reasons for treatment drop-out per study arm.

For participants who do not complete a full treatment course, mean time of exposure to the trial drug will be described per study arm.

10.8 Efficacy Analysis

The primary efficacy endpoint is parasitological response, defined as sustained parasitological clearance at 24 months of follow-up after treatment, determined by serial qualitative PCR results. The primary analysis will be the comparison of the sustained parasitological clearance of each treatment arm. An efficacy interim analysis will be performed when participants complete 12 months from end of treatment. The stopping rule will be applied with no change planned on the sample size. At that point, if the condition of suspending the study is accomplished, participants will be considered as early treatment failures.
Secondary efficacy analyses will further characterize differences in therapeutic response within this trial, namely:

- Parasite clearance at 6, 12 and 18 months.
- Serological response by conventional serology assessed at baseline and at 24 months of follow up after treatment.

For efficacy assessments, the EOT of each treatment arm will be defined according to the duration of the arm dosing regimen.
For PCR analyses, means and 95% confidence intervals will be presented per group and visit. In addition, time to sustained parasite clearance until 6, 12, 18 and 24 months of follow-up visits will be assessed using Kaplan– Meier survival analysis with log rank test for significance. Repeated measures analysis will also be performed. Multivariate analysis including those variables with a p-value<0.20 in the univariate model will be performed to define early and late predictors of a sustained parasitological response. The backwards method will be used to remove the variables that presented p-value greater than 0.05 in the multivariate analysis, until the obtention of the final model.

10.9 Safety Analysis

Safety analyses will include all participants who have received at least one dose of the study medication. The proportion of participants with SAE, AESI and/or AEs leading to treatment discontinuation will be described per study arm and by System Organ Class (using preferred terms defined by MedDRA 13.1), according to the CTCAE, version 5. The proportion of participants presenting at least one AE will be described. Participants with multiple events with the same preferred terms will be counted once. The maximum severity grade for each preferred term and body system will be summarized. If multiple events with the same preferred terms are recorded for a participant, the event with the maximum grade will be

included in the analysis. In addition, a narrative for each of the SAEs and AESI will be developed detailing all aspects related to the medical event. AEs not leading to treatment discontinuation will also be described per study arm using the same classification as presented above.
Incidence rate and 95% confidence interval will be presented per study arm will be presented for SAE and AEs per category and most frequent AEs. Otherwise only descriptive statistics will be presented.
Safety laboratory parameters (hematology and biochemistry) will also be described individually per study arm, showing the proportion of participants by degree of elevation relative to ULN and to baseline values and clinical significance, and blood levels changes over time. Shift tables will be presented. A listing of participants experiencing lab parameters elevation will be included.
EKG abnormalities will be described per treatment arm as the proportion of participants per type of EKG finding, clinical relevance and changes over time. Participants with altered EKG may have an echocardiogram to evaluate cardiac involvement or cardiac progression of CD.

10.10 Exploratory objectives Analysis

Exploratory objectives are to evaluate the incidence and severity of clinically relevant events attributed to CD, measure the QoL of participants, and to assess the cost-effectiveness of two regimens, among participants with chronic CD.

Incidence and severity of clinically relevant events attributed to CD

For the first objective a composite clinical endpoint will be defined considering the more relevant clinical events related with the cardiac progression of CD, such as death, resuscitated cardiac arrest, insertion of a pacemaker or an implantable cardioverter–defibrillator, sustained ventricular tachycardia, cardiac transplantation, new heart failure, stroke or transient ischemic attack. Results will be expressed as proportion of participants in each arm and a comparison between them. Moreover, EKG abnormalities will be described per treatment arm as the proportion of participants per type of EKG finding, clinical relevance and changes over time. Echocardiography will be perfomed at baseline, annually or when new abnormalities on the EKG appear during follow-up period.

Quality of life (QoL)
For the QoL objective, validated questionnaires will be completed by participants during each visit. The WHOQOL Bref will be completed during the baseline visit and at the end of follow up, and the EQ-5D-3L questionnaire will be completed during all visits. Questionnaires must be completed BEFORE any procedures of the proposed visit and must be self-applied. Only a person not related to the research can help the participant during the completion. Longitudinal differences on quality-of-life scores between groups will be fitted using linear mixed models.

EQ-5D-3L questionnaires will be completed to allow the estimation of health utilities (a value between 0 and 1 estimated using country-specific value sets; 0 represents death and 1 full health). The health utilities will be used to calculate quality-adjusted life-years (QALYs) gained. We need EQ-5D-3L to estimate the health utility of each health state within the course of Chagas disease (e.g. cardiomyopathy form, gastrointestinal form, among others). This will be a parameter for our economic analyses. Unfortunately,

EQ-5D is short and includes only 5 dimensions. These questionnaires should preferably be self-administered, but whenever this is not possible, the questionnaire could be read out loud by the interviewer. It is expected to take an average of 5 minutes to fulfill all the items.

WHOQOL Bref has the advantage of being more generic, including more dimensions. It will be very useful as a secondary outcome for the clinical trial. However, it cannot be used to obtain health utilities for cost-effectiveness studies. Additionally, it would be possible to compare and crosswalk the two questionnaires considering the specific study visits when both measures will be completed.
The WHOQOL-BREF should be self-administered if respondents have sufficient ability. If this is not possible, interviewer-assisted or interview-administered forms should be used. Standardized instructions, which are provided on the second page of the WHOQOL-BREF example assessment, should be read out to respondents in instances where the assessment is interviewer-administered. It is expected to take an average of 15 minutes to fulfill all the items.

We have decided to include both measures to estimate impacts on several dimensions of the interventions studied and to generate parameters for our cost-effectiveness study.
Both WHOQOL-BREF and EQ-5D-3L questionnaires have been adapted for each country. For the WHOQOL-BREF are available two versions, Colombian and Chilean, which will be used in Bolivia. For the EQ-5D-3L questionnaires only one available version, the Colombian, which will be used in Colombia and Bolivia.

Cost-effectiveness Analyses
An economic evaluation alongside the primary clinical trial will be conducted following the International Society for Pharmacoeconomics and Outcomes Research, Inc (ISPOR) recommendations. The use of all types of resources within the clinical trial will be collected prospectively at the participant-level to assess the individual direct and indirect costs through study forms and electronic medical records. The collected protocol-driven resource use will be reviewed to allow estimation to reflect the future real-world resource use. Individual participant-level data regarding units of resources (e.g. medical visits, diagnostic tests, drugs, hospitalization) will also include all the procedures associated with the management of AE. There will be an effort to include medical and non-medical resources (e.g. transportation, absence from work). The full list of resource units will be pilot tested in each study site to investigate the feasibility.
A valuation procedure will be conducted to provide country-specific data that will be assigned for all unit costs. The imputation of available information from the most similar setting will be considered should country-specific data not be available.
The obtainment of the number of units used per-participant and the value of each unit will allow the inclusion of costs incurred at participant-level in the database for the study interventions.
Incremental cost-effectiveness ratios (ICER) will be obtained by dividing the incremental cost by the incremental health benefit. This means that the ICER will be calculated using the costs of reduced BZN treatment minus the cost of the standard treatment for chronic CD as numerator, and the effectiveness of reduced BZN treatment minus the effectiveness of the standard treatment as denominator.
The outcome of the cost-effectiveness study will be the cost per QALY gained. The QALYs will be obtained after the multiplication of years lived after each intervention by the utility values of each health state lived within the study duration.

10.11 Joint analyses of NuestroBen and Benlatino trials

For that purpose we have considered the following aspects.
In the NuestroBen trial in Argentina, each of three arms (durations of 60, 30, and 15 days) will enroll 100 participants. In the BENLatino study, 112 patients will be available per country (Bolivia and Colombia) and per arm (60, 30, and 15 days), resulting in 672 total patients. The studies will share overall efficacy results (proportions of patients with sustained clearance at 12 months follow-up) to utilize a dynamic borrowing approach in the analysis, described below.

The primary endpoint is negative serial qualitative PCR results from the end of treatment with the elimination of sustained parasitaemia until the end of 12 months' follow-up from the beginning of treatment.The primary analysis tests whether either of the 30 or 15 day arms is noninferior to the 60 day arm, using a noninferiority margin of 20%. The trials have been powered based on an assumed 80% efficacy/20% failure rate in the 60 day arm. Let p_{60}, p_{30}, and p_{15} be the response rates in each arm. Specifically, we are testing the hypotheses

$H_0 : p_{60} >= p_d + 0.20$ for d=30 and 15

$H_1 : p_{60} < p_d + 0.20$ for either d=30 or d=15.

The primary analysis is a Bayesian test that declares noninferiority if the posterior probability of noninferiority for either arm (either $Pr(p_{60} < p_{30}+0.20)$ or $Pr(p_{60} < p_{15}+0.20)$) exceeds 0.9875.

We calculate both posterior probabilities of noninferiority using a model that incorporates both the data from the NuestroBen trial (300 participants) and the BENLatino trial data (anticipated 672 participants). Let p_{60b}, p_{30b}, and p_{15b} be the response rates for the BENLatino Bolivia patients, and p_{60c}, p_{30c}, and p_{15c} be the response rates for the BENLatino Colombia data. Where needed, we will use p_{60a}, p_{30a}, and p_{15a} interchangeably with p_{60}, p_{30}, and p_{15} to emphasize we are referring to the Argentina parameters.

Within each arm and country, we assume response rates are Binomial. If Y_{dr} is the number of responses within duration d (d=60,30,15) in region r (r=a,b,c for Argentina, Bolivia, and Colombia) and n_{dr} is the total number of participants in that duration and region, then

$Y_{dr} \sim Bin(n_{dr},p_{dr})$

We place a hierarchical model on the 9 arm/country response rates to combine information across countries (no borrowing is conducted across arms). For each d and r let

$$\theta_{dr} = \log\frac{p_{dr}}{1-p_{dr}}$$

Indicating the logit of the corresponding rate.

We first place independent noninformative priors on the response rates in each country for the 60 day duration.

$$\theta_{60a}, \theta_{60b}, \theta_{60c} \sim N(0,10)$$

We then define treatment effects δ_{30r} and δ_{15r} relating the 30 and 15 day durations to the 60 day duration.

$$\theta_{30r} = \theta_{60r} + \delta_{30r}$$

$$\theta_{15r} = \theta_{60r} + \delta_{15r}$$

The treatment effects are then placed in hierarchical model, borrowing information across countries (but not across durations)

$$\delta_{30a}, \delta_{30b}, \delta_{30c} \sim N(\eta_{30}, \tau_{30})$$

$$\delta_{15a}, \delta_{15b}, \delta_{15c} \sim N(\eta_{15}, \tau_{15})$$

with hyperpriors

$$\eta_{30}, \eta_{15} \sim N(0,10)$$

$$\tau_{30}^2, \tau_{15}^2 \sim IGamma(center = 0.3, weight = 0.5)$$

This prior creates dynamic borrowing across the three countries. If the observed treatment effects appear similar across the three countries, the model borrows strength between the countries and obtains more precise treatment effect estimates. If the observed treatment effects are different, then less borrowing is performed and the data from each country will stand more on its own. This hierarchical borrowing methodology improves power and reduces type 1 error when the countries are qualitatively similar (exact equality is unnecessary) in terms of treatment effects, while presenting risks of decreased power and inflated type 1 error if one country differs significantly from the others. Given the apriori belief that the countries are more likely to be similar than they are to be different, the benefits of borrowing are viewed to exceed the risks.

After obtaining data, the posterior distribution of all parameters can be obtained, particularly the posterior distributions of p_{60}, p_{30}, and p_{15}. These will allow the calculation of the posterior probabilities of noninferiority. An arm will be declared to be noninferior if $Pr(p_{60} < p_{30}+0.20)$ or $Pr(p_{60} < p_{15}+0.20)$ exceeds 0.9875.

11. Quality assurance and quality control procedures

11.1 Investigator's file

The investigator in each country must maintain adequate and accurate records to enable the study's full documentation and subsequent verification of the study data. These documents include Investigator's Site File, participant clinical source documents and screening/enrolment logs. The Investigator's Site File will contain the protocol/protocol amendments, CRF and query forms, IEC and regulatory approval with correspondence, sample informed consent, drug accountability records, staff curriculum vitae and authorization forms and other appropriate documents / correspondence.

11.2 Case report forms (CRFs)

Data will be collected by laboratory technicians, medical doctors, clinical officers, and nurses authorized by the investigator in each country. Data collection will be supervised by the investigator and signed by the investigator or by an authorized staff member. Study-specific information will be entered into an electronic Case Report Form (CRF). Data that is entered into the system should be consistent with the source documents and any discrepancies should be explained. All CRF data should be anonymized, i.e. identified by study participant number only. The investigator of each site should ensure the accuracy, completeness, legibility, and timely completion of all data reported to the Sponsor in the CRFs and any other additional information that is required. The investigator is responsible for keeping all consent forms, screening forms, CRF and the completed participant identification code list in a secure location.

11.3 Source documents

The verification of the CRF data must be by direct inspection of source documents. Source documents include participant hospital/clinic records, physicians, and nurse's notes, appointment book, original laboratory reports, EKG and special assessment reports, signed informed consent forms, consultant letters, and participant screening and enrolment logs.
The investigator must maintain source documents (such as laboratory and consultation reports, history, and physical examination reports), for possible review and/or audit by Fiocruz and/or Regulatory Authorities. The Investigator / designee will record the date of each participant's visit together with a summary of their status and progress in the study.

11.4 Record Retention

The investigator must keep all the study documents on file for at least 25 years after the completion or discontinuation of the study. After that period, these documents may be destroyed with prior permission from Fiocruz, participant to local regulations.
Should the investigator wish to assign the study records to another party or move them to another location, Fiocruz must be notified in advance.

11.5 Monitoring, audits, and inspections

Clinical Monitors will conduct regular monitoring visits, during which they will inspect source data, and verify the Informed Consent Forms, medical records, laboratory results, imaging assessments, electronic CRF, drug dispensing logs, and protocol violations.

Monitoring visits to the trial site will be made periodically by Fiocruz representatives or designated clinical monitors to ensure that Good Clinical Practice (GCP) and all aspects of the protocol are followed. Source documents will be reviewed for consistency with data on CRFs/SAEs/AESI forms and any data clarification forms/queries, and the investigator will ensure direct access. It is important that the investigators and their relevant personnel are available during the monitoring visits.

The investigators will permit representatives of Fiocruz and/or designated clinical monitors to inspect all CRFs, medical records, laboratory work sheets and to assess the status of drug storage, dispensing and retrieval at any time during the study. The corresponding source documents for each participant will be made available provided that participant confidentiality is maintained in accordance with local regulations. The inspections are for the purpose of verifying the adherence to the protocol and to ensure the study is conducted according to GCP. It is important that the investigators and other trial site staff are available at these visits.
The monitoring visits will provide Fiocruz with the opportunity to evaluate the progress of the study, verify the accuracy and completeness of CRFs/SAEs/AESI form and data clarification forms / queries, resolve any inconsistencies in the study records, as well as ensure that all protocol requirements, applicable regulations, and investigator's obligations are being fulfilled. Four types of visits are planned: pre-study, study start, during the study, and study end. Visits may also be performed by regulatory authorities.
It will be the clinical monitor's responsibility to inspect the CRF and SAEs/AESI form and data clarification forms/queries at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The investigator agrees to cooperate with the clinical monitor to ensure that any problems detected during these monitoring visits are resolved.

11.6 Audits and inspections

The trial site may also be subject to quality assurance audits by Fiocruz or designated representatives and/or to inspection by regulatory authorities or IECs. It is important that the investigators and their relevant personnel are available for possible audits or inspections.

11.7 Data Management

A CRF must be completed for all participants that have given informed consent. The current clinical trial will use a validated electronic CRF (eCRF). The trial data will be stored in a computer database maintaining confidentiality in accordance with national data legislation. All entries into the CRF are the responsibility of the investigator or a qualified designated staff member. The investigator will attest in writing at the beginning of the trial that their electronic signature is the legally binding equivalent of a written signature. Data will be continuously reviewed by the clinical monitor. Data queries will be generated, documented, and resolved on an ongoing basis during the trial.

11.8 Confidentiality of trial documents and participants' records

The investigator must assure that participants' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the Sponsor, participants should not be identified by their names, but exclusively by an identification code. The investigator should keep a participant enrolment list showing codes, names, and addresses. The investigator should maintain documents for submission to Sponsor authorized representative, and participant's signed written consent forms, in strict confidence.

12. Schedule of Activities

	Screening visit D-21	**Baseline visit D0**	**Treat V S2 D14** (+/-2d)	**Treat V S4 D28** (+/-2d)	**EOT V S8 D56** (+/-7d)	**Follow up Visits 1, 4, 6, 8, 12 and 18 months after EOT** (+/-10 d)	**End Visit 24 months after EOT** (+/-15d)	**Adverse Event Visit** (not scheduled)
Informed Consent Form	X							
Demographic data	X							
Medical History	X							
Toxic habits	X							
Chagas disease interview	X							
Concomitant medication	X	X	X	X	X	X	X	X
Clinical interview	X	X	X	X	X	X	X	X
Physical exam	X		X	X	X	X	X	X
Pregnancy test[1]	X				X			
Hematology[2]	X		X		X			X
Biochemistry[3]	X		X		X			X
EKG	X					X	X	
Echocardiography[4]	X					X	X	
Serology	X						X	
PCR	X					X	X	
Adverse			X	X	X			X

events[5]									
Randomization[6]		X							
Treatment administration[7]		X	X	X					
WHOQOL BREF Questionnaire[8]		X					X		
EQ-5D-3L questionnaire[8]		X	X	X	X	X	X		
Cost-effectiveness Questionnaire		X	X	X	X	X	X		

Treat. V: Treatment visits; **EOT V:** End of treatment visit; **EKG:** Electrocardiogram; **PCR:** Polymerase Chain Reaction.

1. Only women of childbearing age with potential to become pregnant. Baseline visit may be performed at the same time as the screening visit.
2. Red and white cell count.
3. Alanine aminotransferase (ALT); aspartate aminotransferase (AST); urea and creatinine.
4. Echocardiography will be performed in participants with EKG alterations at baseline annually or when new EKG abnormalities appear during follow up.
5. Serious adverse events (SAE) reporting period initiates upon participant enrolment (after signature of informed consent form); adverse event (AE)/ adverse event of special interest (AESI) reporting from administration after first dose of trial medication at Day 1.
6. After completing the screening/baseline visit procedures and evaluating all inclusion/exclusion criteria, participants should be immediately randomized.
7. The first treatment dose under investigation must be administered on the same day of randomization (immediately after randomizing).
8. Questionnaires must be completed BEFORE any procedures of the proposed visit and must be self-applied. Only a person not related to the research can help the participant during the filling. WHOQOL BREF and EQ-5D-3L questionnaires will be distributed and completed by the participant.

13. Protocol Amendments

The PI will ensure that the study protocol is strictly adhered to throughout the study, and that all data are collected and recorded correctly on the CRF.
All protocol modifications must be documented in writing. Any protocol amendment must be approved and signed by the Sponsor and the PI and must to be submitted to the appropriate IEC for information and approval in accordance with local requirements, as well as to any required regulatory agencies and WHO ERB. Approval by IEC (and Regulatory Authority, if applicable) must occur before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial participants, or when the change involves only logistical or administrative aspects of the trial e.g. change in clinical monitor[s], change of telephone number[s]. The protocol amendment can be initiated by the Sponsor or by the study PI.

The investigator will provide the reasons for the proposed amendment in writing and will discuss with the PI and Sponsor.

14. Early termination of the study

Both the Sponsor and the PI reserve the right to terminate the study at any time prior to inclusion of the intended number of participants, but this right will only be exercised for valid scientific or administrative reasons. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, the Sponsor and the PI will assure that adequate consideration is given to the protection of participants interests.
Reasons for termination by the Sponsor(s) may include but is not limited to:

- Too low recruitment rate;
- Protocol violations;
- Inaccurate or incomplete data;
- Unsafe or unethical practices;
- Questionable safety of the test article;
- Suspected lack of efficacy of the test article;
- Administrative decision.

Reasons for early termination by the PI may be:

- Insufficient time or resource to conduct the study;
- Lack of eligible participants.

Stopping rules will be clearly defined for futility:

- Regarding efficacy, a stopping rule is defined by significant difference compare to standard treatment measured through the detection of parasite DNA at 12 months after EOT. The stopping rule will be applied with no change planned on the sample size. Participants will be considered

early treatment failures.

If a study is terminated early either by the Sponsor or by the investigator, the investigator must:

- Complete all CRFs to the greater extent possible;
- Return all test articles, CRF, and related study materials to the Sponsor who provided them;
- Answer all questions of the Sponsors or their representatives related to data of participants enrolled at the site prior to study termination;
- Ensure that participants enrolled in the study who had not yet reached a follow up time point are followed up with the necessary medical care;
- Provide in writing the reasons for his decision to the national health authority and the Sponsor.

15. Ethics

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki and ICH guidelines for GCP (International committee for Harmonization). Fiocruz assures that it will comply with all applicable state, local and regional laws for protecting the rights and welfare of human participants. This protocol and any protocol amendments will be reviewed/approved by the relevant IECs before its implementation.
This study will be submitted for review to WHO Ethics Review Committee (ERC), and to the national Ethical Committee boards in Bolivia and Colombia, shared with each of the collaborating institutions. Any modifications made to the protocol after receipt of the ERC approval must also be submitted by the investigator in writing to the IEC in accordance with local procedures and regulatory requirements.

16. Informed consent process

Inclusion in the study will occur only if the participant gives written informed consent. It is the responsibility of the investigator/designee to obtain voluntary written informed consent from everyone participating in this study, after adequate presentation of aims, methods, anticipated benefits, and potential hazards of the study. The written informed consent document will be translated into the local language, or a language understood by the participant(s). The participant will be given time to discuss the information received with members of the community or family before deciding to consent. The participant will be asked to provide written and signed consent.
If the participant is illiterate or unable to write, a mark will be included (such as a fingerprint), and a literate witness should provide a signature (this person should have no connection to the research team and the Sponsor, and, if possible, should be selected by the participant).
If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All participants (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

17. Participant costs

Participants will be reimbursed for travel to and from the study site and for the lost day(s) of work but will not receive any payment for trial participation. An average cost of 25 dollars per visit per patient has been estimated. Any treatment for trial-related injuries that is required during the trial period will be provided free of charge to the participant.
Trial-related injuries will be considered those adverse events that, according to the investigator or the pharmacovigilance monitor, were classified as related to the experimental drug.
To help investigators with the binary decision tree in the evaluation of causality, investigators will be asked to consider the following before reaching a decision:

- Medical history;
- Lack of efficacy / worsening of existing condition;
- Trial medications;
- Other medications (concomitant or previous);
- Withdrawal of trial medication, especially following trial discontinuation/end of trial medication;
- Erroneous treatment with trial medication (or concomitant);
- Protocol related procedure.

The relationship of an AE to investigational treatment is assessed and determined by the investigator after careful consideration of the event in terms of biological plausibility, possible unrelated causes, any pre-existing medical conditions or concomitant medications, temporal relationship between administration of investigational treatment and the onset (or worsening) of the event and known patterns of response to trial medications in general. The causality assessment of an AE to the investigational medicinal product will be rated as follows:

- *Not related:* There is no reasonable possibility of causal relationship;
- *Related*: There is at least a reasonable possibility of a causal relationship between an AE and an IMP. This means that there are facts (evidence) or arguments to suggest a causal relationship.

18. Funding and Insurance

This study will be financed with funds from Unitaid and Brazilian Ministry of Health to the Project entitled “Project CUIDA Chagas: United Communities for Innovation, Development and Care for Chagas disease - Towards elimination of congenital transmission of Chagas disease in Latin America”. The financial aspects related to the clinical trial will be reflected in a contract between the promoter and the center participating in the trial. The financial report of the study will be made available to the corresponding Ethics Review Committee for its evaluation if required. An insurance will be contracted that will cover possible damages to participants due to participation in the trial, considering the requirements of current regulations in each of the countries where the study will be carried out.

19. Reporting and Publication

All clinical trials will be registered with a recognized clinical trial registry such as www.clinicaltrials.gov.
The results of this study may be published or presented at scientific meetings. The Sponsor agrees to publish the test results whether they are positive or negative. All information related to participants and the study is considered confidential and property of the Fiocruz, the promoter, until its publication. The researchers agree to keep this information confidential, and not to use it for any other purpose without the Sponsor's written authorization. In addition, prior to publication of the results, the approval of the investigators participating in the study will be required.
The results of the study will be shared at major scientific conferences of interest to the CD research community, such as the annual meeting of the respective national Societies of Tropical Medicine and the Chagas Clinical Research Platform meeting. A publication will be authored by the study investigators detailing the findings and submitted to a major medical journal for open access publication. Unitaid and other funding sources will be acknowledged in all publications and conference presentations. Trial participants, study site personnel, and country agencies participating in the CUIDA Chagas project will also be acknowledged. Fiocruz maintains publication and authorship guidelines which will inform the composition and hierarchy of publication authorship.
If the study confirms the viability of the short regimen of BZN, the CUIDA Chagas Consortium will invite regional and country regulatory and health authorities to meetings in which results will be shared and next steps (for regulatory submission/change of label) proposed.
Finally, CUIDA Chagas Consortium members will share the results of the study with the public in a straightforward, non-technical format, in the main languages of the Americas (Spanish, Portuguese, and English) via electronic/social media.

20. Dissemination of the results

The results of the study will be shared at major scientific conferences of interest to the Chagas disease research community, such as the annual meeting of the respective national Societies of Tropical Medicine and the Chagas Clinical Research Platform meeting. A publication will be authored by the study investigators detailing the findings and submitted to a major medical journal for open access publication. UNITAID and other funding sources will be acknowledged in all publications and conference presentations. Trial participants, study site personnel, and country agencies participating in the CUIDA Chagas Consortium project will also be acknowledged. Fiocruz maintain publication and authorship guidelines which will inform the composition and hierarchy of publication authorship.
If the study confirms the viability of the short regimen of benznidazole, the CUIDA Chagas consortium will invite country regulatory and health authorities to share the results and propose next steps, such as regulatory submission and change of label.
Finally, CUIDA Chagas consortium members will share the results of the study with the public in a straightforward, non-technical format, in the main languages of the Americas (Spanish, Portuguese, and English) via electronic/social media.

21. Expected outcomes of the study

The results of the trial will help determine if shorter, 2 or 4-week regimens of BZN have similar efficacy to the current 8-week treatment, and to what extent the shorter regimen will reduce the frequency of adverse effects. Regardless of the trial's outcome, this information will be invaluable from a public health and drug development planning standpoint, to help determine whether or not the current regimen of BZN can be enhanced to improve participant safety and adherence without loss of efficacy.
If the results of this trial confirm that the shorter regimens are non-inferior to the standard regimen, data from the trial can then be used to support regulatory processes for changes in the label/indication of treatment in countries where BZN is currently registered, and for new registration in other countries. BZN is currently registered in all four countries included in the CUIDA Chagas project, as well as in six other countries in the Americas, including Mexico, Argentina, and the United States.
Reducing the current treatment period from 8-weeks to shorter period would greatly facilitate adherence for participants. Currently, participants often prefer to forego treatment due to the long duration and the side effects involved, which can imply lost time from work, difficulty in managing household activities such as care for children, and an inability to participate in community life. Moreover, returning to the clinic for laboratory monitoring during the treatment period can represent additional costs and lost time for participants, many of whom must pay out of pocket for travel, food, and lodging to reach the nearest available clinic.
From a healthcare provider standpoint, a shorter period will greatly facilitate the process by reducing the number of participant visits, the amount of monitoring required, and the frequency of side effects requiring additional management. This in turn should reduce the cost of treatment for health systems. Moreover, training of healthcare personnel in treatment would be simplified.
Treatment coverage is extremely low, reaching only about 1% of estimated cases. A simplified treatment would be a powerful tool enabling scale up of treatment coverage at the level needed to control CD and eliminate congenital transmission.

22. Anticipated problems

Not all trial sites are in close proximity to urban areas. To reduce any difficulties in attending study visits, participants will be compensated for travel.

Some outputs of the clinical trial, including scientific publications, may not be available until after the period of funding due to the duration of the trial. However, the investigators will continue to update Unitaid on activities such as dissemination of trial results.

The coronavirus disease 2019 (COVID-19) pandemic is an ongoing concern, and a specific contingency has been considered under a Standard Operational Procedure. The study will adapt to local conditions and regulations for COVID-19. In situations where significant local outbreaks are underway, and in accordance with local regulations affecting participant travel and non-essential healthcare visits, participant visits may need to be rescheduled, as close as possible to the projected time point, once regulations and conditions permit safe in-person visits. In the event of prolonged restrictions in local travel due to COVID-19, participants may be asked to report AE data via telephone, while sample collection will remain postponed. COVID-19 precautions will be reviewed with investigators and site personnel at the beginning of the study, and information will be provided to participants. Participants will be contacted by site personnel if visits need to be rescheduled. All study sites will have the capability to provide COVID-19 testing.

Discrepancies among PCR results: blood samples for DNA detection will be handled by experienced personnel at referral laboratories under Good Laboratory Practices. Moreover, harmonization panels and internal quality analyses will be performed before and during the clinical trial execution.

23. Collaboration with other scientists or research institutions

The investigators participate in the Chagas Clinical Research Platform, which maintains a network of over 400 investigators and healthcare providers involved in CD research and health care. Founded in 2009, the platform brings together partners, experts, and stakeholders to provide support for evaluation and development of new treatments for CD. The patient-centred platform aims to facilitate clinical research, provide a forum for technical discussions, develop a critical mass of expertise, and strengthen institutional research capacities. In addition, it will identify and review priority needs, works towards standardization of methodology to assess drug efficacy and reviews alternatives for using current approved drugs (new schemes, doses, combination) and special scenarios.

24. Links to other projects

The investigators will remain in close contact with investigators from other ongoing clinical trials in CD, such as BETTY, MULTIBENZ, NUESTROBEN and TESEO clinical trials.

25. Other research activities of the Study Coordinator

The PI declares to have 40% dedication to the proposed research project.

26. Community and Civil Society engagement

The engagement of communities and civil society (CS) is central in any public health intervention, but potentially even more so in settings where there is evidence of inequalities in health. Due to the nature of Chagas disease, the people it affects most, and its classification as a NTD, the focus on community and civil society engagement becomes even more important. Currently, the number of civil society organizations (CSOs) working on CD is limited, especially compared to diseases such as HIV, as is the meaningful engagement of communities and their leaders (traditional, religious, etc.). The aforementioned project CUIDA Chagas will therefore place special emphasis on this topic by executing a number of activities, including a formative research aimed at creating a better understanding of the different municipalities included in the project; information, education and communication campaigns targeting the different communities; a mapping of civil society in each municipality; organizing capacity building sessions with community and CS leaders; and promoting networking between CS and health

managers. This study will benefit from the work that will be done with communities and CS as it will increase awareness and willingness to participate, as well as generate a stream of potential participants.

27. Stakeholders, roles and responsibilities.

The key stakeholders of this clinical trial are:

Stakeholder	Roles & Responsibilities
Fiocruz	- Sponsor. - Execute the clinical trial in accordance with international standards.
The Ministries of Health of Bolivia and Colombia	- Execution of the clinical trial through the public health institutes INLASA and INS. - Incorporation of the results in public policies on the treatment of CD. - Advocacy for regional implementation of the results of the clinical trial.
The research team	- Execute the clinical trial in accordance with international standards. - The responsibilities of each researcher in included on the front page of the protocol.
International researchers	- Provide input at given times on the execution and intermediary and final results of the clinical trial.
Patients with Chagas disease	- Potential participants in the study. Potential beneficiaries of positive research outcomes.
PAHO/WHO	- Approval of the protocol by the Ethical Review Board (WHO). - Inclusion of results and recommendations in regional CD guidelines.
Local, national and international civil society	- Advocacy for the inclusion of the results and recommendation in national policies.
Regulatory agencies in Bolivia and Colombia	- Provide ethical approval for the clinical trial protocol.

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Annex 1 THE WHOQOL-BREF

ABOUT YOU I.D. number

Before you begin we would like to ask you to answer a few general questions about yourself: by circling the correctanswer or by filling in the space provided.

What is your **gender**? Male Female

What is your **date of birth**? _____ /_____ / _____

Day / Month / Year

What is the highest **education** you received?

None at all
Primary school
Secondary school
Tertiary

What is your **marital status**?

Single
Married
Living as married
Separated
Divorced
Widowed

Are you currently **ill**? Yes No

If something is wrong with your health what do you think it is?_____

Instructions

This assessment asks how you feel about your quality of life, health, or other areas of your life. **Please answer all thequestions.** If you are unsure about which response to give to a question, **please choose the one** that appears most appropriate. This can often be your first response.

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the lasttwo weeks.** For example, thinking about the last two weeks, a question might ask:

	Do you get the kind of support from othersthat you need?	Not at all 1	Not much 2	Moderatel y3	A great deal 4	Completely 5

You should circle the number that best fits how much support you got from others over the last two weeks. So youwould circle the number 4 if you got a great deal of support from others as follows.

	Do you get the kind of support from othersthat you need?	Not at all 1	Not much 2	Moderatel y3	A great deal 4	Completely 5

You would circle number 1 if you did not get any of the support that you needed from others in the last two weeks. Please read each question, assess your feelings, and circle the number on the scale for each question that gives the bestanswer for you.

THE WHOQOL-BREF

		Very poor	Poor	Neither poor nor good	Good	Very good
1 (G1)	How would you rate your quality of life?	1	2	3	4	5

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
2 (G4)	How satisfied are you with your health?	1	2	3	4	5

The following questions ask about **how much** you have experienced certain things in the last two weeks.

		Not at all	A little	A moderat eamount	Very much	An extreme amount
3 (F1.4)	To what extent do you feel that (physical) pain prevents you from doing what you need to do?	1	2	3	4	5
4 (F11.3)	How much do you need any medical treatment to function in your daily life?	1	2	3	4	5
5 (F4.1)	How much do you enjoy life?	1	2	3	4	5
6 (F24.2)	To what extent do you feel your life to be meaningful?	1	2	3	4	5

		Not at all	A little	A moderate amount	Very much	Extremely
7 (F5.3)	How well are you able to concentrate?	1	2	3	4	5
8 (F16.1)	How safe do you feel in your daily life?	1	2	3	4	5
9 (F22.1)	How healthy is your physical environment?	1	2	3	4	5

The following questions ask about **how completely** you experience or were able to do certain things in the last twoweeks.

		Not at all	A little	Moderately	Mostly	Completely
10 (F2.1)	Do you have enough energy for everyday life?	1	2	3	4	5
11 (F7.1)	Are you able to accept your bodily appearance?	1	2	3	4	5
12	Have you enough money to meet your	1	2	3	4	5
(F18.1)	needs?					
13 (F20.1)	How available to you is the information thatyou need in your day-to-day life?	1	2	3	4	5
14 (F21.1)	To what extent do you have the opportunity for leisure activities?	1	2	3	4	5

		Very poor	Poor	Neither poor nor good	Good	Very good
15 (F9.1)	How well are you able to get around?	1	2	3	4	5

The following questions ask you to say how **good or satisfied** you have felt about various aspects of your life over thelast two weeks.

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
16 (F3.3)	How satisfied are you with your sleep?	1	2	3	4	5
17 (F10.3)	How satisfied are you with your ability to perform your daily living activities?	1	2	3	4	5
18 (F12.4)	How satisfied are you with your capacity for work?	1	2	3	4	5
19 (F6.3)	How satisfied are you with yourself?	1	2	3	4	5
20 (F13.3)	How satisfied are you with your personal relationships?	1	2	3	4	5
21 (F15.3)	How satisfied are you with your sex life?	1	2	3	4	5
22 (F14.4)	How satisfied are you with the support youget from your friends?	1	2	3	4	5

23 (F17.3)	How satisfied are you with the conditions of your living place?	1	2	3	4	5
24 (F19.3)	How satisfied are you with your access to health services?	1	2	3	4	5
25 (F23.3)	How satisfied are you with your transport?	1	2	3	4	5

The following question refers to **how often** you have felt or experienced certain things in the last two weeks.

		Never	Seldom	Quite often	Very often	Always
26 (F8.1)	How often do you have negative feelings such as blue mood, despair, anxiety, depression?	1	2	3	4	5

Did someone help you to fill out this form?.....

How long did it take to fill this form out?.....

Annex 2

Health Questionnaire

Mark with a cross in the corresponding chart for each group, the answer that best describes your state of health today.

Mobility

I don't have trouble walking ❑

I have some trouble walking ❑

I've got to be in bed ❑

Personal Care

I don't have a problem with self-care ❑

I have some trouble bathing or dressing ❑

I am unable to bathe or dress myself ❑

Activities of Daily Living *(e.g., working, studying, housework, family activities, or recreational activities)*

I have no problem going about my daily activities ❑

I'm having some trouble doing my daily activities ❑

I am unable to carry out my daily activities ❑

Pain / Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I'm in a lot of pain or discomfort ❑

Distress / Depression

I'm not distraught or depressed ❑

I am moderately distressed or depressed ❑

I am very distressed or depressed ❑

To help people describe how good or bad their state of health is, we have drawn a scale similar to a thermometer in which 100 is the best state of health imaginable and 0 is the worst state of health imaginable.

We would like you to tell us on this scale, in your opinion, how good or bad your state of health is today. Please draw a line from the black box where it says "Your health status today" to the point on the thermometer that in your opinion indicates how good or bad your health is today.

Your Health Status Today

The best state of health imaginable

Annex 3: Cost-efectiveness Questionaires

Questionnaire for Survey of Associated Indirect Costs Treatment of Chagas Disease in Adults

1. **General data**

   Education: | | | 1-Unliterate, 2-incomplete elementary school, 3- complete elementary school, 4- High school, 5- Complete higher education, 6- other [specify]:
   99- Not informed

2. **locomotion**
   - Distance between the place of residence and the health center? | | | | | kms
   - Means of locomotion to the health center | |_|1- on foot, 2- bicycle, 3- bus, 4- train, 5-car, 6- other [specify]:
   - Commuting time between the place of residence and the health center | | |hours and
     | |_| minutes
   - Was there spending on displacement between the place of residence and the health center?
     | | 1- Yes, 2- No, 99- Not informed
   - If there were costs, what was the cost of commuting between the place of residence and the health center? (In local currency):

3. **Accommodation for the health center appointment**
   - Did you need an accommodation | | 1- Yes, 2- No, 99- Not informed
   - If you needed a stay, did you have any financial cost with the stay? | | 1- Yes, 2- No, 99- Not informed
   - If you had costs, what is the cost of the stay? (In local currency) :
   - Distance between the place of stay and the health center? | | | | kms
   - Means of locomotion from the place of stay to the health center | | | 1- walking, 2- bike, 3- bus, 4-train, 5-car, 6-other [specify]:
   - Travel time between the place of stay and the health center: | | |hours and | | | minutes.

4. **Food to go to the health center**
   - Did it take any personal spending on food to go to the health center? | | 1- Yes, 2-No, 99- Not informed
   - If there was, what is spent on food (in local currency) :

5. income

- What is your profession?
- What is your average monthly income (in local currency)? | |
- How many people depend on this income? | |
- Do you have any health insurance? | |1- Yes, 2-No, 99-Not informed
- If you have health insurance, have there been any reimbursement for this | |
  1- Yes, 2-No, 99- Not informed
- If there was a refund, what percentage of reimbursement for this service?| | | |
- Have you lost income for your current health situation? | | (1= Yes, 2=No, 99=N/I)
- If lost, how much was average loss of monthly income (in local currency)?
- If you lost income, for how many months? | | | |