

BMJ Open New regimens of benznidazole for the treatment of chronic Chagas disease in adult participants in indeterminate form or with mild cardiac progression (NuestroBen study): protocol for a phase III randomised, multicentre non-inferiority clinical trial

Tayná Marques ¹, Colin Forsyth ¹, Fabiana Barreira,¹ Carola Lombas,² Bethania Blum de Oliveira ¹, Mónica Laserna,¹ Israel Molina,^{3,4,5} Maria del Carmen Bangher,⁶ Rosmiro Javier Fernández,⁷ Susana Lloveras,⁸ Marisa Liliana Fernández,⁹ Pablo Scapellato,¹⁰ Patricia Patterson,¹¹ Wilson Garcia,¹² Lourdes Ortiz,^{13,14} Alejandro Schijman,¹⁵ Otacilio C Moreira,¹⁶ Lineth García,¹⁷ Kert Viele,¹⁸ Silvia Longhi,¹⁵ Michel Vaillant,¹⁹ Craig Tipple,²⁰ Laurent Fraisse,²⁰ Andrea Silvestre-Sousa,²¹ Sergio Sosa-Estani,¹ María-Jesús Pinazo^{1,4}

To cite: Marques T, Forsyth C, Barreira F, *et al.* New regimens of benznidazole for the treatment of chronic Chagas disease in adult participants in indeterminate form or with mild cardiac progression (NuestroBen study): protocol for a phase III randomised, multicentre non-inferiority clinical trial. *BMJ Open* 2025;**15**:e098079. doi:10.1136/bmjopen-2024-098079

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<https://doi.org/10.1136/bmjopen-2024-098079>).



© Author(s) (or their employer(s)) 2025. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ Group.

For numbered affiliations see end of article.

Correspondence to

Dr María-Jesús Pinazo; mpinazo@ndi.org

ABSTRACT

Introduction Chagas disease (CD) is one of the most neglected diseases in the world. In Latin America, CD is endemic in 21 countries, with an estimated 70 million people at risk of infection. Current treatments are limited to two nitroheterocyclic compounds: nifurtimox and benznidazole (BZN). Each has significant limitations, including long duration and safety concerns. However, data from recently completed studies suggest that reduced-duration regimens may be equally effective while enhancing safety.

Methods and analysis NuestroBen is a phase III, randomised, multicentre clinical trial designed to assess whether shorter (2- and 4-week) regimens of BZN are non-inferior to the standard 8-week treatment. A total of 540 adult participants with no evidence of organ damage (the indeterminate form) or with mild cardiac progression (mild electrocardiographic alterations and without systolic dysfunction or symptoms), all in the chronic phase of CD, will be recruited at six study sites in Argentina and two study sites in Bolivia. Participants will be randomised to receive one of the two shortened regimens of BZN (300 mg per day for 2 or 4 weeks) or standard treatment (300 mg per day for 8 weeks). The primary endpoint is sustained elimination of parasitaemia from the end of treatment through 12 months of follow-up. Secondary endpoints will assess sustained clearance of parasitaemia at 1, 4, 6 and 8 months of follow-up from the end of treatment, drug tolerability and adherence to treatment. NuestroBen

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ If non-inferiority is demonstrated by this study, results will be proposed for adoption at regional level, an outcome strengthened by the multicentre design.
- ⇒ Including a non-conventional multiplex serology (multicruzi assay) as an exploratory endpoint will allow for validation of its use as an early serological marker.
- ⇒ Access activities will be implemented to generate an in-depth understanding of the practical implications of new benznidazole regimens, with findings that will inform local public health policies for Chagas disease treatment and support the development of responsible strategies to reduce adverse effects and improve treatment adherence.
- ⇒ An interdisciplinary team composed of clinical doctors, cardiologists, infectious disease specialists, drug development experts, patient representatives, methodologists and statisticians will ensure the rigour of the study by evaluating the data from multiple perspectives.
- ⇒ One limitation of the study is the 12-month follow-up period. Ideally, a longer follow-up would have allowed for a more comprehensive assessment of sustained parasitological response and the opportunity to capture potential clinical progression over time.

will also evaluate whether two shortened regimens of BZN improve drug tolerability and treatment adherence compared with the current standard treatment while maintaining efficacy in participants with the indeterminate form of CD or with mild cardiac involvement.

Ethics and dissemination In Argentina, this study was approved by *Fundación de Estudios Farmacológicos y Medicamentos 'Luis M. Zieher'* for its conduct at the *Instituto de Cardiología de Corrientes 'Juana Francisca Cabral'* (reference: NuestroBen-2020/2021) and the *Instituto Nacional de Parasitología 'Dr. Mario Fatala Chaben'* (reference: NuestroBen-2020/2021) by *Comité Institucional de Ética de Investigación en Salud* for the *Centro de Chagas y Patología Regional de Santiago del Estero* (reference: NuestroBen-2020-088/2021), by *Comité de Ética en Investigación* for the *Hospital de Infecciosas F.J. Muñiz* (reference: NuestroBen-2020-4037) and the *Hospital General de Agudos D.F. Santojanni* (reference: NuestroBen-2020-4039) and by *Comité de Bioética* for the *Fundación Huésped* (reference: NuestroBen-2020/2021). In Bolivia, it was approved by *Comité de Ética en Investigación en Salud* from the *Universidad Autónoma Juan Misael Saracho* (reference: NuestroBen-2020/2025). All participants are asked to provide written informed consent to participate. Recruitment processes started in July 2023, and as of 15 June 2025, 140 participants have been recruited. Findings will be shared with Argentinian and Bolivian public health officials and with the Chagas and tropical medicine communities via international conferences. Findings will also be published in medical journals.

Trial registration number NCT04897516.

INTRODUCTION

Chagas disease (CD), caused by *Trypanosoma cruzi* (*T. cruzi*), ranks among the most neglected diseases in the world. In Latin America, 21 countries are endemic for CD with approximately 70 million people at risk of contracting the disease.¹ The annual global burden is more than 800 000 disability-adjusted life years, US\$600 million in costs to health systems and US\$7 billion in economic damage.² In 2015, the WHO estimated that approximately 6 million people were living with the infection, with an additional 30 000 new cases per year.¹ Mother-to-child transmission during pregnancy is an important source of new cases (8700 per year), and CD is included in a Pan American Health Organization (PAHO) initiative for the elimination of mother-to-child transmission of four diseases of public health importance.³

Argentina is the country with the highest absolute number of people with CD at the global level, with more than 1.5 million individuals living with *T. cruzi* infection and around 2500 new cases per year, the majority due to congenital transmission.¹ Meanwhile, Bolivia is the country with the highest disease prevalence in the world (6.1%), with an estimated 600 000 people living with the infection, and the highest incidence of any country due to vectorial transmission (8087).¹ CD places an economic burden of US\$1.5 billion per year on the country.² The epidemiological profile of CD has transformed in recent decades. While the disease traditionally affected rural populations in endemic areas, especially in the north of the country, large numbers of people with the infection are now found in urban, non-endemic areas, presenting new challenges for the health system.⁴ Whether living in endemic areas or having migrated to urban, non-endemic

settings, most people affected by *T. cruzi* infection live in conditions of socioeconomic vulnerability.

PAHO currently recommends treatment of *T. cruzi* infection with either benznidazole (BZN) or nifurtimox (NFX), the only drugs with efficacy demonstrated against *T. cruzi*. These treatments are strongly recommended for acute and congenital cases, for children and women of childbearing age with the chronic indeterminate form and for chronically infected adults without severe cardiac involvement (conditional recommendation).⁵ While serological cure can be demonstrated in acute cases and chronically infected children, observational studies have suggested that treatment can help prevent progression to more severe complications in people with chronic infection.^{6–8} Another key advantage is the prevention of future congenital transmission in treated girls and women of childbearing age. Despite the well-proved positive effect in mild cardiac cases,⁹ the BENEFIT trial indicated there was no statistically significant advantage to treating adults with advanced cardiomyopathy with BZN, compared with placebo, although treatment decreased the detection of *T. cruzi* DNA in the blood of chronic patients.¹⁰ These assumptions must be carefully interpreted considering study designs, populations and objectives.

Currently, few people affected by CD can access antiparasitic treatment, which furthermore has significant limitations, including lengthy duration of treatment (60 days) and frequent adverse events. Even though most of the adverse events are mild, one in five patients discontinues treatment.¹¹ These concerns complicate administration and adherence because patients require periodic monitoring and laboratory testing to ensure that they tolerate the treatment and may need supportive care due to side effects. Reducing the length of treatment could mitigate significant barriers by decreasing the number of health-care visits and associated costs while making it easier for patients to tolerate and complete treatment.

The doses and duration of the current standard treatment for *T. cruzi* infection are based on pharmacokinetic and pharmacodynamic data from studies performed several decades ago that have not been clinically evaluated in randomised trials.¹² Population pharmacokinetic studies¹³ in adults suggest that the current BZN regimen may cause overexposure in most patients, while evidence in observational studies suggests that shorter and intermittent treatments could still be effective.¹⁴

Consequently, BENznidazole New Doses Improved Treatment and Associations (BENDITA), a phase II proof-of-concept study sponsored by Drugs for Neglected Diseases initiative (DNDi) (CH-E1224-003 and NCT03378661), was conducted in Bolivia to assess the efficacy and safety of several alternative regimens of BZN, both as monotherapy and in combination with E1224 (fosravuconazole), a broad spectrum antifungal triazole compound, in the sustained reduction and elimination of *T. cruzi* in adults with the indeterminate chronic form of CD.¹⁵ The primary efficacy endpoint was sustained parasite clearance, measured by negative results by PCR at

each visit from the end of treatment until the sixth month after treatment. A secondary efficacy endpoint examined sustained parasite clearance through 12 months of follow-up. The trial enrolled 210 participants, who were randomised into one of the seven groups: BZN 300 mg per day for (1) 8 weeks, (2) 4 weeks or (3) 2 weeks; (4) BZN 150 mg per day for 4 weeks; (5) BZN 150 mg per day for 4 weeks in combination with E1224 300 mg per week; (6) BZN 300 mg per day for 4 weeks in combination with E1224 300 mg per week and (7) placebo. In all treatment arms, over 79% of participants exhibited parasite clearance that was sustained for 12 months of follow-up in the intention-to-treat (ITT) population. Furthermore, the safety profile was promising, as there were no treatment discontinuations due to adverse events in the 2-week arm, and only one in the 4-week arm.

While not designed to compare arms, BENDITA's results showed that a short course of treatment with BZN maintained the parasitological response effectively and with good tolerability in participants. Therefore, NuestroBen, a phase III study in Argentina and Bolivia, was planned to assess two of the most promising treatment regimens from the BENDITA study (300 mg per day for 2 and 4 weeks) in comparison with the standard treatment of 300 mg per day for 8 weeks. Meanwhile, several other clinical studies examining shorter or alternative regimens of BZN and NFX in different populations are currently in process or have recently been completed.^{16–19} Once completed, NuestroBen and these other trials, which have comparable and complementary approaches, will add important evidence on the feasibility of different BZN treatment regimens. Reducing treatment duration and the number of adverse events would increase adherence to treatment, improving the overall treatment risk/benefit profile for this disease and significantly increasing treatment access and coverage.

METHODS AND ANALYSIS

Study design

NuestroBen (New Scheme for Treatment with BZN) is a phase III open-label, prospective, randomised controlled, multicentre non-inferiority study to compare the efficacy of 2- and 4-week BZN 300 mg per day regimens (new treatment regimens) with the standard 8-week BZN 300 mg per day regimen (Standard of Care (SoC) treatment regimen) in the treatment of adult participants with chronic CD in the indeterminate form or with mild cardiac progression. The study is sponsored by ELEA Phoenix S.A. and the DNDi. Initially, the study included six different sites in Argentina: *Instituto de Cardiología de Corrientes 'Juana Francisca Cabral'*, Corrientes; *Centro de Chagas y Patología Regional, Hospital Independencia*, Santiago del Estero; *Instituto Nacional de Parasitología 'Dr. Mario Fatala Chaben'*, Buenos Aires; *Hospital Francisco Javier Muñoz*, Buenos Aires; *Hospital Donación Francisco Santojanni*, Buenos Aires and *Fundación Huésped*, Buenos Aires. The initial study design needed to be adapted and amended to include a second

country, Bolivia, with two study sites: *Centro de investigación en enfermedades transmitidas por vectores—SEDES Chiquisaca*, Sucre and *Plataforma de atención integral a los pacientes con enfermedad de Chagas—Universidad Autónoma Juan Misael Saracho*, Tarija.

Recruitment is expected to last approximately 44 months and is being accompanied by a screening programme in coordination with the sites where *T. cruzi* infection testing is offered using rapid tests to identify patients who need further diagnostic confirmation (people with positive results are given information about the study).

The study is designed to evaluate the non-inferiority of the new treatment regimens compared with the SoC treatment regimen in terms of efficacy. Furthermore, the new treatment regimens are expected to reduce the incidence of adverse drug reactions and the rate of definitive discontinuation attributed to the study drug.

Objectives and endpoints

The primary objective is to demonstrate the non-inferiority of treatment with BZN 300 mg per day (given as 100 mg Ter in die /three times a day (TID)) for 2 weeks and 4 weeks compared with the standard treatment of BZN 300 mg per day (given as 100 mg TID) for 8 weeks, in terms of reducing and/or eliminating the *T. cruzi* parasite in adults in the chronic phase of CD with the indeterminate form or mild cardiac progression. This establishes the proportion of participants with sustained clearance of parasitaemia according to the results of qualitative PCR tests from the end of treatment, and up to 12 months of follow-up from the end of treatment.

The secondary objectives are as follows: (1) to describe the proportion of participants with sustained clearance of parasitaemia according to the results of qualitative qPCR at 1, 4, 6 and 8 months of follow-up from the end of treatment; (2) to evaluate the safety of the 2-week and 4-week regimens of BZN (300 mg per day) according to anamnesis (symptom evaluation), physical examination and laboratory tests compared with the standard treatment with BZN of 300 mg per day for 8 weeks and (3) to evaluate the incidence of serious adverse events (SAEs) and/or adverse events that lead to the interruption of treatment with the 2-week and 4-week regimens of BZN at 300 mg per day compared with the standard treatment with BZN of 300 mg per day for 8 weeks; and to describe participant adherence to treatment in each study arm.

Primary efficacy endpoint

The primary endpoint is negative serial qualitative real-time PCR (qPCR) results demonstrating sustained elimination of parasitaemia from the end of treatment until the end of 12-months follow-up, meaning all negative qPCR results on each visit from end of treatment through the final follow-up visit at 12 months (nine qPCR evaluations in each timepoint). Any positive PCR result during that time will be considered to indicate treatment failure and, at the end of the study, participants who remain PCR-positive or who were PCR-positive at any intermediate

evaluation points will be offered the standard treatment with BZN at 5 mg/kg/day, divided into two per day doses for 60 days. Participants who do not tolerate the study treatment will be withdrawn from the study and offered an alternative treatment with NFX at 10–15 mg/kg/day, divided into three per day doses for 60 days.

For efficacy evaluations, the completion of each treatment arm will be defined according to the duration of the assigned treatment, that is, on day 14 of the 2-week regimen of BZN at 300 mg, on day 28 of the 4-week regimen of BZN at 300 mg and on day 56 of the standard treatment of 300 mg per day for 8 weeks.

Although it is well recognised that CD has a slow progression and that parasitaemia can occasionally re-emerge beyond 12-months post-treatment, the choice of a 12-month follow-up period aligns with prior clinical trials evaluating BZN efficacy, which commonly use this timeframe as a primary endpoint for sustained parasitological response.^{7–9} This choice balances the need for capturing early and midterm treatment effects with the practical considerations of patient retention and resource constraints. Nonetheless, longer follow-up would be needed to monitor for late relapses or treatment failures.

Secondary efficacy endpoint

Efficacy evaluation secondary endpoints are negative serial qualitative qPCR results from the end of treatment with sustained elimination of parasitaemia at 1-, 4-, 6- and 8-months' follow-up from the end of treatment.

Exploratory efficacy endpoint

Evaluate the efficacy of 2- and 4-week regimens of BZN (300 mg per day) based on unconventional anti-*T. cruzi* antibody levels (including multicruzi, among other potential biomarkers identified).^{20 21} This is an exploratory endpoint since multicruzi is not yet fully validated.

Statistical analysis

Non-inferiority criteria

The new treatment regimen will be considered non-inferior to the SOC regimen if the upper limit of the 95% Bayesian credible CI of the difference in the 'result' between both arms is $\leq 20\%$ (margin of non-inferiority). This is equivalent to the Bayesian posterior probability of inferiority ($\Pr(\text{SOC rate} - \text{new arm rate} < 0.20) > 97.5\%$).

Sample size justification

We had originally planned to implement a dynamic borrowing approach with another trial, BENLATINO (NCT06339710), an independent clinical trial coordinated by the Oswaldo Cruz Foundation—FIOCRUZ (Ministry of Health/Brazil) as a part of the Unitaïd consortium for CD (CUIDA Chagas Project),²² which was to take place in Bolivia and Colombia. However, since this study ultimately was not launched due to financial constraints, we decided instead to increase recruitment by adding additional sites in Bolivia.

Sample size calculation, considering the inclusion of Bolivia as a second country, was calculated as follows:

a non-inferiority Bayesian assurance for two proportions with beta prior for response rates is implemented for the sample size calculation based on the sustained clearance of parasitaemia until the end of 12 months of follow-up from the end of treatment. A gatekeeping approach is implemented by hierarchically testing non-inferiority of BZN 4 weeks to BZN 8 weeks and only if non-inferiority is verified, testing non-inferiority of BZN 2 weeks to BZN 8 weeks. A prior distribution is assigned to the control group (BZN 300 mg 8 weeks) proportion. The Bayesian assurance of the sustained clearance at 12 months is the unconditional probability that the trial will lead to a specific response rate difference and is a measure of the practical utility of the trial. It is often referred to as 'Bayesian Power' or the 'true probability of success'.

The statistical hypotheses are as follows.

- ▶ Level of significance 0.025
- ▶ BZN standard regimen 300 mg 8 weeks: response rate=80%
- ▶ BZN short regimen 300 mg 2 weeks: response rate=75%
- ▶ BZN short regimen 300 mg 4 weeks: response rate=75%.
- ▶ Non-inferiority margin 20%

A beta distribution of the response rate close to what can be expected for the distribution of a response rate, $B(5,2)$ is used. A two-sample non-inferiority test with a 2.5% significance level and a sample size per group of 163 will have a Bayesian assurance of 0.8, demonstrating that a treatment is non-inferior to a BZN 300 mg 8 weeks, given a non-inferiority limit of -0.2 , and assuming that the proportion of respondents in the treatment group (BZN 300 mg 4 weeks or 2 weeks) is 0.75, and the proportion of respondents in the control group follows a beta distribution, $B(5,2)$. The total minimal sample size is, therefore, 489 and including 10% drop-outs leads to 540 patients to enrol in the study.

The primary analysis will be performed on the per protocol (PP) and ITT populations. It is based on the Bayesian assurance and uses a gatekeeping approach. It will involve the calculation of the proportion of respondents in the treatment group (BZN 300 mg 4 weeks or 2 weeks) in the control group, that is, participants with a sustained parasitaemia clearance among all participants in each group. The difference between the reference treatment group and the experimental treatment groups will be evaluated and confidence intervals will also be calculated.

The lower limit of the credible interval for the difference comparing BZN 4 weeks to BZN 8 weeks will be compared with the non-inferiority margin set at -0.2 . Only if non-inferiority of BZN 4 weeks to BZN 8 weeks is verified, the lower limit of the credible interval for the difference comparing BZN 2 weeks to BZN 8 weeks will be compared with the non-inferiority margin. The alpha level will be set at 2.5% as per parameters set for the sample size calculation.

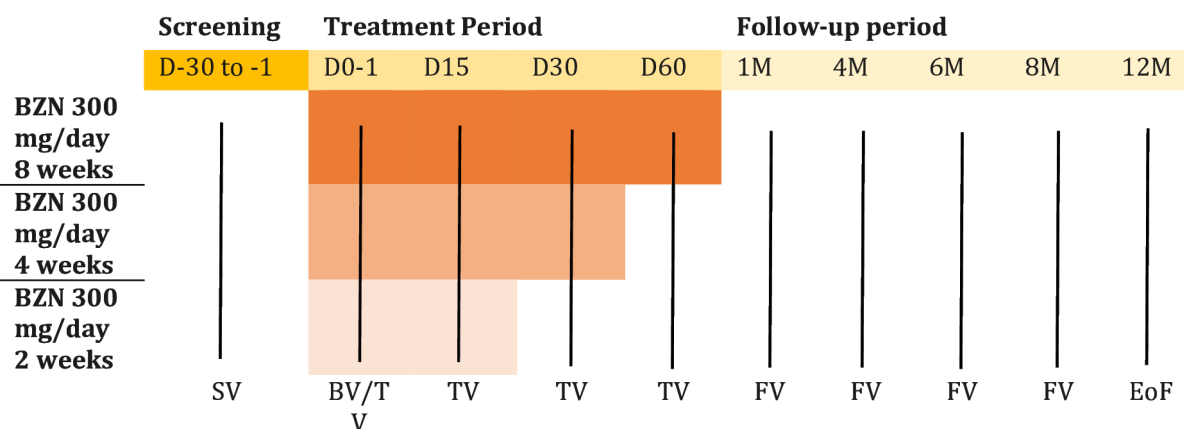


Figure 1 Clinical trial design. BV, baseline visit; BZN, benznidazole; D, day; EoF, end of follow-up; FV, follow-up visit; SV, screening visit; TV, treatment visit.

The secondary analyses will evaluate the different secondary endpoints on the ITT population. The proportion of participants with sustained clearance of parasitaemia according to the results of qualitative qPCR at 1, 4, 6 and 8 months of follow-up from the end of treatment will be compared at each timepoint by using a χ^2 .

The safety of the 2-week and 4-week regimens of BZN (300 mg per day) will be evaluated according to anamnesis (symptom evaluation), physical examination and laboratory tests compared with the standard treatment with BZN of 300 mg per day for 8 weeks. Proportions per patients will be presented and compared. The incidence of SAEs and/or adverse events that lead to the interruption of treatment with the 2-week and 4-week regimens of BZN at 300 mg per day compared with the standard treatment with BZN of 300 mg per day for 8 weeks will be evaluated. Comparison will be carried out by using a χ^2 . Participant adherence to treatment in each study arm will also be evaluated.

Study schedule

Treatment visits are scheduled on days 15, 30 and 60, according to the treatment arms, and follow-up visits will occur at 1, 4, 6, 8 and 12 months after the end of treatment (figure 1).

Participant eligibility and randomisation

Participants aged ≥ 18 and ≤ 60 years with *T. cruzi* infection confirmed by conventional serology (reactive against a minimum of two conventional ELISA, or recombinant ELISA, or indirect immunofluorescence, or indirect haemagglutination or chemiluminescence tests) and serial qualitative qPCR (one blood sample divided into three DNA extractions, at least one of which must be positive), who have not previously received treatment with BZN or NFX (either completely or partially), will be eligible. The study aims to include 300 participants. Inclusion and exclusion criteria are summarised in boxes 1 and 2, respectively.

Considering these criteria, participants will be randomised in a 1:1:1 ratio to one of the three treatment arms (2-week, 4-week or 8-week regimens) using a

centralised, computer-generated randomisation system to ensure allocation concealment and minimise selection bias. Randomisation will be stratified by study site and will employ variable block sizes to maintain balance across them. The allocation sequence will be generated by the study Contract Research Organization (CRO) responsible for the data management activities and the corresponding manuals. Assignment to treatment groups will be revealed only after the participant has been confirmed as eligible.

Study follow-up

The total duration of the study from the start of recruitment (first participant first visit) to the end of follow-up (last participant last visit) is estimated at 56 months. Participants will be followed for 12 months from the end of the assigned treatment. Written informed consent of the participant (and witness, if applicable) will be obtained prior to screening according to local regulations. Participants must be included within 30 days of signing the informed consent form.

After the selection phase, a baseline visit will be performed to review the inclusion/exclusion criteria, concurrent medications and vital signs. A physical exam and a new pregnancy test will also be performed. Random assignments to a study arm will be executed using the Electronic Case Report Form (eCRF) system and according to the randomisation scheme generated by data management prior to the start of the study. Participants will be randomly assigned to any experimental arm with a 1:1:1 allocation as per a computer-generated randomisation scheme without further stratification and using fixed blocks of size equal to 3. The block size will not be disclosed to ensure concealment. Each participant will be assigned a treatment number.

During the treatment period, three visits are scheduled for the standard regimen arm, two visits for the 4-week arm and one visit for the 2-week arm. Any participant may seek consultation at any time for the

Box 1 Inclusion criteria

Participants should meet all criteria.

- ⇒ Signed informed consent form.
- ⇒ Age: ≥ 18 and ≤ 60 years.
- ⇒ Weight: ≥ 50 kg and ≤ 95 kg.
- ⇒ Confirmation of *Trypanosoma cruzi* (*T. cruzi*) infection by conventional serology (a minimum of two tests must be reactive).
- ⇒ Serial qualitative PCR (one blood sample divided in three DNA extractions, at least one of which must be positive).
- ⇒ Women of childbearing potential must have a negative pregnancy result at the time of inclusion, must not be breastfeeding and must use a highly effective method of contraception for 33 days after the last dose, in accordance with local contraceptive regulations for clinical trial participants, or provide proof of sterilisation.
- ⇒ Ability to comply with all exams and specific protocol visits.
- ⇒ Having a permanent address.
- ⇒ ECG criteria: heart rate: 50–100 beats per minute or isolate sinus bradycardia from 41 to 59 beats per minute; QRS ≤ 120 ms and QTc ≥ 350 ms and ≤ 450 ms at screening or following findings belonging to non-severe Chagasic cardiomyopathy: incomplete right bundle branch block, left anterior fascicular block and first-degree atrioventricular block, low voltage. The abnormalities included are not exclusionary.
- ⇒ Normal or minimal structural changes in echocardiogram (left ventricular diastolic diameter ≤ 55 mm, diastolic dysfunction, absence of microaneurysm or tip aneurysm, absence of hypo or generalised akinesia, absence of systolic dysfunction (normal fractional shortening and ejection fraction) and/or absence of mural thrombus).
- ⇒ Not presenting signs or symptoms of moderate–severe chronic cardiac and/or digestive forms of CD (criteria detailed in the study manual and specific standard operational procedure).
- ⇒ No prior history of mental disorders or suicidal tendencies.
- ⇒ No history of acute or chronic diseases that, in the investigator's judgement, may interfere with the assessment of the efficacy or safety of the investigational product (such as acute infections, immunosuppressive conditions or liver or kidney diseases that have required treatment).
- ⇒ Not having received a formal indication not to take benznidazole (BZN) (contraindication, according to the summary of product characteristics).
- ⇒ No prior history of hypersensitivity, allergy or serious adverse reactions to any of the nitroimidazole compounds (including BZN) and/or its components/excipients.
- ⇒ Not having previously undergone antiparasitic treatment for *T. cruzi* infection.
- ⇒ No prior history of drug abuse or alcoholism.
- ⇒ Not suffering from any disease or condition that prevents participants from consuming oral medication.

Mental disorders are limited to severe mental disorders, considered as serious, often enduring psychiatric conditions marked by pronounced symptoms and considerable impairment across major areas of functioning.²⁸

Box 2 Exclusion criteria

Participants should meet none of the criteria.

- ⇒ Participant pregnant or intending to become pregnant during the study or within 30 days after finishing study participation.
- ⇒ Signs or symptoms of the established (moderate–severe) chronic cardiac and/or digestive form of Chagas disease (CD), or any ECG/echocardiographic findings not included in the inclusion criteria.
- ⇒ History of cardiomyopathy, heart failure or severe ventricular arrhythmia.
- ⇒ History of digestive surgery potentially related to CD or megacolon/megaesophagus.
- ⇒ Acute or chronic disease that, in the investigator's discretion, may interfere with the evaluation of the efficacy or safety of the investigational product (such as acute infection, history of immunosuppressive conditions or liver or kidney disease that has required treatment and blood dyscrasias).
- ⇒ Laboratory test values that are considered clinically significant by medical criteria and/or exceed grade 2 limits according to common terminology criteria for adverse events V.5.0 grade 2.
- ⇒ Disease or clinical condition that prevents participants from consuming oral medication.
- ⇒ Participants with a contraindication (known hypersensitivity) to any of the nitroimidazole compound, for example, metronidazole.
- ⇒ Participants with a history of severe non-pharmacological allergy, allergic rash and asthma.
- ⇒ Participants with a history of severe drug allergy or severe intolerance, sensitivity or photosensitivity to any drug.
- ⇒ Concomitant use and/or consumption of allopurinol, antimicrobial and antiparasitic agents, herbal medicines, dietary supplements or energy drinks.
- ⇒ Scheduled surgery that may interfere with the conduct of the trial and/or with the treatment evaluation.
- ⇒ Inability to attend study visits, comply with treatment or cooperate with study procedures.
- ⇒ Previous participation in a trial for the evaluation of the treatment of *Trypanosoma cruzi* infection.
- ⇒ Simultaneous participation in another trial or within 3 months prior to screening for this trial (in accordance with national regulations).

Participants suffering from a serious medical or psychiatric illness that increases the risk associated with study participation or that interferes with the interpretation of study results should not be included.

occurrence of adverse events and may decide unilaterally to interrupt the treatment. The principal investigator can also decide to suspend the medication, after considering the severity, intensity and duration of adverse events. After taking the last dose of treatment, the follow-up phase begins and lasts until 12 months after the end of treatment. A participant has

finished the study when they have completed the treatment and follow-up phases (PP principle). The study may be interrupted voluntarily by the participant or in case of safety interruptions or protocol deviations (ITT principle). A summary of these visits is presented in online supplemental table 3—Schedule of Events (see online supplemental table 3).

PCR methods to evaluate efficacy

PCR techniques yield results for measuring the therapeutic response in clinical trials for *T. cruzi* infection within a reasonable time frame and have been used in most of the *T. cruzi* clinical trials performed in adults in recent decades. In NuestroBen, a methodology developed by Duffy *et al.*^{23 24} and later validated by Ramírez *et al.*²⁵ will be used, in accordance with the technical conclusions from Parrado *et al.*²⁶

In these studies, the kinetics of treatment failure demonstrated by relapse to positive PCR results after the end of treatment and during follow-up were observed. An accelerated increase in failure could be observed between the fourth and sixth month of follow-up, with a slight increase up to 12 months of follow-up, generating a plateau. This observation led the scientific community to assume that 12 months of follow-up are sufficient to determine the relevant failure rate for the different therapeutic alternatives under evaluation.

In NuestroBen, nine qPCR results are analysed per participant for each efficacy timepoint based on triplicate PCR testing from three independent DNA extractions of 5 mL blood sample aliquots treated with 6M guanidine hydrochloride 0.2M Ethylenediaminetetraacetic Acid (EDTA), pH 8.00 (GE) buffer, from a 15 mL blood sample. A commercial kit qPCR, BioMol Chagas, manufactured by Instituto de Biología Molecular de Paraná, Brazil, will be used. This kit has been approved by ANVISA in Brazil as an *in vitro* diagnostic for monitoring treatment response. It is based on the previously published Sat DNA-qPCR procedures.^{23 25} The use of a standardised kit will allow more homogeneous PCR measurements among laboratories located in Bolivia and Argentina (University of San Simon, Cochabamba and INGBI-CONICET, Buenos Aires, respectively), which, in the case of in-house standardised methodologies, have difficulty in purchasing identical versions of key molecular biology reagents.

In order to harmonise the use of this kit, the study will include a first round of qPCR in the above-mentioned qPCR laboratories, using a proficiency panel of samples prepared by a reference laboratory at Oswaldo Cruz Foundation (FIOCRUZ, Ministry of Health/Brazil), Rio de Janeiro/Brazil. The reference laboratory will oversee analysis of the agreement between the qPCR outputs from both laboratories performing qPCR on the clinical samples.

In summary, main aspects related to the qPCR-based monitoring are as follows.

- ▶ The study population will be adults in the chronic phase of CD with the indeterminate form or mild cardiac progression.
- ▶ PCR timepoints during the first 12 months of follow-up at 1, 4, 6, 8 and 12 months after the end of treatment.
- ▶ Nine serial qualitative qPCR results are required to determine negativity: triplicate PCR testing from three independent DNA extractions of 5 mL blood sample aliquots treated with one volume of GE buffer per visit.
- ▶ The commercial qPCR kit (BioMol Chagas) will be used, and quality control of the central laboratories will be coordinated by Fiocruz.
- ▶ The dosage scheme to be used is 100 mg tablet taken three times per day.

Additionally, a new probabilistic model of therapeutic efficacy based on serial qPCR data has been included as an exploratory analysis in the NuestroBen trial. Serial parasite densities estimated from triplicate qPCRs targeting

T. cruzi satellite DNA of all individuals will be pooled, at baseline and in all follow-up visits of the NuestroBen study. Treatment efficacies will be estimated using a hierarchical Bayesian model, taking serial cycle threshold (Ct) data grouped by timepoint, blood draw and technical replicate as inputs (Watson *et al*, 2024, submitted, under review).²⁷ The analysis will be performed in the group of randomised participants who fulfil at least 80% of the allocated treatment duration (adherence), excluding those that received the wrong treatment and those who ingested BZN or NFX outside of what was prescribed in the study, or other prohibited concomitant medications during the study period.

Ethics and dissemination

NuestroBen received ethical approval from Argentina's national ethical committee 'Fundación de Estudios Farmacológicos y Medicamentos (FEFyM) Luis M. Zieher' for its conduct at the *Instituto de Cardiología de Corrientes Juana Francisca Cabral* in January 2021 (NuestroBen-2020/2021) and was subsequently approved by the research ethics committees of the other five participating sites in the country as follows.

- ▶ *Comité Institucional de Ética de Investigación en Salud* for the *Centro de Chagas y Patología Regional de Santiago del Estero* (reference: NuestroBen-2020-088/2021) in May 2021.
- ▶ FEFyM 'Luis M. Zieher' for the *Instituto Nacional de Parasitología 'Dr. Mario Fatala Chaben'* (reference: NuestroBen-2020/2021) in July 2021.
- ▶ *Comité de Ética en Investigación* for the *Hospital de Infecciosas F.J. Muñiz* (reference: NuestroBen-2020-4037) in August 2021.
- ▶ *Comité de Ética en Investigación* for the *Hospital General de Agudos D.F. Santojanni* (reference: NuestroBen-2020-4039) in August 2021.
- ▶ *Comité de Bioética* for the *Fundación Huésped* (NuestroBen-2020/2021) in August 2021.

In Bolivia, the study was approved by the *Comité de Ética en Investigación en Salud* from the *Universidad Autónoma Juan Misael Saracho* (reference: NuestroBen-2020/2025) in May 2025.

In the context of study procedures, the principal investigator will explain to each participant the study objectives, procedures, duration and potential risks. All participants will be asked to provide written informed consent (online supplemental Material). Participants will be assigned a unique identifier by the sponsor. Participant records or datasets will contain the identifier only; participant names or any information, which would make the participant identifiable, will not be provided. The participant will be informed that their personal study-related data will be used by the sponsor in accordance with local data protection law and that their medical records may be examined by clinical quality assurance auditors or other authorised personnel appointed by the sponsor, or ethical committees, and by inspectors from regulatory authorities.

Findings will be shared with Argentinian and Bolivian public health officials, and with the Chagas and tropical medicine scientific communities via international conferences, such as the Congress of the Brazilian Society of Tropical Medicine (MedTrop) and the American Society of Tropical Medicine and Hygiene. At least one peer-reviewed publication of results in an international medical journal is planned.

Trial status

Recruitment commenced in July 2023, and it is estimated to be completed by September 2026. Protocol version V4.0 25 February 2025.

Author affiliations

¹Drugs for Neglected Diseases initiative, Rio de Janeiro, Brazil

²Laboratorio ELEA Phoenix S.A, Buenos Aires, Argentina

³International Health Unit Vall d'Hebron-Drassanes, Infectious Diseases Department, Vall d'Hebron University Hospital, Barcelona, Spain

⁴Centro de Investigación Biomédica en Red Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain

⁵Fundação Oswaldo Cruz, Belo Horizonte, Brazil

⁶Instituto de Cardiología de Corrientes 'Juana Francisca Cabral', Corrientes, Argentina

⁷Centro de Chagas y Patología Regional, Hospital Independencia, Santiago del Estero, Argentina

⁸Hospital de Infecciosas Dr. Francisco Javier Muñoz, Buenos Aires, Argentina

⁹Instituto Nacional de Parasitología 'Dr. Mario Fatała Chaben', Buenos Aires, Argentina

¹⁰Hospital Donación Francisco Santojanni, Buenos Aires, Argentina

¹¹Fundación Huésped, Buenos Aires, Argentina

¹²Centro de Investigación en Enfermedades Transmitidas por Vectores-SEDES Chuquisaca, Sucre, Bolivia

¹³Plataforma de Atención Integral a los Pacientes con Enfermedad de Chagas, Tarija, Bolivia

¹⁴Universidad Autónoma Juan Misael Saracho, Tarija, Bolivia

¹⁵Laboratorio de Biología Molecular de la Enfermedad de Chagas, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular Dr. Héctor N Torres(INGEBI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

¹⁶Laboratório de Virologia e Parasitologia Molecular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

¹⁷Instituto de Investigaciones Biomédicas (IIBISMED), Universidad Mayor de San Simon, Cochabamba, Bolivia

¹⁸Berry Consultants, LLC, Austin, Texas, USA

¹⁹Luxembourg Institute of Health, Strassen, Luxembourg

²⁰Drugs for Neglected Diseases initiative, Geneva, Switzerland

²¹Instituto Nacional de Infectología Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Contributors TM, CF, FB, CL, IM, CT, LF, AS-S, SS-E and M-JP made substantial contributions to the conception and design of the study. MdCB, RJF, SLI, MLF, PS, PP, WG and LO are the principal investigators of the study. TM, FB, ML and BBdO coordinate the trial implementation at the study sites and provided global management of trial activities. Alejandro Gabriel Schijman, Silvia Andrea Longhi and LG were responsible for the PCR method development and implementation. OCM was responsible for the PCR quality assurance. KV and MV were responsible for statistical design. All authors reviewed one or more versions of the manuscript critically for important intellectual content. TM, CF and M-JP led the revision process. Guarantor is M-JP. All authors approved the final version of the manuscript.

Funding The study is supported by the Drugs for Neglected Diseases initiative (DNDi), ELEA-Phoenix S.A., and Fundación Mundo Sano-Argentina. The DNDi is grateful to its donors, public and private, who have provided funding to DNDi since its inception in 2003. A full list of DNDi's donors can be found at <http://www.dndi.org/about/donors/>.

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Tayná Marques <http://orcid.org/0000-0001-7994-4511>

Colin Forsyth <http://orcid.org/0000-0003-1481-8367>

Bethania Blum de Oliveira <http://orcid.org/0009-0005-7234-5213>

REFERENCES

- World Health Organization. Chagas disease in latin america: an epidemiological update based on 2010 estimates. In: *Weekly Epidemiological Record*. 7. World Health Organization, 2015.
- Lee BY, Bacon KM, Bottazzi ME, et al. Global economic burden of Chagas disease: a computational simulation model. *Lancet Infect Dis* 2013;13:342–8.
- Elimination of mother-to-child transmission of hiv, syphilis, perinatal hepatitis b, and congenital chagas disease - opas/oms | organização pan-americana da saúde.
- Pereiro AC, Gold S. Building an innovative Chagas disease program for primary care units, in an urban non- endemic city. *BMC Public Health* 2019;19:904.
- Pan American health organization: guidelines for the diagnosis and treatment of chagas disease. Washington, DC PAHO; 2019.
- Fabbro DL, Streiger ML, Arias ED, et al. Trypanocide treatment among adults with chronic Chagas disease living in Santa Fe city (Argentina), over a mean follow-up of 21 years: parasitological, serological and clinical evolution. *Rev Soc Bras Med Trop* 2007;40:1–10.
- Viotti R, Vigliano C, Lococo B, et al. Long-term cardiac outcomes of treating chronic Chagas disease with benznidazole versus no treatment: a nonrandomized trial. *Ann Intern Med* 2006;144:10:724–34.
- Cardoso CS, Ribeiro ALP, Oliveira CDL, et al. Beneficial effects of benznidazole in Chagas disease: NIH SaMi-Trop cohort study. *PLoS Negl Trop Dis* 2018;12:e0006814.
- Morillo CA, Waskin H, Sosa-Estani S, et al. Benznidazole and Posaconazole in Eliminating Parasites in Asymptomatic T. *Cruzi Carriers The STOP-CHAGAS Trial* 2017;69:939–47.
- Marin-Neto JA, Avezum A, Sosa-Estani S, et al. BENEFIT Investigators. *Randomized Trial of Benznidazole for Chronic Chagas' Cardiomyopathy N Engl J Med* 2015;373:1295–306.
- Kratz JM, Garcia Bournissen F, Forsyth CJ, et al. Clinical and pharmacological profile of benznidazole for treatment of Chagas disease. *Expert Rev Clin Pharmacol* 2018;11:943–57.
- Altcheh J, Moscatelli G, Mastrantonio G, et al. Population pharmacokinetic study of benznidazole in pediatric Chagas disease suggests efficacy despite lower plasma concentrations than in adults. *PLoS Negl Trop Dis* 2014;8:e2907.
- Soy D, Aldasoro E, Guerrero L, et al. Population Pharmacokinetics of Benznidazole in Adult Patients with Chagas Disease. *Antimicrob Agents Chemother* 2015;59:3342–9.
- Alvarez MG, Vigliano C, Lococo B, et al. Seronegative conversion after incomplete benznidazole treatment in chronic Chagas disease. *Trans R Soc Trop Med Hyg* 2012;106:636–8.
- Torrico F, Gascón J, Barreira F, et al. New regimens of benznidazole monotherapy and in combination with fosravuconazole for treatment

- of Chagas disease (BENDITA): a phase 2, double-blind, randomised trial. *Lancet Infect Dis* 2021;21:1129–40.
- 16 Alonso-Vega C, Urbina JA, Sanz S, *et al.* New chemotherapy regimens and biomarkers for Chagas disease: the rationale and design of the TESEO study, an open-label, randomised, prospective, phase-2 clinical trial in the Plurinational State of Bolivia. *BMJ Open* 2021;11:e052897.
- 17 Molina-Morant D, Fernández ML, Bosch-Nicolau P, *et al.* Efficacy and safety assessment of different dosage of benznidazol for the treatment of Chagas disease in chronic phase in adults (MULTIBENZ study): study protocol for a multicenter randomized Phase II non-inferiority clinical trial. *Trials* 2020;21:328.
- 18 Cafferata ML, Toscani MA, Althabe F, *et al.* Short-course Benznidazole treatment to reduce Trypanosoma cruzi parasitic load in women of reproductive age (BETTY): a non-inferiority randomized controlled trial study protocol. *Reprod Health* 2020;17:128.
- 19 Villar JC, Herrera VM, Pérez Carreño JG, *et al.* Nifurtimox versus benznidazole or placebo for asymptomatic Trypanosoma cruzi infection (Equivalence of Usual Interventions for Trypanosomiasis - EQUITY): study protocol for a randomised controlled trial. *Trials* 2019;20:431.
- 20 Francisco AF, Saade U, Jayawardhana S, *et al.* Comparing in vivo bioluminescence imaging and the Multi-Cruzi immunoassay platform to develop improved Chagas disease diagnostic procedures and biomarkers for monitoring parasitological cure. *PLoS Negl Trop Dis* 2022;16:e0010827.
- 21 Granjon E, Dichtel-Danjoy M-L, Saba E, *et al.* Development of a Novel Multiplex Immunoassay Multi-cruzi for the Serological Confirmation of Chagas Disease. *PLoS Negl Trop Dis* 2016;10:e0004596.
- 22 Sousa A de, Vermeij D, Parra-Henao G, *et al.* The CUIDA Chagas Project: towards the elimination of congenital transmission of Chagas disease in Bolivia, Brazil, Colombia, and Paraguay. *Rev Soc Bras Med Trop* 2022;55:e01712022.
- 23 Duffy T, Cura CI, Ramirez JC, *et al.* Analytical Performance of a Multiplex Real-Time PCR Assay Using TaqMan Probes for Quantification of Trypanosoma cruzi Satellite DNA in Blood Samples. *PLoS Negl Trop Dis* 2013;7:e2000.
- 24 Schijman AG, Bisio M, Orellana L, *et al.* International study to evaluate PCR methods for detection of Trypanosoma cruzi DNA in blood samples from Chagas disease patients. *PLoS Negl Trop Dis* 2011;5:e931.
- 25 Ramirez JC, Cura CI, da Cruz Moreira O, *et al.* Analytical Validation of Quantitative Real-Time PCR Methods for Quantification of Trypanosoma cruzi DNA in Blood Samples from Chagas Disease Patients. *J Mol Diagn* 2015;17:605–15.
- 26 Parrado R, Ramirez JC, de la Barra A, *et al.* Usefulness of Serial Blood Sampling and PCR Replicates for Treatment Monitoring of Patients with Chronic Chagas Disease. *Antimicrob Agents Chemother* 2019;63.
- 27 Watson JA, Cruz C, Barreira F, *et al.* Quantifying anti-trypanosomal treatment effects in chronic indeterminate Chagas disease: a secondary analysis of individual patient data from two proof-of-concept trials. *Lancet Microbe* 2025;8:S2666-5247(25)00084-9:101156.
- 28 WHO. Mental disorders. World Health Organization; 2023. Available: <https://www.who.int/news-room/fact-sheets/detail/mental-disorders>