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Benznidazole and Posaconazole in Eliminating Parasites in Asymptomatic *T. Cruzi* Carriers

The STOP-CHAGAS Trial

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ABSTRACT

BACKGROUND Benznidazole is recommended for treatment of Chagas infection. Effects of combination therapy with benznidazole and posaconazole have not been tested in *Trypanosoma cruzi* carriers.

OBJECTIVES The purpose of this study was to determine whether posaconazole alone or combined with benznidazole were superior to benznidazole monotherapy in eliminating *T. cruzi* parasites measured by real time polymerase chain reaction (RT-PCR) in asymptomatic Chagas carriers.

METHODS A prospective, multicenter randomized placebo-controlled study was conducted in 120 subjects from Latin America and Spain who were randomized to 4 groups: posaconazole 400 mg twice a day (b.i.d.); benznidazole 200 mg + placebo b.i.d.; benznidazole 200 mg b.i.d. + posaconazole 400 mg b.i.d.; or placebo 10 mg b.i.d. *T. cruzi* deoxyribonucleic acid was detected by RT-PCR at 30, 60, 90, 120, 150, 180, and 360 days. The primary efficacy outcome is the proportion of subjects with persistent negative RT-PCR by day 180; the secondary outcome was negative RT-PCR at 360 days.

RESULTS Only 13.3% of those receiving posaconazole and 10% receiving placebo achieved the primary outcome, compared with 80% receiving benznidazole + posaconazole and 86.7% receiving benznidazole monotherapy ($p < 0.0001$ vs. posaconazole/placebo). Posaconazole monotherapy or posaconazole combined with benznidazole achieved high RT-PCR conversion rates during treatment (30 days; 93.3% and 88.9% and 60 days; 90%, and 92.3%) that were similar to benznidazole (89.7% and 89.3%); all were superior to placebo or posaconazole (10% and 16.7%, $p < 0.0001$). This was not observed at 360 days; benznidazole + posaconazole and benznidazole monotherapy (both 96%) versus placebo (17%) and posaconazole (16%, $p < 0.0001$). Serious adverse events were rare (6 patients) and were observed in the benznidazole-treated patients. Permanent discontinuation was reported in 19 patients (31.7%) receiving either benznidazole monotherapy or combined with posaconazole.

CONCLUSIONS Posaconazole demonstrated trypanostatic activity during treatment, but it is ineffective long-term in asymptomatic *T. cruzi* carriers. Benznidazole monotherapy is superior to posaconazole, with high RT-PCR conversion rates sustained at 1 year. Side effects lead to therapy discontinuation in 32%. No advantages were observed with combined therapy versus benznidazole monotherapy. (A Study of the Use of Oral Posaconazole [POS] in the Treatment of Asymptomatic Chronic Chagas Disease [P05267] [STOP CHAGAS]: NCT01377480) (J Am Coll Cardiol 2017;69:939–47)

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ABBREVIATIONS AND ACRONYMS

b.i.d. = twice a day

BNZ = benznidazole

CI = confidence interval

DNA = deoxyribonucleic acid

POS = posaconazole

RT-PCR = real-time polymerase chain reaction

Chagas disease is due to infection with *Trypanosoma cruzi* and carries a significant tropical disease burden in the Western hemisphere (1,2). It is estimated that between 5.7 and 9.4 million people are infected, of whom 20% to 30% will develop cardiomyopathy (3). Recent estimates project that 200,000 people with Chagas disease will die from cardiovascular complications in the next 5 years (4). However, other projections provide lower estimates of 12,500 deaths annually, highlighting the uncertainty surrounding precise prevalence estimates (3,4). The Global Burden of Disease Study, performed between 1990 and 2013, estimated a decrease of potential years of life lost from 343,000 to 245,000 years (5).

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Current treatments reportedly have less efficacy in adults than in younger individuals, and low tolerability often compromises treatment (3). Determining treatment response is challenging, due to the long-lasting antibody response, but the real-time polymerase chain reaction (RT-PCR) has recently been developed as an alternative method to document infection and monitor response to therapy (6–8). Recently, the BENEFIT (Benznidazole Evaluation for Interrupting Trypanosomiasis) trial reported modest and nonsustained trypanocidal activity in subjects with established cardiomyopathy, further supporting the need for more effective treatment strategies (9).

Benznidazole (BNZ) and nifurtimox are the only available therapies for treating *T. cruzi* infection, but whether adult patients with long-standing *T. cruzi* infection should be treated is not clear, given the limited clinical evidence and the lack of reliable biomarkers of therapeutic efficacy (10,11). Studies of combination therapy with BNZ and ketoconazole in a murine model of acute *T. cruzi* infection demonstrated a substantially higher response rate with combination therapy than with monotherapy with either agent, suggesting possible synergy of combination therapy, but this has not been evaluated in humans (12). Posaconazole (POS) is considered a good candidate for treatment of the intracellular amastigote form of *T. cruzi*, given its mechanism of action and favorable pharmacokinetic profile (13,14). A recent trial assessing the efficacy of 2 doses of POS (100 mg twice a day [b.i.d.] and 400 mg b.i.d.) alone showed high rates of treatment failure compared with BNZ (5 mg/kg/day), but combination therapy was not tested (15).

We designed the STOP-CHAGAS (Study of Oral Posaconazole in the Treatment of Asymptomatic Chronic Chagas Disease) trial to evaluate the efficacy and safety of POS monotherapy and POS in combination with BNZ, given orally for 60 days to eliminate parasitemia, as measured by RT-PCR, in subjects with chronic indeterminate Chagas infection.

METHODS

STUDY DESIGN. We conducted the STOP-CHAGAS trial, in which POS was given in a randomized single-blinded fashion, whereas BNZ was given as an open-label treatment, in 19 centers from 5 countries in Latin America and Spain. The trial was designed by a steering committee and data was managed, analyzed, and coordinated by the Population Health Research Institute at McMaster University. The study protocol was designed and registered in 2011, when RT-PCR-based efficacy estimates for BNZ were limited. The protocol was approved by the national and institutional review board or ethics committees of each recruiting center (8 in Argentina, 1 in Chile, 3 in Colombia, 3 in Guatemala, 1 in Mexico, and 3 in Spain). All patients enrolled provided written informed consent. Full details of the protocol are provided in the Online Appendix.

STUDY POPULATION. Treatment was administered for 60 days. Eligible patients were ≥18 and ≤50 years of age, weighing >60 kg, with evidence of *T. cruzi* infection given by a positive serology result (2 of 3 conventional tests) and duplicate positive RT-PCR for *T. cruzi*. All subjects required a normal 12-lead electrocardiogram, 2-dimensional echocardiogram, and a 24-h Holter monitor, with no evidence of

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rhythm disorders, including nonsustained ventricular tachycardia (detailed information is provided in Online Table 1). Patients were randomly assigned to 1 of 4 groups: 1) POS 400 mg b.i.d.; 2) BNZ 200 mg + placebo b.i.d.; 3) BNZ 200 mg b.i.d. + POS 400 mg b.i.d.; or 4) placebo 10 mg b.i.d.

STUDY PROCEDURES. All patients were followed at days 15, 30, 45, and 60 (end of treatment) and at days 90, 120, 150, 180, and 360 after randomization. All patients underwent 12-lead electrocardiogram and liver function tests at baseline and during each follow-up visit during the 60-day treatment period. RT-PCR samples to detect *T. cruzi* deoxyribonucleic acid DNA were collected at baseline, and at 30, 60, 90, 120, 150, 180, and 360 days. Blood samples to assess pharmacokinetics of POS were obtained to determine drug adherence and levels at treatment visits on days 30 and 60. Full details of the study protocol and procedures are provided in the Online Appendix.

POLYMERASE CHAIN REACTION METHODOLOGY. Blood samples were blindly analyzed by the Translational Medicine Biomarker group at Merck (Rahway, New Jersey). Before DNA extraction, 5 ml of the whole blood specimens collected in PAXgene DNA tubes (Qiagen, Hilden, Germany) were first lysed with 5 ml of ZR Viral DNA Buffer from Zymo Research (Irvine, California). TaqMan-based (Beckman Coulter, Brea, California) quantitative PCR assay was custom-designed in-house to detect and quantify minicircle kinetoplast DNA from 2 strains of *T. cruzi*, CL-Brener and K98, representing the 2 lineages of *T. cruzi*. For RT-PCR detection of *T. cruzi* kinetoplast DNA, each DNA sample was analyzed 3 times with 3 PCR runs, with 4 technical replicates per RT-PCR run. Only samples with 0 or 1 positive amplification signal for the 12 RT-PCR reactions were considered negative (16).

PHARMACOKINETIC SAMPLING. POS plasma trough concentrations were measured pre-dose on days 30 and 60. As the study design was blinded to the assignment of POS therapy, samples were collected on all subjects and analyzed for POS plasma concentration. The pharmacokinetic evaluation and target range are summarized in the Online Appendix.

STUDY OUTCOMES. The primary efficacy outcome is the proportion of subjects with persistent negative RT-PCR by day 180 in all treatment groups. Secondary outcomes included the long-term sustainability of response by RT-PCR at the end of follow-up (day 360), and safety and tolerability of POS versus placebo, and of monotherapy or combined therapy of either BNZ or POS. A successful response is defined by both a negative RT-PCR on day 180 (or day 150 if day 180 was missing), and on at least the 2 preceding samples.

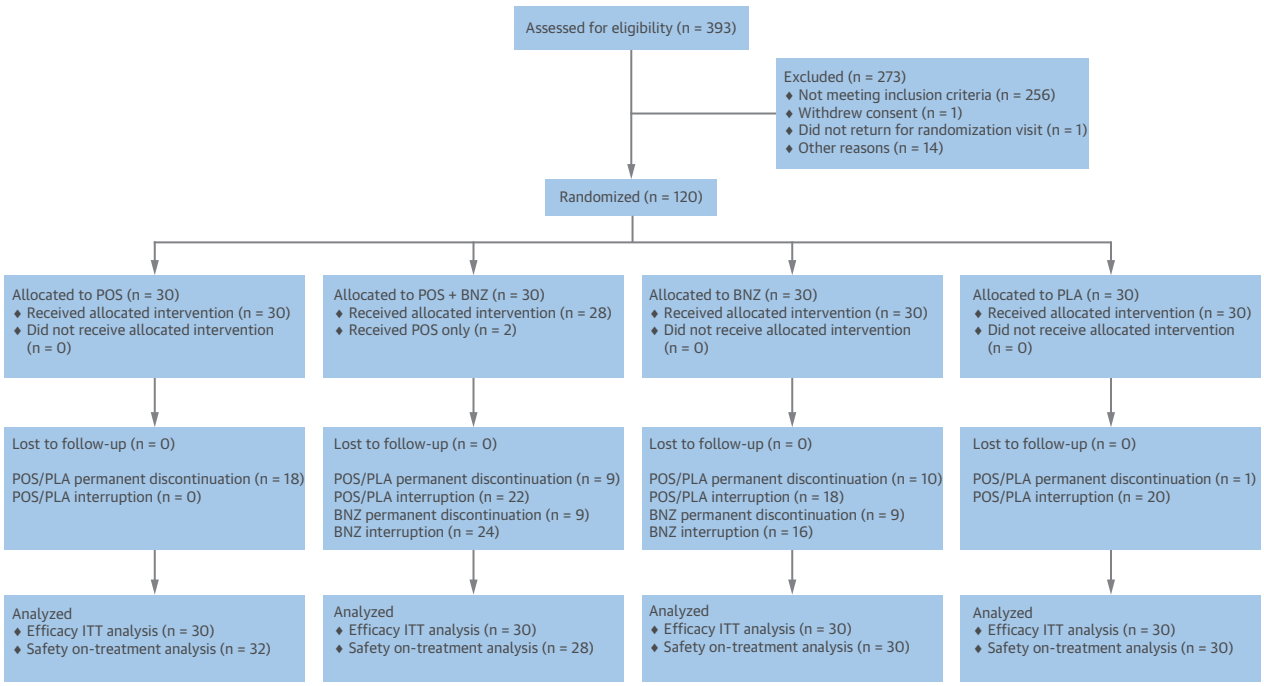
STATISTICAL ANALYSIS. The primary efficacy outcome analyses were performed on all randomized subjects in their assigned treatment arms (intention-to-treat) and were defined as the proportion of subjects responding on the basis of RT-PCR conversion (negative) on day 180 (or day 150 if the previous sample was missing), in addition to a negative RT-PCR in at least 2 preceding samples (i.e., days 120 and 150, or days 90 and 120 if the day-180 sample was missing). On review of blinded data prior to database lock, an additional consideration for the determination of treatment response was made for subjects with missing data patterns different from that described in the preceding text (i.e., at least 3 consecutive assessments, including 1 in follow-up) of RT-PCR–negative results (i.e., a subject would be considered to have responded to treatment if RT-PCR was negative at days 30, 60, and 180, with all other time points missing in-between). In contrast, a subject would not be considered to have a treatment response if any RT-PCR results were positive at days 120, 150, or 180. Per-protocol analysis included patients who completed treatment and follow-up.

Secondary outcomes included the long-term sustainability of the RT-PCR response by the end of follow-up (day 360). A successful response was defined by a negative RT-PCR on day 180 (or day 150 if day 180 was missing) on at least both of the 2 preceding samples. A median of 5 RT-PCR measurements were obtained after treatment was completed by 180 days. Safety and tolerability of POS versus placebo, and monotherapy or combined therapy of either BNZ or POS, were also assessed during the 60-day treatment period, and were reported.

The frequencies and percentages of subjects with a successful response are presented for the comparison of the monotherapy treatment arm versus the placebo arm, and the proportions of successful response between arms was compared with the risk difference and its 95% confidence intervals (CIs) using the method of Miettinen and Nurminen (17). The frequencies and percentages of subjects reporting adverse and serious adverse events were tabulated by treatment arm and compared by using the Fisher exact test. All laboratory values, QT interval (electrocardiogram), and laboratory data are summarized as mean ± SD by treatment arm. Analyses were performed using SAS software (version 9.4, SAS Institute, Cary, North Carolina).

SAMPLE SIZE CALCULATION. We initially estimated that 40 subjects should be randomized into each treatment arm in an equal randomization ratio. During the trial, a randomized comparison of 2 doses of

FIGURE 1 STOP-CHAGAS Consort Diagram



A total of 393 patients were screened and 120 were randomized into the 4 study arms (30 per arm). All patients completed follow-up to 360 days. Two patients in the posaconazole (POS) + benznidazole (BNZ) arm did not receive BNZ and were included in the intention-to-treat (ITT) analysis, but not in the per-protocol analysis. PLA = placebo; STOP-CHAGAS (Study of the Use of Oral Posaconazole in the Treatment of Asymptomatic Chronic Chagas Disease).

POS versus BNZ in a trial with study patients similar to those in STOP-CHAGAS was published, demonstrating 81% treatment failure with the same POS dose our trial was testing (15). On the basis of these external data, the sample size was recalculated to require 30 patients per arm, which would still retain sufficient power.

We assumed that if POS and placebo had response rates of 40% and 5%, respectively, the sample size would provide >95% power to show significant differences in response rates between the 2 arms (at alpha < 0.05). A response rate of 40% in the POS arm was selected as the minimally acceptable point estimate of response, as measured by RT-PCR, that would be clinically meaningful relative to a response rate in the placebo arm of 5% and relative to a point estimate of expected response of 10% in the BNZ arm (18).

TABLE 1 Baseline Characteristics of Study Population

	POS (n = 30)	BNZ (n = 30)	POS + BNZ (n = 30)	Placebo (n = 30)	p Value
Male	50.0	43.3	53.3	76.7	0.0524
Mean age, yrs	38.7	38.1	37.6	38.7	
BMI, kg/m^2	28.0	28.5	27.6	27.8	
Country					
Argentina	80.0	86.7	63.3	80.0	
Chile	10.0	6.7	10.0	10.0	
Colombia	0.0	0.0	10.0	0.0	
Guatemala	3.3	0.0	0.0	3.3	
Mexico	0.0	0.0	0.0	3.3	
Spain	6.7	6.7	16.7	3.3	0.3429
LVEF, %	64.6 ± 7.0	65.2 ± 7.2	63.9 ± 8.7	66.9 ± 8.3	0.5136
PR interval, ms	158.6 ± 22	155.2 ± 21.1	159.8 ± 19.2	161.7 ± 19.2	0.6617
QTc (Bazzett), ms	419.4 ± 19.4	421.1 ± 18.6	420 ± 14.7	412.3 ± 20.7	0.2253

Values are % or mean ± SD, unless otherwise indicated.

BMI = body mass index; BNZ = benznidazole; LVEF = left ventricular ejection fraction; POS = posaconazole; QTc = QT interval corrected for heart rate.

RESULTS

Between July 27, 2011 and December 24, 2013, 393 patients were screened and 120 were randomized (Figure 1). Thirty patients were randomized to each study arm. The majority of the patients (93 [77.5%]) were recruited in Argentina, followed by Chile (11 [9.1%]), and Spain (10 [8.3%]), with Colombia, Guatemala, and Mexico recruiting the remaining 6 patients (5%). All patients were in the indeterminate

stage of Chagas disease. The mean age was 38.6 ± 8.1 years. Baseline characteristics were well balanced among treatment groups (Table 1). All subjects were followed post-treatment for up to 300 days (day 360). Two subjects who were randomized to receive BNZ + POS did not receive BNZ in error.

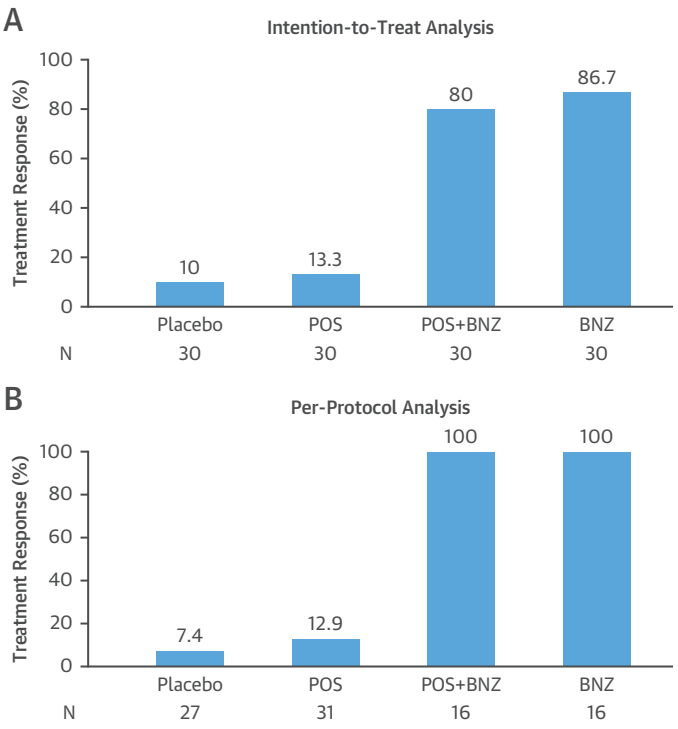
PRIMARY OUTCOME. The primary efficacy outcome was defined as the proportion of subjects with persistent negative RT-PCR by day 180 (or day 150 if day 180 was missing) in all treatment groups. A successful response was defined by both a negative RT-PCR on day 180 and for at least the 2 preceding samples. The RT-PCR response rate for POS was 13.3% (95% CI: 1.2% to 25.5%) versus 10% (95% CI: 0% to 20.7%) for placebo. RT-PCR response rates of 80% (95% CI: 65.7% to 94.3%) for POS + BNZ and 86.7% (95% CI: 74.5% to 98.8%) for BNZ monotherapy were observed. The latter 2 response rates were significantly higher than for placebo (10% risk difference between POS + BNZ and placebo 70% [95% CI: 48% to 84%]; BNZ vs. placebo risk difference 77% [95% CI: 56% to 89%] and POS 13.3%, risk difference between POS + BNZ and POS 67% [95% CI: 44% to 81%]; and between BNZ and POS 73% [95% CI: 56% to 86%]; $p < 0.0001$) (Figure 2A). Similar results were seen in a per-protocol analysis (Figure 2B).

A high RT-PCR conversion rate response was observed during treatment, both at 30 days and at the end of treatment (day 60), for all active arms. POS showed significant RT-PCR conversion rates of 93.3% and 90%, respectively, compared with 10% and 16.7% in the placebo group ($p < 0.001$). BNZ and POS as monotherapy or in combination had significantly higher RT-PCR conversion rates during treatment at days 30 and 60 (end of treatment); POS monotherapy 93% and 90%, POS + BNZ 88.9% and 92.3%, and BNZ 89.7% and 89.3%, respectively, compared with placebo (10% and 16.7%, $p < 0.0001$).

SECONDARY OUTCOMES. Sustainability of the RT-PCR response was observed only in the BNZ monotherapy or BNZ + POS arms at all time points (90, 120, 150, 180, and 360 days) compared with placebo (16.7%) and POS (23.3%) at day 360, respectively ($p < 0.0001$) (Central Illustration).

Serious adverse events were reported in 6 patients and were primarily observed in the study arms receiving BNZ monotherapy or BNZ in combination with POS (Table 2). The most frequent serious adverse event reported on BNZ therapy was cutaneous reactions (7%) (Table 2). The frequency of adverse events was higher in all study groups receiving BNZ, particularly cutaneous reaction (52% vs. 8%), followed by nervous system disorders (33% vs. 11%), and gastrointestinal signs and symptoms (31% vs. 27%). Overall, the most frequently reported adverse events were headache (14%), nausea (10%), and rash (10%). No significant effects on QT interval were observed with BNZ alone or in combination with POS.

FIGURE 2 Primary Efficacy Outcome

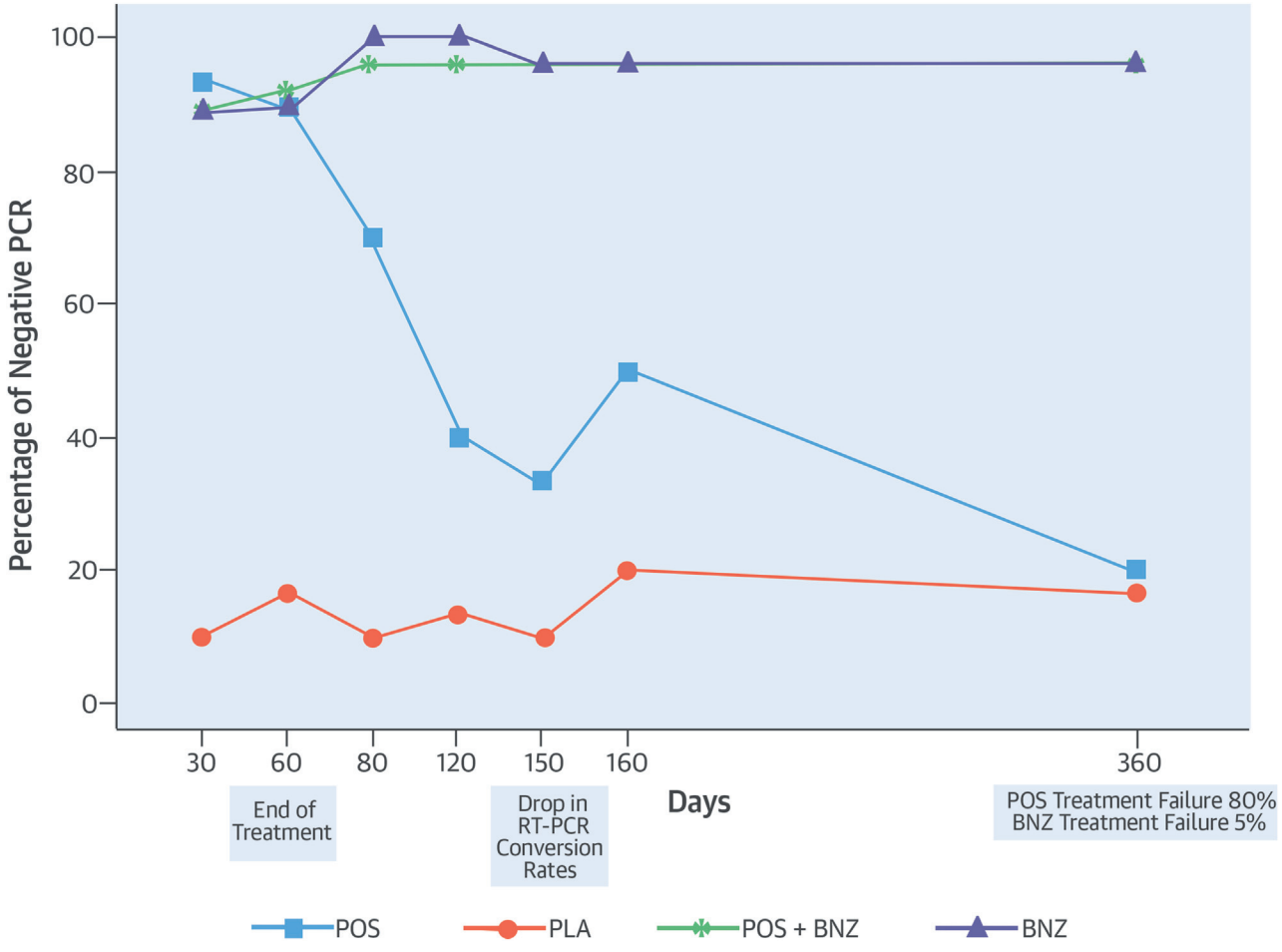


(A) Intention-to-treat analysis including all randomized patients. The rate of conversion to (-) real-time polymerase chain reaction (RT-PCR) at day 180 (treatment response) is shown. POS monotherapy had a treatment failure rate of 86.7%, similar to placebo, and in contrast to only 20% and 13.3% in the combination and BNZ therapy arms, respectively. **(B)** Per-protocol analysis included patients who completed treatment and follow-up. The rate of conversion to (-) RT-PCR at day 180 (treatment response) shows a similar response as the intention-to-treat analysis with POS monotherapy. In contrast, no treatment failures were observed in either the combination or BNZ monotherapy arms. Abbreviations as in Figure 1.

PHARMACOKINETICS. Steady-state exposures for POS ranged from 291 ng/ml to 2,395 ng/ml, but there was no relationship between exposure and response in the short or long terms.

ADHERENCE. Overall, 98 patients (81.6%) completed the assigned treatment. All patients in the POS arm completed treatment and 97% in the placebo arm completed treatment. In the arms receiving BNZ, a temporary discontinuation was reported in 40 patients (66.7%) and permanent discontinuation was reported in 19 patients (31.7%) receiving BNZ or BNZ + POS.

CENTRAL ILLUSTRATION **BNZ Versus POS Versus Their Combination in Asymptomatic *T. Cruzi* Infection**



Morillo, C.A. et al. J Am Coll Cardiol. 2017;69(8):939–47.

Proportion of real-time polymerase chain reaction (RT-PCR) negative rates after 30 days and end of treatment (60 days), and at all follow-up visits per treatment allocation. RT-PCR negative conversion rates were significantly higher in all treatment arms compared with placebo (PLA) at 30 and 60 days (end of treatment), with only 10% treatment failure rates. However, once treatment was finished at 60 days, a marked drop in RT-PCR conversion rates was observed in the posaconazole (POS) monotherapy arm. This was similar to placebo rates, which continued to drop at 360 days to an overall RT-PCR–negative conversion rate of only 20% (80% treatment failure) compared with 95% (5% treatment failure) in the combination and benznidazole (BNZ) monotherapy arms. *T. cruzi* = *Trypanosoma cruzi*.

DISCUSSION

In subjects with chronic asymptomatic Chagas infection, POS administered for 60 days had significant short-term trypanostatic activity, leading to RT-PCR negative conversion in 90% of patients; however, this effect was not sustained at 1 year. In contrast, BNZ demonstrated strong trypanocidal activity and high efficacy rates during treatment that were sustained at the end of follow-up at 1 year. The combination of BNZ and POS had no added benefits compared with those of BNZ monotherapy. Permanent treatment discontinuation of BNZ was frequent and was reported in 32% of patients.

Exploring novel therapeutic approaches for the treatment of Chagas disease is a priority, given the high rates of adverse effects and therapy discontinuation rates with currently available therapies (19,20). Similarly, reducing the burden of disease and progression to cardiomyopathy is critical, as 5.7 to 9.4 million people around the world are currently infected with *T. cruzi*, and it is projected that

40,000 annual deaths associated with cardiac complications of Chagas infection will occur within the next 5 years (4). *T. cruzi* transmission has also been recently documented in the United States, with evidence of Chagas cardiac manifestations among Texas blood donors (21,22). There is presently no agreement on whether all patients with chronic asymptomatic Chagas infection should receive treatment with trypanocidal therapy (23), which justified using a placebo arm. Several observational studies and a meta-analysis of nonrandomized studies suggest that BNZ is an efficacious trypanocidal agent (24–26). However, there is little evidence that BNZ affects progression to the cardiomyopathic stage, and the recently published BENEFIT trial did not show any evidence of delay in the progression of cardiomyopathy with trypanocidal treatment for 60 days in patients with established cardiomyopathy (9,27). Nonetheless, given the putative evidence supporting the role of parasites in determining progression to cardiomyopathy (28,29), trypanocidal therapy in infected individuals appears reasonable if an efficacious and safe agent is available. Recent cross-sectional studies correlating *T. cruzi* parasite DNA detection by RT-PCR with the rate of progression to cardiomyopathy have been conflicting, and further studies are needed to establish the role for etiologic treatment (30–32). Similarly, in women of gestational age, trypanocidal treatment poses a clear benefit by significantly preventing congenital transmission (33).

Our trial demonstrated a significant trypanostatic effect of POS during treatment, but this effect was not sustained. This finding suggests that POS trypanostatic activity is probably related to its blood concentration levels and inversely to levels of trypomastigotes in the peripheral blood. Higher concentrations may be required to sustain trypanocidal activity and prolonged therapy, or a booster dose may be needed to achieve parasite elimination. In contrast, BNZ had high rates of RT-PCR conversion at 30 days of treatment that were maintained throughout the follow-up to 360 days. The reason for the limited trypanostatic activity with POS is unclear, and the discrepancy with the murine models may be related to differences in *T. cruzi* genotype and their sensitivity to treatment, different pharmacokinetic properties in an animal model, or a different level of complexity of the *T. cruzi* cycle in humans (34–36).

Our findings are consistent with the CHAGASAZOL (Clinical Trial for the Treatment of Chronic Chagas Disease With Posaconazole and Benznidazole), which studied similar patients with both the indeterminate and early cardiomyopathy forms of Chagas disease, and in which the same dose of POS tested in our trial resulted in an 81% rate of treatment failure (defined as persistent positive RT-PCR), compared with 38% in the BNZ group ($p < 0.01$) (16). Per our definition, this translates into 19% and 62% treatment response rates in the POS and BNZ groups, respectively. Our superior RT-PCR conversion rates may be related to the higher dose of BNZ used in our trial (200 mg b.i.d.) compared with 150 mg b.i.d. in the CHAGASAZOL trial (16). Additionally, the CHAGASAZOL trial did not have a placebo arm, so the true rate of positive RT-PCR is unknown.

Alternative effective and safe regimens to eliminate *T. cruzi* are needed, given the high rates of side effects of current treatments. In our study, 32% of patients had to permanently discontinue therapy due to side effects. This rate is higher than in the CHAGASAZOL and BENEFIT trials, which reported 19% and 13% permanent discontinuation rates, respectively, is likely related to the higher BNZ dose used in our trial (9,16). The median time to discontinuing therapy in our trial was 40 days, which appears to have been enough to clear *T. cruzi* DNA, as supported by the 90% RT-PCR conversion rate at 30 days, with the benefits sustained at 1 year. These findings raise questions as to whether a shorter treatment course is sufficient (30 days), as previously reported by Viotti et al. (27) and Fabbro et al. (33). Intermittent BNZ regimens were recently proposed by Álvarez et al. (37), who reported similar efficacy and fewer side effects.

TABLE 2 Adverse Events and Laboratory Abnormalities

	POS	PLA	POS + BNZ	BNZ + PLA	Difference BNZ - No BNZ
Treated patients	32	30	28	30	
Any serious adverse event	0 (0.0)	1 (3.3)	2 (7.1)	3 (10.0)	
Hepatitis	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)	
Head injury	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	
Peripheral neuropathy	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)	
Abortion spontaneous	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)	
Rash	0 (0.0)	0 (0.0)	1 (3.6)	1 (3.3)	
Any adverse event	20 (62.5)	15 (50.0)	22 (78.6)	26 (86.7)	26.0 (10 to 41)
Cutaneous reactions	2 (6.3)	3 (10.0)	12 (42.9)	18 (60.0)	44.0 (29 to 57)
Gastrointestinal disorders	12 (37.5)	5 (16.7)	10 (35.7)	8 (26.7)	4.0 (-13 to 20)
Nervous system disorders	4 (12.5)	3 (10.0)	9 (32.1)	10 (33.3)	22.0 (7.0 to 36)
Randomized patients	30	30	30	30	
Discontinuation of treatment drugs	0 (0.0)	1 (3.3)	9 (30.0)	10 (33.3)	
Lab abnormalities					
Alanine aminotransferase >3× ULN	1 (3.3)	1 (3.3)	2 (6.7)	2 (6.7)	
Alanine aminotransferase >5× ULN	0 (0.0)	1 (3.3)	2 (6.7)	0 (0.0)	

Values are n, n (%), or % (95% CI).

CI = confidence interval; PLA = placebo; ULN = upper limit of normal; other abbreviations as in Table 1.

Detection of parasite DNA by RT-PCR was used as a marker of therapeutic response. This approach seems reasonable, given the intermittent circulation of parasites in individuals with asymptomatic chronic *T. cruzi* infection (38,39). Our study used a rigorous validation and standardized approach to RT-PCR, in addition to having a placebo arm that provided us with the true response rate to therapy. In our study, the RT-PCR conversion rate in the placebo group was 10%, and it was observed consistently at multiple time points throughout the 1-year study period. These findings support the use of RT-PCR as a marker of therapeutic response in individuals with chronic asymptomatic *T. cruzi* infection.

STUDY LIMITATIONS. Follow-up was limited to only 1 year and therefore the effect of trypanocidal treatment on progression to cardiomyopathy is unknown.

CONCLUSIONS

In individuals with chronic asymptomatic *T. cruzi* infection, POS has significant short-term trypanostatic therapy; however, this effect is not sustained. Monotherapy with BNZ is superior to POS monotherapy and achieved RT-PCR conversion of *T. cruzi* in all subjects on treatment by 30 days, which was sustained for at least 1 year. Permanent discontinuation of BNZ was frequent, and combination therapy did not provide any further advantages compared to BNZ monotherapy.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: In asymptomatic carriers of *T. cruzi*, which causes Chagas disease, treatment for 60 days with BNZ was superior to POS alone or POS + BNZ, eliminating detectable circulating parasite DNA in >90% of patients by 30 days, and the effect was sustained for at least 1 year.

TRANSLATIONAL OUTLOOK: More work is needed to overcome the side effects of BNZ, which resulted in discontinuation of by one-third of the patients, including evaluation of shorter courses of therapy and the development of better tolerated antiparasitic agents.

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KEY WORDS Chagas disease, intention-to-treat analysis, parasitemia, polymerase chain reaction, treatment failure, trypanocidal agents

APPENDIX For study committee and investigator information, inclusion and exclusion criteria, and an extended Methods section, please see the online version of this paper.