Treatment of adult chronic indeterminate Chagas disease with benznidazole and three E1224 dosing regimens: a proof-of-concept, randomised, placebo-controlled trial

Faustino Torrico, Joaquim Gascon*, Lourdes Ortiz, Cristina Alonso-Vega, María-Jesús Pinazo, Alejandro Schijman, Igor C Almeida, Fabiana Alves, Nathalie Strub-Wourgaft, Isabela Ribeiro, on behalf of the E1224 Study Group†*

Summary

Background Chagas disease is a major neglected vector-borne disease. In this study, we investigated the safety and efficacy of three oral E1224 (a water-soluble ravuconazole prodrug) regimens and benznidazole versus placebo in adult chronic indeterminate Chagas disease.

Method In this proof-of-concept, double-blind, randomised phase 2 clinical trial, we recruited adults (18–50 years) with confirmed diagnosis of *Trypanosoma cruzi* infection from two outpatient units in Bolivia. Patients were randomised with a computer-generated randomisation list, which was stratified by centre and used a block size of ten. Patients were randomly assigned (1:1:1:1:1) to five oral treatment groups: high-dose E1224 (duration 8 weeks, total dose 4000 mg), low-dose E1224 (8 weeks, 2000 mg), short-dose E1224 (4 weeks + 4 weeks placebo, 2400 mg), benznidazole (60 days, 5 mg/kg per day), or placebo (8 weeks, E1224-matched tablets). Double-blinding was limited to the E1224 and placebo arms, and assessors were masked to all treatment allocations. The primary efficacy endpoint was parasitological response to E1224 at the end of treatment, assessed by PCR. The secondary efficacy endpoints were parasitological response to benznidazole at end of treatment, assessed by PCR; sustainability of parasitological response until 12 months; parasite clearance and changes in parasite load; incidence of conversion to negative response in conventional and non-conventional (antigen trypomastigote chemiluminescent ELISA [AT CL-ELISA]) serological response; changes in levels of biomarkers; and complete response. The primary analysis population consisted of all randomised patients by their assigned treatment arms. This trial is registered with ClinicalTrials.gov, number NCT01489228.

Findings Between July 19, 2011, and July 26, 2012, we screened 560 participants with confirmed Chagas disease, of whom 231 were enrolled and assigned to high-dose E1224 (n=45), low-dose E1224 (n=48), short-dose E1224 (n=46), benznidazole (n=45), or placebo (n=47). Parasite clearance was observed with E1224 during the treatment phase, but no sustained response was seen with low-dose and short-dose regimens, whereas 13 patients (29%, 95% CI 16·4–44·3) had sustained response with the high-dose regimen compared with four (9%, 2·4–20·4) in the placebo group (p<0·0001). Benznidazole had a rapid and sustained effect on parasite clearance, with 37 patients (82%, 67·9–92·0) with sustained response at 12-month follow-up. After 1 week of treatment, mean quantitative PCR repeated measurements showed a significant reduction in parasite load in all treatment arms versus placebo. Parasite levels in the low-dose and short-dose E1224 groups gradually returned to placebo levels. Both treatments were well tolerated. Reversible, dose-dependent liver enzyme increases were seen with E1224 and benznidazole. 187 (81%) participants developed treatment-emergent adverse events and six (3%) developed treatment-emergent serious adverse events. Treatment-emergent adverse events were headaches, nausea, pruritus, peripheral neuropathy, and hypersensitivity.

Interpretation E1224 is the first new chemical entity developed for Chagas disease in decades. E1224 displayed a transient, suppressive effect on parasite clearance, whereas benznidazole showed early and sustained efficacy until 12 months of follow-up. Despite PCR limitations, our results support increased diagnosis and access to benznidazole standard regimen, and provide a development roadmap for novel benznidazole regimens in monotherapy and in combinations with E1224.

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*Contributed equally

†Members listed at the end of the paper

Universidad Mayor de San Simón, Faculty of Medicine, Cochabamba, Bolivia (Prof F Torrico PhD); **Fundación CEADES, Cochabamba, Bolivia** (Prof F Torrico); **ISGlobal, Barcelona Centre for International Health Research, Barcelona, Spain** (J Gascon PhD, M-J Pinazo MD); **Universidad Autónoma Juan Misael Saracho de Tarija, Faculty of Medicine, Tarija, Bolivia** (L Ortiz MD); **Drugs for Neglected Diseases initiative, Geneva, Switzerland** (C Alonso-Vega MD, F Alves PhD, N Strub-Wourgaft MD, I Ribeiro MD); **Laboratorio de Biología Molecular de la Enfermedad de Chagas, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr Héctor N Torres"—INGEBI-CONICET, Buenos Aires, Argentina** (A Schijman PhD); **and Border Biomedical Research Center, Department of Biological Sciences, University of Texas at El Paso, El Paso, TX, USA** (I C Almeida DSc)

Correspondence to:
Dr Isabela Ribeiro, Drugs for Neglected Diseases initiative, 1202 Geneva, Switzerland
iribeiro@dndi.org

Introduction

Chagas disease is a major endemic vector-borne disease and global public health problem, with an estimated 70 million people at risk in Latin America and approximately 6 million people infected worldwide.[1] Substantial mortality and morbidity are observed in 20–30% of those chronically affected, with development of target organ involvement 10–30 years after initial infection.[2]

Clinical development in Chagas disease is fraught with difficulties relating to the evaluation of therapeutic response and long delays in showing clinical effects.[3]

Research in context

Evidence before this study

The only two medicines available for the treatment of Chagas disease—benznidazole and nifurtimox—are known to cause some toxicity, with insufficient data on therapeutic response, especially when used in adult patients with chronic Chagas disease. Novel antifungal triazole derivatives, including ravuconazole prodrug E1224, are alternative treatments for Chagas disease. Ravuconazole inhibits *Trypanosoma cruzi* ergosterol biosynthesis, which is essential for parasite growth and survival, and has pharmacokinetic properties suitable for treatment of disseminated intracellular infection. Before this study, no trials had been done on the activity of E1224 in human Chagas disease. Data were available on safety, tolerability, and pharmacokinetics in healthy volunteers, and from clinical trials of E1224 for the treatment of onychomycosis. We searched MEDLINE and the Cochrane CENTRAL register for articles published up to Dec 31, 2015, reporting randomised controlled trials of trypanocidal drug treatments of Chagas disease. This review was cross-referenced to published meta-analyses of randomised controlled trials and non-randomised studies of aetiological treatment of Chagas disease. We identified five randomised controlled trials with benznidazole, three of them placebo-controlled: two placebo-controlled trials in children with acute and early chronic Chagas disease and one placebo-controlled trial in adult chronic symptomatic Chagas disease. In the two placebo-controlled paediatric trials, benznidazole was superior to placebo in producing negative seroconversion of specific antibodies in children. The only published placebo-controlled trial in adults with advanced chronic cardiac Chagas disease concluded that benznidazole treatment did not affect the clinical progression of Chagas cardiomyopathy, despite significant reduction of circulating parasite load. Serological testing was not available in that study. To date, no other placebo-controlled randomised trials of benznidazole have been done in adult patients with chronic indeterminate Chagas disease, thus with no target organ involvement.

Added value of this study

E1224 is the first new chemical entity in decades to be evaluated for Chagas disease in a double-blind, placebo-controlled trial, in this case with benznidazole as a comparator. In addition, this study is, to the best of our knowledge, the first randomised controlled trial to evaluate a benznidazole regimen in rigorously assessed adult patients with chronic Chagas disease with no evidence of cardiac involvement, using (triplicate) PCR as the primary outcome. We also assessed other biological markers of therapeutic response, including conventional ELISA, trypanolytic anti-α-Gal antibodies, and conventional serological methods. The size, placebo-controlled design, and primary endpoints enabled demonstration at high significance ($p<0·0001$) that benznidazole is highly efficacious, with substantial early and sustained trypanocidal effect, corroborated by significant effect on reduction of titres of trypanolytic anti-α-Gal antibodies. E1224 displayed a transient, suppressive effect on parasite DNA clearance. Despite some known limitations of PCR in Chagas disease, our study found sensitivity to be sufficient as an early indicator of therapeutic response, provided that the technique is standardised and multiple samples and serial examination are used for increased sensitivity. In addition, our study corroborates the safety and pharmacokinetic–pharmacodynamic results of benznidazole and provides the first results of E1224 in human beings with Chagas disease.

Implications of all the available evidence

This study offers support for scaling up of diagnosis and access to standard regimens of benznidazole, and provides a roadmap for the development and registration of novel, alternative treatment regimens of benznidazole in monotherapy and in combinations with E1224 for the treatment of adults with chronic indeterminate Chagas disease.

The pharmacokinetic–pharmacodynamic relationships of treatments for Chagas disease are not fully understood[4] and, to date, no markers can adequately predict patients at risk of progression to chronic disease. During the chronic phase, diagnosis depends on serology, with varying degrees of sensitivity.[2,3,5] Despite the disease having been described over a century ago,[6] only two drugs are available for Chagas disease treatment, benznidazole and nifurtimox, and very little data are available from randomised, controlled studies on their use in adults with chronic indeterminate disease. These patients are believed to require prolonged treatment that is often associated with safety concerns.[7,8] Development of alternative treatments is urgently needed to improve disease morbidity. The generation of data that would help to fill existing scientific gaps and inform future drug development is paramount.

Ravuconazole is an ergosterol biosynthesis inhibitor with potent in-vitro and in-vivo activities against Chagas disease in animal models.[9–11] Encouraging data has raised hopes that E1224, a water-soluble ravuconazole prodrug, could be a priority candidate for clinical development in Chagas disease, and the first new chemical entity developed in over three decades. Overall, ravuconazole systemic exposures are substantially higher in human beings with E1224 prodrug administration leading to increased bioavailability and longer plasma terminal half-life. With a E1224 loading dose strategy, steady-state of ravuconazole is achieved within a week and allows for once-weekly dosing for maintenance of target plasma concentrations.

We present the results of a proof-of-concept randomised phase 2 study assessing three oral E1224 dosing regimens and benznidazole versus placebo for the treatment of adult chronic indeterminate Chagas disease.

Methods

Study design

The study was done in two outpatient units in Bolivia (Cochabamba and Tarija). The trial was a placebo-controlled, randomised, prospective, assessor-blind, comparative, dose-finding, and proof-of-concept study of superiority, testing five parallel groups, which received one of three different E1224 dose regimens, or placebo as the negative control, or benznidazole as the positive control for the treatment of Chagas disease in adults. Double-blinding was limited to the E1224 and placebo arms. Patients were enrolled randomly and equally into each of the five oral treatment groups, which were high-dose E1224 (8 weeks) of E1224 loading dose (400 mg once a day for days 1–3), followed by 400 mg once a week (starting on day 8) for 7 weeks (total dose 4000 mg); low-dose E1224 (8 weeks) of E1224 loading dose (200 mg once a day for days 1–3) plus placebo, followed by 200 mg E1224 and placebo once a week (starting on day 8) for 7 weeks (total dose 2000 mg); short-dose E1224 (4 weeks) of E1224 loading dose (400 mg once a day for days 1–3), followed by 400 mg once a week (starting on day 8) for 3 weeks, followed by placebo for 4 weeks (total dose 2400 mg); placebo (8 weeks) of four E1224-matched placebo tablets once a day for days 1–3 followed by four placebo tablets once a week (starting on day 8) for 7 weeks; and benznidazole (100 mg tablet), 5 mg/kg per day divided in two daily doses, for 60 days. E1224 was manufactured by Eisai (Toyko, Japan) and benznidazole by Laboratório do Estado de Pernambuco—LAFEPE (Recife, Brazil).

Patients who did not tolerate treatment were withdrawn and received standard nifurtimox treatment. After unblinding at the end of the study, patients in the placebo arm and those on E1224 with no parasitological clearance were offered benznidazole treatment, whereas patients allocated to receive benznidazole who showed no parasitological clearance were offered nifurtimox treatment.

This study was implemented in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki after the approval by the ethical committees of the participating institutions (Universidad Mayor San Simón, CEADES, and Hospital Clínic Barcelona).

Study participants

Participants eligible for randomisation were aged 18–50 years and weighed at least 40 kg. Women could not be pregnant or breastfeeding, and were required to use non-hormonal contraception for 4 months. Inclusion criteria were confirmed diagnosis of *Trypanosoma cruzi* infection by at least two of three conventional serological tests and serial positive qualitative PCR (at least one of three samples collected over 7 days). Included participants had a normal screening electrocardiogram (ECG), and no contraindication to study drugs or any azole. They had not received prior benznidazole or nifurtimox treatment, or systemic treatment with azoles, allopurinol, or any concomitant antimicrobial and immunosuppressant agents. Participants who had signs or symptoms of chronic Chagas disease, acute or chronic health conditions, abnormal screening laboratory tests, or history of alcohol abuse or other drug addiction were excluded.

The full study protocol with further details is available in the appendix. All patients were required to provide written informed consent before inclusion.

See Online for appendix

Randomisation and masking

A computer-generated randomisation list was prepared by an external provider. The list was stratified by centre and used a block size of ten. Treatment packages for the three E1224 regimen and placebo groups were prepared and labelled with numbers corresponding to the randomisation list. Placebo tablets were identical to E1224 tablets. Each centre received a list of randomisation numbers and the corresponding treatment packages. After confirmation that the patient met all entry criteria, the next available randomisation number in the corresponding centre (in chronological order) was assigned by the study pharmacist at each site, who then delivered the corresponding package for the masked arms of the study (E1224 or placebo) or prepared the package with an adequate number of tablets for the benznidazole arm and identified it with the patient's number. Study participants, investigators, and the medical and nursing team remained masked to treatment allocation. All parasitological, laboratory testing, and statistical analyses for all groups were done masked to treatment allocation.

Procedures

We chose the PCR assay method on the basis of the results of a multicentre study for the standardisation and laboratory validation of qualitative PCR testing for *T cruzi*.[12] A multiplex TaqMan real-time quantitative PCR assay, aiming to quantify *T cruzi* satellite DNA and an internal amplification control in a single-tube reaction, was further developed and validated. The method limit of detection is 0·6979 parasite equivalents (pEq)/mL and limit of quantification is 1·531 pEq/mL.[13] Each PCR experiment included positive and negative controls. External quality control panels for PCR were evaluated during the study period, using blinded seronegative blood samples spiked with serial dilutions of cultured *T cruzi*. PCR positivity was defined as a positive result in at least one of the replicates of the three different samples.[14]

For the serological diagnosis, we used two ELISAs, one based on recombinant antigen (Chagatest ELISA recombinante, Wiener Lab, Rosario, Argentina) and another on a crude antigen (Chagatest ELISA lisado, Wiener Lab). We also used a highly sensitive and specific chemiluminescent ELISA (antigen trypomastigote [AT] CL-ELISA), on the basis of the reactivity of sera to mucin glycoproteins purified from infective *T cruzi*

trypomastigote forms,[15–17] with titres calculated as previously described.[17]

We measured blood concentrations of ravuconazole and benznidazole on the first day of treatment (day 0, pre-dose), during steady state (days 3–59), at the end of treatment, and at the 4-month follow-up visit. We did pharmacokinetic sample analysis using liquid chromatography-electrospray ionisation-tandem mass spectrometry. The calibration curve was linear in the concentration range of 50–30 000 ng/mL for benznidazole and 50–20 000 ng/mL for ravuconazole (see appendix for details). Population pharmacokinetic parameters included area under the curve (AUC), maximum concentration (C_{max}), minimum concentration (C_{min}), clearance (C_L), volume of distribution (V_d), and plasma terminal half-life ($t_{1/2}$). We evaluated age, body-mass index, and baseline parasite load as covariates.

We evaluated safety through routine monitoring of adverse events. Patients were strictly monitored for liver and cardiac safety. If alanine aminotransferase (ALT) or aspartate aminotransferase (AST) exceeded three-to-eight times the upper limit of normal (ULN), treatment was continued if the patient was asymptomatic and did not show bilirubin elevation greater than twice the ULN. In case of any abnormality in QT interval corrected for heart rate (QTc), patients were re-dosed only following review of available ECGs. We did echocardiography or Holter monitoring on patients with cardiac adverse events or clinically significant ECG changes. Patients were withdrawn from the study based on specified liver and cardiac safety criteria. Safety was monitored by an independent data safety monitoring board and by cardiac safety experts on an ongoing basis.

Outcomes

Following expert consultation, we defined the primary efficacy endpoint as parasitological response at the end of treatment, determined by serial negative qualitative standardised PCR: three negative PCR results, from three samples of 10 mL collected over 7 days at the end of treatment. We used serial examination for increased sensitivity. The secondary efficacy endpoints were sustainability of parasitological clearance (negative qualitative PCR results at the end of treatment, and at 4, 6, and 12 months of follow-up); parasite clearance and changes in parasite load (measured by qualitative PCR and quantitative PCR [qPCR] on days 8, 15, 36, end of treatment, and at 4, 6, and 12 months of follow-up); incidence of conversion to negative response in conventional and non-conventional (lytic anti-α-Gal antibodies measured by AT CL-ELISA) serological response (assessed at end of treatment, and at 4, 6, and 12 months after treatment initiation); changes in levels of biomarkers, both alone and combined with parasite clearance; and complete response, defined as parasite clearance combined with consistent serological and biomarker response.

Safety endpoints were treatment-emergent adverse events, laboratory variables (mean change from baseline for each timepoint of days 2, 3, 15, 36, and 65), and ECG measurements (ventricular rate [VR], PR interval, RR interval, QRS interval, QT interval, QTcF, and QTcB at each timepoint).

Statistical analysis

The study was powered to provide evidence of superior efficacy of each of the E1224 regimens versus placebo and of benznidazole versus placebo as a secondary endpoint. 46 patients per treatment group were required for 90% power at a global 5% significance level (two-sided), if the proportion of patients with clearance of parasitaemia at the end of treatment in the E1224 treatment groups or benznidazole is 60%, and the proportion in the placebo group is 20%, with an estimated dropout rate of 15%.

The intention-to-treat (ITT) population comprised all randomised patients by their assigned treatment arms (primary analysis set) whereas the full analysis set (FAS) comprised all patients by their actual treatment arms. The per-protocol population was composed of all ITT patients without any major protocol deviations. A safety population was defined as all patients randomised and having received at least one dose of study therapy. We first did the primary efficacy analysis on the ITT population and secondarily on FAS and per-protocol populations as sensitivity analyses for the primary and secondary endpoints. The safety population was used for the safety analyses.

We used several missing data replacement strategies. In case of missing PCR evaluations, the following imputation rule was used. The outcome was imputed as success if and only if one of the following occurred: one unevaluable PCR result and two consecutive negative PCR results at day 65, two unevaluable PCR results and one negative PCR result at day 65, or three unevaluable PCR results at day 65 and three consecutive negative results at day 36. Patients with missing end of treatment results for any other reason were to be considered as failures for the primary analysis (IMPUT1). We used a second, more conservative, method of imputation on the ITT and FAS populations to assess the sensitivity of the results to the imputation of missing data: all patients with at least one missing PCR result at the end of treatment were analysed as failures (IMPUT2).

For demographics and baseline characteristics, we present descriptive statistics on both the per-protocol and ITT populations. Patient disposition and study discontinuations and their frequencies were tabulated.

For the analysis of the primary endpoint, we used a one-sided Fisher's exact test of the proportion of patients with serial negative qualitative standardised PCR on the ITT (primary analysis), FAS, and per-protocol (secondary analysis) populations to do pairwise comparisons

between treatment arms and placebo. We used Hochberg procedure[18] to keep the global type I error at the 2·5% level and to address the multiplicity issue. We did three sensitivity analyses: ITT with strategy IMPUT1, and FAS and per protocol with strategy IMPUT2.

We analysed secondary efficacy endpoints on the ITT and per-protocol as well as FAS (for most important endpoints) populations by treatment arm. We analysed sustainability of parasitological clearance relative to placebo at 12 months with Fisher's exact test. We used repeated measures model contrasts (dependent variable: parasite density at all available timepoints; treatment arm included as a fixed effect) to evaluate the reduction in parasite load over time at the end of treatment and at 4, 6, and 12 months of follow-up.

We evaluated the time to parasite clearance and the time to first relapse for patients who had cleared parasitaemia at the end of treatment using the Kaplan-Meier survival method with a log-rank test. All tests were done pairwise and we used a Hochberg procedure for the four comparisons of E1224 and benznidazole arms with placebo. We modelled time to first relapse with a proportional hazard Cox model and a backward stepwise procedure including age, sex, serological markers, and biomarkers. We transformed serology markers data using geometric mean ratios at each timepoint and used a repeated measure linear model.

We analysed safety in the safety population. We evaluated and tabulated numbers and percentages of patients with at least one reported adverse event and the number of events by treatment group for all treatment-emergent adverse events, all of those judged to be related to the treatment, and all of those leading to drug discontinuation; all treatment-emergent severe adverse events and all of those judged to be related to the treatment; and all serious adverse events and deaths. We calculated confidence intervals of proportions (exact two-sided 95%) for each system organ class and for preferred terms with a prevalence of more than 10% in any study arm.

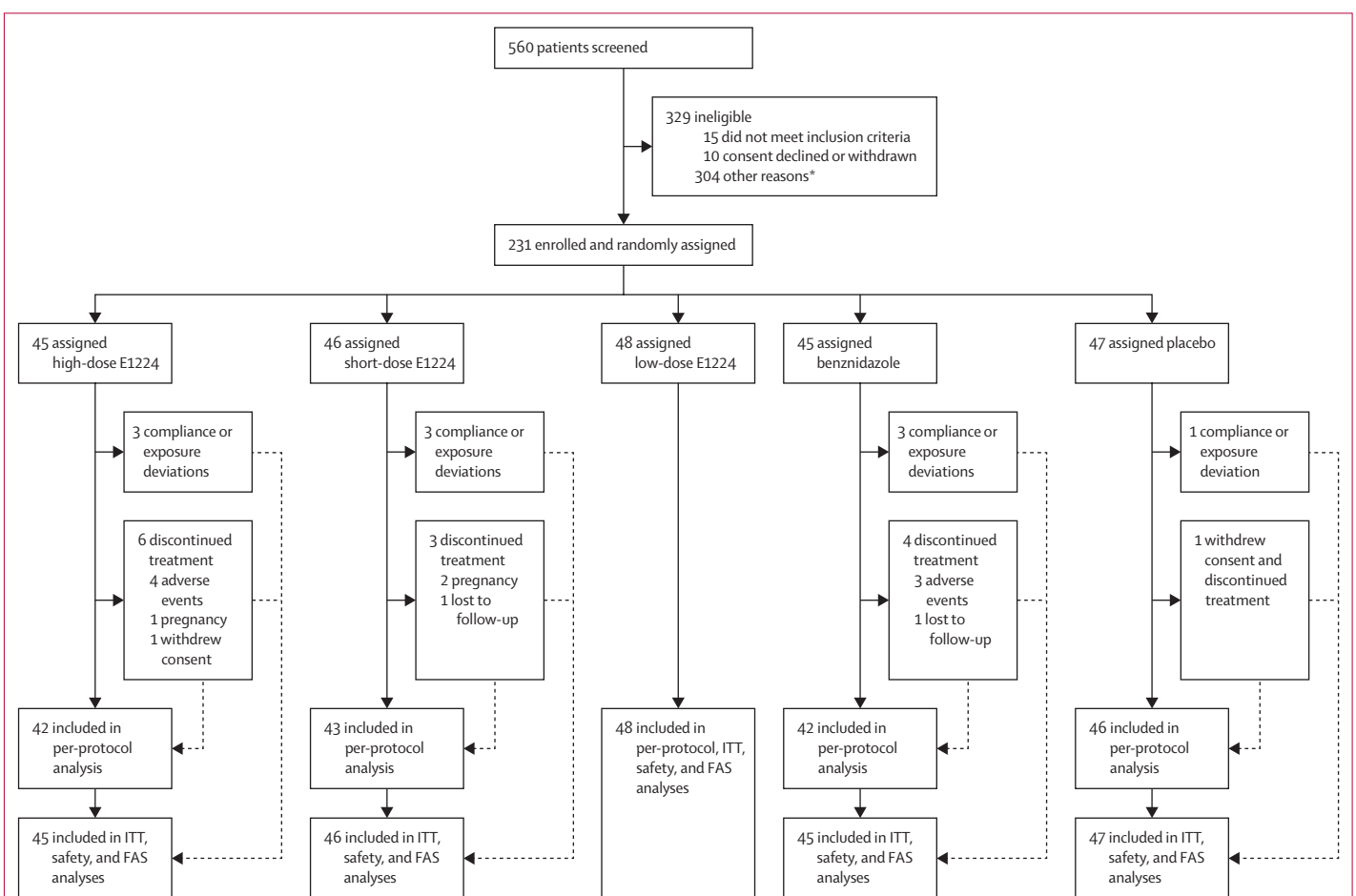

Figure 1: **Trial profile**
Compliance or exposure deviation defined as less than 80% of the planned cumulative dose. ITT=intention-to-treat. FAS=full analysis as treated set. *Other reasons were 46 patients with electrocardiogram abnormalities, one with digestive Chagas, 110 with laboratory abnormalities, 91 with negative PCRs, nine pregnancies, 24 with out-of-window visits, three with clinical conditions, and 20 patients with more than one reason (negative PCR, electrocardiogram or laboratory abnormalities, pregnancy, and weight).

	Placebo (n=47)	Low-dose E1224 (n=48)	Short-dose E1224 (n=46)	High-dose E1224 (n=45)	Benznidazole (n=45)	Total (n=231)
Age at screening, years	31·0 (9·1)	31·3 (8·7)	27·7 (8·3)	30·1 (8·8)	30·7 (9·0)	30·2 (8·8)
Male	10 (21%)	11 (23%)	11 (24%)	13 (29%)	14 (31%)	59 (26%)
Female	37 (79%)	37 (77%)	35 (76%)	32 (71%)	31 (69%)	172 (74%)
Conventional ELISA, optical density	2·4046 (0·4214)	2·2192 (0·5652)	2·3248 (0·5569)	2·2421 (0·5705)	2·1981 (0·6339)	2·2783 (0·5537)
Recombinant ELISA, optical density	2·9409 (0·1397)	2·8659 (0·3362)	2·9598 (0·1197)	2·9049 (0·3000)	2·8816 (0·3554)	2·9105 (0·2691)
Total white blood cells per µL	5538·3 (1352·2)	5235·4 (847·6)	5619·6 (1286·1)	5413·3 (1285·3)	5571·1 (1139·7)	5473·6 (1191·7)
Neutrophils, cells per µL	3134·8 (1003·2)	2849·2 (699·4)	3127·4 (901·5)	3038·8 (898·9)	3244·8 (1120·9)	3076·7 (934·3)
Lymphocytes, cells per µL	1988·6 (634·4)	2046·6 (487·0)	2058·5 (660·0)	2029·2 (597·9)	1998·9 (464·6)	2024·5 (569·7)
Aspartate transaminase, U/L	20·5 (4·7)	20·7 (3·3)	21·7 (5·0)	21·8 (4·5)	21·7 (4·0)	21·3 (4·3)
Alanine transaminase, U/L	21·0 (7·7)	21·7 (6·9)	21·5 (7·1)	24·0 (8·8)	23·7 (7·9)	22·4 (7·7)
GGT, U/L	0·680 (0·155)	0·646 (0·181)	0·676 (0·234)	0·706 (0·198)	0·689 (0·196)	0·679 (0·193)
Ventricular rate, beats per min	63·1 (6·9)	64·1 (6·5)	64·5 (8·7)	62·4 (7·4)	63·6 (9·0)	63·6 (7·7)
QT interval, ms	411·9 (27·7)	414·0 (21·6)	408·6 (26·1)	416·4 (25·4)	415·5 (28·0)	413·2 (25·8)
Quantitative PCR, pEq/mL	0·9539 (2·3714)	1·1921 (1·8874)	0·5853 (0·8722)	0·7521 (1·1655)	0·6170 (0·8315)	0·8251 (1·5640)

Data are mean (SD) or n (%). GGT=γ-glutamyltranspeptidase.

Table 1: **Baseline characteristics**

	Placebo (n=47)	Low-dose E1224 (n=48)	Short-dose E1224 (n=46)	High-dose E1224 (n=45)	Benznidazole (n=45)
Participants with parasite clearance at day 65, end of treatment	12 (26%, 14–40)	43 (90%, 77–96)	41 (89%, 76–96)	34 (76%, 60–87)	41 (91%, 79–98)
p value for comparison against placebo	..	<0·0001	<0·0001	<0·0001	<0·0001
Participants with sustainability of parasitological clearance until 12 months of follow-up	4 (9%, 2–20)	4 (8%, 2–20)	5 (11%, 4–24)	13 (29%, 16–44)	37 (82%, 68–92)
p value for comparison against placebo	..	0·6547	0·6547	0·0343	<0·0001
Time to first relapse, days					
Kaplan-Meier estimate of time to 50% reappearance	84·0 (55·0–not estimable)	111·0 (60·0–112·0)	56·0 (56·0–60·0)	287·0 (111·0–not estimable)	Not estimable (305·0–not estimable)
Log-rank test p value for comparison against placebo	..	0·302	0·203	0·412	<0·0001
Hazard ratio from Cox model adjusted on baseline qPCR*	..	1·17 (0·53–2·56)	1·68 (0·77–3·67)	0·60 (0·26–1·37)	0·06 (0·02–0·21)

Data in parentheses are 95% CI or %, 95% CI. qPCR=quantitative PCR. *Hazard ratio of parasitological relapse 1·10 (95% CI 1·03–1·16) for a higher parasite load (ie, doubling of parasite equivalents per µL) at baseline.

Table 2: **PCR assessment at end of treatment and until 12 months of follow-up**

We did exploratory post-hoc pairwise comparisons of the three E1224 regimens versus benznidazole for parasitological response (proportion of patients with sustained response at 12 months and parasite DNA load decrease at 12 months) using the same methods already described for the comparisons with placebo. We did post-hoc pairwise adjustment for multiplicity using the Tukey-Kramer method.

We analysed laboratory safety variables as mean changes from baseline with each timepoint up to day 65 (appendix). We described and analysed ECG outcomes (VR, PR interval, RR interval, QRS interval, QT interval, QTcF, and QTcB) as mean changes from the last pre-baseline ECG with each timepoint up to 4 months (appendix).

We did a population pharmacokinetic analysis on time-log-concentration data by using a two-compartmental model. Owing to sparse data, we used a model-based simulation to estimate total AUC and AUC at steady state. We created a pharmacokinetic–pharmacodynamic analysis dataset based on qPCR measurements obtained from patients treated with E1224. We imputed positive but not quantified PCR measurements by half the limit of quantification and negative PCR measurements by half the limit of detection.

We developed an empirical and a semimechanistic pharmacokinetic–pharmacodynamic model to describe the observed timecourse of (quantitative and qualitative) PCR and the proportion of patients with relapse within 1 year as a function of E1224 exposure (steady-state plasma concentrations) and treatment duration, respectively. Individual estimated pharmacokinetic parameters were based on a previous population-pharmacokinetic analysis. The semimechanistic model consisted of an indirect response component describing the parasite load based on exposure-dependent parasite proliferation rate and a constant parasite clearance, in combination with a mixture component categorising patients as sustained responders or relapsers.

Statistical tests were one-sided for analyses on the primary endpoint and two-sided for all other analyses. p<0·025 (one-sided) or p<0·05 (two-sided) was considered significant. We used SAS version 9.4 (SAS Institute, Cary, NC, USA) for all analyses.

An amendment of the study protocol (Feb 22, 2013) included an interim blinded efficacy analysis of sustained parasitological response of all patients at 6 months to allow administrative decisions of the sponsor by an independent statistician. The principal investigators (FT, JG, and LO) and study personnel remained unequivocally masked until database lock.

This trial is registered with ClinicalTrials.gov, number NCT01489228.

Role of the funding source

DNDi, a not-for-profit product development organisation, sponsored this study. DNDi received funding for this study from different sources, which had no role in study design, data collection, data analysis, data interpretation, or writing of the report. 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

Between July 19, 2011, and July 26, 2012, we screened 560 participants with confirmed Chagas disease, among whom 231 participants were eligible for randomisation. 45 participants were allocated to receive benznidazole, 45 high-dose E1224, 48 low-dose E1224, 46 short-dose E1224, and 47 placebo (figure 1). Follow-up was completed on June 13, 2013. During treatment, 14 (6%) patients discontinued treatment due to adverse events (n=7), consent withdrawal (n=2), loss to follow-up (n=2), or pregnancy (n=3; figure 1). We documented ten significant protocol deviations, resulting in a per-protocol population very close in number to the ITT and safety populations (figure 1). The only substantial protocol deviation was related to reduced compliance or exposure, defined as less than 80% of the planned cumulative dose (figure 1).

Treatment groups seemed well balanced in terms of age, sex, and baseline ECG values, serology, haematology, liver function, and parasite load (table 1). The total study population had a 4:1 female-to-male ratio. The primary endpoint, parasite DNA clearance at the end of treatment, was significantly different for all active treatment arms compared with placebo (p<0·0001), with the highest clearance observed in the benznidazole group (secondary endpoint; table 2). Analyses in the FAS and per-protocol populations obtained similar results, as did sensitivity analyses (appendix).

The proportions of patients achieving sustainability of parasite DNA clearance at 12 months in the E1224 short-dose and low-dose groups were similar to the placebo value (table 2). We found significant differences when comparing the high-dose E1224 and benznidazole groups with placebo, corrected for multiplicity (table 2).

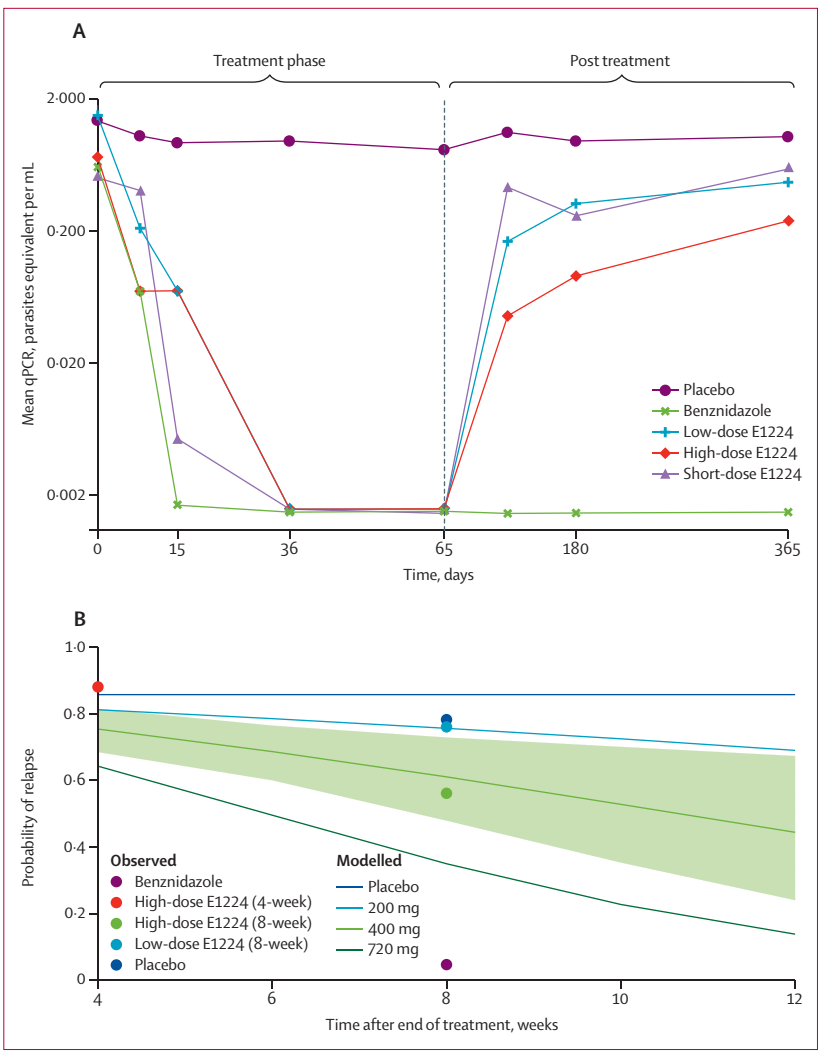

Figure 2: **Sequential qPCR measurements of *Trypanosoma cruzi* DNA and pharmacokinetic–pharmacodynamic model of predicted probability of relapse**

(A) The mean of qPCR-based responses was derived during the treatment phase and over time up to 12 months post treatment. (B) The continuous lines in the graph are model predictions of the probability of relapse using E1224 at different treatment regimens. For clarity, the 95% CI (shaded area) for these predictions is only shown for the high-dose E1224 cohort. Circles represent the observed population of patients with relapse. qPCR=quantitative PCR.

After 1 week of treatment, mean qPCR repeated measurements showed a significant reduction in parasite load in all treatment groups compared with placebo (figure 2; appendix). All benznidazole-treated patients cleared circulating parasite DNA after 2 weeks of treatment. After the end of treatment, the eight patients in the benznidazole group that had at least one positive PCR until 12 months post treatment (no sustained response) presented low parasite load. After end of treatment, parasite concentrations in the low-dose and short-dose E1224 groups gradually returned to placebo levels (figure 2). The parasite load in the high-dose E1224 group remained significantly lower than in the placebo group (figure 2, table 3), with no difference from the benznidazole group on adjusted post-hoc comparison (p=0·97, adjusted).

	Placebo (n=47)	Low-dose E1224 (n=48)	Short-dose E1224 (n=46)	High-dose E1224 (n=45)	Benznidazole (n=45)
Changes from baseline in parasite load by qPCR, geometric mean ratio					
Day 8 of treatment	1·30 (0·78 to 2·16)	0·17 (0·10 to 0·28)	0·09 (0·06 to 0·16)	0·13 (0·08 to 0·22)	0·10 (0·06 to 0·17)
Day 15 of treatment	0·81 (0·54 to 1·20)	0·11 (0·07 to 0·23)	0·07 (0·05 to 0·11)	0·09 (0·06 to 0·14)	0·07 (0·05 to 0·10)
Day 36 of treatment	0·77 (0·56 to 1·06)	0·06 (0·05 to 0·09)	0·07 (0·05 to 0·09)	0·07 (0·05 to ·009)	0·07 (0·05 to 0·09)
End of treatment, day 65	0·70 (0·49 to 0·96)	0·06 (0·05 to 0·09)	0·07 (0·05 to 0·09)	0·07 (0·05 to 0·09)	0·07 (0·05 to 0·09)
4-month follow-up	0·67 (0·40 to 1·12)	0·12 (0·07 to 0·20)	0·45 (0·27 to 0·76)	0·11 (0·06 to 0·18)	0·07 (0·04 to 0·11)
6-month follow-up	1·08 (0·61 to 1·92)	0·31 (0·18 to 0·55)	0·25 (0·14 to 0·46)	0·19 (0·11 to 0·36)	0·07 (0·04 to 0·12)
12-month follow-up	0·91 (0·50 to 1·66)	0·69 (0·38 to 1·23)	1·05 (0·58 to 1·92)	0·22 (0·12 to 0·42)	0·07 (0·04 to 0·12)
p value for comparison against placebo at 12-month follow-up*	..	0·499	0·744	0·0015	<0·0001
Changes from baseline in conventional ELISA, mean difference					
Day 36 of treatment	−0·05 (−0·17 to 0·08)	−0·03 (−0·15 to 0·09)	0·12 (−0·01 to 0·24)	−0·06 (−0·19 to 0·07)	−0·119 (−0·24 to 0·01)
End of treatment, day 65	−0·07 (−0·19 to 0·05)	−0·02 (−0·14 to 0·1041)	0·08 (−0·05 to 0·20)	−0·086 (−0·21 to 0·04)	−0·08 (−0·20 to 0·05)
4-month follow-up	−0·14 (−0·26 to −0·02)	−0·031 (−0·15 to 0·09)	−0·03 (−0·15 to 0·09)	0·018 (−0·11 to 0·15)	−0·05 (−0·18 to 0·08)
6-month follow-up	−0·03 (−0·15 to 0·08)	−0·042 (−0·15 to 0·07)	0·02 (−0·10 to 0·13)	−0·037 (−0·16 to 0·08)	−0·09 (−0·21 to 0·02)
12-month follow-up	−0·15 (−0·28 to −0·03)	−0·192 (−0·31 to −0·07)	−0·05 (−0·18 to 0·08)	−0·044 (−0·18 to 0·09)	−0·14 (−0·27 to −0·01)
p value for comparison against placebo at 12-month follow-up*	..	0·670	0·254	0·233	0·868
AT CL–ELISA					
Changes from baseline, mean difference					
Day 36 of treatment	0·04 (−0·02 to 0·11)	−0·00 (−0·06 to 0·06)	0·02 (−0·04 to 0·09)	−0·01 (−0·07 to 0·05)	0·05 (−0·01 to 0·11)
End of treatment, day 65	0·01 (−0·06 to 0·08)	−0·02 (−0·09 to 0·05)	−0·01 (−0·08 to 0·06)	−0·04 (−0·11 to 0·03)	0·02 (−0·05 to 0·09)
4-month follow-up	−0·01 (−0·07 to 0·05)	0·03 (−0·02 to 0·09)	0·02 (−0·05 to 0·08)	−0·03 (−0·09 to 0·03)	−0·01 (−0·07 to 0·05)
6-month follow-up	−0·02 (−0·09 to 0·05)	0·02 (−0·04 to 0·09)	−0·02 (−0·09 to 0·05)	−0·05 (−0·13 to 0·02)	0·02 (−0·05 to 0·09)
12-month follow-up	−0·02 (−0·08 to 0·05)	−0·02 (−0·09 to 0·04)	0·03 (−0·03 to 0·09)	0·04 (−0·02 to 0·11)	−0·01 (−0·07 to 0·05)
p value for comparison against placebo at 12-month follow-up*	..	0·835	0·321	0·192	0·894
Estimated geometric mean at 12 months	3·01 (2·60 to 3·47)	2·85 (2·47 to 3·27)	2·85 (2·46 to 3·29)	2·55 (2·19 to 2·96)	2·44 (2·11 to 2·83)
Estimated geometric mean ratio of treatment:placebo	..	0·95 (0·77 to 1·16)	0·95 (0·77 to 1·16)	0·85 (0·69 to 1·04)	0·81 (0·66 to 1·0)
p value for ratio to placebo*	..	0·593	0·596	0·121	0·049

Data in parentheses are 95% CIs. qPCR=quantitative PCR. AT CL-ELISA=antigen trypomastigote chemiluminescent ELISA. *Estimates from repeated measures linear model with treatment, baseline, sample, treatment by sample, and baseline by sample interactions.

Table 3: **Changes from baseline until 12 months of follow-up in parasite load by qPCR, conventional serology, and AT CL-ELISA**

In a stepwise Cox model, a lower risk of relapse was observed with benznidazole (hazard ratio 0·06, 95% CI 0·02–0·21) compared with placebo. An increased hazard of parasitological relapse (1·10, 1·03–1·16) was independently associated with a significantly higher baseline parasite load (table 3).

Among patients after 12 months of follow-up, the analyses of conventional serology showed no significant differences between active treatment and placebo at any timepoint (table 3). However, we found a small but significant reduction in the titres of trypanolytic anti-α-Gal antibodies, as measured by AT CL-ELISA, among benznidazole-treated patients versus placebo-treated patients at 12 months (table 3, figure 3). At the end of the study follow-up, five (11%) patients in the benznidazole group had a negative AT CL-ELISA result compared with two (4%) patients in the placebo group. Four (9%) of 44 benznidazole-treated patients negatively seroconverted for the AT CL-ELISA at the end of the follow-up. Two (4%) patients in the benznidazole group and one (2%) in the placebo group showed inconclusive results (0·9≤titre<1·0).

No deaths occurred during the trial. Overall, 187 (81%) participants developed treatment-emergent adverse events (table 4). The benznidazole treatment group had the highest proportion of treatment-emergent adverse events considered related to treatment (table 4). Nine participants, exclusively in the high-dose E1224 and benznidazole groups, experienced 14 treatment-emergent adverse events resulting in treatment suspension or discontinuation (table 4). Among these, six were of grade 3 or worse, and included ALT, AST, and γ-glutamyl transferase increases, and infective cholecystitis.

Six patients experienced serious adverse events, two of which occurred within 75 days from the start of treatment (one in the high-dose E1224 group and one in the benznidazole group; table 4). Three serious adverse events occurred in the high-dose E1224 group (infective

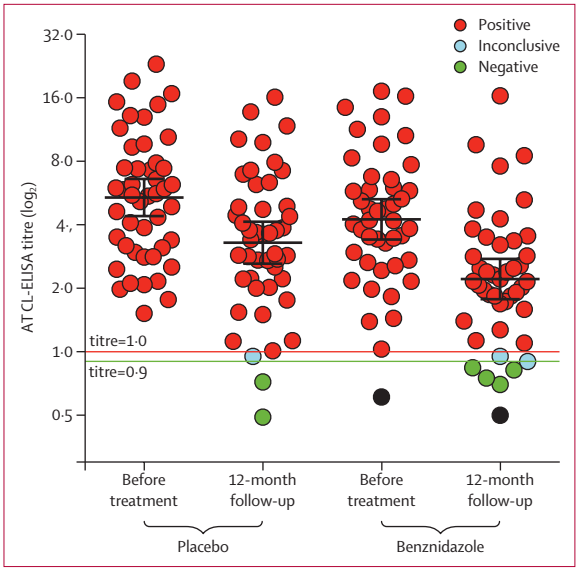

Figure 3: **AT CL-ELISA titres before treatment and after 12 months of follow-up in placebo-treated and benznidazole-treated patients.** Results are divided into positive (titre ≥1·0), negative (titre <0·9), and inconclusive (0·9≤titre<1·0). Black circles show serum samples from the same patient with negative AT CL-ELISA results at months 0 and 12. Only patients with serum samples at 0 and 12 months were considered for this analysis. The placebo group had 43 (93%) positive, two (4%) negative , and one (2%) inconclusive result. The benznidazole group had 37 (84%) positive, five (11%) negative, and two (4%) inconclusive. In each group, the geometric mean (long black horizontal line) is shown with 95% CI (short black horizontal lines). AT CL-ELISA= antigen trypomastigote chemiluminescent ELISA.

	Placebo (n=47)	Low-dose E1224 (n=48)	Short-dose E1224 (n=46)	High-dose E1224 (n=45)	Benznidazole (n=45)
Any treatment-emergent adverse events	38 (81%); 95 events	37 (77%); 116 events	40 (87%); 116 events	33 (73%); 131 events	39 (87%); 165 events
Judged related to treatment	14 (30%); 17 events	15 (31%); 28 events	24 (52%); 39 events	20 (44%); 48 events	29 (64%); 89 events
Resulting in treatment discontinuation	0	0	0	5 (11%); 8 events*	4 (9%); 6 events†
Any treatment emergent serious adverse events	0	0	1 (2%); 1 event	3 (7%); 3 events	2 (4%); 2 events
Judged related to treatment	0	0	0	2 (4%); 2 events	0

Data are number of patients (%); number of events. No deaths occurred. ALT=alanine aminotransferase. AST=aspartate aminotransferase. *One patient had infective cholecystitis that also showed increased ALT, AST, and γ-glutamyl transferase; two patients presented increased ALT and two patients presented increased AST. †Four patients had a total of five events of hypersensitivity; one patient also experienced an ALT increase.

Table 4: **Summary of adverse events**

cholecystitis and two spontaneous abortions), two in the benznidazole group (bronchitis and blighted ovum), and one in the short-dose E1224 group (appendicitis). The spontaneous abortions were considered possibly related to study treatment. All other serious adverse events recovered completely.

Among the most common adverse events, we observed a higher frequency in the benznidazole group compared with the E1224 groups of headaches (17 [38%] patients in the benznidazole group, 13 [27%] in the low-dose E1224 group, 16 [35%] in the short-dose E1224 group, 11 [24%] in the high-dose E1224 group, and eight [17%] in the placebo group); nausea (ten [22%] benznidazole, two [4%] low-dose E1224, three [7%] short-dose E1224, seven [16%] high-dose E1224, and four [9%] placebo); pruritus (nine [20%] benznidazole, two [4%] low-dose E1224, three [7%] short-dose E1224, and one [2%] placebo); peripheral neuropathy (five [11%] benznidazole, one [2%] low-dose E1224, and one [2%] placebo); and hypersensitivity (ten [22%] benznidazole, one [2%] high-dose E1224, and two [4%] placebo; appendix). Patients randomised to receive high-dose E1224 had more frequent treatment-related hepatic toxicity and diarrhoea. No hepatic safety signal was observed with E1224 at lower doses. ECG outcomes appeared comparable across treatment groups, with no clinically significant increases in QTcF during treatment.

Results showed that the E1224 loading schedule is appropriate to quickly reach steady state and stable trough ravuconazole concentrations, compatible with dosing simulations done on earlier human pharmacokinetic studies, and several times higher than published in-vitro minimal inhibitory concentrations against most strains of *T cruzi*. In the range of E1224 doses studied, peak and trough ravuconazole concentrations were proportional to dose. We observed no evidence of accumulation. Benznidazole pharmacokinetic results were compatible with previously reported pharmacokinetic studies.[19]

Exploratory evaluations indicated an exposure-dependent effect of E1224 on the dynamics of parasite load (figure 2). The pharmacokinetic–pharmacodynamic model confirmed that the predicted probability of relapse decreases with E1224 treatment duration and dose. Model-based simulations indicated that an increase in high-dose E1224 treatment duration would significantly reduce the probability of relapse from 61% (95% CI 48–73) with 8 weeks to 44% (24–67) with 12 weeks. Similarly, model-based calculations with the maximum observed average concentrations showed that if the E1224 dose were increased and treatment duration prolonged to, for instance, 12 weeks, the probability of relapse would fall below 20% (figure 2).

Discussion

Our proof-of-concept study generated key human pharmacokinetic and pharmacodynamic data for benznidazole, together with the first available data for E1224.

Following a long wait for novel Chagas disease drugs, we provide initial evidence that E1224 has manageable toxicity and shows antitrypanosomal activity during treatment. Disappointingly, but consistent with data on posaconazole,[20,21] sustained response after 12 months of follow-up was partial or incomplete after 8 weeks of treatment. Given this absence of a prolonged effect, E1224 will not be further investigated as monotherapy.

However, because of its favourable safety profile, combinations of E1224 with existing drugs, such as benznidazole or nifurtimox, should be considered, especially given that animal model studies show combination therapy has the potential to improve treatment response and shorten treatment duration.[22,23] Two spontaneous abortions were documented in the study, despite contraceptive measures. Such findings represent a known class effect of azole treatments and indicate the continued need for highly effective contraceptive methods during future clinical trials.

By contrast, benznidazole was found to have a rapid and sustained effect on parasite DNA clearance with parasite counts dropping significantly after 1 week of treatment and with 82% of patients with sustained response until 12 months of follow-up. This study provides the first placebo-controlled data on parasite DNA clearance and trypanolytic anti-α-Gal antibodies (AT CL-ELISA) in adult chronic indeterminate Chagas disease, with unique evidence on the dynamics of parasite DNA clearance during treatment and its correlation with exposure. Post-treatment follow-up in this study was limited to 12 months. Late parasite relapses post treatment with benznidazole in patients with advanced Chagas cardiomyopathy have been reported; however, such data are scarce in chronic indeterminate Chagas disease.[24] The pattern of treatment-related adverse events was similar to those observed in other studies.[8] These results, together with those seen for benznidazole in recently published studies,[20,21] support increased use of the existing treatment, and the evaluation of alternative regimens of benznidazole, in particular short-course and combination treatments.

Expert opinion has highlighted that aetiological treatment should be offered in adult chronic Chagas disease patients.[25,26] These results should be placed in context of recent placebo-controlled trial[24] data showing no clinical effect of treatment with benznidazole in patients with different stages of Chagas cardiomyopathy. This study was not sufficiently powered to show smaller effect (<20%) in cardiac outcome, but it is clear that existing strategies for antiparasitic chemotherapy need to be revisited for patients with chronic Chagas cardiac involvement. Similarly, early treatment intervention before established cardiac damage needs to be considered, as in our trial population that consisted largely of young adults.

Double-blind, placebo-controlled paediatric studies in early chronic *T cruzi* infection have shown that a 60-day benznidazole course was safe and effective in producing reduction of titres and negative seroconversion of specific antibodies (including trypanolytic anti-α-Gal antibodies), which had a key effect on treatment policy—based on serology rather than clinical benefit—and in justifying recommendation of treatment in seropositive children.[16,27] However, to date, in the short term, no significant changes in conventional serology have been documented in chronic infected adults. Quite importantly, our study in patients with indeterminate Chagas disease provides placebo-controlled data showing an effect of treatment with benznidazole on trypanolytic anti-α-Gal antibodies, as measured by a significant decrease in AT CL-ELISA titres at 12 months post treatment. 9% of treated patients negatively seroconverted for the AT CL-ELISA at the end of the follow-up compared with 4% of the placebo-treated group. Higher AT CL-ELISA negative seroconversion (58% at 3 years post treatment and 89% at 6 years post treatment, by per-protocol analysis) was observed in *T cruzi*-infected children treated with benznidazole.[16,28] Because the follow-up in our study was only for 12 months, we postulate that a higher number of negative seroconversions could have been detected with a longer follow-up period.[28]

Finally, due to an absence of early biomarkers of therapeutic efficacy,[29] the need for a long follow-up using conventional serology, and the inability to use the clinical symptoms for this purpose, PCR has emerged as a useful tool.[12] Our study found PCR sensitivity to be sufficient as an indicator of therapeutic response, provided that the technique is standardised and multiple samples and serial examination are used for increased sensitivity. Other studies have also shown that PCR is a useful tool for drug development, for revealing therapeutic failure on a short-term basis, and for the follow-up of patients after specific treatment.[5,13,30,31] However, a negative PCR does not exclude the presence of parasites in tissues or circulating in levels below those of detection, and long-term follow-up data correlating PCR with clinical benefit are needed.

In conclusion, this study provides information on the first new chemical entity to be developed for Chagas disease in over three decades. E1224 displayed a transient, suppressive effect, whereas benznidazole showed early and sustained efficacy by PCR and AT CL-ELISA. These results provide support to the scaling up of diagnosis and access to standard regimens of benznidazole, and provide a roadmap for the development and registration of novel, alternative treatment regimens of benznidazole in monotherapy and in combinations with E1224 for the treatment of adults with chronic indeterminate Chagas disease.

Contributors

IR, FT, JG, LO, NS-W, and FA conceived and designed the study. FT, JG, and LO were the principal investigators of the study. CA-V and M-JP coordinated the trial implementation in the study sites. IR and FA provided global coordination of trial activities. AS was responsible for the PCR method development and quality assurance. ICA was responsible for coordination of the AT CL-ELISA analyses. All authors participated in data acquisition, analysis, and interpretation, and drafting and critical revision of the manuscript for important intellectual content. All the authors have read and approved the final manuscript.

Declaration of interests

We declare no competing interests.

E1224 Study Group

Drugs for Neglected Diseases initiative, Geneva, Switzerland: Glaucia Santina, Bethania Blum, Erika Correia. ISGlobal: Luis Izquierdo,

Silvia Sanz Sender. Hospital Clinic Barcelona, Barcelona, Spain: Joan Carlos Reverter, Manuel Morales, Wladimiro Jimenez. CEADES—Platform for Comprehensive Care of Patients with Chagas Disease, Bolivia: Cochamba Roxana Challapa Quechover, Maria Yurly Escobar Caballero, Daniel Franz Lozano Beltrán, Helmut Ramón Magne Anzoleaga, Albert Mendoza Zalabar, Nilce Mendoza, Jimena Ramos Morales, Gimena Rojas Delgadillo, Lizeth Rojas Panozo, Jimy José Pinto Rocha, Dunia Torrico Terceros. Tarija Alejandro Palacios López, Letty Cardozo, Gabriela Cuellar, Violeta Alejandra Fernández Gálvez, Isabel Gonzales, Rudy Nelson Vasco Arenas. Molecular Biology Laboratory, Universidad Mayor de San Simón, Cochabamba, Bolivia: Lineth Garcia, Rudy Parrado, Anabelle de la Barra, Nair Montaño, Sandro Villarroel. Universidad Juan Misael Saracho, Tarija, Bolivia: Carlos Florencio Hoyos Delfin. INGEBI/CONICET, Buenos Aires, Argentina: Tomás Duffy, Margarita Bisio, Juan Carlos Ramirez. Eisai, Tokyo, Japan: Fred Duncanson, Michael Everson, Antonia Daniels, Makoto Asada. Hospital de Niños Ricardo Gutierrez, Buenos Aires, Argentina: Facundo Garcia-Bournisen (pharmacokinetics). Competence Center in Methodology and Statistics, Luxembourg: Michel Vaillant (statistical analyses). Quantitative Solutions, Breda, Netherlands: Eugene Cox, David Wesche, Paul Matthias Diderichsen. Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX, USA, and Universidade Federal de Minas Gerais, Instituto de Ciencias Biologicas, Departamento de Parasitologia, Pampulha, Belo Horizonte, Minas Gerais, Brazil: Alexandre F Marques (AT CL-ELISA).

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