New regimens of benznidazole monotherapy and in combination with fosravuconazole for treatment of Chagas disease (BENDITA): a phase 2, double-blind, randomised trial

Faustino Torrico*, Joaquim Gascón*, Fabiana Barreira, Bethania Blum, Igor C Almeida, Cristina Alonso-Vega, Tayná Barboza, Graeme Bilbe, Erika Correia, Wilson Garcia, Lourdes Ortiz, Rudy Parrado, Juan Carlos Ramirez, Isabela Ribeiro, Nathalie Strub-Wourgaft, Michel Vaillant, Sergio Sosa-Estani, on behalf of the BENDITA study group†

Summary

Background Current treatment for Chagas disease with the only available drugs, benznidazole or nifurtimox, has substantial limitations, including long treatment duration and safety and tolerability concerns. We aimed to evaluate the efficacy and safety of new benznidazole monotherapy regimens and combinations with fosravuconazole, in the treatment of Chagas disease.

Methods We did a double-blind, double-dummy, phase 2, multicentre, randomised trial in three outpatient units in Bolivia. Adults aged 18–50 years with chronic indeterminate Chagas disease, confirmed by serological testing and positive qualitative PCR results, were randomly assigned (1:1:1:1:1:1:1) to one of seven treatment groups using a balanced block randomisation scheme with an interactive response system. Participants were assigned to benznidazole 300 mg daily for 8 weeks, 4 weeks, or 2 weeks, benznidazole 150 mg daily for 4 weeks, benznidazole 150 mg daily for 4 weeks plus fosravuconazole, benznidazole 300 mg once per week for 8 weeks plus fosravuconazole, or placebo, with a 12-month follow-up period. The primary endpoints were sustained parasitological clearance at 6 months, defined as persistent negative qualitative PCR results from end of treatment, and incidence and severity of treatment-emergent adverse events, serious adverse events, and adverse events leading to treatment discontinuation. Primary efficacy analysis was based on the intention-to-treat and per-protocol populations and secondary efficacy analyses on the per-protocol population. Safety analyses were based on the as-treated population. Recruitment is now closed. This trial is registered with ClinicalTrials.gov, NCT03378661.

Findings Between Nov 30, 2016, and July 27, 2017, we screened 518 patients, and 210 were enrolled and randomised. 30 patients (14%) were assigned to each treatment group. All 210 randomised patients were included in the intention-to-treat population, and 190 (90%) were included in the per-protocol population. In the intention-to-treat analysis, only one (3%) of 30 patients in the placebo group had sustained parasitological clearance at 6 months of follow-up. Sustained parasitological clearance at 6 months was observed in 25 (89%) of 28 patients receiving benznidazole 300 mg daily for 8 weeks (rate difference *vs* placebo 86% [95% CI 73–99]), 25 (89%) of 28 receiving benznidazole 300 mg daily for 4 weeks (86% [73–99]), 24 (83%) of 29 receiving benznidazole 300 mg daily for 2 weeks (79% [64–95]), 25 (83%) of 30 receiving benznidazole 150 mg daily for 4 weeks (80% [65–95]), 23 (85%) of 28 receiving benznidazole 150 mg daily for 4 weeks plus fosravuconazole (82% [67–97]), and 24 (83%) of 29 receiving benznidazole 300 mg weekly for 8 weeks plus fosravuconazole (79% [64–95]; p<0·0001 for all group comparisons with placebo). Six patients (3%) had ten serious adverse events (leukopenia [n=3], neutropenia [n=2], pyrexia, maculopapular rash, acute cholecystitis, biliary polyp, and breast cancer), eight had 12 severe adverse events (defined as interfering substantially with the patient's usual functions; elevated alanine aminotransferase [n=4], elevated gamma-glutamyltransferase [n=2], elevated aspartate aminotransferase [n=1], neutropenia [n=3], leukopenia [n=1], and breast cancer [n=1]), and 15 (7%) had adverse events that led to treatment discontinuation (most of these were in the groups who received benznidazole 300 mg daily for 8 weeks, benznidazole 300 mg once per week for 8 weeks plus fosravuconazole, and benznidazole 150 mg daily for 4 weeks plus fosravuconazole). No adverse events leading to treatment discontinuation were observed in patients treated with benznidazole 300 mg daily for 2 weeks or placebo. There were no treatment-related deaths.

Interpretation Benznidazole induced effective antiparasitic response, regardless of treatment duration, dose, or combination with fosravuconazole, and was well tolerated in adult patients with chronic Chagas disease. Shorter or reduced regimens of benznidazole could substantially improve treatment tolerability and accessibility, but further studies are needed to confirm these results.

Funding Drugs for Neglected Diseases initiative (DNDi).

Correspondence to:
Dr Sergio Sosa-Estani, DNDi
Latin America, Rio de Janeiro
20010-903, Brazil
ssosa@dndi.org

Research in context

Evidence before this study

Only two drugs, nifurtimox and benznidazole, are currently known to be effective against *Trypanosoma cruzi*, the protozoan which causes Chagas disease. Both drugs were developed decades ago, involve long treatment durations of 60 days or more, and have frequent side effects, especially in adults. Side effects from benznidazole and nifurtimox are estimated to cause 15–20% of patients to discontinue treatment. The majority of patients with Chagas disease are untreated, with concerns from both physicians and patients about potential side effects representing an important barrier to treatment initiation. Nonetheless, preclinical and clinical research indicates benznidazole maintains antiparasitic efficacy even at lower cumulative doses.

We searched LILACS, the Cochrane Library, Embase, ClinicalTrials.gov, and the WHO International Clinical Trials Registry from Jan 1, 1995, to June 30, 2020, with no language restrictions, and identified nine published randomised controlled trials involving benznidazole for the treatment of Chagas disease. Published results were not yet available for five additional trials, of which two were still recruiting. In two placebo-controlled trials, benznidazole treatment was superior to placebo in producing seroreversion in children aged 6–12 years with acute or early chronic Chagas disease. However, benznidazole was no more effective than placebo in reducing morbidity and mortality in older adults (mean age 55 years [SD 11]) who had already developed moderate to severe cardiomyopathy. Two trials found that benznidazole was more efficacious than posaconazole, as determined by a persistent absence of *T cruzi* DNA in multiple PCR tests, in treating chronically infected adults with indeterminate disease or mild cardiomyopathy. In a previous placebo-controlled clinical trial comparing fosravuconazole with benznidazole for the treatment of adults with indeterminate chronic Chagas disease, both drugs were effective at clearing the parasite, measured by continuously negative PCR results, but only benznidazole sustained the effect over 12 months of follow-up. Another pilot study tested an intermittent regimen of benznidazole administered once (divided into two daily doses) every 5 days for 60 days; 11 (65%) of 17 patients had positive PCR results at the beginning of treatment, whereas only three (18%) of 17 were positive following treatment and 36 months of follow-up.

Added value of this study

This is, to our knowledge, the first completed trial to assess whether shorter or reduced regimens of benznidazole, alone or in combination therapy with fosravuconazole, could be efficacious and safe for treating adults with chronic Chagas disease. The trial consisted of seven groups, all but two of which had daily dosing of benznidazole: the current standard treatment (benznidazole 300 mg daily for 8 weeks); shortened or reduced regimens of benznidazole (300 mg daily for 2 or 4 weeks, or 150 mg daily for 4 weeks); combination therapies (fosravuconazole plus benznidazole 150 mg daily for 4 weeks or 300 mg once per week for 8 weeks); and placebo. Treatment efficacy, as measured by continuously negative PCR, was 89% in the standard treatment group, and was similar (83–89%) in the shortened monotherapy and combination therapy groups, all of which were significantly higher than in the placebo group (3%). Incidence of adverse events was lower in the shortened monotherapy regimen groups than in the standard treatment group. There were no serious or severe (defined as interfering substantially with the patient's usual functions) adverse events, or adverse events leading to discontinuation of treatment, in patients treated with benznidazole 300 mg daily for 2 weeks.

Implications of all the available evidence

Our results suggest alternative regimens of benznidazole with shorter treatment durations and lower dose maintain similar efficacy to the current standard regimen, with superior safety. These findings have important implications for improving the accessibility and availability of treatment. The use of fosravuconazole in combination with benznidazole did not significantly affect efficacy. A larger, multicentric trial of benznidazole regimens with shorter treatment duration is urgently needed to confirm these findings. Reducing the current 8-week standard treatment regimen of benznidazole to a shorter 2-week regimen could greatly simplify the treatment of Chagas disease and facilitate better adherence for patients, improving access to treatment for patients with this long-neglected disease.

Introduction

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is endemic in 21 Latin American countries, and is spread mostly via vectorial transmission.[1] More than 6 million people are currently infected worldwide, including 5·7 million people in Latin America, and more than 70 million people are at risk of infection.[1–3] In the past two decades, Chagas disease has emerged as a global health problem, with the recognition of affected populations residing in non-endemic areas. The disease manifests with an initial acute phase with high parasitaemia. Following the acute phase, *T cruzi* lodges in organ tissue (most often the heart or gastrointestinal tract), and a lifelong chronic phase ensues. Initially, the chronic phase has an indeterminate form characterised by the presence of antibodies to *T cruzi*, but with normal electrocardiogram (ECG) and echocardiogram, and normal features at examination of the digestive system. Although most people remain asymptomatic, after several years or decades Chagas disease ultimately leads to clinical progression, typically the development of cardiac and gastrointestinal disorders with substantial mortality and morbidity, in 30–40% of chronically infected individuals.

Current treatment for Chagas disease is limited to two nitroheterocyclic drugs, nifurtimox and benznidazole,[4] which have substantial limitations, including long treatment duration, concerns regarding safety and tolerability, and insufficient efficacy in chronic Chagas disease.[5] Side effects of both drugs cause 15–20% of patients to discontinue treatment; and both drugs are better tolerated in children, particularly before adolescence, than in adults, as the frequency and severity of side effects tend to increase with age.[6–9] Accordingly, new treatments with improved safety and efficacy are needed. Clinical trials in the past decade have generated a good level of evidence for treatment of chronic indeterminate Chagas disease with benznidazole.[10–12] However, the current recommended dosing for benznidazole of 5–8 mg/kg per day, given in two doses each day for 60 days,[13] is likely to represent the maximum dose and treatment duration. The long treatment regimen and frequent occurrence of adverse drug reactions, mainly dermatological and gastrointestinal, necessitates ongoing monitoring and laboratory testing, and is demanding for both patients and health-care personnel. Nonetheless, experimental and clinical studies indicate that benznidazole exposure could be reduced without loss of efficacy.[14–19] Combination therapy with compounds targeting different pathways has been suggested as a way to improve efficacy.[14]

Fosravuconazole, a prodrug of ravuconazole, has been evaluated in patients with chronic indeterminate Chagas disease in a proof-of-concept study reported in 2018.[12] Fosravuconazole was shown to have a favourable safety profile. Transient parasite clearance was found at end of treatment but was not sustained until 12 months of follow-up in most patients. Therefore, further development of fosravuconazole as monotherapy was stopped. Due to the rapid initial clearance of parasitaemia and the favourable safety profile of fosravuconazole in the proof-of-concept study, as well as preclinical research in mice indicating that combinations of benznidazole and fosravuconazole were more effective than benznidazole monotherapy against drug-resistant *T cruzi* strains, the combination of fosravuconazole with benznidazole has been investigated, initially in a drug–drug interaction study,[20] to improve treatment response, shorten treatment duration, and address benznidazole-resistant parasite strains.

We aimed to evaluate the efficacy and safety of two approaches to optimise treatment of chronic indeterminate Chagas disease in adults: new benznidazole monotherapy regimens with reduced exposure to improve tolerability while maintaining efficacy, and combination regimens of benznidazole with fosravuconazole to improve efficacy.

Methods

Study design

We did a double-blind, double-dummy, phase 2, multicentre, randomised, proof-of-concept trial in three outpatient units in Bolivia that specialised in Chagas disease, in the Cochabamba, Tarija, and Sucre locations of the Platform of Comprehensive Care for Patients with Chagas Disease (appendix 2 p 2). The trial was done in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines after approval from the applicable ethics committees. The trial protocol can be accessed on the Drugs for Neglected Diseases initiative (DNDi) website.

Participants

Adults aged 18–50 years were eligible if they had a confirmed diagnosis of *T cruzi* infection by serial qualitative PCR (three samples collected during a single day, at least one of which was to be positive) and conventional serology (a minimum of two positive tests; ELISA, recombinant ELISA, or indirect immunofluorescence); no acute or chronic health conditions that, in the opinion of the principal investigator, might have interfered with the efficacy or safety evaluation of the trial drugs; and no signs or symptoms of the chronic cardiac or digestive form of Chagas disease. Cardiac and digestive involvement were assessed through a physical examination and review of patient medical history, and all potential participants had an ECG at baseline. Only patients with normal ECG results (PR interval ≤200 ms, QRS interval <120 ms, and QTc interval 350–450 ms in men and ≤470 ms in women) were eligible to participate. For the purposes of the trial, these measures were considered appropriate to ensure inclusion of patients with the indeterminate form of chronic Chagas disease. All patients provided written informed consent. Full eligibility criteria are provided in appendix 2 (pp 3–6).

The first patient was enrolled on Nov 30, 2016, and recruitment was finalised on July 27, 2017. The trial was registered on Dec 20, 2017. Due to low resources and internal administrative delays at the sponsor organisation, it was not possible to register the trial earlier than this. Although registration is not required by regulatory authorities in Bolivia, best practice would have been to register the trial before commencement of patient recruitment.

Randomisation and masking

Participants were enrolled by principal investigators and their teams at each site and were randomly assigned (1:1:1:1:1:1:1) by a statistician to one of seven treatment groups using a balanced block randomisation scheme with an interactive response system and randomisation list generated in SAS version 9.3. Using the interactive response system, each participant was assigned an identification code corresponding to a study treatment group. Participants, investigators, and sponsor staff were masked to treatment allocation and the randomisation list.

Procedures

The treatment regimens were: benznidazole 300 mg daily for 8 weeks, benznidazole 300 mg daily for 4 weeks,

benznidazole 300 mg daily for 2 weeks, benznidazole 150 mg daily for 4 weeks, benznidazole 150 mg daily for 4 weeks plus fosravuconazole, benznidazole 300 mg once weekly for 8 weeks plus fosravuconazole, and placebo. Benznidazole or benznidazole-matched placebo were administered orally in two doses each day. Fosravuconazole was administered orally at a loading dose of 300 mg once daily for 3 days, followed by 300 mg once per week. Fosravuconazole-matched placebo was administered in the same dosing schedule (appendix 2 p 9).

Follow-up visits took place daily on days 1–3, then once per week at weeks 2–8, at weeks 10 and 12, and then at 4 months, 6 months, and 12 months. Based on a previously validated method for detection of *T cruzi* DNA, three blood samples were assayed in triplicate by quantitative realtime PCR (qrtPCR) at each visit.[21,22] qrtPCR results were expressed qualitatively for the primary efficacy endpoint and quantitatively for analysis of parasite load, which was analysed using a standard curve based on a *T cruzi* stock from the discrete typing unit *T cruzi* V. Details of qrtPCR, conventional serology (ELISAs), and nonconventional serology (chemiluminescent ELISA for lytic anti-α-galactosyl [anti-α-Gal] antibodies to trypomastigote derived glycosylphosphatidylinositol-anchored mucins [tGPI-mucins]) are provided in appendix 2 (pp 6–7).

Adverse events were monitored at each follow-up visit. The timing for haematology, blood chemistry, vital signs, physical examinations, and ECGs is specified in appendix 2 (pp 6–7).

Outcomes

The primary efficacy endpoint was sustained parasitological clearance at 6 months of follow-up. Sustained parasitological clearance was defined as persistent negative qualitative PCR results from end of treatment until a specified timepoint. Primary safety endpoints were the incidence and severity of treatment-emergent adverse events, serious adverse events, and adverse events leading to treatment discontinuation. Secondary efficacy endpoints included sustained parasitological clearance at 12 weeks and at 12 months; parasite clearance at 1, 2, 3, 4, 6, 10, and 12 weeks and at 4, 6, and 12 months; time to parasite DNA clearance; time to sustained parasitological clearance; change in parasite load over time; conventional serology; and non-conventional serology (appendix 2 p 7).

Statistical analysis

A sample size of 11 patients per group was required for comparison of two independent binomial proportions of sustained parasitological clearance using Pearson's χ^2 statistic with a χ^2 approximation with a Bonferroni adjusted (six comparisons with placebo) two-sided significance level of 0·006, assuming a balanced design achieves a power of at least 0·8. With an estimated dropout rate of 10%, 12 patients per group were needed. A sample size of 30 patients per treatment group

provided a 99% probability of observing at least one peripheral neuropathy or paresthesia, aminotransferase increase, and hypersensitivity, and an 85% probability of observing at least one treatment discontinuation per group. A sample size of 30 patients per group (a total sample of 210 patients) would therefore enable efficacy and safety analyses.

The intention-to-treat population included all randomised patients, the per-protocol population included all patients who received the allocated treatment without major protocol deviation or treatment discontinuation, and the as-treated population included all patients who received at least one dose of treatment. The primary efficacy analysis was based on the intention-to-treat and per-protocol populations and secondary efficacy analyses on the per-protocol population. Safety analyses were based on the as-treated population. Unless otherwise stated, percentages were calculated using the total number of patients per treatment group or population.

The overall qualitative PCR outcome at each visit was classified as positive if at least one sample had a positive result (details on imputation are provided in appendix 2 pp 7–8). The proportion of patients with sustained parasitological clearance in each active-treatment group was compared with that of the placebo group using the χ^2 method. A Benjamini-Hochberg procedure was planned to adjust for the six comparisons with placebo, but could not be done as the statistical software did not calculate the false-discovery-rate-adjusted p values, given that the crude p values were all less than 0·0001. The time to sustained parasitological clearance was determined by Kaplan-Meier analysis of survival time. Each of the active-treatment groups was compared with placebo by log-rank test, or Gehan-Wilcoxon test if Kaplan-Meier curves overlapped. For serology, changes from baseline, defined as the last assessment collected before dosing on day 1, were summarised. Apart from PCR values, missing data were not replaced. A p value of less than 0·05 was considered significant. All statistical analyses were done using SAS version 9.3.

Role of the funding source

The funder of the study was involved in study design, data collection, data interpretation, and review of the report.

Results

Between Nov 30, 2016, and July 27, 2017, we screened 518 patients, and 210 were enrolled and randomised. 30 patients (14%) were assigned to each treatment group, and baseline characteristics were similar between treatment groups (table 1, figure 1). 202 (96%) of 210 patients completed the study, and the last follow-up visit took place on July 19, 2018. The intention-to-treat population included all 210 patients and the per-protocol population included 190 patients (90%). Compliance with study treatment was high, with mean compliance ranging from 86% to 100%.
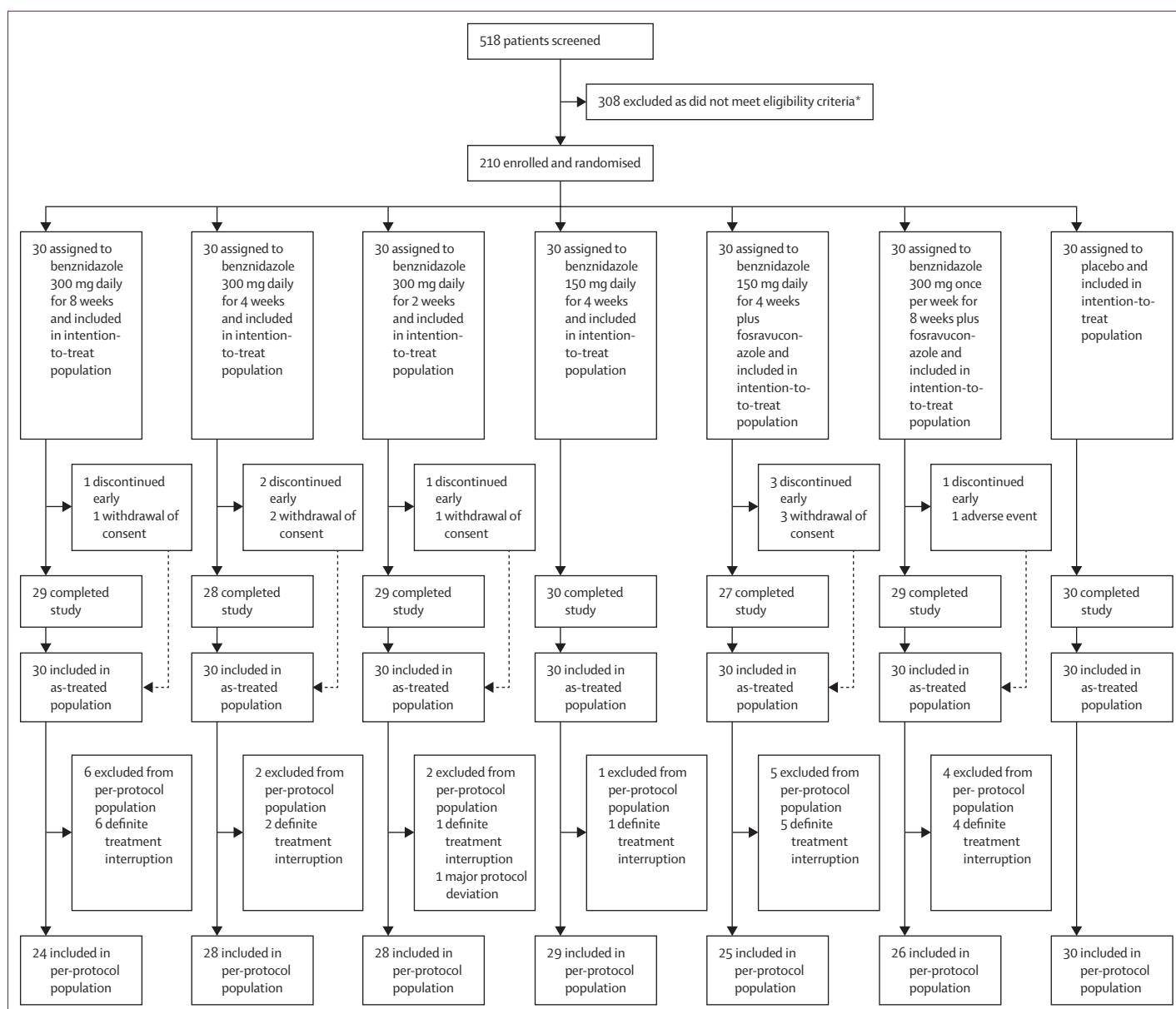
Figure 1: Trial profile

*Reasons for screening failure were negative PCR (n=158), abnormal laboratory values (n=99), and other (n=51), which included presence of Chagas cardiomyopathy or other health condition, pregnancy, or inability to attend regular appointments.

In the intention-to-treat population, 146 (85%) of 171 patients in all active-treatment groups, compared with only one (3%) of 30 patients in the placebo group, had sustained parasitological clearance from end of treatment until 6 months of follow-up (table 2, figure 2): 25 (89%) of 28 patients receiving benznidazole 300 mg daily for 8 weeks (rate difference *vs* placebo 86% [95% CI 73–99]), 25 (89%) of 28 receiving benznidazole 300 mg daily for 4 weeks (86% [73–99]), 24 (83%) of 29 receiving benznidazole 300 mg daily for 2 weeks (79% [64–95]), 25 (83%) of 30 receiving benznidazole 150 mg daily for

4 weeks (80% [65–95]), 23 (85%) of 27 receiving benznidazole 150 mg daily for 4 weeks plus fosravuconazole (82% [67–97]), and 24 (83%) of 29 receiving benznidazole 300 mg weekly for 8 weeks plus fosravuconazole (79% [64–95]; p<0·0001 for all group comparisons with placebo). Similar results were found in the per-protocol population (table 2). Most patients in the intention-to-treat population with sustained parasitological clearance at 6 months maintained it until 12 months; only one patient from each of the groups benznidazole 300 mg daily for 8 weeks, benznidazole 300 mg daily for 2 weeks, and

	Benznizadole 300 mg daily for 8 weeks group (n=30)	Benznizadole 300 mg daily for 4 weeks group (n=30)	Benznizadole 300 mg daily for 2 weeks group (n=30)	Benznizadole 150 mg daily for 4 weeks group (n=30)	Benznizadole 150 mg daily for 4 weeks plus fosravuconazole group (n=30)	Benznizadole 300 mg once per week for 8 weeks plus fosravuconazole group (n=30)	Placebo group (n=30)	Overall (n=210)
Age, years	32·7 (8·10)	32·9 (8·78)	31·9 (7·18)	34·0 (8·20)	30·4 (9·30)	34·0 (6·78)	33·2 (8·50)	32·7 (8·13)
Sex								
Male	11 (37%)	8 (27%)	8 (27%)	11 (37%)	11 (37%)	8 (27%)	5 (17%)	62 (30%)
Female	19 (63%)	22 (73%)	22 (73%)	19 (63%)	19 (63%)	22 (73%)	25 (83%)	148 (70%)
Bolivian nationality	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	210 (100%)
Body-mass index, kg/m²	27·28 (3·59)	26·28 (2·85)	25·24 (3·01)	26·59 (3·15)	25·92 (3·84)	27·27 (4·43)	26·82 (3·10)	26·49 (3·48)
Parasite load*	1·46 (1·68)	1·22 (0·99)	1·07 (0·79)	1·43 (1·52)	1·17 (0·72)	1·34 (1·28)	1·38 (1·52)	1·30 (1·25)
Conventional ELISA								
Positive	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	210 (100%)
Optical density	2·28 (0·49)	2·21 (0·38)	2·11 (0·41)	2·07 (0·40)	2·17 (0·40)	2·30 (0·40)	2·23 (0·50)	2·20 (0·43)
Recombinant ELISA								
Positive	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	210 (100%)
Optical density	3·09 (0·14)	3·07 (0·10)	3·03 (0·15)	3·04 (0·14)	3·01 (0·20)	3·04 (0·15)	3·01 (0·12)	3·04 (0·15)
QT interval, ms	400·0 (23·05)	412·2 (21·62)	404·7 (23·06)	416·3 (20·77)	397·6 (21·89)	400·7 (24·52)	410·5 (19·65)	406·0 (22·78)
Mean heart rate, beats per min	59·1 (5·63)	61·1 (8·21)	62·6 (6·38)	59·3 (6·59)	62·8 (7·65)	64·2 (7·64)	62·1 (6·07)	61·6 (7·06)
Total white blood cells, 10⁹/L	5·55 (1·10)	5·48 (1·09)	5·42 (1·04)	5·51 (1·05)	5·35 (1·23)	5·23 (1·09)	5·93 (0·94)	5·49 (1·09)
Neutrophils, 10⁹/L	3·22 (0·83)	3·16 (0·83)	3·09 (0·73)	3·21 (0·98)	3·01 (1·14)	3·67 (1·22)	3·72 (0·90)	3·39 (1·02)
Lymphocytes, 10⁹/L	2·10 (1·16)	2·02 (0·53)	1·91 (0·55)	1·99 (0·42)	1·98 (0·54)	1·89 (0·51)	1·99 (0·60)	1·93 (0·55)
Aspartate aminotransferase, U/L	22·68 (3·97)	24·92 (6·41)	23·12 (5·15)	24·39 (6·31)	23·32 (5·08)	22·64 (4·98)	23·48 (6·15)	23·51 (5·48)
Alanine aminotransferase, U/L	23·52 (7·01)	23·23 (7·56)	21·47 (5·95)	25·61 (8·73)	24·56 (8·84)	23·87 (8·83)	25·19 (7·89)	23·92 (7·89)
Gamma-glutamyltransferase, U/L	24·93 (9·41)	23·52 (10·87)	25·00 (10·14)	25·96 (7·82)	24·05 (9·24)	26·31 (13·54)	26·22 (12·72)	25·14 (10·59)

Data are n (%) or mean (SD). *Parasite equivalents per 1 mL of blood.

Table 1: Baseline characteristics of the as-treated population

benznidazole 150 mg daily for 4 weeks had sustained parasitological clearance at 6 months but not at 12 months. Parasite clearance over time was high and similar in all active-treatment groups. Kaplan-Meier estimates for the median time to sustained parasitological clearance ranged from 9 to 16 days with active treatments versus 358 days with placebo (p<0·0001 for all group comparisons with placebo; table 2).

Parasite load was quantifiable in 20% of samples at baseline (appendix 2 p 10). After the start of treatment, the number of patients with parasite load greater than the limit of quantification (1·5 parasite equivalents per mL) decreased rapidly, and the mean parasite load in most patients remained consistently below the limit of quantification in all active-treatment groups throughout the follow-up period. Although qualitative data indicated a meaningful decrease in parasite load, no meaningful quantitative information could be derived.

As expected, no meaningful changes in *T cruzi* antibody levels from baseline were found with conventional serology (appendix 2 p 14). By contrast, decreases in non-conventional serology with tGPI-mucins were detected in the groups receiving active treatment. At 12 months, mean lytic anti-α-Gal antibody titre changes from baseline ranged from −3·29 to −4·97

with active treatments compared with 1·03 with placebo (appendix 2 p 14).

Overall, 70% of patients experienced treatment-emergent adverse events, with a similar incidence between treatment groups (table 3). The most adverse events reported with any treatment were nervous system disorders, skin and subcutaneous tissue disorders, and gastrointestinal disorders. Most treatment-emergent adverse events were mild or moderate in intensity. Severe adverse events (defined as interfering substantially with the patient's usual functions) were only reported in the group receiving benznidazole 300 mg daily for 8 weeks and in the benznidazole and fosravuconazole combination therapy groups. The most common severe adverse events were blood and lymphatic system disorders and liver enzyme increases. Severe adverse events were reported in eight patients: four receiving benznidazole 300 mg for 8 weeks plus fosravuconazole (neutropenia [n=1], elevated alanine aminotransferase [n=2], elevated gamma-glutamyl transferase [n=1], breast cancer [n=1]), two receiving benznidazole 300 mg weekly for 8 weeks (neutropenia [n=2], leukopenia [n=1]), and two receiving benznidazole 150 mg for 4 weeks plus fosravuconazole (elevated alanine aminotransferase [n=2], elevated gamma-

	Benznizadole 300 mg daily for 8 weeks group	Benznizadole 300 mg daily for 4 weeks group	Benznizadole 300 mg daily for 2 weeks group	Benznizadole 150 mg daily for 4 weeks group	Benznizadole 150 mg daily for 4 weeks plus fosravuconazole group	Benznizadole 300 mg once per week for 8 weeks plus fosravuconazole group	Placebo group
Sustained clearance until 6 months of follow-up (intention-to-treat population)							
N	28	28	29	30	27	29	30
n (%)	25 (89%)	25 (89%)	24 (83%)	25 (83%)	23 (85%)	24 (83%)	1 (3%)
Rate difference vs placebo (95% CI)	86% (73–99)	86% (73–99)	79% (64–95)	80% (65–95)	82% (67–97)	79% (64–95)	..
p value*	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	..
Sustained clearance until 6 months of follow-up (per-protocol population)							
N	22	27	28	29	24	25	30
n (%)	19 (86%)	24 (89%)	23 (82%)	25 (86%)	20 (83%)	21 (84%)	1 (3%)
Rate difference vs placebo (95% CI)	83% (67–99)	86% (72–99)	79% (63–94)	83% (69–97)	80% (64–96)	81% (65–96)	..
p value*	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	..
Sustained clearance until 12 months of follow-up (intention-to-treat population)							
N	29	28	29	30	27	29	30
n (%)	24 (83%)	25 (89%)	23 (79%)	24 (80%)	23 (85%)	24 (83%)	1 (3%)
Rate difference vs placebo (95% CI)	79% (64–95)	86% (73–99)	76% (60–92)	77% (61–92)	82% (67–97)	79% (64–95)	..
p value*	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	..
Sustained clearance until 12 months of follow-up (per-protocol population)							
N	23	27	28	29	24	25	30
n (%)	18 (78%)	24 (89%)	22 (79%)	24 (83%)	20 (83%)	21 (84%)	1 (3%)
Rate difference vs placebo (95% CI)	75% (57–93)	86% (72–99)	75% (59–92)	79% (64–95)	80% (64–96)	81% (65–96)	..
p value*	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	..
Time to sustained clearance† (per-protocol population)							
Median time, days (95% CI)	9 (8–16)	16 (9–16)	12 (9–64)	15 (9–16)	9 (9–16)	9 (8–16)	358 (358–365)
p value	<0·0001‡	<0·0001§	<0·0001‡	<0·0001§	<0·0001‡	<0·0001§	..

All p values are for comparison of treatment group versus placebo. *χ² method. †Kaplan-Meier estimates. ‡Gehan-Wilcoxon test. §Log-rank test.

Table 2: **Parasitological clearance based on qualitative PCR**

glutamyltransferase [n=1], elevated aspartate aminotransferase [n=1]). Treatment-related adverse events were reported in a similar proportion of patients in each of the active-treatment groups (63–73%) and slightly fewer were reported in the placebo group (47%). Six patients (3%) had ten serious adverse events: two patients receiving benznidazole 300 mg daily for 8 weeks had leukopenia and one also had neutropenia; one patient receiving benznidazole 300 mg weekly plus fosravuconazole had neutropenia and leukopenia, and one patient had breast cancer; one patient receiving benznidazole 300 mg for 4 weeks had moderate serious pyrexia and maculopapular rash; and one patient receiving benznidazole 150 mg for 4 weeks plus fosravucazole had acute cholecystitis and a biliary polyp. All serious adverse events except for breast cancer, acute cholecystitis, and biliary polyp were considered treatment-related, most of them resolved, and none resulted in death. No serious adverse events were reported in patients receiving benznidazole 300 mg daily for 2 weeks, benznidazole 150 mg daily for 4 weeks, or placebo. Adverse events of special interest, defined as non-serious adverse events of scientific and medical concern specific to the study drugs, were only reported in the benznidazole 300 mg daily for 8 weeks group, benznidazole and fosravuconazole combination therapy groups, and placebo groups, in one patient (3%) in each group (mostly liver enzyme increases).

The most common treatment-related adverse event was headache, reported in 30% of patients overall. Other common treatment-related adverse events included pruritus, upper abdominal pain, and nausea. Treatment-related skin and subcutaneous tissue disorders, blood and lymphatic system disorders, and liver enzyme increases were reported less frequently with placebo than with active treatments. In patients receiving benznidazole 300 mg daily for 8 weeks, treatment-related adverse events of headache, neutropenia, and leukopenia were reported more frequently than with the other benznidazole treatment regimens.

15 patients (7%) experienced a total of 30 adverse events that led to treatment discontinuation: most of these were in the groups who received benznidazole 300 mg daily for 8 weeks (six patients [20%]), benznidazole 300 mg once per week for 8 weeks plus fosravuconazole (four patients [13%]), and benznidazole 150 mg daily for 4 weeks plus fosravuconazole (three patients [10%]), and none in the benznidazole 300 mg daily for 2 weeks or placebo groups. The most common adverse events leading to treatment discontinuation were abnormal liver laboratory values (4%), blood and lymphatic system disorders (2%), and skin and subcutaneous tissue disorders (1%).

There were no meaningful safety signals from standard haematology, biochemistry, and ECG assessments.

	Benznidazole 300 mg daily for 8 weeks group (n=30)	Benznidazole 300 mg daily for 4 weeks group (n=30)	Benznidazole 300 mg daily for 2 weeks group (n=30)	Benznidazole 150 mg daily for 4 weeks group (n=30)	Benznidazole 150 mg daily for 4 weeks plus fosravuconazole group (n=30)	Benznidazole 300 mg once per week for 8 weeks plus fosravuconazole group (n=30)	Placebo group (n=30)	Overall (n=210)
Adverse events	24 (80%)	20 (67%)	21 (70%)	20 (67%)	22 (73%)	21 (70%)	19 (63%)	147 (70%)
Severe adverse events*	2 (7%)	0	0	0	2 (7%)	4 (13%)	0	8 (4%)
Treatment-related adverse events	22 (73%)	19 (63%)	21 (70%)	19 (63%)	22 (73%)	19 (63%)	14 (47%)	136 (65%)
Adverse events of special interest	1 (3%)	0	0	0	1 (3%)	1 (3%)	1 (3%)	4 (2%)
Serious adverse events	2 (7%)	1 (3%)	0	0	1 (3%)	2 (7%)	0	6 (3%)
Adverse events resulting in death	0	0	0	0	0	0	0	0
Adverse events leading to treatment interruption	5 (17%)	8 (27%)	10 (33%)	5 (17%)	3 (10%)	1 (3%)	1 (3%)	33 (16%)
Adverse events leading to treatment discontinuation	6 (20%)	1 (3%)	0	1 (3%)	3 (10%)	4 (13%)	0	15 (7%)
Adverse events by system organ class†								
Nervous system disorders	13 (43%)	7 (23%)	11 (37%)	4 (13%)	11 (37%)	9 (30%)	8 (27%)	63 (30%)
Headache	13 (43%)	7 (23%)	11 (37%)	4 (13%)	11 (37%)	8 (27%)	8 (27%)	62 (30%)
Dizziness	3 (10%)	0	1 (3%)	1 (3%)	2 (7%)	2 (7%)	0	9 (4%)
Hypersomnia	0	0	1 (3%)	1 (3%)	0	2 (7%)	2 (7%)	6 (3%)
Skin and subcutaneous tissue disorders	6 (20%)	13 (43%)	12 (40%)	11 (37%)	5 (17%)	4 (13%)	3 (10%)	54 (26%)
Pruritus	4 (13%)	10 (33%)	8 (27%)	10 (33%)	4 (13%)	1 (3%)	2 (7%)	39 (19%)
Urticaria	1 (3%)	7 (23%)	3 (10%)	5 (17%)	1 (3%)	1 (3%)	0	18 (9%)
Rash maculopapular	1 (3%)	3 (10%)	4 (13%)	0	0	0	0	8 (4%)
Rash papular	2 (7%)	1 (3%)	1 (3%)	0	2 (7%)	0	0	6 (3%)
Erythema	2 (7%)	0	0	2 (7%)	0	0	0	4 (2%)
Pruritus generalised	0	0	0	0	1 (3%)	2 (7%)	1 (3%)	4 (2%)
Gastrointestinal disorders	9 (30%)	4 (13%)	10 (33%)	5 (17%)	10 (33%)	7 (23%)	6 (20%)	51 (24%)
Abdominal pain upper	5 (17%)	1 (3%)	8 (27%)	2 (7%)	4 (13%)	2 (7%)	4 (13%)	26 (12%)
Nausea	5 (17%)	4 (13%)	4 (13%)	1 (3%)	3 (10%)	5 (17%)	1 (3%)	23 (11%)
Vomiting	1 (3%)	0	3 (10%)	0	2 (7%)	1 (3%)	0	7 (3%)
Abdominal pain	2 (7%)	0	1 (3%)	0	1 (3%)	1 (3%)	1 (3%)	6 (3%)
Dyspepsia	0	1 (3%)	1 (3%)	1 (3%)	2 (7%)	0	0	5 (2%)
Blood and lymphatic system disorders	10 (33%)	6 (20%)	2 (7%)	3 (10%)	5 (17%)	3 (10%)	1 (3%)	30 (14%)
Neutropenia	8 (27%)	2 (7%)	0	2 (7%)	4 (13%)	2 (7%)	0	18 (9%)
Leukopenia	5 (17%)	2 (7%)	0	0	1 (3%)	2 (7%)	0	10 (5%)
Lymphopenia	3 (10%)	3 (10%)	1 (3%)	1 (3%)	1 (3%)	0	0	9 (4%)
Investigations‡	4 (13%)	2 (7%)	4 (13%)	4 (13%)	5 (17%)	7 (23%)	2 (7%)	28 (13%)
Alanine aminotransferase increased	4 (13%)	1 (3%)	2 (7%)	4 (13%)	4 (13%)	3 (10%)	0	18 (9%)
Gamma-glutamyltransferase increased	1 (3%)	1 (3%)	0	1 (3%)	5 (17%)	7 (23%)	0	15 (7%)
Aspartate aminotransferase increased	2 (7%)	1 (3%)	1 (3%)	0	1 (3%)	3 (10%)	1 (3%)	9 (4%)
Infections and infestations	6 (20%)	2 (7%)	3 (10%)	1 (3%)	4 (13%)	3 (10%)	6 (20%)	25 (12%)
Influenza	2 (7%)	0	2 (7%)	1 (3%)	2 (7%)	1 (3%)	1 (3%)	9 (4%)
Musculoskeletal and connective tissue disorders	4 (13%)	1 (3%)	1 (3%)	2 (7%)	2 (7%)	0	3 (10%)	13 (6%)
Myalgia	4 (13%)	0	1 (3%)	0	1 (3%)	0	1 (3%)	7 (3%)
Arthralgia	2 (7%)	0	0	2 (7%)	0	0	0	4 (2%)

(Table 3 continues on next page)

	Benznizadole 300 mg daily for 8 weeks group (n=30)	Benznizadole 300 mg daily for 4 weeks group (n=30)	Benznizadole 300 mg daily for 2 weeks group (n=30)	Benznizadole 150 mg daily for 4 weeks group (n=30)	Benznizadole 150 mg daily for 4 weeks plus fosravuconazole group (n=30)	Benznizadole 300 mg once per week for 8 weeks plus fosravuconazole group (n=30)	Placebo group (n=30)	Overall (n=210)
(Continued from previous page)								
General disorders and administration site conditions	3 (10%)	1 (3%)	3 (10%)	1 (3%)	1 (3%)	1 (3%)	1 (3%)	11 (5%)
Pyrexia	1 (3%)	1 (3%)	2 (7%)	0	0	1 (3%)	0	5 (2%)
Asthenia	2 (7%)	0	0	1 (3%)	0	0	1 (3%)	4 (2%)
Hepatobiliary disorders	0	0	1 (3%)	0	3 (10%)	1 (3%)	0	5 (2%)
Injury, poisoning, and procedural complications	0	1 (3%)	2 (7%)	1 (3%)	0	0	1 (3%)	5 (2%)
Reproductive system and breast disorders	2 (7%)	1 (3%)	0	0	0	1 (3%)	1 (3%)	5 (2%)

Data are n (%). Adverse events were analysed in the as-treated population. *Defined as interfering substantially with the patient's usual functions. †System organ classes with adverse events reported in more than two patients (≥10%) in any group were included; patients with more than one event of the same coded term were counted only once per system organ class and per preferred term. ‡Mostly liver enzyme increases; adverse events in the investigations system organ class included the preferred terms alanine aminotransferase increased (n=18), gamma-glutamyltransferase increased (n=15), aspartate aminotransferase increased (n=9), blood bilirubin increased (n=2), blood alkaline phosphatase increased (n=1 patient), and cortisol decreased (n=1).

Table 3: **Adverse events**

Discussion

Despite experimental and clinical data suggesting the potential for improvement of benznidazole dosing,[14–19] no randomised, controlled trials have yet systematically evaluated alternative therapy regimens. This randomised trial evaluated benznidazole 300 mg daily for 8 weeks, which is the current standard treatment, different monotherapy regimens with reduced benznidazole exposure, and combination therapy regimens with fosravuconazole, versus placebo, in terms of efficacy and safety. In the primary efficacy analysis, more than 80% of patients had sustained parasitological clearance in all active-treatment groups, compared with 3% in the placebo group. Results with benznidazole 300 mg daily for 8 weeks were in line with previous studies.[12] All active-treatment groups had significantly greater response rates than the placebo group at 6 months and 12 months of follow-up, and shorter time to sustained parasitological clearance. Treatment effects were also observed in terms of lytic antibody titres. Of note, patient numbers in this study were small, and the study was not powered for comparisons between the active-treatment groups. Other currently ongoing trials will add important evidence on the safety and efficacy of treatment regimens with shorter duration or lower dosage of benznidazole and nifurtimox (NCT03981523, NCT03672487, NCT03191162), and a study reported in 2020 showed that 14 (82%) of 17 patients treated with an intermittent scheme of benznidazole sustained negative PCR results after 36 months of follow-up.[18] Further studies are needed to corroborate whether shorter regimens of benznidazole have similar potency to the current standard regimen for interrupting congenital transmission in treated women, treating acute cases including reactivations, and reducing progression to chronic Chagas cardiomyopathy.
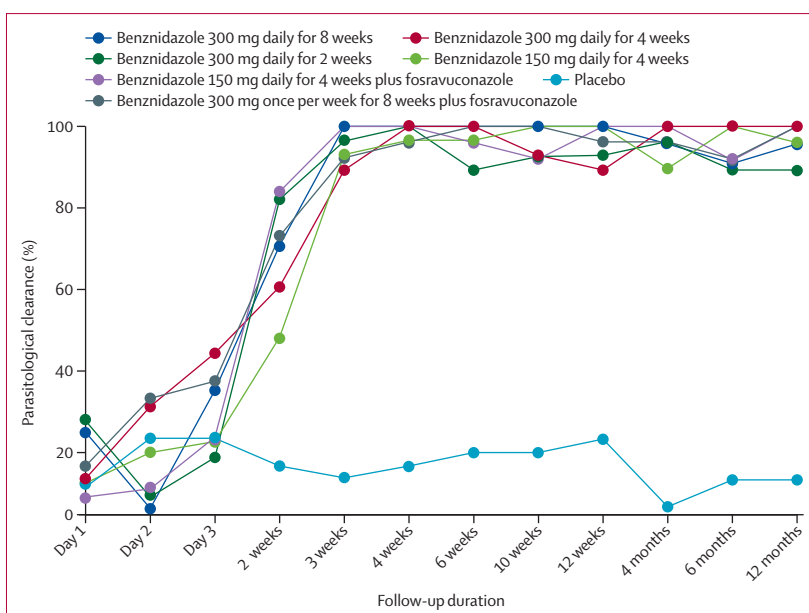

Figure 2: **Parasitological clearance over time**
Data are presented for the per-protocol population.

The effects of a fosravuconazole and benznidazole combination had been shown to be promising in preclinical studies,[23] but fosravuconazole monotherapy resulted in only a transient response and no sustained effect in a previous phase 2 study.[12] Similarly disappointing results have been observed for posaconazole, another candidate drug for combination treatment of Chagas disease.[10,11,14] Both fosravuconazole and benznidazole combinations regimens in this study were significantly more efficacious than placebo, but more research is needed to determine potential combinations that might improve safety or efficacy compared with benznidazole monotherapy.

All treatments were well tolerated, with a low incidence of serious or severe adverse events, or adverse events leading to discontinuation of treatment. Compliance with study treatment was high, with no major differences between groups. The pattern of observed adverse events was as expected based on the literature for benznidazole. Known adverse reactions such as skin and haematological disorders and liver enzyme increases were reported slightly more often with active treatments than with placebo. Even though there were no major differences in adverse events incidence with lower benznidazole exposure compared with the standard treatment, known adverse reactions to benznidazole such as neutropenia and leukopenia,[13] and headache[24] occurred slightly more often with benznidazole 300 mg daily for 8 weeks than in the other groups. Also, most adverse events leading to treatment discontinuation were reported with the standard benznidazole treatment regimen or combination therapies, and patients with low benznidazole exposure in monotherapy had fewer serious or severe adverse events. Due to the small group sizes and low numbers of adverse events, these observations should be interpreted cautiously.

The evaluation of parasitological cure after aetiological Chagas disease treatment has been done previously in studies with conflicting results due to differences in the applied methods and cure criteria.[25] Published studies support the use of PCR in the monitoring of therapeutic responses in chronic Chagas disease.[25,26] The usefulness of qrtPCR for detection of treatment failure in patients with chronic Chagas disease was shown in a study from 2019.[21]

The absence of meaningful changes in conventional serology in this study was expected, as seroconversion to negative does not occur until several years after treatment in patients with chronic indeterminate Chagas disease.[27] By contrast, in all active-treatment groups, antibodies to tGPI-mucins, which are biomarkers for lytic, protective anti-α-Gal antibodies,[12,28,29] tended to decrease, whereas only negligible changes were observed in the placebo group. This finding supports the efficacy of the active treatments, as lytic anti-α-Gal antibody titres have been identified as a sensitive and specific method for early assessment of cure of *T cruzi* infections following chemotherapy.[29,30,31]

Our study has several limitations. Registration of the trial occurred shortly after finalisation of patient recruitment, whereas it should have preceded recruitment to adhere to international best practices. The small number of patients in each treatment group precludes making firm conclusions regarding efficacy and safety data, necessitating further research in larger studies to confirm our findings. Although PCR is currently the best available endpoint for Chagas disease clinical trials, a large portion of patients with serological diagnosis are consistently PCR-negative and thus excluded from trial participation. There is a great need for improved biomarkers for assessing therapeutic efficacy. The study took place in Bolivia, where *T cruzi* V is the dominant parasite genotype, but further research is needed to confirm the applicability of the results for infections from other strains (especially *T cruzi* I and *T cruzi* II), which are more prevalent in other parts of the Americas. More than 70% of participants in the trial were women, and Chagas disease pathology might vary by sex. Different social and cultural factors affecting participation of men and women in clinical trials should be taken into account in recruitment strategies to ensure more balanced representation.

New regimens of reduced dose or shorter course benznidazole and combinations with fosravuconazole induced effective antiparasitic responses in patients with chronic indeterminate Chagas disease, with clearance of parasitaemia and decrease in lytic antibody titres, and were well tolerated. Positive results in efficacy, safety, and treatment compliance were observed with all active treatments. The 2-week benznidazole treatment regimen appears particularly promising because, in addition to being substantially shorter than the standard treatment regimen, no patients in this group had serious or severe adverse events, adverse events of special interest, or adverse events leading to treatment discontinuation. The proportion of people with Chagas disease who receive antitrypanosomal treatment is very low. If confirmed in phase 3 trials, a shorter benznidazole treatment regimen could help expand access to treatment for people with Chagas disease, and reduce demands and costs for health-care systems, by decreasing the number of office visits, laboratory tests, drug intakes, and supportive treatments for adverse reactions, compared with the current standard treatment regimen. As the study had a small sample size and groups were only powered to be compared against placebo, these results should be interpreted cautiously and confirmatory studies evaluating the efficacy of reduced-exposure benznidazole regimens compared with the standard benznidazole regimen with larger patient numbers are required. Adopting a shorter benznidazole regimen could have an important public health impact by reducing demands on health-care systems and facilitating patient adherence to treatment, helping to remove barriers to treatment access for Chagas disease.

References

1 WHO. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. *Wkly Epidemiol Rec* 2015; **90:** 33–43.

2 Manne-Goehler J, Umeh CA, Montgomery SP, Wirtz VJ. Estimating the burden of Chagas disease in the United States. *PLoS Negl Trop Dis* 2016; **10:** e0005033.

3 Basile L, Jansa JM, Carlier Y, et al. Chagas disease in European countries: the challenge of a surveillance system. *Euro Surveill* 2011; **16:** 19968.

4 Pan American Health Organization, WHO. Guidelines for the diagnosis and treatment of Chagas disease. 2018. http://iris.paho.org/xmlui/handle/123456789/49653 (accessed Aug 15, 2019).

5 Kratz JM, Garcia Bournissen F, Forsyth CJ, Sosa-Estani S. Clinical and pharmacological profile of benznidazole for treatment of Chagas disease. *Expert Rev Clin Pharmacol* 2018; **11:** 943–57.

6 Altcheh J, Moscatelli G, Moroni S, Garcia-Bournissen F, Freilij H. Adverse events after the use of benznidazole in infants and children with Chagas disease. *Pediatrics* 2011; **127:** e212–18.

7 Hasslocher-Moreno AM, do Brasil PE, de Sousa AS, Xavier SS, Chambela MC, Sperandio da Silva GM. Safety of benznidazole use in the treatment of chronic Chagas' disease. *J Antimicrob Chemother* 2012; **67:** 1261–66.

8 Tornheim JA, Lozano Beltran DF, Gilman RH, et al. Improved completion rates and characterization of drug reactions with an intensive Chagas disease treatment program in rural Bolivia. *PLoS Negl Trop Dis* 2013; **7:** e2407.

9 Jackson Y, Wyssa B, Chappuis F. Tolerance to nifurtimox and benznidazole in adult patients with chronic Chagas' disease. *J Antimicrob Chemother* 2020; **75:** 690–96.

10 Molina I, Gómez i Prat J, Salvador F, et al. Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. *N Engl J Med* 2014; **370:** 1899–908.

11 Morillo CA, Waskin H, Sosa-Estani S, et al. Benznidazole and posaconazole in eliminating parasites in asymptomatic *T. cruzi* carriers: the STOP-CHAGAS trial. *J Am Coll Cardiol* 2017; **69:** 939–47.

12 Torrico F, Gascon J, Ortiz L, et al. Treatment of adult chronic indeterminate Chagas disease with benznidazole and three E1224 dosing regimens: a proof-of-concept, randomised, placebo-controlled trial. *Lancet Infect Dis* 2018; **18:** 419–30.

13 Laboratorios Liconsa. Package insert: benznidazole tablets, for oral use. 2017. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209570lbl.pdf (accessed Sept 16, 2019).

14 Bustamante JM, Craft JM, Crowe BD, Ketchie SA, Tarleton RL. New, combined, and reduced dosing treatment protocols cure *Trypanosoma cruzi* infection in mice. *J Infect Dis* 2014; **209:** 150–62.

15 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.

16 Soy D, Aldasoro E, Guerrero L, et al. Population pharmacokinetics of benznidazole in adult patients with Chagas disease. *Antimicrob Agents Chemother* 2015; **59:** 3342–49.

17 Álvarez MG, Vigliano C, Lococo B, Petti M, Bertocchi G, Viotti R. Seronegative conversion after incomplete benznidazole treatment in chronic Chagas disease. *Trans R Soc Trop Med Hyg* 2012; **106:** 636–38.

18 Álvarez MG, Ramírez JC, Bertocchi G, et al. New scheme of intermittent benznidazole administration in patients chronically infected with *Trypanosoma cruzi*: clinical, parasitological, and serological assessment after three years of follow-up. *Antimicrob Agents Chemother* 2020; **64:** e00439–20.

19 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:** 724–34.

20 Ribeiro I, Blum B, Fernandes J, et al. Drug-drug interaction study of benznidazole and E1224 in healthy male volunteers. *Antimicrob Agents Chemother* 2021; published online Feb 8. https://doi.org/10.1128/AAC.02150-19.

21 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:** e01191–18.

22 Ramírez 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.

23 Diniz LF, Mazzeti AL, Caldas IS, Ribeiro I, Bahia MT. Outcome of E1224–benznidazole combination treatment for infection with a multidrug-resistant *Trypanosoma cruzi* strain in mice. *Antimicrob Agents Chemother* 2018; **62:** e00401–18.

24 Pinazo M-J, Muñoz J, Posada E, et al. Tolerance of benznidazole in treatment of Chagas' disease in adults. *Antimicrob Agents Chemother* 2010; **54:** 4896–99.

25 Machado-de-Assis GF, Silva AR, Do Bem VAL, et al. Post-therapeutic cure criteria in Chagas' disease: conventional serology followed by supplementary serological, parasitological, and molecular tests. *Clin Vaccine Immunol* 2012; **19:** 1283–91.

26 Pinazo M-J, Thomas M-C, Bustamante J, Almeida IC, Lopez MC, Gascon J. Biomarkers of therapeutic responses in chronic Chagas disease: state of the art and future perspectives. *Mem Inst Oswaldo Cruz* 2015; **110:** 422–32.

27 Sguassero Y, Roberts KN, Harvey GB, et al. Course of serological tests in treated subjects with chronic *Trypanosoma cruzi* infection: a systematic review and meta-analysis of individual participant data. *Int J Infect Dis* 2018; **73:** 93–101.

28 Almeida IC, Ferguson MA, Schenkman S, Travassos LR. Lytic anti-α-galactosyl antibodies from patients with chronic Chagas' disease recognize novel O-linked oligosaccharides on mucin-like glycosyl-phosphatidylinositol-anchored glycoproteins of *Trypanosoma cruzi*. *Biochem J* 1994; **304:** 793–802.

29 de Andrade AL, Zicker F, de Oliveira RM, et al. Randomised trial of efficacy of benznidazole in treatment of early *Trypanosoma cruzi* infection. *Lancet* 1996; **348:** 1407–13.

30 Almeida IC, Krautz GM, Krettli AU, Travassos LR. Glycoconjugates of *Trypanosoma cruzi*: a 74 kD antigen of trypomastigotes specifically reacts with lytic anti-alpha-galactosyl antibodies from patients with chronic Chagas disease. *J Clin Lab Anal* 1993; **7:** 307–16.

31 Moscatelli G, Moroni S, García Bournissen F, et al. Longitudinal follow up of serological response in children treated for Chagas disease. *PLoS Negl Trop Dis* 2019; **13:** e0007668.