Efficacy and safety of fexinidazole for treatment of chronic indeterminate Chagas disease (FEXI-12): a phase 2, double-blind, multicenter randomized trial

Maria-Jesus Pinazo, PhD[1-3], Colin Forsyth, PhD [2], Irene Losada, MsC[1], Elena Trigo Esteban, PhD[4], Magda García Rodríguez, PhD[5], Maria Luz Villegas, MD[6], Israel Molina, PhD[3,7], Clara Crespillo-Andújar, PhD[4], Montserrat Gállego, PhD[1,3,8], Cristina Ballart, PhD [1], Juan Carlos Ramirez, PhD [9], Tilman Aden CMT [10], Achim Hoerauf MD [10,11], Kenneth Pfarr, PhD [10,11], Michel Vaillant PhD[12], Tayná Marques MsC[2], Jayme Fernandes MD[2], Bethania Blum, PhD [2], Isabela Ribeiro, MD[2], Sergio Sosa-Estani, PhD [2, 13], Fabiana Barreira, MD[2], Joaquim Gascón, PhD [1,3], on behalf of the FEXI-12 Study Team*

* FEXI study team members are listed in the acknowledgments.

1. Barcelona Institute for Global Health, ISGlobal-Hospital Clinic, Universitat de Barcelona, Barcelona, Spain
2. Drugs for Neglected Diseases initiative, Rio de Janeiro, Brazil
3. CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III (CIBERINFEC, ISCIII), Spain
4. Unidad de Patología Importada y Salud Internacional. Hospital Universitario La Paz, Madrid, Spain
5. Hospital General Universitario de Valencia, Valencia, Spain
6. Hospital General de L'Hospitalet. Complex Hospitalari Universitari Moisès Broggi. Barcelona, Spain
7. Hospital Universitario Vall d'Hebron, Barcelona, Spain
8. Parasitology section, Department of Biology, Health and Environment, Faculty of Pharmacy and Food Science, Universitat de Barcelona, Barcelona, Spain.
9. Instituto Multidisciplinario de Investigaciones en Patologías Pediátricas (IMIPP-CONICET-GCBA), Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina
10. Institute of Medical Microbiology, Immunology, and Parasitology, University Hospital Bonn, Germany
11. German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, Bonn, Germany
12. Luxembourg Institute of Health, Strassen, Luxembourg
13. Centro de Investigaciones en Epidemiología y Salud Pública (CIESP-CONICET), Buenos Aires, Argentina.

Summary

Background: More than six million people worldwide are infected with *Trypanosoma cruzi*, causative agent of Chagas disease (CD), which particularly impacts vulnerable communities in Latin America. Only a small portion have accessed diagnosis and treatment. Both drugs used to treat this chronic, neglected infection were developed over fifty years ago, and adverse drug reactions during treatment pose a major barrier, causing one in five patients to discontinue drug therapy. Fexinidazole proved efficacious in an earlier, interrupted clinical trial, but the doses evaluated were not well tolerated. The present study evaluated fexinidazole at lower doses and for shorter treatment durations.

Methods: In this double-blind, Phase II, randomized clinical trial, we evaluated three regimens of fexinidazole with 15 patients per arm: 600 mg once daily for 10 days (6.0 g total dose); 1,200 mg daily for three days (3.6 g), and 600 mg daily for three days followed by 1,200 mg daily for four days (6.6 g), compared to a historical placebo control group (n=46). The primary endpoint was sustained negative results by PCR at end of treatment and on each visit up to four months of follow-up.

Findings: Only 8/43 (18.6%) fexinidazole-treated patients attained the primary endpoint, compared to 6/46 (13.0%) in the placebo group. Mean parasite load decreased sharply following treatment but rebounded beginning 10 weeks post-treatment. Tolerability was good, with only two treatment discontinuations due to adverse effects unrelated to fexinidazole.

Interpretation: The fexinidazole regimens in this study were well tolerated but did not prove effective against *T. cruzi* infection. Development of fexinidazole for treating *T. cruzi* infection in monotherapy is stopped.

Funding: Through the Drugs for Neglected Diseases initiative.

Research in Context

Evidence before this study

Chagas disease is one of the world's most neglected diseases, with <1% of the number of estimated patients obtaining treatment. The World Health Organization has targeted Chagas disease for elimination as a public health problem by 2030. Only two drugs, benznidazole and nifurtimox, are known to be effective against *Trypanosoma cruzi*, the protozoan that causes the disease. Current Pan American Health Organization guidelines based on the GRADE methodology recommend either drug for acute and congenital infections and chronically infected children (strong, moderate), girls and women of childbearing age to prevent future congenital transmission (strong, moderate), and chronically infected adults without specific organ damage (conditional, low) to prevent or delay the onset of clinical complications.

Clinical trials in pediatric populations have demonstrated high cure rates for infants and chronically infected children after treatment with benznidazole and nifurtimox. Evidence is more limited in chronically infected adults. Observational studies have indicated treatment of asymptomatic or mildly symptomatic adults may discourage the development of clinical complications, but the BENEFIT trial did not demonstrate any advantage to treatment of adults with established cardiological chronic disease, observing the progression to moderate and severe cardiomyopathy. Moreover, both drugs have significant limitations, including long treatment periods and frequent adverse events (AEs), particularly in adult patients. This complicates their administration and limits patient adherence, causing one in five patients to discontinue drug therapy and posing a major barrier to increasing access to treatment.

Another important barrier is the difficulty of measuring treatment success. Diagnosis of chronic Chagas disease relies on serological methods, but it may take years or decades for chronically infected patients to serorevert following treatment. Polymerase chain reaction has low sensitivity for detecting *T. cruzi* DNA, although positive results are considered a good indication of treatment failure.

Two strategies are being pursued to improve treatment of Chagas disease: optimization of current treatments to minimize adverse reactions, and development of new drug candidates. Several recently completed and ongoing studies are investigating whether shorter or alternative regimens of benznidazole and nifurtimox can improve safety and maintain high efficacy. However, few clinical trials have evaluated new chemical entities for treatment of *T. cruzi* infection.

We searched the Cochrane Library and National Library of Medicine (Pubmed), with no language restrictions, for studies of new drug treatments for Chagas disease in its chronic indeterminate phase published from January 1, 2000 to December 31, 2022. One previous clinical trial evaluated fexinidazole, while other studies assessed posaconazole (n=2), fosravuconazole (n=2), allopurinol (n=2), and itraconazole and allopurinol (n=1). In the FEXI-001 trial of fexinidazole, parasite clearance, sustained for 12 months after treatment, was observed

in all treated patients with data available (n=31), but the study was halted due to concerns regarding AEs. Both posaconazole trials compared the drug to standard treatment with benznidazole, and one was placebo controlled; despite an initial antiparasitic effect in posaconazole-treated patients, most had positive PCR tests during the follow-up period. Similar findings were reported in a clinical trial that compared fosravuconazole to benznidazole and placebo. In all three studies, only patients receiving standard treatment with benznidazole had consistently negative PCR results from end of treatment to the end of follow-up (12-18 months). The BENDITA clinical trial evaluated a fosravuconazole and benznidazole combination, but there was no improvement in safety or efficacy compared to the standard treatment. A study in Chile found that 88/109 (80.7%) patients treated with itraconazole or allopurinol were positive on at least one parasitological test 11 years after treatment, while in another study in Brazil, all 17 patients treated with allopurinol had positive results on xenodiagnoses during follow-up. In a pilot study with 11 patients, treatment with allopurinol followed by 30 days of treatment with benznidazole was well tolerated and some *T. cruzi*-specific antibodies declined during follow-up.

Added value of this study

The FEXI-001 trial indicated strong antiparasitic efficacy, yet limited tolerability of fexinidazole. In this Phase II trial, we evaluated lower doses and shorter regimens based on collected preclinical and clinical information related to pharmacology and safety of previous studies. We aimed to determine whether such regimens could still yield the high efficacy suggested in FEXI-001 while improving safety. Patients (15 per arm) were randomized to three different fexinidazole treatments: 600 mg once daily for 10 days (6.0 g total dose), 1,200 mg daily for three days (3.6 g), and 600 mg daily for three days followed by 1,200 mg daily for four days (6.6 g); a placebo arm was based on historical data from a prior Chagas disease trial. Overall, 28/43 (65%) fexinidazole-treated patients had negative PCR results at end of treatment compared to 12/46 (26%) in the historical placebo control group. Nonetheless, the effect was transient, and only 8/43 (18.6%) fexinidazole-treated patients achieved the primary endpoint of sustained PCR results during four months of follow-up, not significantly different from the 6/46 (13.0%) in the historical placebo group. Safety results improved compared to previous studies with longer fexinidazole regimens, with most adverse events being mild, and only two treatment discontinuations were observed due to adverse events, both considered unrelated to the study drug.

Implications of all the available evidence

Despite antiparasitic efficacy at end of treatment, most patients treated with fexinidazole did not have sustained negative PCR results during the follow-up period. The doses and durations of fexinidazole evaluated in this study do not appear viable for treatment of chronic indeterminate CD in monotherapy. A fexinidazole dosing regimen which is safe, well tolerated, and effective against *T. cruzi* remains elusive. The need remains for a safer alternative to the current treatment, either through improved regimens of benznidazole and nifurtimox, or via

new drug candidates. Meanwhile, although PCR is a viable method for detecting treatment failure, a better test of treatment success is urgently needed to strengthen future clinical research.

Introduction

Chagas disease (CD) is a neglected parasitic infection caused by the protozoan *Trypanosoma cruzi*, affecting 6-7 million people worldwide.[1-3] The most common transmission route is still via haematophagous triatomine insects whose habitat ranges from the Southern United States to the Southern Cone of South America. In rural areas of Latin America, housing is often constructed of natural materials such as adobe or thatch, which provide an ideal habitat for the insect vector. Consequently, CD disproportionately affects vulnerable populations with limited access to healthcare, including rural and indigenous communities, and migrant communities in non-endemic and urban areas. Other important routes include congenital transmission, consumption of contaminated food or beverages, and transfusions or transplants using untested material. CD has an acute phase, which is usually asymptomatic or easily confused with other infections, so rates of diagnosis are extremely low. The infection then enters a chronic phase which is lifelong in the absence of treatment. Of those infected, 30-40% eventually develop cardiomyopathy and/or complications of the digestive or nervous systems.[4]

Only two drugs, benznidazole and nifurtimox, are known to be effective against *T. cruzi*, curing the infection in infants, children, and acute cases; preventing future congenital transmission; and eventually reducing the development of cardiomyopathy and other complications in adults with chronic indeterminate CD, even if this evidence reflects observational studies and needs to be strengthened .[5-11] However, several challenges complicate their administration, including long treatment periods of 60-90 days, and adverse drug reactions (AEs), which cause about 20% of adult patients to discontinue treatment.[12] Healthcare providers and patients are often reluctant to initiate treatment due to concerns about AEs. Moreover, treatment requires periodic laboratory monitoring and multiple appointments, creating a burden on the healthcare system and entailing travel, missed work, and other indirect costs for patients. These and other barriers contribute to <1% of the estimated number of people with *T. cruzi* infection accessing treatment.[13] Developing a safer, more effective treatment for people with chronic indeterminate CD – most patients – represents a crucial step toward achieving the World Health Organization (WHO) objective of eliminating CD as a public health concern by 2030. Clinical trials in the past decade assessed posaconazole and fosravuconazole, antifungal compounds which had demonstrated promise in preclinical research, but these drugs only had a transient effect against the infection, with frequent resurgence of parasitemia post-treatment.[14-16] Previous studies examined allopurinol and itraconazole, with a similar recurrence of positive parasitological results post-treatment.[17-19]

Fexinidazole is a nitroimidazole, developed as a broad spectrum antimicrobial, which exhibited antitrypanosomal potential in preclinical studies in the 1970's and 1980's before its

development was abandoned.[20] It was later rediscovered by the Drugs for Neglected Diseases initiative (DNDi) through screening of several hundred nitroheterocyclic compounds from various sources, and found to be safe and effective for treatment of sleeping sickness in a Phase II/III study.[21] Moreover, fexinidazole was more efficacious than benznidazole or nifurtimox in *T. cruzi*-infected mice, including against benznidazole-resistant strains, while having an acceptable safety profile.[22]

Therefore, a phase II, proof-of-concept study (FEXI-001) assessed six different dosing regimens of fexinidazole in chronically infected adults with CD in Bolivia.[23] The primary endpoint was sustained clearance of *T. cruzi* in peripheral blood as evidenced by serial negative results by real-time polymerase chain reaction (PCR) from end of treatment to six months; with a total follow-up of 12 months. The trial was interrupted with 47 patients enrolled due to safety concerns, including transient asymptomatic grade 3 and 4 neutropenia and significant neuropsychiatric and hepatic adverse events (AEs). Nonetheless, all 31 fexinidazole-treated patients with 12 months' follow-up data exhibited sustained clearance of *T. cruzi* DNA, including 11 patients with treatment durations <7 days. None of the seven placebo-treated patients attained the primary endpoint.

Pharmacokinetic-pharmacodynamic (PKPD) analysis of the FEXI-001 trial and consultations with experts in liver safety indicated liver toxicity was related to dose and duration of exposure, with the most significant events occurring at high doses with treatment periods >14 days. Simulations suggested that lower doses administered over a shorter treatment period could still be efficacious, while reducing the incidence of hepatic and neuropsychiatric AEs. A new Phase II trial, FEXI-12, was therefore conducted to assess lower doses of fexinidazole at short (3-10 days) treatment periods for treatment of chronic indeterminate CD and is presented below.

Methods

Study design

FEXI-12 (NCT03587766, EUDRA-CT 2016-004905-15) was a double-blind, randomized, prospective proof-of-concept trial with three-parallel groups and a historical placebo control in patients with chronic indeterminate CD. The trial was conducted at five hospitals in Spain with expertise in caring for patients with CD: Hospital Clínic, Hospital General de L'Hospitalet, and Hospital Universitario Vall d'Hebron in Barcelona; Hospital Universitario La Paz in Madrid; and Hospital General Universitario in Valencia.

The study was sponsored by DNDi, while the national coordinating center in Spain was the Barcelona Institute of Global Health (ISGlobal). The trial adhered to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines, and the study protocol was approved by the Spanish Agency of Medicines and Medical Products (AEMP), the Spanish Independent Ethics Committee, and the Institutional Ethical Committees of Hospital Clínic (HCB/2017/0431), Hospital Vall D'Hebron, Hospital General de L'Hospitalet (CSI_17/28), Hospital Universitario La Paz and Hospital General Universitario in Valencia.

Participants

The trial enrolled adult patients 18-60 years old with confirmed *T. cruzi* infection using two different serological tests (conventional or recombinant enzyme linked immunosorbent assay (ELISA), chemiluminescence immunoassay, or indirect immunofluorescence) and a positive PCR result for at least one of three samples (each assessed in triplicate) collected on a single day. Patients with normal electrocardiogram (heart rate 50 to 100 bpm; PR ≤200 msec, QRS ≤120 msec, and QT interval corrected for heart rate ≥350 msec and ≤450 msec interval), and without clinically relevant arrhythmias on 24-hour Holter monitoring were included. Women of childbearing age were required to have a negative serum pregnancy test at screening and to use an effective contraceptive method during treatment and for the estimated time for clearance of fexinidazole metabolites (21 days). Women currently breastfeeding were not included. Patients with signs or symptoms of organ involvement from the cardiac or digestive forms of CD, a history of prior etiological treatment of CD, and/or known hypersensitivity or allergic reactions to nitroimidazole compounds were excluded. Other exclusion criteria included: history of cardiomyopathy, ventricular arrhythmia, heart failure, digestive surgery or megasyndrome; Hospital Anxiety and Depression Scale (HADS) self-assessment score ≥11 in any of the sub-scales; and known hypersensitivity to nitroimidazoles or history of allergic skin rash, asthma, intolerance, sensitivity, or photosensitivity to any drug. Full inclusion and exclusion criteria are available in the study protocol (Supplementary File 1).

Historical rather than concurrent controls were chosen for this trial to decrease the burden on patients of a new RCT and to accelerate enrolment. The controls were all previously enrolled in Chagas disease clinical trials with very similar timepoints and procedures, hence ensuring minimum differences with the treated patients in the current trial.

Randomisation and masking

Site investigators enrolled patients, who were then randomized to one of three treatment groups in a 1:1:1 ratio. Patients, investigators, and study team members were blinded to the treatment allocation throughout the duration of the study. However, following an interim analysis with a futility stopping rule, the results of the primary endpoint were disclosed unblinded to the study data monitoring committee and, subsequently, to the sponsor, who determined to continue with the trial until completion of all planned follow-up.

The historical placebo control group consisted of data from a previous clinical trial.[16]

Procedures

Three different treatment regimens of fexinidazole were assessed: 600 mg once daily (QD; one 600 mg tablet of fexinidazole plus a matching placebo tablet) for 10 days (6.0 g total dose); 1,200 mg QD consisting of two 600 mg fexinidazole tablets for three days (3.6 g total dose), followed by two matching placebo tablets for seven days; and 600 mg QD for three days (one 600 mg fexinidazole tablet plus one matching placebo tablet) followed by 1,200 mg (two 600 mg fexinidazole tablets) QD for four days, and two matching placebo tablets for three additional days (6.6 g total dose) (Figure 1). Fexinidazole or matching placebo tablets were

taken orally once daily within 30 minutes of starting a regular meal. Patients were asked to record the exact time of treatment intake in a diary.
Study visits took place during the first three days of treatment and at two, three, four, six, ten, and 12 weeks and four, six, and twelve months after study initiation. During these visits and at screening, three 5 ml blood samples were collected and mixed with an equal volume of 6M guanidine hydrochloride 0.2M EDTA pH 8.0 buffer for analysis by PCR, for evaluation of the primary efficacy endpoint using the method described by Duffy et al.[24]

Adverse events were monitored at each visit. On selected visits, haematological, blood chemistry, electrocardiogram (ECG), physical examinations, and/or pregnancy tests were performed (see details in Supplementary File 1), as well as specific evaluation of the psychological status of the subject through a Hospital Anxiety and Depression Scale (HADS).

Outcomes

The primary efficacy endpoint was parasitological clearance measured by serial negative qualitative PCR results (three negative qualitative PCR results from three samples collected on the same day) at end of treatment, and sustained during the follow up period at 3, 4, 6, 8, and 10 weeks, and 3 and 4 months after treatment initiation. A positive result during any of these visits was considered a treatment failure. As a secondary efficacy endpoint, sustained parasitological clearance through 12 months (all visits assessed in the primary endpoint, plus 6 and 12 months' follow-up) was ascertained, along with reduction in parasite load measured by quantitative PCR. Safety endpoints included the frequency and severity of adverse events (clinical, laboratory, and ECG) and the incidence of adverse events causing treatment discontinuation, serious adverse events (SAEs), or adverse events of special interest (AESI). The latter group included neuropsychiatric signs and symptoms requiring specialized therapeutic intervention, neutrophil counts <1000 cells/µL, and persistent or acute increases in alanine aminotransferase (ALT) or raised aspartate aminotransferase (AST).

Statistical analysis

Sample size was determined by comparing each treatment arm to the historical placebo control group, for two independent binomial proportions using Pearson's Chi-square statistic with a Chi-square approximation and a two-sided significance level of 0.0167. With expected proportions of 0.9 in the treatment group and 0.082 in the placebo group, a sample size of eight per arm would achieve 82% power. Further, a sample size of 15 patients per arm would allow a 90% probability of observing at least one safety event with 5% frequency and 5% precision.
The primary efficacy endpoint was analyzed in the intention-to-treat (ITT) population, which included all patients randomized to a treatment group, regardless of whether treatment was received. Per-protocol (PP) analysis, consisting of patients randomized to a treatment group without permanent treatment discontinuation or major protocol deviations, was also performed. The all-treated set included every patient who took at least one dose of fexinidazole.
For the efficacy analysis, if there was a positive PCR result for any of the three samples collected per visit, the overall outcome for that visit was deemed positive. Any positive PCR

result between end of treatment and the primary endpoint (at four months) was considered a treatment failure. Sustained parasitological clearance meant every PCR result from end of treatment throughout the follow-up period remained negative. The proportion of patients with sustained parasite clearance in each arm was compared to that of the placebo group for the primary efficacy endpoint at four months follow-up, and at 12 months follow-up. Chi square or Fisher's exact tests were performed, and the Hochberg procedure was used to account for multiple comparisons. Reported p values reflect comparison to the placebo group.

For quantitative PCR analyses, means and 95% confidence intervals were calculated by group and visit. Kaplan-Meier survival analysis was used to describe time to parasite clearance with a log rank test for significance.

Safety analyses were performed in the all-treated set, comprising all patients who received at least one dose of treatment. Descriptive analyses included the number and percentage of patients with AEs, SAEs, AESIs and/or treatment-emergent AEs leading to treatment discontinuation, by study arm and system organ class (SOC). The severity of adverse events was assessed using the Common Terminology Criteria for Adverse Events (v 4.03).

Role of the funding source

As the study sponsor, DNDi had a role in oversight of data collection, analysis, and interpretation, and was involved in the writing of the manuscript and decision to submit. The other funders had no role in the design of the study, collection and interpretation of data, or production and review of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication

Results

Study recruitment commenced on October 16, 2017; 150 patients were screened, and 45 were enrolled and randomized, with 15 to each treatment arm (Figure 1). Among 93 patients who did not meet study entry criteria, the main reasons were negative PCR (n=53), clinically significant abnormal laboratory (n=29), Holter (n=12), or ECG (n=3) values; and score >3.1 on the HADS (n=3). The last patient visit occurred on August 28, 2019; 43 patients (96%) completed the study. All but two patients (one from Chile and one from Paraguay) were of Bolivian origin (Table 1).

The ITT population included all 45 randomized patients, while the PP population comprised 35/45 randomized patients (78%). All results are reported for the ITT population unless otherwise stated. For assigned treatment administration, 41/45 patients (91%) were 100% compliant. Of the remaining four, two patients took 50% (5/10 tablets) of the assigned treatment but discontinued due to adverse effects, another patient withdrew consent and was lost to follow-up after taking 20% (2/10 tablets) of assigned medication, and another patient was incorrectly randomized and did not receive medication. The latter two patients were treated as missing in the intention to treat analyses.

Most patients (28/43, 65%) achieved parasite clearance at end of treatment compared to 12/46 (26%) in the historical placebo control group. The highest clearance (12/14, 85.7%, p<0.001) was observed in the 6.0 g arm, followed by the 3.6 g arm (9/15, 67%, p=0.020), and the 6.6g arm (7/14, 50%, p=0.089). However, during post-treatment follow-up, most patients relapsed (registering at least one positive PCR result) before four months (Table 2). After four months' follow-up, only 8/43 patients treated with fexinidazole showed sustained parasite clearance: 3/15 (20%, p=0.168) in the 3.6 g arm, 4/14 (29%, p=0.387) in the 6.0 g arm, and 1/14 (7%, p=0.861) in the 6.6 g arm, compared with 6/46 (13%) in the historic placebo group. None of the treatment groups were significantly different from the placebo. During 12 months' follow-up, an additional two patients in the 3.6 g arm relapsed, and in total 6/43 (14%) fexinidazole-treated patients had sustained parasite clearance, only slightly more than the 4/46 (9%) in the placebo group. No statistically significant differences were observed in the time to relapse from parasite clearance for patients treated with fexinidazole compared to the historical placebo control. Mean parasite load declined sharply following treatment, with most values below the detectable limit, but resurgence was noted after ten weeks follow-up in all three study arms (Figure 2, Supplementary Table 1).

The all-treated set included 44 patients. There were 290 AEs and 43/44 (97.7%) patients experienced at least one AE (Table 3). AEs occurred more frequently in the 3.6 g arm (142 AEs) compared to the 6.0 g (71) and 6.6 g (77) arms. The most frequently reported AEs were headache (n=23, 52.3%) and nausea (n=14, 31.8%), followed by back pain (n=10, 22.7%) and pruritus (n=7, 15.9%). Overall, 89 AEs affecting 29/44 (65.9%) patients were considered related to the study drug. There were no notable safety concerns related to electrocardiograms or haematology and biochemistry assessments. Most AEs (243/290, 83.8%) were mild, while 40 (13.8%) were moderate and seven (2.4%) were severe. Severe AEs were not deemed treatment-related and comprised cases of carpal tunnel, sciatica, device infection, pneumonia, staphylococcal infection, and joint and device dislocation.

Two patients, both in the 6.6 g arm, were obliged to discontinue treatment due to AEs. One patient suffered from moderate anxiety and depression, which were initially considered treatment-related by the investigator. Because symptoms persisted for several months despite withdrawal of treatment, the sponsor later determined the AEs were not related to the study drug. Another patient experienced mild paresthesia, nausea, and insomnia; all were initially deemed treatment related. There were no temporary treatment interruptions. Three patients suffered four serious adverse events: severe device and joint dislocation in one patient; and in the other two patients, severe pneumonia and moderate cholecystectomy, all considered unrelated to the study drug. There were no fatal or life-threatening AEs. Two patients experienced AEs of special interest: the events of anxiety and depression described above, and one patient with elevated transaminases in the 6.0 g arm, which was of mild intensity and considered unrelated to the study drug.

Discussion

The doses and regimens evaluated in the FEXI-12 trial were generally well tolerated. They exhibited efficacy at end of treatment, but this effect was transient, and most patients relapsed by assessment of the primary endpoint at four months post-treatment. Only 6/43 patients had sustained negative PCR results at 12 months of follow-up, which was not significantly different from the historical placebo group. These results contrast with those of the FEXI-001 clinical trial, where higher doses of fexinidazole were effective against *T. cruzi* , but serious safety concerns including severe neutropenia and psychiatric AEs prompted a halt of FEXI-001.[23] The initial selection of higher doses in the FEXI-001 study was based on higher cure rates following treatment with fexinidazole in animals infected with different *T. cruzi* strains, and subsequently by the fexinidazole metabolites fexinidazole sulfoxide and fexinidazole sulfone, compared with the same doses of benznidazole. Experiments showed that extended exposure throughout the dosing interval were necessary to achieve cures.[22,25] (ref) Both fexinidazole metabolites were well tolerated at higher doses, having a longer half-life, which was consistent with previous evidence. (The No Observed Adverse Event Level (NOAEL) in 4-weeks repeated dose toxicokinetics studies in dogs and rats was set at 200 mg/kg/day).[20] Accumulation with repeated administration was unlikely for either the sulfoxide or the sulfone, consistent with previous reports on the metabolites following repeated administration of fexinidazole.[22]

After an extensive analysis of safety events observed, low doses and durations selected for the FEXI-12 trial were based on both PK modelling after the FEXI-001 trial, and the fact that even the lowest doses and shortest-durations of fexinidazole had proved effective in a clinical context, albeit in a small sample.[23] Evaluating short durations was also considered important because of the potential for facilitating patient adherence and access. In practice, safety and tolerability was greatly improved in the regimens evaluated in FEXI-12 compared to those in FEXI-001, but the tradeoff appears to be limited antiparasitic effect. One area that deserves further research is the role of dormant parasites. In one study, dormant *T. cruzi* amastigotes were highly resistant to trypanocidal treatment in “in vivo” and “in vitro” experiments, with the capacity to reestablish a strong infection in the host within 30 days of treatment.[26] Although persister forms of *T. cruzi* parasites are only beginning to be understood and could even be drug dependant, the inability of some drugs to target these forms may help explain the failure of posaconazole and other antiparasitics to sustainably eliminate *T. cruzi* after treatment. On the other hand, benznidazole appears to be more effective at eliminating different forms of the parasite through multiple pathways.[27,28] More research is needed to determine what implications this may have for future compounds in development. Another hypothesis is that rather than completely eliminating the parasite, drug treatment simply reduces *T. cruzi* parasitemia to a lower steady state, which hovers near or just below the limit of detection.[29]

Some differences in adverse events were noted between the FEXI-12 study and the clinical trial for fexinidazole for patients with late-stage human African trypanosmiasis (HAT) caused by *Trypanosoma brucei gambiense* .[21] In that study, among 264 patients treated with fexinidazole, headache was the most frequent AE, as in FEXI-12. However, other frequent AEs in the HAT trial included insomnia (28%), asthenia (23%), tremor (22%), vomiting (28%), and decreased

appetite (21%), whereas none of these events affected more than 12% of FEXI-12 patients. This disparity may reflect the moderately higher mean total dose in the HAT trial (10.2 g) compared with FEXI-12 (5.4 g) and/or different disease dynamics, environmental factors, concomitant treatments, as well as genotypic and phenotypic characteristics of the at-risk population. All these factors deserve further investigation to better understand differences in the safety of fexinidazole in the different studies.

The results of this trial do not support the further evaluation of any of these regimens in a Phase III study. Although the higher doses evaluated in the FEXI-001 trial had shown evidence of effectiveness, the tradeoff was a high risk of serious adverse events. Moreover, when considering investigation of potential combinations with other drugs, because its mode of action is the inhibition of *T. cruzi* ergosterol biosynthesis and due to other characteristics of the molecule, we do not consider combining it with benznidazole and nifurtimox. Nonetheless, DNDi and collaborators continue searching and developing new therapeutic approaches based on new chemical entities. In this scenario fexinidazole could eventually be included in drug combinations.

CD remains severely neglected, with <1% of the estimated number of patients able to access treatment,[13] and the development of new treatments which are safer and more effective than current standard treatments will probably take many more years. In different clinical trials, the standard treatment with benznidazole has proven effective against *T. cruzi,* as measured by negative PCR results, with the effect sustained for 12 months follow-up in about 80% of patients.[15,16,30]

At present, the optimization of current treatments by using alternative regimens remains a promising avenue. Following the completion of the BENDITA trial, which suggested shorter benznidazole regimens could maintain a high antiparasitic efficacy,[30] other trials evaluating new regimens of benznidazole and nifurtimox have recently been completed, are in progress, or are poised to begin recruitment (Multibenz-NCT03191162, TESEO- NCT03981523, BETTY-NCT03672487, and EQUITY- NCT02369978). These trials should provide clearer evidence on the viability of alternative regimens and whether they can improve tolerability and adherence while reducing the incidence of adverse effects. Meanwhile, drug discovery efforts continue and promising new chemical entities are advancing to Phase I and II trials.[27]

CD continues to be one of the world's most neglected diseases, with few patients obtaining diagnosis, and for those few who initiate treatment, there is a high discontinuation rate due to adverse effects. Finding a safer treatment which is effective against *T. cruzi*, facilitates administration for healthcare providers, and makes adherence easier for patients is key to achieving the WHO objective of eliminating CD as a public health problem.

Our study has some limitations. Because of population-level differences and/or genetic variation in *T. cruzi*, our results might not be generalizable outside of Bolivian patients. It also should be noted that the trial took place in a non-endemic area. Because of low numbers of circulating parasites during chronic infection, parasite DNA levels are often below the quantifiable limit on PCR, so conclusions regarding parasite load should be interpreted with

caution. PCR is currently the best available method for measuring antitrypanosomal efficacy of new drugs for CD, and is valuable for detecting therapeutic failure, but development of new tools which can provide a definitive indication of treatment success – linked to clinical outcomes – will be of great value for both clinical research and care of patients. The limited sample size in the study limits our ability to make robust comparisons between the different arms.

Contributors

MJP, FB, SSE, IR and JG developed the study concept and design. JG was the national coordinating investigator, and MJP, ETE, MGR, MLV, and IM were principal investigators. IL, CC, FB, BB, TM, and JF were involved in study coordination and implementation; FB, BB, and CF provided global coordination of trial and post-trial activities. MG, CB, TA, AH, and KP developed and conducted PCR analyses, and JCR conducted quality control activities for PCR. MV was responsible for statistical analyses. CF, FB and MV accessed and verified study data. MJP and CF drafted the original manuscript. All authors participated in data collection and interpretation, and in critical review and editing of the manuscript. All authors read and approved the final manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

AH reports being Chair of the German Network against NTDs, a German advocacy group to fight NTDs. The other authors report no competing interests.

Data Sharing

Following publication, deidentified data underlying the results of this study are only available upon request, because they contain potentially sensitive information. Interested researchers can contact DNDi, the sponsor of this study, for data access requests via email at ctdata@dndi.org. Researchers can also request data by completing the form available at https://www.dndi.org/ category/clinical-trials/. The protocol, statistical analysis plan, and clinical study report will also be made available. All requests will be evaluated between DNDi and ELEA. In this data request form, researchers must confirm that they will share data and results with DNDi and ELEA and will publish any results open access.

Acknowledgments

We would first and foremost like to thank the patients who participated in this study for their commitment to improving treatment options for all people affected by Chagas disease.
We would also like to thank the members of the FEXI-12 Study Team: Eva Muñoz Valda, Gladys Carranza, Julio Alonso-Padilla, Nieves Martínez Peinado, Diana Barrios, Núria Cortés, Alba Abras, Anna Fernández-Arévalo, Albert Arnau Vila, Leonardo de la Torre, Elizabeth Posada, Marta Arsuaga, Eugenia Márquez, and Núria Pons. This study was funded through DNDi and was supported by grants from the Associação Bem-Te-Vi Diversidade; the Dutch Ministry of Foreign Affairs (DGIS); the Federal Ministry of Education and Research (BMBF) through KfW, Germany; Fundación Mundo Sano; Médecins Sans Frontières International; the Swiss Agency for Development and Cooperation (SDC); and UK Aid. The opinions expressed in this article do not necessarily reflect the position or policies of any of the funding bodies.

Table 1. Baseline characteristics

	FEXI 6.0 g (N=15)	FEXI 3.6 g (N=15)	FEXI 6.6 g (N=15)	ALL (N=45)
Age (years), mean (SD)	39.9 (9.53)	40.7 (6.99)	40.5 (6.79)	40.4 (7.70)
Male, n (%)	5 (33.3)	2 (13.3)	3 (20.0)	10 (22.2)
Female, n (%)	10 (66.7)	13 (86.7)	12 (80.0)	35 (77.8)
Nationality, Bolivian n (%)	14 (93.3)	14 (93.3)	15 (100.0)	43 (95.6)
BMI (kg/m^2)	27.23 (3.00)	28.06 (3.17)	25.95 (3.58)	27.08 (3.31)
T. cruzi parasite load, (eq-p/mL)*, mean (SD)	3.67 (9.56)*	1.29 (2.56)	3.83 (7.24)	2.92 (6.95)
Prior medical history				
Diabetes mellitus	1 (6.7)	0 (0.0)	0 (0.0)	1 (2.2)
Dyslipidemia	2 (13.3)	1(6.7)	0 (0.0)	3 (6.7)
Hypercholesterolemia	2 (13.3)	1(6.7)	0 (0.0)	3 (6.7)
Obesity	1 (6.7)	0 (0.0)	0 (0.0)	1(2.2)
Hypertension	0 (0.0)	1 (6.7)	0 (0.0)	1 (2.2)
Anxiety	1 (6.7)	1 (6.7)	1 (6.7)	3 (6.7)
Insomnia	1 (6.7)	0 (0.,0)	0 (0.0)	1 (2.2)

* One patient did not have a screening sample due to being incorrectly randomized. All data are n (%).

564 **Table 2. Sustained *T. cruzi* clearance after treatment with fexinidazole**
565

	FEXI 6.0 g (N=15)	FEXI 3.6 g (N=15)	FEXI 6.6 g (N=15)	Placebo
Sustained parasite clearance until Month 4 (ITT)				
N	14	15	14	46
Sustained parasite clearance n (%)	4 (28.6)	3 (20.0)	1 (7.1)	6 (13.0)
P-value*	0.168	0.387	0.861	
Sustained parasite clearance until Month 12 (ITT)				
N	14	15	14	46
Sustained parasite clearance n (%)	4 (28.6)	1 (6.7)	1 (7.1)	4 (8.7)
P-value*	0.077	0.770	0.749	
Sustained parasite clearance until Month 4 (PP)				
N	11	13	11	46
Sustained parasite clearance n (%)	3 (27.3)	3 (23.1)	1 (9.1)	6 (13.0)
P-value*	0.231	0.310	0.798	
Sustained parasite clearance until Month 12 (PP)				
N	11	13	11	46
Sustained parasite clearance n (%)	3 (27.3)	3 (23.1)	1 (9.1)	6 (13.0)
P-value*	0.231	0.310	0.798	

566
567
568 *against placebo; sustained parasite clearance means negative serial qualitative PCR (three
569 negative PCR results from three samples collected on the same day) results on all assessments
570 from end of treatment through the follow-up period.
571 ITT=Intention to treat; PP=per protocol

572 Table 3. Summary of treatment-emergent adverse events (TEAEs)
573

Description	FEXI 6.0 g (N=14)	FEXI 3.6 g (N=15)	FEXI 6.6 g (N=15)	All (N=44)	
TEAEs		**13 (92.9)**	**15 (100.0)**	**15 (100.0)**	**43 (97.7)**
Study drug-related TEAE	9 (64.3)	10 (66.7)	10 (66.7)	29 (65.9)	
TEAEs leading to treatment interruption	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
TEAEs leading to permanent treatment discontinuation	0 (0.0)	0 (0.0)	2 (13.3)	2 (4.5)	
TEAEs of moderate intensity	6 (42.9)	7 (46.7)	5 (33.3)	18 (40.9)	
TEAEs of severe intensity	2 (14.3)	0 (0)	2 (13.3)	5 (11.4)	
TEAEs of life-threatening intensity	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
TEAEs of fatal intensity	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Serious TEAEs	1 (7.1)	1 (6.7)	1 (6.7)	3 (6.8)	
TEAEs of special interest	1 (0.0)	0 (0.0)	1 (6.7)	1 (2.3)	
Gastrointestinal disorders		**9 (64.3)**	**10 (66.7)**	**10 (66.7)**	**29 (65.9)**
Nausea	3 (21.4)	8 (53.3)	3 (20.0)	14 (31.8)	
Abdominal pain	3 (21.4)	3 (20.0)	0 (0.0)	6 (13.6)	
Dyspepsia	2 (14.3)	2 (13.3)	2 (13.3)	6 (13.6)	
Abdominal pain upper	2 (14.3)	1 (6.7)	1 (6.7)	4 (9.1)	
Flatulence	1 (7.1)	2 (13.3)	1 (6.7)	4 (9.1)	
Vomiting	2 (14.3)	2 (13.3)	0 (0.0)	4 (9.1)	
Diarrhoea	0 (0.0)	1 (6.7)	2 (13.3)	3 (6.8)	
Toothache	1 (7.1)	1 (6.7)	1 (6.7)	3 (6.8)	
Nervous system disorders	**6 (42.9)**	**13 (86.7)**	**10 (66.7)**	**29 (65.9)**	
Headache	3 (21.4)	13 (86.7)	7 (46.7)	23 (52.3)	
Dizziness	2 (14.3)	1 (6.7)	2 (13.3)	5 (11.4)	
Paraesthesia	0 (0.0)	2 (13.3)	1 (6.7)	3 (6.8)	
Musculoskeletal and connective tissue disorders	**5 (35.7)**	**7 (46.7)**	**4 (26.7)**	**16 (36.4)**	
Back pain	2 (14.3)	5 (33.3)	3 (20.0)	10 (22.7)	
Arthralgia	0 (0.0)	4 (26.7)	0 (0.0)	4 (9.1)	
Pain in extremity	2 (14.3)	2 (13.3)	1 (6.7)	5 (11.4)	
Infections and infestations	**7 (50.0)**	**7 (46.7)**	**5 (33.3)**	**19 (43.2)**	
Nasopharyngitis	2 (14.3)	2 (13.3)	1 (6.7)	5 (11.4)	
Psychiatric disorders	**3 (21.4)**	**4 (26.7)**	**6 (40.0)**	**13 (29.5)**	
Insomnia	0 (0.0%)	1 (6.7)	4 (26.7%)	5 (11.4)	
Nervousness	0 (0.0)	3 (20.0)	2 (13.)	5 (11.4)	
Anxiety	2 (14.3)	1 (6.7)	1 (6.7)	4 (9.1)	
Restlessness	0 (0.0)	1 (6.7)	2 (13.3)	3 (6.8)	
General disorders and administration site conditions	**2 (14.3)**	**7 (46.7)**	**4 (26.7)**	**13 (29.5)**	

Asthenia	1 (7.1)	2 (13.3)	1 (6.7)	4 (9.1)
Chest pain	1 (7.1)	2 (13.3)	0 (0.0)	3 (6.8)
Fatigue	0 (0.0)	1 (6.7)	2 (13.3)	3 (6.8)
Skin and subcutaneous tissue disorders	**2 (14.3)**	**6 (40.0)**	**3 (20.0)**	**11 (25.0)**
Pruritus	1 (7.1)	3 (20.0)	3 (20.0)	7 (15.9)
Dry skin	1 (7.1)	2 (13.3)	0 (0.0)	3 (6.8)
Ear and labyrinth disorders	**1 (7.1)**	**3 (20.0)**	**1 (6.7)**	**5 (11.4)**
Vertigo	1 (7.1)	3 (20.0)	1 (6.7)	5 (11.4)
Blood and lymphatic system disorders	**1 (7.1)**	**2 (13.3)**	**2 (13.3)**	**5 (11.4)**
Iron deficiency anaemia	1 (7.1)	2 (13.3)	1 (6.7)	4 (9.1)

574

575 All data presented are number (percentage) of patients in the all treated set.

576 TEAEs=treatment-emergent adverse events

577 * SOCs reported in >2 patients (≥10%) in any group; patients with more than 1 event of the

578 same coded term were counted only once per SOC and per preferred term.

579 **Table 4. TEAEs reported in >2 patients by System Organ Class (SOC)***
580

System Organ Class Preferred Term	FEXI 6.0 g (N=14)	FEXI 3.6 g (N=15)	FEXI 6.6 g (N=15)	All (N=44)
Any TEAE	**13 (92.9%)**	**15 (100.0%)**	**15 (100.0%)**	**43 (97.7%)**
Gastrointestinal disorders	**9 (64.3%)**	**10 (66.7%)**	**10 (66.7%)**	**29 (65.9%)**
Nausea	3 (21.4%)	8 (53.3%)	3 (20.0%)	14 (31.8%)
Abdominal pain	3 (21.4%)	3 (20.0%)	0 (0.0%)	6 (13.6%)
Dyspepsia	2 (14.3%)	2 (13.3%)	2 (13.3%)	6 (13.6%)
Abdominal pain upper	2 (14.3%)	1 (6.7%)	1 (6.7%)	4 (9.1%)
Flatulence	1 (7.1%)	2 (13.3%)	1 (6.7%)	4 (9.1%)
Vomiting	2 (14.3%)	2 (13.3%)	0 (0.0%)	4 (9.1%)
Diarrhoea	0 (0.0%)	1 (6.7%)	2 (13.3%)	3 (6.8%)
Toothache	1 (7.1%)	1 (6.7%)	1 (6.7%)	3 (6.8%)
Nervous system disorders	**6 (42.9%)**	**13 (86.7%)**	**10 (66.7%)**	**29 (65.9%)**
Headache	3 (21.4%)	13 (86.7%)	7 (46.7%)	23 (52.3%)
Dizziness	2 (14.3%)	1 (6.7%)	2 (13.3%)	5 (11.4%)
Paraesthesia	0 (0.0%)	2 (13.3%)	1 (6.7%)	3 (6.8%)
Musculoskeletal and connective tissue disorders	**5 (35.7%)**	**7 (46.7%)**	**4 (26.7%)**	**16 (36.4%)**
Back pain	2 (14.3%)	5 (33.3%)	3 (20.0%)	10 (22.7%)
Arthralgia	0 (0.0%)	4 (26.7%)	0 (0.0%)	4 (9.1%)
Pain in extremity	2 (14.3%)	2 (13.3%)	1 (6.7%)	5 (11.4%)
Infections and infestations	**7 (50.0%)**	**7 (46.7%)**	**5 (33.3%)**	**19 (43.2%)**
Nasopharyngitis	2 (14.3%)	2 (13.3%)	1 (6.7%)	5 (11.4%)
Psychiatric disorders	**3 (21.4%)**	**4 (26.7%)**	**6 (40.0%)**	**13 (29.5%)**
Insomnia	0 (0.0%)	1 (6.7%)	4 (26.7%)	5 (11.4%)
Nervousness	0 (0.0%)	3 (20.0%)	2 (13.3%)	5 (11.4%)
Anxiety	2 (14.3%)	1 (6.7%)	1 (6.7%)	4 (9.1%)
Restlessness	0 (0.0%)	1 (6.7%)	2 (13.3%)	3 (6.8%)
General disorders and administration site conditions	**2 (14.3%)**	**7 (46.7%)**	**4 (26.7%)**	**13 (29.5%)**
Asthenia	1 (7.1%)	2 (13.3%)	1 (6.7%)	4 (9.1%)
Chest pain	1 (7.1%)	2 (13.3%)	0 (0.0%)	3 (6.8%)
Fatigue	0 (0.0%)	1 (6.7%)	2 (13.3%)	3 (6.8%)
Skin and subcutaneous tissue disorders	**2 (14.3%)**	**6 (40.0%)**	**3 (20.0%)**	**11 (25.0%)**
Pruritus	1 (7.1%)	3 (20.0%)	3 (20.0%)	7 (15.9%)
Dry skin	1 (7.1%)	2 (13.3%)	0 (0.0%)	3 (6.8%)
Ear and labyrinth disorders	**1 (7.1%)**	**3 (20.0%)**	**1 (6.7%)**	**5 (11.4%)**
Vertigo	1 (7.1%)	3 (20.0%)	1 (6.7%)	5 (11.4%)
Blood and lymphatic system disorders	**1 (7.1%)**	**2 (13.3%)**	**2 (13.3%)**	**5 (11.4%)**

System Organ Class Preferred Term	FEXI 6.0 g (N=14)	FEXI 3.6 g (N=15)	FEXI 6.6 g (N=15)	All (N=44)
Iron deficiency anaemia	1 (7.1%)	2 (13.3%)	1 (6.7%)	4 (9.1%)

*Data are n (%). System organ classes with adverse events reported in more than two patients in any group were included. Patients with more than one event of the same type were counted only once per system organ class.

Figure Legends

Figure 1. Flowchart of patient disposition in the FEXI-12 study.

Figure 2. Mean parasite load at each visit by dosing regimen of fexinidazole: 6.0 g (blue line), 3.6 g (orange line) and 6.6 g (green line). One outlier was removed (extremely high value in the 6.6g arm at Day 3) to facilitate visual interpretation. EOT (end of treatment) was at day 8 for 3.6 g and day 15 for the 6.0 g and 6.6 g arms. Blood samples collected in triplicate per patient at the indicated times were extracted for DNA and parasite load quantified by real-time PCR of the *T. cruzi* satDNA sequence. Values show the mean satDNA copies/µL of the three samples drawn at each time point per patient.

Figure 1. Patient disposition

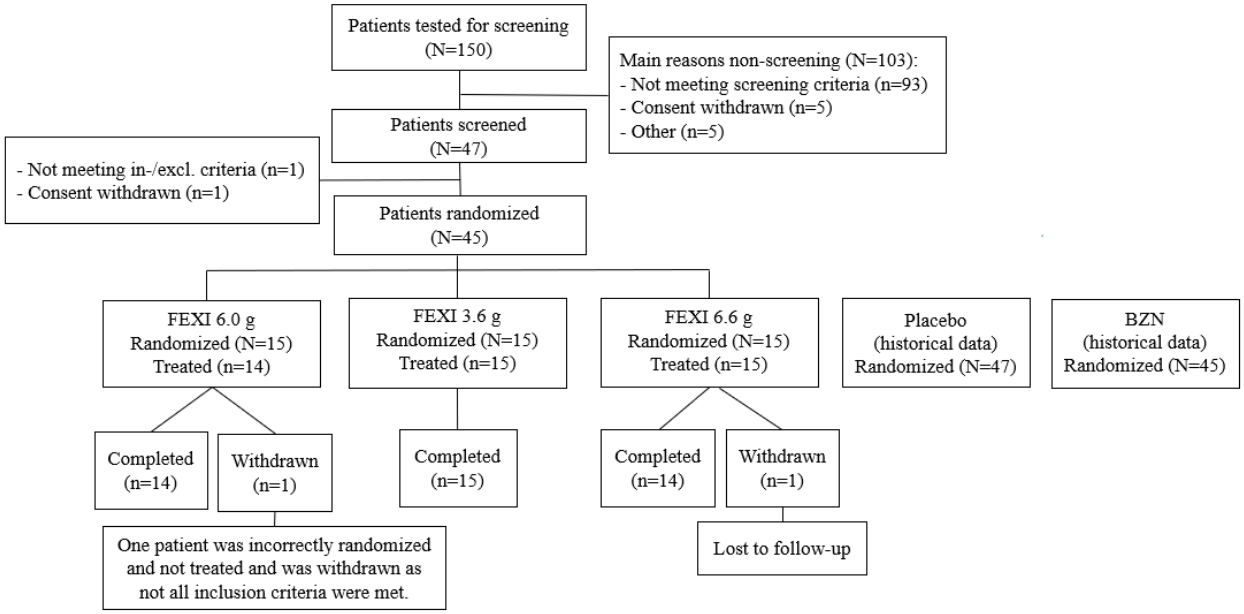
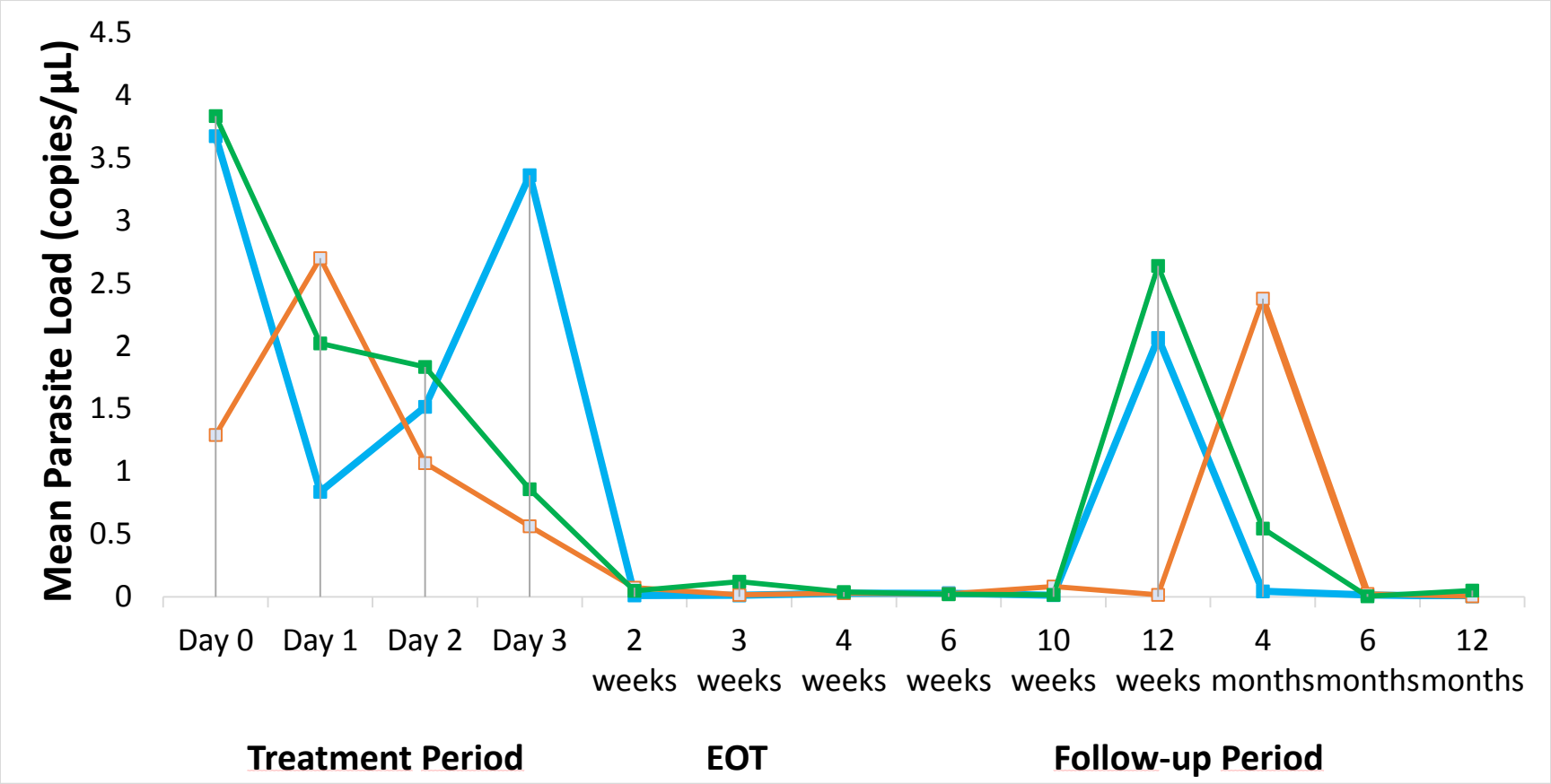


Figure 2. Mean parasite load by arm at each visit (ITT), FEXI-12 study



References

1. Chagas disease in Latin America: An epidemiological update based on 2010 estimates: World Health Organization, 2015.
2. Manne-Goehler J, Umeh CA, Montgomery SP, Wirtz VJ. Estimating the Burden of Chagas Disease in the United States. *PLoS neglected tropical diseases* 2016; **10**(11): e0005033.
3. Basile L, Jansa JM, Carlier Y, et al. Chagas disease in European countries: the challenge of a surveillance system. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2011; **16**(37).
4. Rassi A, Jr., Rassi A, Marin-Neto JA. Chagas disease. *Lancet (London, England)* 2010; **375**(9723): 1388-402.
5. Viotti R, Vigliano C, Bertocchi G, et al. Long-term outcomes of treating chronic Chagas disease with benznidazole versus no treatment. *Annals of internal medicine* 2006; **144**: 10.
6. Fabbro DL, Streiger ML, Arias ED, Bizai ML, del Barco M, Amicone NA. 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. *Revista da Sociedade Brasileira de Medicina Tropical* 2007; **40**: 1-10.
7. Fabbro DL, Danesi E, Olivera V, et al. Trypanocide treatment of women infected with Trypanosoma cruzi and its effect on preventing congenital Chagas. *PLoS neglected tropical diseases* 2014; **8**(11): e3312.
8. Sosa-Estani S, Cura E, Velazquez E, Yampotis C, Segura EL. Etiological treatment of young women infected with Trypanosoma cruzi, and prevention of congenital transmission. *Revista da Sociedade Brasileira de Medicina Tropical* 2009; **42**(5): 484-7.
9. Sosa Estani S, Segura EL, Ruiz AM, Velazquez E, Porcel BM, Yampotis C. Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas' disease. *The American journal of tropical medicine and hygiene* 1998; **59**(4): 526-9.
10. Alonso-Vega C, Billot C, Torrico F. Achievements and challenges upon the implementation of a program for national control of congenital Chagas in Bolivia: results 2004-2009. *PLoS neglected tropical diseases* 2013; **7**(7): e2304.
11. Moscatelli G, Moroni S, García-Bournissen F, et al. Prevention of congenital Chagas through treatment of girls and women of childbearing age. *Mem Inst Oswaldo Cruz* 2015; **110**(4): 507-9.
12. Kratz JM, Garcia Bournissen F, Forsyth CJ, Sosa-Estani S. Clinical and pharmacological profile of benznidazole for treatment of Chagas disease. *Expert review of clinical pharmacology* 2018; **11**(10): 943-57.
13. Cucunubá ZM, Manne-Goehler JM, Díaz D, et al. How universal is coverage and access to diagnosis and treatment for Chagas disease in Colombia? A health systems analysis. *Social science & medicine (1982)* 2017; **175**: 187-98.
14. Molina I, Gomez i Prat J, Salvador F, et al. Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. *The New England journal of medicine* 2014; **370**(20): 1899-908.
15. 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**(8): 939-47.

16. 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**(4): 419-30.
17. Apt W, Arribada A, Zulantay I, Rodríguez J, Saavedra M, Muñoz A. Treatment of Chagas' disease with itraconazole: electrocardiographic and parasitological conditions after 20 years of follow-up. *Journal of Antimicrobial Chemotherapy* 2013; **68**(9): 2164-9.
18. Rassi A, Luquetti AO, Rassi A, Jr., et al. Specific treatment for Trypanosoma cruzi: lack of efficacy of allopurinol in the human chronic phase of Chagas disease. *The American journal of tropical medicine and hygiene* 2007; **76**(1): 58-61.
19. Perez-Mazliah DE, Alvarez MG, Cooley G, et al. Sequential combined treatment with allopurinol and benznidazole in the chronic phase of Trypanosoma cruzi infection: a pilot study. *The Journal of antimicrobial chemotherapy* 2013; **68**(2): 424-37.
20. Torreele E, Bourdin Trunz B, Tweats D, et al. Fexinidazole--a new oral nitroimidazole drug candidate entering clinical development for the treatment of sleeping sickness. *PLoS neglected tropical diseases* 2010; **4**(12): e923.
21. Mesu V, Kalonji WM, Bardonneau C, et al. Oral fexinidazole for late-stage African Trypanosoma brucei gambiense trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. *Lancet (London, England)* 2018; **391**(10116): 144-54.
22. Bahia MT, Andrade IMd, Martins TAF, et al. Fexinidazole: A Potential New Drug Candidate for Chagas Disease. *PLoS neglected tropical diseases* 2012; **6**(11): e1870.
23. Torrico F, Gascón J, Ortiz L, et al. A phase-2, randomized, multicenter, placebo-controlled, proof-of-concept trial of oral fexinidazole in adults with chronic indeterminate Chagas disease. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2022.
24. Duffy T, Bisio M, Altcheh J, et al. Accurate real-time PCR strategy for monitoring bloodstream parasitic loads in chagas disease patients. *PLoS neglected tropical diseases* 2009; **3**(4): e419.
25. Bahia MT, Nascimento AF, Mazzeti AL, et al. Antitrypanosomal activity of fexinidazole metabolites, potential new drug candidates for Chagas disease. *Antimicrobial agents and chemotherapy* 2014; **58**(8): 4362-70.
26. Sánchez-Valdéz FJ, Padilla A, Wang W, Orr D, Tarleton RL. Spontaneous dormancy protects Trypanosoma cruzi during extended drug exposure. *eLife* 2018; **7**.
27. De Rycker M, Wyllie S, Horn D, Read KD, Gilbert IH. Anti-trypanosomatid drug discovery: progress and challenges. *Nature reviews Microbiology* 2023; **21**(1): 35-50.
28. Kratz JM, Gonçalves KR, Romera LM, et al. The translational challenge in Chagas disease drug development. *Mem Inst Oswaldo Cruz* 2022; **117**: e200501.
29. De Salazar PM, Sosa-Estani S, Salvador F, et al. Human Trypanosoma cruzi chronic infection leads to individual level steady-state parasitemia: Implications for drug-trial optimization in Chagas disease. *PLoS neglected tropical diseases* 2022; **16**(11): e0010828.

30. Torrico F, Gascon J, Barreira F, Blum B, Almeida I, Alonso-Vega C, Barboza T, Bilbe G, Correia E, Garcia W, Ortiz L, Parrado R, Ramirez JC, Ribeiro I, Strub-Wourgaft N. Randomized Trial of New Regimens of Benznidazole and Fosravuconazole for Chagas Disease: The BENDITA Study. *The Lancet Infectious Diseases* 2020.