Clinical Infectious Diseases

MAJOR ARTICLE

A Phase 2, Randomized, Multicenter, Placebo-Controlled, Proof-of-Concept Trial of Oral Fexinidazole in Adults With Chronic Indeterminate Chagas Disease

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Background. Chagas disease (CD) has significant global health impact, but safe, effective treatments remain elusive. The nitroimidazole fexinidazole is a potential treatment.

Methods. This double-blind, randomized, placebo-controlled, dose-finding, proof-of-concept study was conducted in Bolivia. Adults with serologically confirmed chronic indeterminate CD and positive PCR were randomly assigned to 1 of 6 fexinidazole regimens (1200 or 1800 mg/day for 2, 4, or 8 weeks) or placebo. Target recruitment was 20 patients/arm. The primary endpoint was sustained parasitological clearance by serial negative qPCR from end of treatment (EOT) until 6 months follow-up in the intention-to-treat (ITT) population. Follow-up was extended to 12 months.

Results. Enrollment was interrupted after 4/47 patients presented with transient asymptomatic grade 3 and 4 neutropenia. Treatment of ongoing patients was stopped in all patients administered >2 weeks. A total of 40 patients received treatment with fexinidazole from 3 days to 8 weeks. Delayed-onset neutropenia (n = 8) and increased liver enzymes (n = 8) were found in fexinidazole patients vs none in the placebo arm. In the ITT analysis, sustained parasitological clearance from EOT to 12 months follow-up varied between 66.7% (1200 mg–2 week) and 100.0% (1800 mg–2 week). Rapid, sustained clearance of parasitemia was observed in all treated patients with available data, but not in any patients in the placebo group, at 12 months ($P = .0056$). Further exploratory exposure-response analysis suggested low dosages of fexinidazole may be safe and effective.

Conclusions. Further evaluation is needed to establish fexinidazole's minimum effective dosage and risk–benefit relationship. Results suggest potential for effective treatment regimens <10 days.

Clinical Trials Registration. NCT02498782.

Keywords. Chagas disease; *Trypanosoma cruzi*; neglected tropical diseases; fexinidazole.

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is a neglected infection endemic to Latin America [1], where it causes a major health and economic burden, with an estimated 5.7 million people infected, 70 million at risk of infection, and more than 10 000 deaths annually [1, 2]. It is also a significant public health challenge in the United States and Europe. Morbidity and mortality from CD in Latin America exceed other parasitic infections, including malaria [1, 3]. It develops into a chronic condition with an indeterminate asymptomatic form, with 30–40% of infected individuals progressing to cardiomyopathy and/or gastrointestinal disease [4]. The only 2 medicines available, benznidazole and nifurtimox, have serious safety and tolerability concerns, particularly due to their prolonged treatment period (2–3 months) [5, 6].

Fexinidazole is a 2-substituted 5-nitroimidazole, broad-spectrum antiprotozoal drug that was not pursued into development [7]. The compound was "rediscovered" by the Drugs for Neglected Diseases *initiative* (DND*i*), after a systematic review and profiling of hundreds of nitroheterocyclic compounds [8]. As a candidate drug for sleeping sickness, fexinidazole's safety profile was extensively characterized in regulatory preclinical testing and early clinical development, showing a promising safety and efficacy profile [7–11]. In a phase II/III trial, fexinidazole proved safe and effective for treatment of sleeping sickness [12]. In animal testing [7, 13], fexinidazole

Received 15 March 2022; editorial decision 02 July 2022; published online 4 August 2022

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Clinical Infectious Diseases® 2023;76(3):e1186–e94

https://doi.org/10.1093/cid/ciac579

had dose-dependent cure rates in mice infected with different (including benznidazole-resistant) *T. cruzi* strains and was superior to benznidazole or nifurtimox. Fexinidazole has 2 metabolites: the sulfoxide M1 and the sulfone M2; both were as effective as fexinidazole in curing *T. cruzi* infection in mice. In vivo simulations for dose prediction suggested the need for a high dose and prolonged treatment courses, as with existing benznidazole regimens, to reach exposure levels efficacious and curative in mouse models [7, 13]. With the food effect observed in phase I trials for sleeping sickness [10], such an exposure could be obtained with doses no lower than 20 mg/kg.

Based on preclinical efficacy data and lack of genotoxicity in preclinical tests [9], a placebo-controlled proof-of-concept trial was initiated in Bolivia in adults with chronic indeterminate CD. While enrollment of 140 patients was initially planned, recruitment was interrupted after the inclusion of 47 patients due to emerging safety concerns. Consequently, treatment was halted but patients remained in the trial until completion of follow-up study visits. Here we present an evaluation of the pharmacokinetic (PK) basis of the risk–benefit of fexinidazole treatment for CD.

METHODS

Study Design and Participants

This was a double-blind, multicenter, randomized, placebo-controlled, prospective, dose-finding, proof-of-concept clinical trial of 6 regimens of fexinidazole treatment of adult chronic indeterminate CD, including population PK analyses and PK-pharmacodynamic assessments (study registration: ClinicalTrials.gov, NCT02498782). It was undertaken in Cochabamba and Tarija, Bolivia, where CD is prevalent and there is significant clinical expertise in CD management [14] and good clinical practice (GCP)–compliant trial investigations. The study obtained approval from the Ethical Committees of the Bolivian Foundation for Applied Science and Studies for Health and Environmental Development (CEADES), the Hospital Clinic of Barcelona, and Universidad Mayor de San Simon (UMSS). Regulatory approval was obtained from the Bolivian Agency of Medications and Health Technologies (AGEMED) on 15 April 2014.

The study enrolled adult patients with chronic indeterminate CD confirmed by serology and serial qualitative polymerase chain reaction (PCR) (see Supplementary Appendix 1, Trial Protocol).

Randomization and Masking

Eligible participants were randomly assigned to study treatment arms through an interactive web-based response system (IWRS) and a back-up telephone interactive voice response system (IVRS). Placebo tablets were identical to fexinidazole tablets.

Procedures

Patients received 1 of 7 oral treatment regimens: 2 fexinidazole doses (1200 and 1800 mg) for durations of 2, 4, and 8 weeks or a placebo treatment. All patients received 3 tablets of fexinidazole and/or placebo daily for the total of 8 weeks. The test drug was an oral tablet formulation (600 mg) of fexinidazole with the active pharmaceutical ingredient manufactured at Sanofi-Chinoin Pharmaceutical and Chemical Works Private Co. Ltd. (Budapest, Hungary).

Patient follow-up visits were planned for treatment days 1, 2, and 4 (±2-day window), at weekly intervals (±3 days) from week 2 to 10 (±3 days) and at 4 (±7 days), 6 (±7-day window), and 12 (+120 days) months post-treatment. Qualitative PCR and quantitative PCR (qPCR) tests were used as surrogate markers of therapeutic response. Detection of *T. cruzi* infection used qPCR, using an internationally validated analytical method [15–17]. Each PCR experiment included positive and negative controls. External quality-control panels for PCR were evaluated using blinded seronegative blood samples spiked with serial dilutions of cultured *T. cruzi*. PCR positivity was defined as a positive result in at least 1 of the replicates of the 3 different samples. For serological diagnosis, we used 2 enzyme-linked immunosorbent assays (ELISAs), one based on recombinant antigens (Chagatest ELISA recombinante; Wiener Lab, Rosario, Argentina) and another on crude antigens (Chagatest ELISA lisado; Wiener Lab).

Population PK analysis for fexinidazole, M1, and M2 included the parameters area under the curve (AUC), maximum concentration (C_{max}), minimum concentration (C_{min}), clearance (CL), volume of distribution (Vd) and plasma terminal half-life ($t_{1/2}$), with age, body mass index, and parasite load at baseline as covariates. Quantitative analysis of fexinidazole and metabolites was performed using the liquid chromatography–tandem mass spectrometry (LC/MS-MS) system (Supplementary Appendix 2).

We evaluated safety through routine monitoring of adverse events (AEs). All reported laboratory and safety assessments were performed at baseline and all follow-up visits. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE v 4.03). Patients who did not tolerate treatment were withdrawn. After unblinding at the end of the study, all patients were offered benznidazole treatment.

Efficacy and safety were monitored by an external independent data-monitoring committee (DMC) with cardiac, liver safety, and CD experts. A harm stopping rule was applied to safety criteria by the DMC, and a futility stopping rule was set for lower-than-expected efficacy (Supplementary Appendix 1). Due to multiple comparisons, the Hochberg procedure was applied.

Outcomes

The primary objective was to determine whether any of 6 dosing regimens of fexinidazole were efficacious and safe, compared with placebo, in eliminating *T. cruzi* parasitemia in

patients with chronic indeterminate CD. Elimination of *T. cruzi* was indicated by sustained conversion of a positive to a negative PCR result (3 negative qualitative [q]PCR) results from 3 samples per visit, from the end of treatment and sustained at each time point (2, 3, 4, 6, and 10 weeks, and 4, 6, and 12 months). Safety was determined as the incidence and severity of AEs. Secondary efficacy endpoints included parasite clearance at 2, 3, 4, 6, and 10 weeks and at 4, 6, and 12 months' follow-up, as measured by qPCR.

Statistical Analysis

We assumed the proportion of patients with sustained parasitemia clearance at 6 months follow-up of fexinidazole treatment would be 0.85 compared with 0.2 with placebo. Twenty patients per treatment arm was calculated, sufficient for 90% power at a global 5% significance level (2-sided) and an estimated drop-out rate of 8%.

The primary endpoint analyses are presented for the intention-to-treat (ITT) population. Secondary analyses were also conducted for the per-protocol (PP) and full analysis set (FAS) populations; efficacy data are described in patients with available PCR results and presented by treatment duration. For quantitative PCR analyses, descriptive statistics were presented by group and visit. Repeated-measures analysis evaluated sustained parasitological clearance at 6 and 12 months.

After finalization, a pooled analysis of AE results was conducted, including all subjects treated with any fexinidazole treatment regimen, to better understand the fexinidazole safety profile. Population PK were modeled using nonlinear mixed-effects with NONMEM VI software (Icon Development Solutions) (Supplementary Appendix 2). Post hoc exploratory analyses of clinically defined events relating to safety and exposure parameters were performed. These analyses assessed exposure–response relationships from the safety data set (all patients who received at least 1 dose of fexinidazole and/or placebo).

RESULTS

The trial commenced in July 2014. Recruitment was temporarily interrupted on 17 October 2014, and permanently on 11 December 2014. Forty-seven patients were randomized and 42 completed the 12-month follow-up (Figure 1). Baseline demographic and clinical characteristics (Table 1) did not significantly differ across trial arms.

Efficacy

All fexinidazole-treated patients with available data showed sustained parasite clearance at 6 and 12 months irrespective of treatment duration or dose (Table 2 and Figure 2). However, patients receiving placebo failed to show sustained clearance of parasitemia at 12 months. Notably, time to parasite DNA clearance measured by PCR negativization was less than 8 days in all fexinidazole-treated patients.

Safety

After the inclusion of 47 patients, 4 cases of grade III and IV neutropenia were identified around day 63 of the study. The cases were unblinded; all occurred in patients receiving more than 2 weeks of fexinidazole treatment. Recruitment was suspended and treatment interrupted in all patients with more than 2 weeks of treatment (blinded); all included patients were closely followed through 12 months. Recruitment was formally stopped afterwards, and no new patient was recruited.

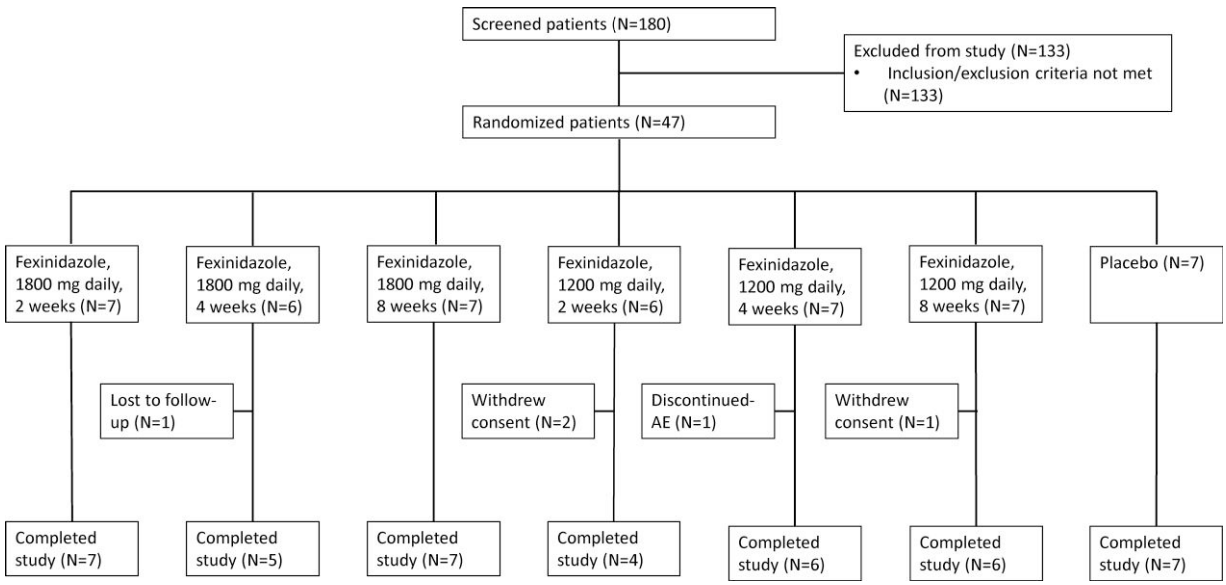

Figure 1. Trial profile. Abbreviation: AE, adverse event.

Table 1. Patient Baseline Clinical and Demographic Characteristics

	Fexinidazole Dosage						Placebo	Total	*P*
	1800 mg/d			1200 mg/d					
Treatment duration, weeks	8	4	2	8	4	2	8	…	…
n	7	6	7	7	7	6	7	47	…
Age, mean ± SD, y	36.9 ± 11.1	30.6 ± 6.68	34.8 ± 7.41	37.2 ± 10.5	33.9 ± 9.55	34.0 ± 9.24	31.1 ± 5.44	34.2 ± 8.56	.7540
Range	33.1–50.8	24.6–39.9	22.8–48	25–49.7	23–47.7	23.8–47.6	25.5–38.8	21.5–50.8	…
Males, n	3	1	3	2	0	3	2	14	.4472
Body weight, mean ± SD, kg	64.9 ± 8.73	66.7 ± 21.21	67.6 ± 6.63	65.0 ± 8.01	64.4 ± 9.71	71.9 ± 18.91	68.5 ± 11.85	66.9 ± 12.18	·9461
Hb, mean ± SD, g/dL	16.9 ± 1.8	15.3 ± 2.61	15.9 ± 1.2	15.6 ± 1.43	15.1 ± 1.04	16.4 ± 1.56	15.8 ± 1.24	15.9 ± 1.61	.4048
Neutrophils, mean, n/μL	3964	3309	2903.7	3392.4	3386.7	3511.5	3963.7	3493.6	.6007
Range	2810–6480	2650–4230	2110–4275	2250–5041	2640–4870	2304–5467	2090–6930	2090–6930	…
AST, mean ± SD, U/L	24.4 ± 7.04	23.5 ± 4.7	22.5 ± 3.28	22.3 ± 4.02	21.5 ± 3.7	24.7 ± 6.38	23.0 ± 4.38	22.5 ± 4.72	.8990
ALT, mean ± SD, U/L	23.8 ± 5.12	23.8 ± 7.84	22.7 ± 7.29	23.6 ± 6.85	22.8 ± 6.11	24.1 ± 8.54	24.2 ± 9.93	23.6 ± 7.01	.9996

Abbreviations: ALT, alanine transferase; AST, aspartate transferase; Hb, hemoglobin; SD, standard deviation.

There was 1 fatal, serious AE (SAE) in the fexinidazole arm: a case of severe depression leading to suicide. Twenty-four SAEs were reported, all in the fexinidazole arms (Table 3). Sixteen were assessed as treatment related. Of fexinidazole-treated patients, 10 of 40 (25%) stopped treatment permanently due to AEs. Adverse events by treatment group are listed in Supplementary Appendix 3.

Serious AEs of neutropenia and hepatic abnormalities occurred after treatment termination and formed the basis for exposure–response analysis (see below). Other AEs occurred mostly during treatment and time-to-event analyses were performed (data not shown). A total of 8 cases (8/40; 20%) of grade 3 (n = 6) and grade 4 (n = 2) neutropenia were reported. Nadir occurred in all patients around day 63 post-treatment, irrespective of date of treatment discontinuation. All cases were exposed to fexinidazole for longer than 14 days, remained asymptomatic, and resolved without specific treatment. A decrease in platelet counts was documented among fexinidazole-treated patients, although no patients presented clinically significant abnormalities. There was a significant association between decreased neutrophil and platelet count.

In 16 of the 47 (34%) recruited patients, serum levels of alanine transferase (ALT) and aspartate transferase (AST) were elevated (3 times the upper limit of normal). Six of these cases showed a pattern of acute hepatocellular liver injury (elevated ALT and AST, normal alkaline phosphatase [AP] levels, with a ratio of ALT or AST to AP of >5). Acute cholestatic liver injury (ALT or AST:AP of <2) or mixed liver injury (ALT or AST:AP of 2–5) was evident in 2 patients. Biological signs of chronic disease (persisting abnormalities beyond 3 months) were observed in 9 cases. The transaminase elevations were asymptomatic without development of signs of global liver dysfunction, and all cases resolved after 12 months of follow-up. No chronic liver safety finding was observed with cumulative fexinidazole doses of less than 12.6 g or in the placebo arm.

Many (30/40, 75%) fexinidazole-treated patients experienced a neuropsychiatric or nervous system AE, mostly between 2 and 7 days after beginning treatment. These included insomnia (n = 18), anxiety (n = 10), depression (n = 15), and neuropathy

Table 2. Number of Patients Attaining Efficacy Endpoints (Sustained Undetectable qPCR Values for Plasma Parasite DNA) for Each Treatment Group: Intention-to-Treat Population

	Fexinidazole Dosage						Placebo
	1800 mg/d			1200 mg/d			
Planned treatment duration, wks	8	4	2	8	4	2	…
Number of patients	7	6	7	7	7	6	7
Primary endpoint							
Patients with sustained response at 6 mo,[a] n (%)	5 (71.4)	5 (83.3)	7 (100)	5 (71.4)	5 (71.4)	4 (66.7)	0 (0)
Secondary endpoint							
Patients with sustained response at 12 mo, n (%)	5 (71.4)	5 (83.3)	7 (100)	5 (71.4)	5 (71.4)	4 (66.7)	0 (0)
Patients with missing qPCR data,[b] n (%)	2 (28.6)	1 (16.7)	0 (0)	2 (28.6)	2 (28.6)	2 (28.6)	0 (0)

Abbreviations: PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction.

[a]Defined as serial negative PCR results from end of treatment through 6 months' follow-up.

[b]A total of 9 fexinidazole-treated patients had missing qPCR samples post-treatment due to treatment discontinuation or consent withdrawal: 4 patients had an isolated qPCR sample missing (1 patient on day 8, 1 patient on day 15, 1 patient on day 22 and 1 patient on day 36); 5 patients had more than 1 sample missing (1 patient had no PCR samples after the screening, 2 patients had no qPCR samples from day 15 onwards, 1 patient had no PCR samples from day 64 onwards, and 1 patient had no samples from 4-month follow-up visit).

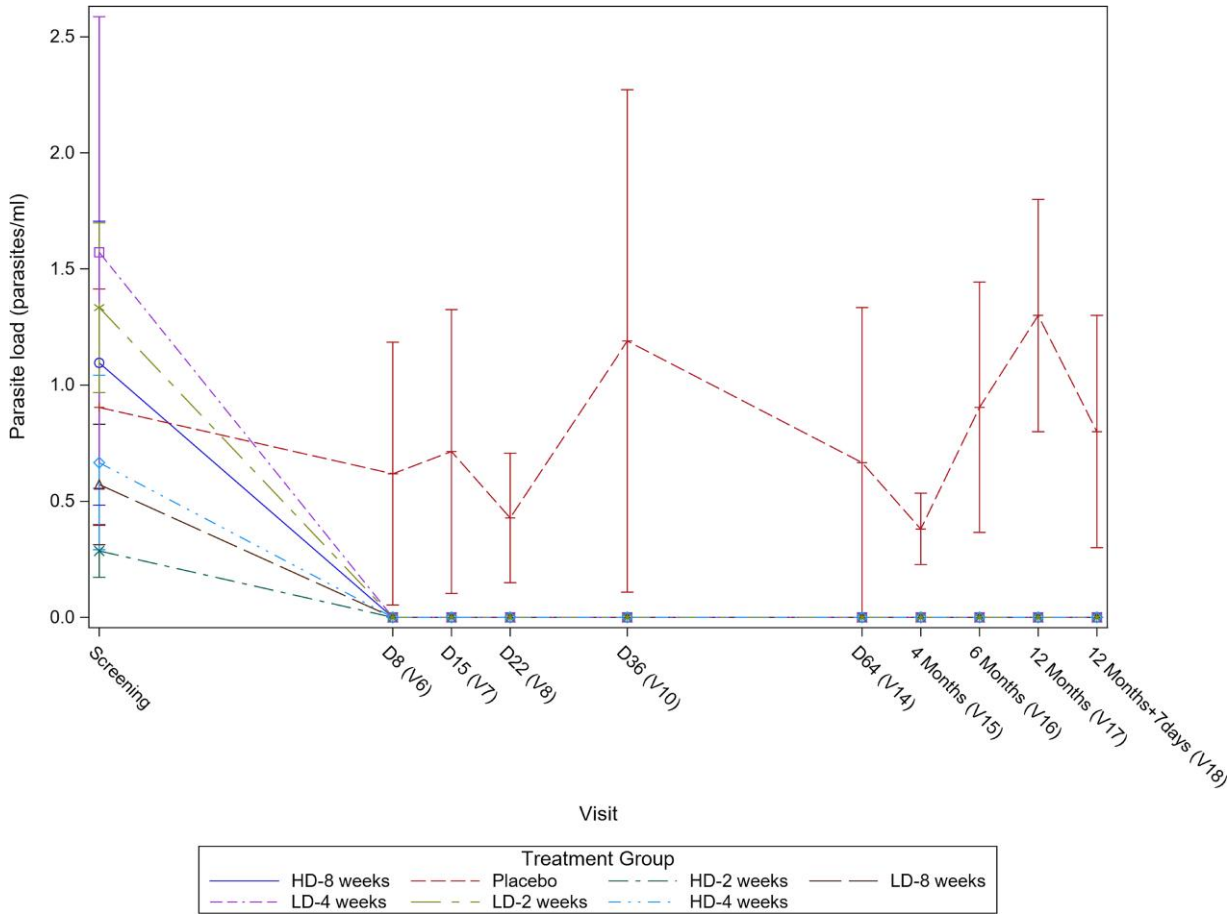

Figure 2. qPCR data for repeated measures of samples collected during 16 days after treatment start—fexinidazole- vs placebo-treated patients. Average values for each group during follow-up are indicated by the blue, purple, and green (solid or dashed) lines for the fexinidazole arms, and red dashed line for placebo. All the samples from the fexinidazole-treated patients provided qPCR values below the detection limit, indicating absence of *Trypanosoma cruzi* DNA. Abbreviations: D, day; HD, high dose (1800 mg daily); LD, low dose (1200 mg daily); qPCR, quantitative polymerase chain reaction; V, visit.

(n = 15). Time-to-event analyses (data not shown) revealed a trend towards earlier AE occurrence with the higher dose (1800 mg/day). No neuropsychiatric or nervous system AEs were reported in the placebo arm.

An exposure–response analysis was performed (Table 4). Patients experiencing at least 1 event of neutropenia (neutrophil count <1000/mm^3) were exposed to doses of 1200 mg or 1800 mg/day of fexinidazole between 14 and 56 days.

Table 3. Numbers of Adverse Events, Serious Adverse Event, Total Treatment Interruptions, and Treatment Interruptions Due to an Adverse Event

	High-Dose Fexinidazole			Low-Dose Fexinidazole			All Fexinidazole	Placebo	Total
Treatment duration, wks	8	4	2	8	4	2	…	8	…
Number of patients	7	6	7	7	7	6	40	7	47
Number of AEs	90	60	68	109	76	48	451	10	461
Number of patients with AEs (%)	7 (100)	6 (100)	7 (100)	7 (100)	7 (100)	6 (100)	40 (100)	3 (43)	43 (91.5)
Number of SAEs	6	2	2	10	4	0	24	0	24
Number of patients with SAEs (%)	4 (57)	2 (33)	2 (33)	5 (71)	2 (29)	0	15 (32)	0	15 (32)
Number of interruptions	…	…	…	…	…	…	…	…	…
Temporary	10	14	22	5	11	9	71	0	71
Number due to AE (%)	3 (42.9)	3 (50.0)	3 (42.9)	2 (28.6)	3 (42.9)	2 (33.3)	16 (34)	…	16 (34)
Permanent	4	1	1	4	9	0	19	0	19
Number due to AE (%)	2 (28.6)	1 (16.7)	1 (14.3)	4 (57.1)	2 (28.6)	0	10 (21.3)	0	10 (21.3)

Abbreviations: AE, adverse event; SAE, serious adverse event.

Table 4. Summary Estimate of Probability of Events According to Fexinidazole Regimen

Exposure	Neutrophil Count <1000 (Yes/No)	ALT >3× ULN (Yes/No)	AST >3× ULN (Yes/No)	Persistent ALT >1× ULN (Yes/No)	Persistent AST >1× ULN (Yes/No)	SAE (Yes/No)	Treatment-Related SAE (Yes/No)
Cumulative dose of fexinidazole							
Prob. 600 mg × 3 d	.000124	.02689	.04133	.004830	<.0001	.01856	.01187
Prob. 600 mg × 5 d	.000180	.03116	.04520	.005555	<.0001	.02313	.01446
Prob. 600 mg × 10 d	.000458	.04491	.05645	.007879	<.0001	.03990	.02361
Prob. 600 mg × 14 d	.000968	.05988	.06730	.01041	<.0001	.06118	.03481
Prob. 1200 mg × 3 d	.000217	.03354	.04727	.005958	<.0001	.02582	.01595
Prob. 1200 mg × 5 d	.000458	.04491	.05645	.007879	<.0001	.03990	.02361
Prob. 1200 mg × 10 d	.002967	.09128	.08722	.01579	<.0001	.1134	.06164
Prob. 1200 mg × 14 d[a]	.01312	.1557	.1220	.02740	<.0001	.2393	.1275
Prob. 1200 mg × 28 d[a]	.7145	.6070	.3401	.1681	<.0001	.8800	.7058
Prob. 1200 mg × 56 d[a]	1.0000	.9909	.8764	.9121	.02044	.9997	.9985
Prob. 1800 mg × 14 d[a]	.1543	.3479	.2111	.07015	<.0001	.6031	.3719
Prob. 1800 mg × 28 d[a]	.9979	.9283	.6566	.5915	.000017	.9942	.9752
Prob. 1800 mg × 56 d[a]	1.0000	.9999	.9899	.9981	1.0000	1.0000	1.0000
Duration of exposure to fexinidazole							
Prob. 3 d	.002052	.03054	.05424	.009075	<.0001	.03929	.01999
Prob. 5 d	.003921	.04440	.06581	.01206	<.0001	.06183	.03121
Prob. 10 d	.01957	.1093	.1054	.02442	<.0001	.1784	.09180
Prob. 14 d	.06815	.2107	.1510	.04256	<.0001	.3606	.2015
Prob. 28 d[a]	.8732	.8021	.4289	.2492	<.0001	.9408	.8611
Prob. 56 d[a]	1.0000	.9989	.9305	.9488	.9998	.9999	.9997

Abbreviations: ALT, alanine transferase; AST, aspartate transferase; Prob., probability; SAE, serious adverse event; ULN, upper limit of normal.

[a]Regimen tested in the present study.

Cumulative fexinidazole dose and cumulative plasma concentration before dosing (C_0) for fexinidazole, and metabolites M1 and M2, were found to be related to neutropenia events (positive slopes of 0.0003 and 0.033 [for M2], respectively, in logistic regression). The probability of experiencing a neutropenia event was estimated as negligible (≤1%) for dosing of fexinidazole for up to 14 days at 1200 mg/day, but at greater exposures, probability values were 15% or higher. The probability of a neutropenia event was estimated as less than 5% for up to 10 days of treatment, irrespective of dose. Similar estimates were obtained for elevated blood levels of the liver enzymes, ALT and AST, and SAEs.

Analysis and interpretation of population pharmacokinetics data were limited due to several factors: discontinuation of treatment reduced the sample size, duration of treatment varied from 2 to 66 days, and some patients interrupted and restarted treatment during the trial. A total of 245 samples were collected and analyzed. Median plasma fexinidazole concentration was 0.227 μg/mL on day 1 and ranged from 0 to 1.37 μg/mL during week 2 to 10. Median plasma M1 concentration was 4.68 μg/mL on day 1 and ranged from 0 below the limit of quantification (BLQ) to 6.92 μg/mL during weeks 1 to 10. Median plasma M2 concentration was 3.20 μg/mL on day 1 and ranged from 0 (BLQ) to 30.0 μg/mL during weeks 1 to 10. It was not possible to estimate all fixed model parameters with a sufficient precision because of large residual errors (44–55%) and intersubject variance. Pharmacokinetics of fexinidazole showed a limited variability, with a between-subject variance for clearance of 17% and 21% for apparent central volume of distribution.

DISCUSSION

All fexinidazole-treated patients had early and complete *T. cruzi* clearance, which was sustained for 12 months, despite some patients having brief treatment durations (<5 days in 9

patients, <7 days in 11 patients). This is noteworthy because, although research is limited, clearance within 8 days has not previously been reported in adults with chronic CD antiparasitic treatments. This is important in light of our exposure–response findings, which suggest that shorter treatment regimens and lower doses may improve safety outcomes.

The unexpected AEs recorded in this study indicate that the fexinidazole treatment regimens tested are not sufficiently safe for treating patients with chronic CD. Therefore, no further evaluation of these regimens should be pursued. However, efficacy findings and exposure–response analyses suggest that further proof-of-concept trials of fexinidazole at lower doses and shorter durations are warranted.

Unexpectedly, neutropenia occurred over the follow-up period, affecting 20% (8/40) of fexinidazole-treated patients. Fexinidazole trials for sleeping sickness did not show similar findings [12]. Although the mechanism of drug-induced neutropenia is unclear, the association with platelet decrease suggests a bone marrow suppression effect. Although an unexpected event, neutropenia has been reported in benznidazole-treated patients with CD [6, 18–20] and may be a nitroimidazole group effect associated with high doses. The total dose of fexinidazole administered to the cases of neutropenia in our trial was between 27 and 100.8 g, compared with maximal total doses in other human trials of 14.4 g in adults [10] and 10.8 g in children (recently registered trial: NCT02184689). A subsequent population PK analysis of clinical trials of fexinidazole indicated a dose-dependent relationship between fexinidazole and neutropenia, platelet count reductions, and increases in liver transaminases in patients with CD [21].

Experts who were consulted on the high rate of elevated liver transaminases opined that, given the degree of delay in onset, it was unlikely that a direct toxic effect of fexinidazole had occurred. An adaptive immune attack on the liver was considered more likely, as has been reported for other drugs, with a delayed onset of weeks or months post-treatment. A prerequisite for such a process is a liver cell stress reaction, with inherited or environmental factors for increased susceptibility to both *T. cruzi* infection and liver damage. No obvious signs of liver abnormalities have so far been reported in other patient trials of fexinidazole, but in healthy volunteers high and transient elevated transaminases were reported after 3600 mg/day for 14 days in 1 subject [10]. It may be a nitroimidazole group effect since their potential for liver toxicity is well documented [22–24], albeit at lower frequency from what we observed [25–27].

Other significant unexpected safety findings were high rates of anxiety, insomnia, and headache. Anxiety has been reported in fexinidazole trials, but our trial had a substantially higher rate. However, symptoms developed mostly during the first week of treatment and were of less than 7 days' duration. Two patients (including the reported patient depression SAE) required antidepressive treatment. There were several reports in our trial of transient digestive disturbances. Nervous system–related and gastrointestinal adverse effects are also reported in benznidazole-treated patients with CD [6, 19, 20], so these may be nitroimidazole group effects.

Several factors could explain the discrepant frequency and pattern of AEs in this trial compared with the clinical experience of fexinidazole in other indications. Patients with CD may have increased susceptibility to liver toxicity from fexinidazole. Release of damage-associated molecular patterns (DAMPs) and activation of Kupffer cells may be occurring in patients with CD independent of drug treatment due to liver involvement or secondary liver effects from systemic inflammation. Patients with certain diseases are more likely to develop adverse immune reactions to drugs (eg, sulfonamides in patients with human immunodeficiency virus [HIV]). Other host factors, such as the association between susceptibility to fexinidazole liver injury and specific HLA risk alleles, can be considered. Ultimately, however, the different treatment regimens of fexinidazole seem to be one of the determinant factors.

We assessed the possible relationship between fexinidazole exposure and various safety outcomes. For safety endpoints of delayed onset, cumulative dose and cumulative C_0 appeared to be the most relevant parameters—M2 exposure was significantly related to most safety endpoints. These data provide evidence on the safety of cumulative exposure to fexinidazole and M2 that could inform dose regimen determination in future studies.

Fexinidazole has high efficacy in chronic *T. cruzi* infection, even at the lowest tested dose, 1200 mg/day, and at less than 3 days of treatment. Treatment response at such short durations is unprecedented in adult patients with *T. cruzi* infection. Significant treatment-related safety concerns (increased liver enzymes, neutropenia) were associated with high doses administered for more than 14 days but were absent at low doses for short treatment durations. Further evaluation of fexinidazole in CD is warranted to establish the minimum effective dose and, through further investigations of safety, the risk–benefit balance. Current treatment regimens for CD are lengthy and involve frequent side effects in adults and older patients, contributing to low coverage of antiparasitic treatment for the over 6 million people with the disease. A treatment duration of less than 10 days would be a paradigm shift in CD therapeutics.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors dedicate this article to the memory of Dr Rodolfo Viotti, a leading Chagas expert and member of the Data Safety Monitoring Committee of this trial. They express their sincere and

profound thanks to the patients who took part in this trial and to the nurses and laboratory staff who contributed to its successful and safe execution. They thank their Data Safety Monitoring Board who reviewed the trial data and provided very valuable and timely responses and advice: Drs Oscar Noya (Chairperson), Rodolfo Viotti, Dominique Larrey, Jean-Louis Paillasseur, and Pascal Voiriot. They also thank Dr Frank Andersohn for expert review of the hematological toxicity findings and Dr Paul Watkins for review of liver safety findings. They extend their thanks to Dr Gerard McGregor, OmniScience SA, Geneva, who provided medical writing services on behalf of DND*i*, and to Drs Fabiana Alves, Susan Wells, Colin Forsyth, and Louise Burrows (DND*i*) for editing the manuscript. The authors are fully responsible for the contents and editorial decisions for this manuscript. They are grateful for the work and contribution of all the service providers (in particular, the teams from JSS Research, SGS Life Science Services, and CardiaBase by Banook Group). The data sets used and/or analyzed during the current study are available from the corresponding author, according to DND*i*'s institutional guidelines.

Financial support. This work was supported by the Drugs for Neglected Diseases *i*nitiative through funding by the following donors: Associação Bem-Te-Vi Diversidade, Brazil; UK aid, United Kingdom; Federal Ministry of Education and Research (BMBF) through KfW, Germany; Médecins Sans Frontières (MSF) International; Rockefeller Foundation, USA; Swiss Agency for Development and Cooperation (SDC), Switzerland; and other private foundations and individuals. The Platform for Comprehensive Care of Patients with Chagas disease in Bolivia is a collaborative project between CEADES; Universidad Mayor de San Simon in Cochabamba, Bolivia; Juan Misael University Saracho, Tarija, Bolivia; and Institut de Salut Global de Barcelona (ISGLOBAL; Barcelona Institute for Global Health, Spain), with the support of the National Chagas Control Program in Bolivia. The Platform is funded by the Spanish Agency for Cooperation and Development (Agencia Española de Cooperación Internacional para el Desarrollo-AECID; grant number 10-CO1-039). ISGLOBAL Research group receives funds from the Agència de Gestiód' Ajuts Universitarisi de Recerca (AGAUR; grant number 2017SGR924), and from Instituto de Salud Carlos III (Carlos III Health Institute), subprogram Redes temáticas de investigación cooperativa en salud (Thematic networks of collaborative health research) co-funded by the European Regional Development Fund (grant number RD16/0027/0004). DNDi, CEADES, and ISGLOBAL are members of the Ibero-American NHEPACHA network (New tools for patients with Chagas disease). DND*i*, a not-for-profit product development organization, sponsored this study. DND*i* 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 of the study data and had final responsibility for the decision to submit for publication.

Potential conflicts of interest. F. T. reports receiving technical and scientific support from ISGlobal relevant to the study. I. R. reports having unpaid roles on the scientific advisory boards of the University of Dundee Drug Discovery Unit for the Kinetoplastid portfolio and the Wellcome Centre for Anti-infective Research, and the Data Safety Monitoring Committee for a clinical trial of favipiravir for the treatment of coronavirus disease 2019 (COVID-19) (SAKURA). The Ministry of Science, Technology, and Innovation provided funding to A. G. S.'s institution (Consejo Nacional de Investigaciones en Ciencia y Tecnica [CONICET]-Argentina) for work on the manuscript, and A.G.S. reports receiving salary support from CONICET for work as a scientific researcher. J. G. reports receiving salary support for the work as a scientific researcher during the conduct of the study from Institut de Salut Global de Barcelona (ISGlobal).M. V. reports participation on a Data Safety Monitoring Board or Advisory Board for the University of Texas at El Paso and DND*i*. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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