Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Morillo CA, Marin-Neto JA, Avezum A, et al. Randomized trial of benznidazole for chronic Chagas' cardiomyopathy. N Engl J Med 2015;373:1295-306. DOI: 10.1056/NEJMoa1507574

This supplement contains the following items:

1. BENEFIT Trial Protocol - Original protocol
2. BENEFIT Trial Protocol – Final Version 4
3. Summary of Protocol Changes
4. BENEFIT Trial Final Statistical Analysis Plan (version 1.0)

The BENEFIT Trial

BENznidazole **E**valuation **F**or **I**nterrupting
Trypanosomiasis

A randomized double-blind placebo controlled clinical trial of Benznidazole in patients with chronic Chagas´ heart disease

A COLLABORATIVE STUDY OF THE
CHAGAS LATIN AMERICAN DISEASE (CLAD) WORK-GROUP
&
POPULATION HEALTH RESEARCH INSTITUTE (PHRI)
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COORDINATING CENTRES: **South America: Instituto Dante Pazzanese de Cardiologia**
Sao Paulo, Brasil
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CLINICAL TRIAL SUMMARY

TITLE	**Benznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT)Trial** A randomized double-blind placebo-controlled trial of benznidazole in patients with chronic Chagas' heart disease
COORDINATING TRIAL LOCATION	Population Health Research Institute, CCC Project Office Hamilton General Hospital, McMaster Clinic 237 Barton Street East Hamilton, Ontario L8L 2X2 CANADA
STUDY OBJECTIVE (S)	**1. Primary objectives** • To evaluate whether 60 days of therapy with benznidazole, an antiparasitic drug, will reduce mortality and morbidity in patients with chronic Chagas' heart disease. **2. Secondary objectives** • To evaluate whether benznidazole reverses or halts the deterioration of LV function • To evaluate whether benznidazole prevents the development of new ECG changes. • To evaluate the safety of benznidazole in patients with chronic Chagas' heart disease
STUDY DESIGN	A multicentre, prospective, double-blind, randomized evaluation of benznidazole in patients with chronic Chagas' heart disease.

STUDY POPULATION **Inclusion criteria:**	**BENEFIT:** • Serologic evidence of Chagas' infection (indirect immunofluorescence, indirect hemagglutination, or ELISA) – any combination of 2 positive tests • Age ≥ 18 yrs and ≤ 75 yrs **AND any ONE or MORE of the following:** • Abnormal Electrocardiogram: at least **two** of the following 1) Complete right bundle branch block 2) Complete left bundle branch block 3) Left anterior fascicular block 4) Left posterior fascicular block 5) Ventricular premature beat 6) First degree AV block > 220 ms, in the absence of drugs that slow AV conduction 7) Mobitz type I AV block, in the absence of drugs that slow AV 8) Sinus bradycardia < 50 bpm or sinus pauses > 3.0s, in the absence of sinus node blocking drugs 9) Primary ST-T changes 10) Abnormal Q waves 11) Low voltage of QRS 12) Atrial fibrillation **OR** • Abnormal ECG: one of the following 1) Mobitz type II, advanced or third degree AV block **OR** • Increased cardiothoracic ratio (> 0,50) **OR** • Complex ventricular arrhythmias (multiform > 10/hour, couplets or NSVT) on 24 hr. ambulatory ECG monitoring **OR** • Evidence of regional wall-motion abnormality or reduced (< 50%) global left ventricular systolic function (2D-Echo, RNA, contrast ventriculography) or increased left ventricular end diastolic diameter (> 55 mm) on 2D-Echo **PLUS** • Informed consent

Exclusion criteria:	• NYHA heart failure class IV or decompensated heart failure • Evidence of concomitant CAD or other etiology of dilated cardiomyopathy. • Previous treatment with antitrypanosomal agents or an accepted indication for antiparasitic therapy (e.g. reactivation of Chagas infection due to immunosuppression by several diseases or treatment with steroids) • Inability to comply with follow-up • History of severe alcohol abuse within 2 years • Known chronic renal insufficiency (serum creatinine > 2.5 mg/dl or 200µmol) or hepatic insufficiency (AST/ALT > 3x normal) • Pregnancy or breast feeding • Megaesophagus with swallowing impairment • History of severe alcohol abuse within 2 years • Other severe disease significantly curtailing life expectancy
Total expected number of patients:	**3000**
Expected number of centers:	75

	BENEFIT
Formulation:	Benznidazole: 100 mg tablet and matching placebo
Route of administration:	Oral
Dose regimen:	5mg/kg/day (maximal daily dose = 400mg) vs. matching placebo, divided in two daily doses for 60 days.
Duration of Recruitment **Duration of Follow-Up**	24 months Minimum 4 years Maximum 6 years Mean 5 years
EVALUATION CRITERIA	**-PRIMARY OUTCOME-** The first occurrence of any component of the following cluster over the duration of follow-up: • Death • Resuscitated cardiac arrest • Documented sustained ventricular tachycardia requiring cardioversion • Insertion of pacemaker or implantable cardiac defibrillator • Thromboembolic phenomena (stroke, pulmonary or systemic embolism) • New development of symptomatic heart failure (HF) characterized by 2 out of 4 of the following: a) signs and symptoms of HF (orthopnea, paroxysmal nocturla dyspnea, shortness of breath, edema); b) Chest X-Ray: pulmonary congestion; c) need for intravenous therapy (diuretics, inotropes); d) hospital admission due to HF

EVALUATION CRITERIA	**-SECONDARY OUTCOMES-** – Additional data will be collected in subgroups of patients to investigate other hypotheses: • New development of any of the following echo changes; segmental wall motion abnormalities, ventricular aneurysm, reduction in LV ejection fraction > 5%, increase in LVDD > 5,0 mm compared with baseline. • New 12 lead ECG alterations (complete bundle branch block, fascicular block, advanced atrio-ventricular block, atrial fibrillation, etc). • Progression of NYHA functional class by at least one category.
VISIT SCHEDULE/STUDY PROCEDURES	• Screening & randomization visit • Baseline and initiation visit (Day 0) • Follow-up visits at 11 ± 2 days, 3 weeks ± 3 days and 2 months ± 1 week (end of therapy), 6 months ± 1 week, 12 months ± 1 week and yearly thereafter until study termination. • ECG recordings at baseline, 24 months and study end (all patients) • 2D-echo at baseline, and final follow-up visit – all patients • Blood sample for hematological changes (WBC) and liver and renal function tests (AST, ALT, creatinine) during the treatment period (three-weeks and 2-months visits) • Blood sample for central analysis (quantitative serological tests) at baseline and at final follow-up visit (substudy) • Drops of blood sample on filter papers for PCR at baseline, end of at 24 months, and final follow-up visit (substudy)
STATISTICAL CONSIDERATIONS	• Intention-to-treat analysis • **BENEFIT**: Survival analysis using log rank test on primary outcome to show a difference in active versus placebo groups with p value < 0.05. • For a 90% power 2-sided $\alpha = 0.05$ assuming 30% event rate of the composite primary outcome after a mean follow up of 5 years, the sample size needed is 3000 patients for a RRR of 20.0% • Two interim analyses are planned at one-half and two-thirds of expected events.

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1.1.Rationale

Patients with Chagas' infection documented by a positive *T. cruzi* serology test have a 20%-30% chance of progressing to dilated cardiomyopathy. Recent data indicate persistence of the parasite in chronic Chagas' disease (CCD) and suggest the possibility that antitrypanosomal therapy may prevent the progression of Chagas' cardiomyopathy. However, this hypothesis has not been tested in a prospective randomized intervention trial[1].

1.2.Introduction

Chagas' disease (CD) represents the fourth largest tropical disease burden only after malaria, tuberculosis and schistosomiasis[2]. Cardiomyopathy secondary to CD is by far the most common form of non-ischemic cardiomyopathy worldwide[3]. CD, first described almost a century ago, is a zoonosis caused by the hemoflagellate protozoan *Trypanosoma cruzi*[4].

It has been estimated that approximately 20 million are currently infected, and 20% to 30% of infected subjects will eventually develop cardiomyopathy with 50,000 deaths are expected to occurr annually, which are attributed to CD[2, 3, 5, 6].

1.3.Transmission of CD

Transmission of CD is primarily vector-borne through the sting of bloodsucking insects of the Reduviidae family (triatominae subfamily). Control of blood transfusion transmission by obligatory Chagas serology in all blood donors, although cost-effective, is still not implemented in several Latin American countries[7, 8, 9, 10].

1.4.Epidemiology and burden of CD

The most recent figures provided by the World Health Organization, indicating that 100 million persons are exposed to the disease, and that > 550,000 new cases occur each year, may be an underestimate, due to lack of reports from highly endemic remote rural communities, [11]. *T. cruzi* infection leads to chronic symptomatic CD in about 1/5 of persons resulting in a major burden of disability and mortality,[12]. In the early 1990s it was estimated that in Brazil the yearly cost of medical care, including pacemaker and surgery for gastrointestinal CD, as well as early pension costs and time lost from work due to Chagas' associated disability, totaled several billion dollars. Despite the reduction in vector-borne and transfusion-associated transmissions of *T. cruzi* accomplished in many regions by successful interventions, this burden will remain a threat as millions of individuals currently infected by *T. cruzi* gradually develop symptomatic disease[2, 6].

1.5.Clinical features of CD

Chagas disease has two clinical phases; 1) acute, and 2) chronic[13]. The acute phase is undiagnosed in more than 90% of vector transmission cases. Clinically overt acute myocarditis develops in approximately 1% of cases, of which about one-tenth are fatal.

Following the acute phase, the great majority of infected people remain asymptomatic and with no clinical evidence of structural disease during the so-called indeterminate form of CD, that may last two or more decades before clinical signs of chronic disease appear[14], [15]. Most patients remain with this form of CD for life, but are carriers of the infective agents unless treated with

antiparasitic drugs[16]; although there is no clinical evidence of end-organ disease. However, positive serology and low-grade parasitemia persist. Moreover, there is evidence from autopsy and biopsy studies indicating that parasite related myocarditis is present in > 60% of subjects in this stage of CD[17], [18]

In the chronic phase, 10%-30% of infected patients manifest symptoms and signs of heart failure (usually with prominent systemic congestion), ventricular dysrhythmias, and atrioventricular block[1,12,16]. Chest pain, felt by 15-20% of patients, is usually atypical for myocardial ischemia but, in a subgroup of chagasic patients, may mimic an acute coronary syndrome[19]. However, epicardial coronary arteries are angiographically normal[20].

Typical ECG abnormalities include right bundle branch with left anterior fascicular block. Episodes of non-sustained VT are present in approximately 40% of patients with wall-motion abnormalities, and in 90% of those with heart failure[21]. Sustained VT is inducible by programmed ventricular stimulation in a substantial proportion of patients[22], [23], [24]. Not infrequently, complex ventricular rhythm disturbances coexist with bradyarrhythmias[25], [26], and, when associated with impaired left ventricular function, constitute a major risk factor for sudden cardiac death.

Striking segmental wall motion abnormalities in both ventricles[27], [28], [29], [30] occur early in the development of CD. The most characteristic lesion is the apical aneurysm[31], but it is the posterior basal dysynergy that best correlates with the occurrence of malignant ventricular arrhythmia[24, 32]. The aneurysms are also sources of emboli[33].

1.6. Natural History of CCD

Mortality in patients with CCD is primarily ascribed to sudden cardiac death, progressive heart failure and thromboembolic events Sudden cardiac death occurs in 55% to 65% of cases, progressive heart failure in 20 to 25% and stroke in 10% to 15%[34]. Sudden death is more frequent in young patients with isolated segmental wall motion abnormalities[35], but its real frequency is probably underestimated particularly in rural areas. Reported causes of death in CCD vary widely depending on the population studied and duration of follow-up[34].

The experience reported on recent studies of the natural history of CCD in outpatient cohorts which included both rural and urban populations is informative[36, 37].

Rassi et al.[36] followed for a mean period of 7.9 ± 3.2 years 424 patients with Chagas cardiomyopathy determined by a positive serology and either ECG or 2D-echo abnormalities. Approximately half of the subjects were asymptomatic at baseline, palpitations and dyspnea being the most frequent clinical manifestations. Eighty one per cent of patients were in NYHA class I, and another 15% in Class II/III. A wide range of ECG abnormalities was documented, but complete right bundle branch block, left fascicular anterior block, and ventricular premature beats were the most frequent findings, detected in 40%, 37.5%, and 37.3% of the patients, respectively. Advanced ($\geq 2^{nd}$ degree) AV block occurred in 4.2% of cases only. Echocardiogram was available at baseline in 354 patients and normal left ventricular function was described in 52%, with global dysfunction ranging from mild to severe in 41%. Apical aneurysm was reported in 10.5% of the cases and an intracavitary thrombus was identified in 1.7%. Holter monitoring documented frequent ventricular premature complexes (> 1000/24 hrs) in 45% as well as nonsustained ventricular tachycardia in 46% of the patients.

There were 130 deaths (31%) of which cardiovascular deaths accounted for 88%. The primary cause of cardiovascular death was sudden cardiac death (71%), progressive heart failure (18%) and thromboembolic events(10%). Multivariate Cox analysis identified six independent predictors of mortality: male gender, NYHA functional class III/IV, low QRS voltage, cardiomegaly on the chest x-ray, left ventricular dysfunction on the echocardiogram, and non-sustained ventricular tachycardia on Holter monitoring.

Salles et al.[37] followed 738 outpatients in the chronic phase of Chagas` disease (403 with abnormal ECG) during a mean of 4.8 ± 3.2 years. Sixty-two (8.4%) patients died, Chagas` disease related deaths occurred in 54/62 (87%). Sudden cardiac death occurred in 40 (74%) patients, congestive heart failure in 12 (22%), and embolic stroke in two (4%). Multivariate Cox survival analysis revealed that QT-interval dispersion calculated from the 12-lead ECG and left ventricular end-systolic dimension measured by echocardiography were the strongest independent predictors for mortality.

1.7. Pathophysiology of CD

Organ damage arising during the acute phase is closely related to high grade parasitemia and parasite presence in target organs (gastrointestinal tract, central nervous system and heart)[38, 39]. As the parasitemia abates and the systemic inflammatory reaction subsides, silent relentless focal myocarditis ensues during the indeterminate phase[40]. In predisposed hosts, encompassing approximately 30-50% of the infected population, this chronic myocarditis evolves to cumulative destruction of cardiac fibers and marked reparative fibrosis[41, 42].

Apart from the possible ancillary role of neuronal depopulaton and microvascular derangements as mechanisms of CCM, evidence gathered from pathophysiological studies in animal models and in humans is consistent with two prevailing hypotheses to explain the pathogenesis of CCD : 1) *T. cruzi* infection induces immune responses which are targeted at self-tissues and are independent of the persistence of the parasite, so called autoimmune hypothesis[43, 44, 45, 46, 47]; and 2) the persistence of the parasite at specific sites in tissues of the infected host results in chronic inflammatory reactions, the parasite persistence hypothesis[48, 49, 50, 51].

Finding parasites in cardiac tissues from patients in the chronic stages of CD had been very difficult using classical histological techniques. This has been taken as evidence that parasites were not involved in the progressive nature of myocardial damage seen in CCD[52], [53]. However, persistent serologic positivity is found in virtually all patients with end-organ disease even with small numbers of parasites recovered from blood cultures[54]. Moreover, the introduction of more sensitive methods for parasite detection, such as polymerase chain reaction (PCR), in-situ hybridization, and immunoperoxidase techniques, has provided indisputable evidence of parasite persistence in tissues obtained from patients with CCD, topographically related to inflammatory foci[48, 49, 50, 55]. Of note, *T. cruzi* DNA was consistently detected by PCR in heart specimens from patients with CCM but not in the heart tissues from seropositive cadavers without evidence of CCM. Patients with *T. cruzi* DNA detected by PCR also have increased CD8+ cell numbers, and are at a higher risk of progression of cardiomyopathy[56]. Furthermore, from experimental reports using trypanocidal therapy, there is evidence of microbiologic cure and a halt in the progression of the disease, with regression of cardiac inflammation and fibrosis[57, 58]

Collectively these findings support the hypothesis that direct parasitic damage indeed plays a role in the progression of CCM, and lend further support to the notion that antiparasitic therapy in the chronic stages of CD may arrest the progression of disease.

1.8 Antiparasitic Therapy for CD

Clinical experience has been almost exclusively assessed in the acute and indeterminate phases. Experience with nifurtimox (Lampit™, Bayer 2503, Leverkusen, Germany, currently not on the market) and benznidazole (Rochagan™, Roche 7-1051, Sao Paulo, Brazil) was acquired primarily in Argentina and Brazil in the mid 1960s and 1970s[59, 60, 61, 62]. Long-term retrospective observational studies have been the rule and only a handful of randomized clinical trials (RCT's)

have been carried out primarily in children and subjects in the indeterminate phase, with outcomes usually been based on clearance of parasitemia and disappearance of antibodies [9,63, 64].

1.8.1 Acute Phase

Antiparasitic therapy is currently recommended in all acute phase patients, irrespective of the mechanism of transmission, including congenital transmission and accidental infection. A cure (serology and parasitologic negativation) rate of 60 to 80% has been attained in most studies. Very few studies evaluated the effect of antiparasitic therapy on the prevalence of chronic cardiac and/or digestive alterations after therapy in the acute stages. Rassi et al.[65] treated 43 patients in the acute phase of Chagas´ disease with benznidazole or nifurtimox and followed them for several years. The appearance of chronic manifestations was higher in the "non cured" patients (36%) when compared to the "cured" group of patients (7%).

1.8.2 Chronic Phase

Among the several observational trials evaluating the effect of trypanocidal therapy on parasitologic and serologic tests, some have described ECG changes and clinical progression of cardiac disease[66,67,68,69,70]. The results of such studies are discordant, due to differences in populations, methods of evaluation, therapeutic schemes, duration of follow up, cure evaluation criteria, and interpretation of results.

Macedo et al.[66] (1987) studied 171 adults with chronic Chagas disease (103 received nifurtimox or benznidazole, 68 placebo) and followed them for up to 7 years; they reported progression of ECG changes in 6 of treated against 8 placebo patients (5.8 vs. 11.8%, difference not significant).

Ianni et al.[67] (1993) studied 33 adults in the indeterminate form for 8 years and reported ECG evolution in 13,3% (2 out of 15) patients treated with benznidazole and 0% of the cases that received placebo (n = 18). The small study population and the fact that 1 supraventricular extrasystole was considered as ECG evolution in one patient limit the interpretation of their results.

Miranda et al.[68] (1994) described the clinical, ECG and radiographic evolution of 76 patients in the indeterminate or with mild cardiac or digestive alterations treated with benznidazole, and compared with that of 44 untreated matched patients. After 10 to 16 years of follow up, disease progression was significantly higher in the untreated patients (63.6% versus 10.5%, $p < 0, 001$).

Viotti et al.[69] (1994) also assessed the effects of benznidazole, 5mg/kg/day for 30 days on ECG changes and clinical progression in a non-randomized clinical trial of 201 unselected patients with chronic Chagas`disease. After 131 patients received therapy with benznidazole and the remaining 70 were untreated. By the end of the study (mean follow-up 8 years), a higher percentage of three negative serologic reactions was documented in the treated group compared with the untreated group: 19.1% vs. 6%, respectively. A significant reduction in new ECG changes was reported in the group treated with benznidazole, 4.2% compared to 30% in the untreated group; also, a lower frequency in the deterioration of clinical status 2.1% vs 17% was reported. Treatment was discontinued due to side effects in 12%. The most frequent side effect was a moderate allergic dermatitis (77% of patients with side effects) that disappeared after treatment with antihistaminic drugs. No peripheral neuropathy or significant neutropenia was reported in this observational trial.

Fragata Filho et al.[70] (1995) retrospectively compared 71 chagasic patients (58% with mild and 42% without cardiomyopathy) treated with benznidazole to 49 untreated subjects (51% with mild and 49% without cardiomyopathy). Lower incidence of clinical, ECG or radiologic progression of disease was reported in the treated group (7% vs 14.3%, $p < 0.01$) after 7 to 8 years of follow up.

More recently, a few observational studies reported on the long term effects of etiological treatment with three trypanocidal agents (benznidazole, nifurtimox and allopurinol) on hard clinical outcomes (heart failure and/or all-cause mortality) [71,72,73,74]

Analysis of pooled data from these observational studies involving a total of 2.096 chronic chagasic patients with and without cardiomyopathy and followed for 5 to 14 years was performed by Villar[75] (2002), who showed a non-significant reduction of clinical outcomes in the treated patients (2.3 vs 5.0%, OR = 0.55, 95% CI 0.17-1.80 for all-cause mortality, and 1.2 vs 3.8%, OR = 0.28, 95% CI 0.06-1.43 for appearance of heart failure). The heterogeneity of the results, the small number of events recorded and the methodological concerns inherent to observational studies, are limiting factors precluding any reasonable interpretation of the results from such trials.

Three randomized clinical trials (RCT's) have tested benznidazole in the chronic phase of CD, before development of any overt cardiac disease[76,77,78]. Two of them were conducted in school children from Brazil and Argentina and the remaining trial compared nifurtimox to benznidazole in adults. The two studies on children reported on the development of ECG changes, whereas negative seroconversion was assessed in all three trials as a surrogate for parasitic clearance.

Andrade et al.[76] (1996) randomized 64 school children aged 7 to 12 years to benznidazole 7.5 mg/kg/day, and 65 to placebo for 60 days, and followed them during 3 years The primary outcome for efficacy was the disappearance of specific antibodies (negative seroconversion). At the end of follow-up, 37 (58%) of the benznidazole treated subjects and 3 (5%) of those assigned to placebo were negative for *T. cruzi* antibodies. The efficacy of benznidazole treatment estimated by intention-to-treat analysis was 55.8% (95% CI 40.8-67.0).

Sosa-Estani et al.[77] (1998), also conducted a double-blind randomized trial in school children aged 6 to 12 years and randomized 55 to benznidazole 5mg/kg/day and 51 to matching placebo during 60 days. After 48 months, 62% of the benznidazole treated children became seronegative to *T. cruzi antigens* compared to none of the placebo assigned children. Follow up xenodiagnosis was positive in 4.7% of treated children compared to 51.2% in the placebo treated group.

Regarding clinical outcomes, Andrade et al[76] (1996) reported 5 new cases of complete right bundle branch block during follow-up, 4 of which were in the placebo group. Sosa-Estani et al.[77] documented changes in ECG in 2.5% in the benznidazole group compared to 2.4% in the placebo group during follow up. Of note, changes documented were merely ventricular ectopic beats so that no relevant conclusion could be drawn regarding the effect of benznidazole on progression of cardiomyopathy, because of the limited follow-up in very low risk groups of patients. In these two RCTs testing benznidazole in children adverse effects leading to discontinuation of therapy occurred in 7 of 119 patients treated with benznidazole.

Coura et al.[78] (1997) followed 77 patients for 1 year with the indeterminate form of CD, who were randomized to three different treatments: 26 to benznidazole 5mg/kg/day, 27 to nifurtimox 5/mg/kg/day, and 24 to matching placebo during 30 days, and followed during 1 year. Of the 77 patients randomized 64 (83.1%) completed therapy. Withdrawal from the study due to side effects occurred in 11.5% in the benznidazole group, 29.6% in the nifurtimox group and 8.3% in the placebo group. The primary outcome was parasite clearance assessed by xenodiagnosis, which was positive in only 1.8% of the benznidazole, in 9.6% of nifurtimox, and 34.3% of the placebo group.

Villar et al.[79] conducted a metanalysis of 5 small RCTs, and concluded that antitrypanosomal drugs have a beneficial effect on sero-conversion of patients with chronic CD and no overt cardiac manifestations, with an acceptable tolerance, particularly with benznidazole. Overall, benznidazole reduced the proportion of positive xenodiagnosis in both children and adults by about 80%, and led to a 11-fold increase in the rate of negative seroconversion.

When trypanocidal treatment is used for patients with the late chronic phase, a very slow decrease in antibody titers is observed in those who later show evidence of serological cure. Decline in titers may start after 15 or more years. "Cure" rates (completely negative serology)

between 8% and 26% have been reported in the late chronic phase by investigators who were able to follow this group of patients for such a long period of time (nearly 20 years)[80,81].

1.8.3. Benznidazole; Mechanism of Action, Efficacy and Safety in patients with CD

Benznidazole (N-benzil-2nitro-1imidazole-acetamide) has direct action against both the trypomastigotes and intracellular amastygotes, probably by affecting reductive stress, which involves covalent modification of macromolecules by nitroreduction intermediates. The recent demonstration that *T cruzi* strains which over-express superoxide dismutase have increased sensitivity to benznidazole[82] lends support to this mechanism. The activity of these compounds may be enhanced by selectively interfering with the parasite's redox state. This possibility is also supported by the recent discovery of trypanothione and trypanothione reductase, a unique system which replace glutathione and glutathione reductase in trypanosomatids as the main intracellular thiol-redox system[83]. Although there is an active search for specific trypanothione reductase inhibitors as potential antitrypanosomal drugs[84] none of these new class of enzyme inhibitors have shown significant anti-parasitic activity, either *in vitro* or *in vivo*. Thus, benznidazole remains as the only adequate compound to test in appropriately designed clinical trials.

The efficacy of benznidazole in the chronic stages of CD can only be determined by testing the hypothesis that trypanocidal treatment can impact the evolution of disease, preventing or delaying the development of defined clinical forms, no matter what happens with the results of the serological and parasitological tests in the treated and untreated patients. An investigation designed for this purpose should also clarify the unclear safety profile of treatment with benznidazole.

1.9 Study Justification

CD remains an important public health issue as one of the leading causes of cardiomyopathy and sudden cardiac death in most Latin American countries. Although efforts to interrupt transmission of the disease have been successful in several countries these measures have no effect on carriers and patients with symptomatic disease[2, 11, 85, 86,87,88,89,90].

Based on the hypothesis that CCD may indeed be triggered by persistent parasitic infection it appears plausible that trypanocidal therapy may delay or reduce the progression of CCD. This hypothesis needs to be tested in a randomized clinical trial. Therefore a clinical trial determining the role of a trypanocidal agent such as benznidazole, using clinical outcomes to determine the effects on the progression of cardiomyopathy and mortality in CCD, is urgently needed.

2. STUDY OBJECTIVES

2.1 Primary Objective

- To evaluate by means of a double-blind placebo controlled randomized clinical trial whether the use of antitrypanosomal therapy with benznidazole reduces mortality and progression of Chagas cardiomyopathy.

Hypothesis: In patients with evidence of chronic Chagas' heart disease, treatment for 60 days with benznidazole will reduce the rate of clinical disease progression.

2.2 Secondary Objectives

- To evaluate whether benznidazole reverses or halts the deterioration of LV function in patients with CD
- To evaluate whether benznidazole prevents the development of new ECG changes (complete bundle branch block, fascicular block, advanced atrio-ventricular block, atrial fibrillation, etc)
- To evaluate the safety of benznidazole in patients with chronic CD

3. PARTICIPANT ELEGIBILITY

This is a prospective, multicenter, international double blind randomized study evaluating benznidazole versus placebo. Subjects with serological and clinical evidence of cardiac CD will be recruited to determine if benznidazole prevents mortality and progression of CCM.

Participants will be randomly assigned to receive either benznidazole 5mg/kg/day bid or placebo during 60 days. The study will last 6 years. Patient recruitment will take 24 months with an average follow-up time of 5 years per patient. The study will be initiated in early 2004 and patient enrolment will be complete by December 2006. All patients will be followed for 4 years after the last patient has been randomized, giving a mean follow up of approximately 5 years.

3.1 Inclusion Criteria

PATIENTS MUST BE ≥ 18 AND ≤ 75 YEARS
AND HAVE TWO POSITIVE SEROLOGICAL TESTS FOR CHAGAS DISEASE (INDIRECT IMMUNOFLUORESCENCE, INDIRECT HEMMAGLUTINATION, OR ELISA) and

ANY ONE OR MORE of the following markers of cardiac involvement:

a. Abnormal 12 lead ECG: at least two of the following**:**

i. Complete right bundle branch block
ii. Complete left bundle branch block
iii. Left anterior fascicular block
iv. Left posterior fascicular block
v. Ventricular premature beat
vi. First degree AV block > 220 ms, in the absence of drugs that slow AV conduction
vii. Mobitz type I AV block, in the absence of drugs that slow AV
viii. Sinus bradycardia < 50 bpm or sinus pauses > 3.0s, in the absence of sinus node blocking drugs
ix. Primary ST-T changes
x. Abnormal Q waves
xi. Low voltage of QRS
xii. Atrial fibrillation

OR

- **Abnormal 12 lead ECG**: one of the following

1) Mobitz type II, advanced or third degree AV block

OR

b. increased cardio thoracic ratio > 0.50 at baseline on upright chest X ray

OR

c. Evidence of regional wall motion abnormality (hypokinesis, akinesis or dyskinesis) or reduced global left ventricular systolic function LVEF < 50% (2D-Echo, RNA, LV ventriculography) or incresed left ventricular dyastolic diameter (> 55 mm) on 2D-Echo

OR

d. complex ventricular arrhythmias (multiform > 10/hour, couplets or NSVT) on 24 hour ambulatory ECG monitoring

3.2 Exclusion Criteria

a) NYHA heart failure class IV or decompensated heart failure
b) Evidence of concomitant CAD or other etiology of dilated cardiomyopathy.
c) Previous treatment with antitrypanosomal agents or an accepted indication for antiparasitic therapy (e.g. reactivation of Chagas infection due to immunosuppression by several diseases or treatment with steroids)
d) Inability to comply with follow-up
e) History of severe alcohol abuse within 2 years
f) Known chronic renal insufficiency (serum creatinine > 2.5 mg/dl or 200µmol) or hepatic insufficiency (AST/ALT > 3x normal)
g) Pregnancy or breast feeding
h) Megaesophagus with swallowing impairment
i) History of severe alcohol abuse within 2 years
j) Other severe disease significantly curtailing life expectancy

Patients living in inadequate housing conditions that may predispose to *t. cruzi* re-infection will not be excluded; instead this condition will be appropriately documented and further analysis will be performed.

4. STUDY DESIGN AND TREATMENTS

4.1 Sample Size Calculation

The sample size calculation for this trial is based on observational data indicating that the five year rate for the time to event of the composite endpoint of death, resuscitated cardiac arrest, development of new heart failure, life threatening non-fatal arrhythmias, thromboembolic phenomena, and need for pacemaker implantation or ICD in the placebo group will be around 30%. Event rate calculations were based on pooled data from longitudinal population studies in patients with CD[35, 36]. These studies indicate a 5% annual mortality with sudden cardiac death being responsible for 60% of the mortality. However, event rates among patienta entered into trials is generally lower so we have assumed a 3% -4% annual mortality. It is expected that the annual morbidity rates will also be similar givingh a composite event rate of 6%-8%/yr.

In order to have 90% power at the 5% significance level (two sided) with an overall drop-out rate of 20%, to detect a 20% risk reduction the trial will require the enrolment of at least 3000 patients. Analysis of the primary and secondary outcomes will be performed according to the intention-to-treat principle.

A sensitivity analysis wil be performed in patients with prior sustained ventricular tachycardia, thromboembolic phenomena or heart failure hospitalization. This analysis will allow that the mentioned events are not included as a primary outcome. Also, in patients with advanced AV block at onset, pacemaker insertion will not be counted as an endpoint.

4.2 Treatment

4.2.1 Study Drug

Study drug supply will be coordinated by the the Brazilian Coordinating Center with support by the PHRI-BENEFIT Project Office. Benznidazole 100 mg tablets and matching benznidazole-placebo tablets will be supplied. Non-study medications such as ACE inhibitors, diuretics, digoxin, beta-blockers, amiodarone, ASA or warfarin, will not be supplied. Patients will be encouraged to remain adherent to concomitant therapy according to current guidelines.

4.2.2 Randomization

Patients will be screened and if inclusion criteria are fulfilled immediately randomized in a double blinded fashion to receive either benznidazole 5 mg/kg/day (maximal dose 400 mg) divided in two doses or matched placebo (equal administration regimen) . Active therapy and placebo will be administered for 60 days. Subjects will be followed for detection of side effects that include, dermatitis, leukopenia, peripheral neuropathy and gastro-intestinal symptoms. Toxic effects will be assessed with white-cell count, liver and renal function tests (AST, ALT, creatinine) and clinical assessment prior to initiation of treatment, and during the treatment period (three-weeks and 2-months visits)

4.2.3 Method of assigning patients to treatment group

The patient identification (ID) and treatment numbers will be provided at randomization by a centralized randomization service located at the Brazilian Coordinating Center. A system similar to that used in the CREATE and HOPE trials conducted by the PHRI will be designed. Alterbatively if all sites have access to a computer with internet access, local randomization may be performed in a secure way. Randomization will be 1:1 with stratification according to centre using a random block size. Subsequent treatment numbers for study drug re-supply will be provided.

4.2.4 Permitted concomitant therapy

Any concomitant therapy, including treatments demonstrated to be effective in the study population is permitted.

4.2.5 Treatment adherence

Study continuation is recommended unless clear contraindications arise, study drug should be continued or only briefly interrupted ($\leq$ less than 1 week). The only clear criteria for permanent discontinuation of benznidazole study medication are: (i) significant leukopenia (< 2,500), (ii) serious gastrointestinal symptoms (iii) severe allergic dermopathy, and (iv) peripheral sensitive neuropathy. In other situations, discontinuation of study drug will be discouraged as much as possible. Symptomatic treatment for mild symptoms (nauseas, vomiting, allergy) is allowed. Study drug accountability, as well as all pre-specified concomitant medications, must be appropriately recorded at each scheduled visit.

– Additional data will be collected in subgroups of patients to investigate other hypotheses:

5. OUTCOMES

5.1 Primary Outcome

The first occurrence of any of the following clinically significant outcomes:

1. Death
2. Cardiac arrest, requiring defibrillator or cardioversion
3. Documented sustained ventricular tachycardia requiring cardioversion
4. New development of symptomatic congestive heart failure fulfilling at least two of the following: a) signs and symptoms of CHF, b) Chest X-ray findings compatible with CHF, c) need for intravenous therapy, and d) hospital admission due to CHF
5. Pacemaker or implantable cardiac defibrillator indication.
6. Stroke or any other thromboembolic event in patients with no prior thromboembolic phenomena

5.2 Secondary Outcomes

a) New development of any of the following echo changes; segmental wall motion abnormalities, ventricular aneurysm, reduction in LV ejection fraction > 5%, increase in LVDD > 5.0 mm compared with baseline.
b) New 12 lead ECG alterations (complete bundle branch block, fascicular block, advanced atrio-ventricular block, atrial fibrillation, etc).
c) Progression of NYHA functional class by at least one category.

6. FOLLOW-UP

6.1 Follow-up Schedule

The follow-up visit schedule will initially be targeted to assess safety by detecting side effects of the assigned interventions. For this purpose the first visit will be scheduled 11 ± 2 days after initial randomization, when the allergic dermopathy usually starts. The second follow-up visit will be scheduled 3 weeks ± 3 days after randomization (in order to search for leukopenia), with a final follow-up for safety and detection of side effects at the end of therapy administration at 8 weeks (60 days). Adherence to study drugs will be assessed by counting the remaining pills during follow-up visits at 11 ± 2 days, 3 weeks and 8 weeks. Reasons for poor adherence will be detected and patients will be appropriately counselled. If reduction of the benznidazole dose is believed to likely increase adherence the dosage may be temporarily reduced. Only in the presence of serious adverse events will the study medication be withdrawn. Follow-up visits will be scheduled 6 months after randomization and yearly thereafter until study termination.

7. STUDY ORGANIZATION

The PHRI-BENEFIT project office at McMaster University in Hamilton, Canada will coordinate the study. BENEFIT is a collaborative effort between the PHRI, and the recently established Chagas' Latin American Disease Work-Group (CLAD). CLAD currently has members from Argentina, Brazil, Colombia, and Venezuela, and will also aggregate Peru and Bolivia. CLAD will provide the study centers and investigators with experience in CD from Argentina, Brazil, Colombia and Venezuela. It is expected that 75 centers are necessary to enroll 3000 patients (at least 40 patients per centre).

National coordinators will be responsible for recruitment and implementation of the trial. National coordinators will select the study sites and principal investigators in each country. It is estimated that Argentina and Brazil will enroll 2000 patients (1000 each) and 25 centres are needed in each country. Colombia and Venezuela will enroll another 1000 patients (500 each) with another 25 centres between the two countries. To enroll 3000 patients in 24 months we will need to recruit 125 patients monthly. This will be approximately 2 patients per month per center. These estimates suggest that the enrolment phase for BENEFIT is feasible. Drug distribution and labeling will be coordinated from the Brazilian Coordinating Centre.

7.1 BENEFIT Operations Committee

The Operations Committee will consist of a select group of Steering Committee members chosen for their specific expertise, time availability and experience. This group will be responsible for ensuring that study execution and management are of the highest quality. The Operations Committee will convene regularly by teleconference and/or face-to-face meeting (at least every 2 months) to discuss and report on the ongoing supervision of the study.

7.2 Steering Committee

The Steering Committee is composed of a group of national coordinators who will mostly be cardiologists, since patients with CD are most often cared for by cardiologists. This group will be supplemented with experts in CD, and electrophysiology. The committee has the overall responsibility for producing and conducting a scientifically sound design and ensuring accurate reporting of the study. In that capacity, the Steering Committee must address and resolve scientific issues encountered during the study. This committee will meet at least twice a year.

All proposed ancillary research investigations on patients enrolled in BENEFIT must be approved by the Steering Committee. The primary scientific publication reporting the study results is the responsibility of the Steering Committee. Collaborating Investigators or members of the various study committees wishing to prepare secondary publications must submit proposals and manuscripts to the Steering Committee for approval. However, the final decision on the contents of all publications will be the responsibility of the BENEFIT Operations Committee.

7.3. Data Safety Monitoring Board

The Data Safety Monitoring Committee Board (DSMB) will include an epidemiologist, 2 prominent cardiologists, as well as a statistician. The DSMB provide on-going review of the safety of all the investigational treatments. To facilitate its responsibilities, the DSMB will have an Associated Statistician who will receive study data directly from the Central Study Database and who will remain independent of the trial management team.

The DSMB Associated Statistician, being unblinded, will not be able to edit/alter any part of the Central Study Database. Routine access to the treatment code will be restricted to the Chairman of the DSMB, except for emergency unblinding on a case-by-case basis.

DSMB Responsibilities

Primary:

1) Regular review of safety data and serious adverse events
2) Formal interim analyses of efficacy data
3) Feedback to the Operations Committee

Secondary:

1) Respond to special requests from regulatory authorities or IRBs
2) Recommendations for protocol amendments
3) Verification of the final analysis of the study will be done by the DSMB Associated Statistician

Safety Review

Recommendation to stop a trial early for safety reasons is, by definition, a qualitative judgement. The DSMB is composed of eminent clinicians and methodologists who are experienced with clinical trials and can be relied upon to exercise good judgment in weighing the potential risks and benefits to patients as data accumulate in this trial.
Safety aspects, and more specifically severe dermatitis and/or neuropathy will be monitored. No formal boundaries will be proposed for safety, but clear, consistent, and persistent evidence of net harm that overwhelms any benefit should be apparent. A recommendation to stop the trial will be based on the pattern of treatment effect across all endpoints, as well as the benefit/risk ratio. Two interim analyses to assess futility are scheduled at approximately 1/2 and 3/4 of the total of anticipated events.

The trial may be stopped for efficacy if a reduction in events by four standard deviations, or a three standard deviation excess occur in the first half of the trial, or if a reduction in events by three standard deviations or a 2-standard deviation excess is detected in the second half of the trial. If the upper limit of the 95% CI for the conditional power for the primary outcome falls below 15%, then, all other things being equal, the DSMB may recommend early termination**.**

7.4. Adjudication Committee

This committee, composed of experts in the relevant fields will review, in a blinded manner; all reported outcome events to provide consistency and validity in the assessment of outcomes. Their decisions will be based on blind clinical data and they will consider the impressions of the clinical investigator. Their decisions will be used for the final analysis.

8. TIMETABLE

- **Mid 2002 through November 2003 : protocol development and finalization**
- **November 2003 through February 2004 : site selection and preparation**
- **March 2004 through February 2006 : recruitment**
- **March 2006 through February 2010 : follow-up**
- **March 2010 through February 2012 : analysis of data and publication**

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The BENEFIT Trial

BENznidazole **E**valuation **F**or **I**nterrupting
Trypanosomiasis

A randomized double-blind placebo controlled clinical trial of Benznidazole in patients with chronic Chagas´ heart disease

&

POPULATION HEALTH RESEARCH INSTITUTE (PHRI)
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COORDINATING CENTRES: **South America: Instituto Dante Passaneze**
Sao Paulo, Brasil
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CLINICAL TRIAL SUMMARY

TITLE	**Benznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT)Trial** A randomized double-blind placebo-controlled trial of benznidazole in patients with chronic Chagas' heart disease
COORDINATING TRIAL LOCATION	Population Health Research Institute, CCC Project Office Hamilton General Hospital, McMaster Clinic 237 Barton Street East Hamilton, Ontario L8L 2X2 CANADA
STUDY OBJECTIVE (S)	**1. Primary objectives** • Evaluate if benznidazole, an antiparasite drug, given at a dose calculated as 5mg/kg/day for 60 days, now administered as a fixed daily dose of 300mg during 40to 80 days of treatment – period adjusted according to the patient's body weight to a total minimum dose of 12g (corresponding to 40kg) and a total maximum dose of 24g (corresponding to 80kg) – reduces morbidity and mortality in patients with Chronic Chagas' Cardiomyopathy (CCC). **2. Secondary objectives** • To evaluate whether benznidazole reverses or halts the deterioration of LV function • To evaluate whether benznidazole prevents the development of new ECG changes. • To evaluate the safety of benznidazole in patients with chronic Chagas' heart disease
STUDY DESIGN	A multicentre, prospective, double-blind, randomized evaluation of benznidazole in patients with chronic Chagas' heart disease.

STUDY POPULATION **Inclusion criteria:**	**BENEFIT:** • Serologic evidence of Chagas' infection (indirect immunofluorescence, indirect hemagglutination, or ELISA) – any combination of 2 positive tests • Age ≥ 18 yrs and ≤ 65 yrs **AND any ONE or MORE of the following:** • Abnormal Electrocardiogram: at least **two** of the following 1) Complete right bundle branch block 2) Complete left bundle branch block 3) Left anterior fascicular block 4) Left posterior fascicular block 5) Ventricular premature beat 6) First degree AV block > 220 ms, in the absence of drugs that slow AV conduction 7) Mobitz type I AV block, in the absence of drugs that slow AV 8) Sinus bradycardia < 50 bpm or sinus pauses > 3.0s, in the absence of sinus node blocking drugs 9) Primary ST-T changes 10) Abnormal Q waves 11) Low voltage of QRS 12) Atrial fibrillation **OR** • Abnormal ECG: one of the following 1) Mobitz type II, advanced or third degree AV block **OR** • Increased cardiothoracic ratio (> 0,50) **OR** • Complex ventricular arrhythmias (multiform > 10/hour, couplets or NSVT) on 24 hr. ambulatory ECG monitoring **OR** • Evidence of regional wall-motion abnormality or reduced (< 50%) global left ventricular systolic function (2D-Echo, RNA, contrast ventriculography) or increased left ventricular end diastolic diameter (> 55 mm) on 2D-Echo **PLUS** • Informed consent

Exclusion criteria:	• NYHA heart failure class IV or decompensated heart failure • Evidence of concomitant CAD or other etiology of dilated cardiomyopathy. • Previous treatment with antitrypanosomal agents or an accepted indication for antiparasitic therapy (e.g. reactivation of Chagas infection due to immunosuppression by several diseases or treatment with steroids) • Inability to comply with follow-up • History of severe alcohol abuse within 2 years • Known chronic renal insufficiency (serum creatinine > 2.5 mg/dl or 200µmol) or hepatic insufficiency (AST/ALT > 3x normal) • Pregnancy or breast feeding • Megaesophagus with swallowing impairment • History of severe alcohol abuse within 2 years • Other severe disease significantly curtailing life expectancy
Total expected number of patients:	**3000**
Expected number of centers:	75

	BENEFIT
Formulation:	Benznidazole: 100 mg tablet and matching placebo
Route of administration:	Oral
Dose regimen:	Therapeutic goal: 300mg daily dose vs placebo, divided in two daily doses, being 100mg in the morning and 200mg in the evening for 40 to 80 days, according to the following simple scheme (Appended Table):
Duration of Recruitment **Duration of Follow-Up**	24 months Minimum 4 years Maximum 6 years Mean 5 years
EVALUATION CRITERIA	**-PRIMARY OUTCOME-** The first occurrence of any component of the following cluster over the duration of follow-up: • Death • Resuscitated cardiac arrest • Documented sustained ventricular tachycardia requiring cardioversion • Insertion of pacemaker or implantable cardiac defibrillator. • Thromboembolic phenomena (stroke, pulmonary or systemic embolism) • New development of symptomatic heart failure (HF) characterized by 2 out of 4 of the following: a) signs and symptoms of HF (orthopnea, paroxysmal nocturnal dyspnea, shortness of breath, edema); b) Chest X-Ray: pulmonary congestion; c) need for intra-venous therapy (diuretics, inotropes); d) hospital admission due to HF • Heart transplant

EVALUATION CRITERIA	**-SECONDARY OUTCOMES-** – Additional data will be collected in subgroups of patients to investigate other hypotheses: • PCR response to treatment • New development of any of the following echo changes; segmental wall motion abnormalities, ventricular aneurysm, reduction in LV ejection fraction > 5%, increase in LVDD > 5,0 mm compared with baseline. • New 12 lead ECG alterations (complete bundle branch block, fascicular block, advanced atrio-ventricular block, atrial fibrillation, etc). • Progression of NYHA functional class by at least one category.
VISIT SCHEDULE/STUDY PROCEDURES	• Screening & randomization visit • Baseline and initiation visit (Day 0) • Follow-up visits after 11± 2 days, 3 weeks± 3 days, 40 to 90 days (end of treatment period with a maximum tolerance of 10 days after the last day of drug or placebo administration), 6 months, 12 months and annually from then on, until the end of the study. • ECG recordings at baseline, 24 months and study end (all patients) • 2D-echo at baseline, and final follow-up visit – all patients • Blood sample for hematological changes (WBC) and liver and renal function tests (AST, ALT, creatinine) during the treatment period (three-weeks and 2-months visits) • Blood sample for central analysis (quantitative serological tests) at baseline and at final follow-up visit – (substudy) • Drops of blood sample/ blood sample 5-10ml on filter papers for PCR at baseline, at 24 months, and final follow-up visit (substudy)
STATISTICAL CONSIDERATIONS	• Intention-to-treat analysis • **BENEFIT**: Survival analysis using log rank test on primary outcome to show a difference in active versus placebo groups with p value < 0.05. • For a 90% power 2-sided $\alpha = 0.05$ assuming 30% event rate of the composite primary outcome after a mean follow up of 5 years, the sample size needed is 3000 patients for a RRR of 20.0% • Two interim analyses are planned at one-half and two-thirds of expected events.

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1.1.Rationale

Patients with Chagas' infection documented by a positive *T. cruzi* serology test have a 20%-30% chance of progressing to dilated cardiomyopathy. Recent data indicate persistence of the parasite in chronic Chagas' disease (CCD) and suggest the possibility that antitrypanosomal therapy may prevent the progression of Chagas' cardiomyopathy. However, this hypothesis has not been tested in a prospective randomized intervention trial[1].

1.2.Introduction

Chagas' disease (CD) represents the fourth largest tropical disease burden only after malaria, tuberculosis and schistosomiasis[2]. Cardiomyopathy secondary to CD is by far the most common form of non-ischemic cardiomyopathy worldwide[3]. CD, first described almost a century ago, is a zoonosis caused by the hemoflagellate protozoan *Trypanosoma cruzi*[4].

It has been estimated that approximately 20 million are currently infected, and 20% to 30% of infected subjects will eventually develop cardiomyopathy with 50,000 deaths are expected to occurr annually, which are attributed to CD[2, 3, 5, 6].

1.3.Transmission of CD

Transmission of CD is primarily vector-borne through the sting of bloodsucking insects of the Reduviidae family (triatominae subfamily). Control of blood transfusion transmission by obligatory Chagas serology in all blood donors, although cost-effective, is still not implemented in several Latin American countries[7, 8, 9, 10].

1.4.Epidemiology and burden of CD

The most recent figures provided by the World Health Organization, indicating that 100 million persons are exposed to the disease, and that > 550,000 new cases occur each year, may be an underestimate, due to lack of reports from highly endemic remote rural communities[2, 11]. *T. cruzi* infection leads to chronic symptomatic CD in about 1/5 of persons resulting in a major burden of disability and mortality[1, 12]. In the early 1990s it was estimated that in Brazil the yearly cost of medical care, including pacemaker and surgery for gastrointestinal CD, as well as early pension costs and time lost from work due to Chagas' associated disability, totaled several billion dollars[8]. Despite the reduction in vector-borne and transfusion-associated transmissions of *T. cruzi* accomplished in many regions by successful interventions, this burden will remain a threat as millions of individuals currently infected by *T. cruzi* gradually develop symptomatic disease[2, 6].

1.5.Clinical features of CD

Chagas disease has two clinical phases; 1) acute, and 2) chronic[13]. The acute phase is undiagnosed in more than 90% of vector transmission cases. Clinically overt acute myocarditis develops in approximately 1% of cases, of which about one-tenth are fatal[12].

Following the acute phase, the great majority of infected people remain asymptomatic and with no clinical evidence of structural disease during the so-called indeterminate form of CD, that may last two or more decades before clinical signs of chronic disease appear[14, 15]. Most patients remain with this form of CD for life, but are carriers of the infective agents unless treated with

antiparasitic drugs[16]; although there is no clinical evidence of end-organ disease. However, positive serology and low-grade parasitemia persist. Moreover, there is evidence from autopsy and biopsy studies indicating that parasite related myocarditis is present in > 60% of subjects in this stage of CD[17], [18]

In the chronic phase, 10%-30% of infected patients manifest symptoms and signs of heart failure (usually with prominent systemic congestion), ventricular dysrhythmias, and atrioventricular block[1,12,16]. Chest pain, felt by 15-20% of patients, is usually atypical for myocardial ischemia but, in a subgroup of chagasic patients, may mimic an acute coronary syndrome[19]. However, epicardial coronary arteries are angiographically normal[20].

Typical ECG abnormalities include right bundle branch with left anterior fascicular block. Episodes of non-sustained VT are present in approximately 40% of patients with wall-motion abnormalities, and in 90% of those with heart failure[21]. Sustained VT is inducible by programmed ventricular stimulation in a substantial proportion of patients[22], [23], [24]. Not infrequently, complex ventricular rhythm disturbances coexist with bradyarrhythmias[25], [26], and, when associated with impaired left ventricular function, constitute a major risk factor for sudden cardiac death[21].

Striking segmental wall motion abnormalities in both ventricles[27], [28], [29], [30] occur early in the development of CD. The most characteristic lesion is the apical aneurysm[31], but it is the posterior basal dysynergy that best correlates with the occurrence of malignant ventricular arrhythmia[24, 32]. The aneurysms are also sources of emboli[33].

1.6. Natural History of CCD

Mortality in patients with CCD is primarily ascribed to sudden cardiac death, progressive heart failure and thromboembolic events Sudden cardiac death occurs in 55% to 65% of cases, progressive heart failure in 20 to 25% and stroke in 10% to 15%[34]. Sudden death is more frequent in young patients with isolated segmental wall motion abnormalities[35], but its real frequency is probably underestimated particularly in rural areas. Reported causes of death in CCD vary widely depending on the population studied and duration of follow-up[34].

The experience reported on recent studies of the natural history of CCD in outpatient cohorts which included both rural and urban populations is informative[36, 37].

Rassi et al.[36] followed for a mean period of 7.9 ± 3.2 years 424 patients with Chagas cardiomyopathy determined by a positive serology and either ECG or 2D-echo abnormalities. Approximately half of the subjects were asymptomatic at baseline, palpitations and dyspnea being the most frequent clinical manifestations. Eighty one per cent of patients were in NYHA class I, and another 15% in Class II/III. A wide range of ECG abnormalities was documented, but complete right bundle branch block, left fascicular anterior block, and ventricular premature beats were the most frequent findings, detected in 40%, 37.5%, and 37.3% of the patients, respectively. Advanced ($\geq 2^{nd}$ degree) AV block occurred in 4.2% of cases only. Echocardiogram was available at baseline in 354 patients and normal left ventricular function was described in 52%, with global dysfunction ranging from mild to severe in 41%. Apical aneurysm was reported in 10.5% of the cases and an intracavitary thrombus was identified in 1.7%. Holter monitoring documented frequent ventricular premature complexes (> 1000/24 hrs) in 45% as well as nonsustained ventricular tachycardia in 46% of the patients.

There were 130 deaths (31%) of which cardiovascular deaths accounted for 88%. The primary cause of cardiovascular death was sudden cardiac death (71%), progressive heart failure (18%) and thromboembolic events(10%). Multivariate Cox analysis identified six independent predictors of mortality: male gender, NYHA functional class III/IV, low QRS voltage, cardiomegaly on the chest x-ray, left ventricular dysfunction on the echocardiogram, and non-sustained ventricular tachycardia on Holter monitoring.

Salles et al.[37] followed 738 outpatients in the chronic phase of Chagas` disease (403 with abnormal ECG) during a mean of 4.8 ± 3.2 years. Sixty-two (8.4%) patients died, Chagas` disease related deaths occurred in 54/62 (87%). Sudden cardiac death occurred in 40 (74%) patients, congestive heart failure in 12 (22%), and embolic stroke in two (4%). Multivariate Cox survival analysis revealed that QT-interval dispersion calculated from the 12-lead ECG and left ventricular end-systolic dimension measured by echocardiography were the strongest independent predictors for mortality.

1.7. Pathophysiology of CD

Organ damage arising during the acute phase is closely related to high grade parasitemia and parasite presence in target organs (gastrointestinal tract, central nervous system and heart)[38, 39]. As the parasitemia abates and the systemic inflammatory reaction subsides, silent relentless focal myocarditis ensues during the indeterminate phase[40]. In predisposed hosts, encompassing approximately 30-50% of the infected population, this chronic myocarditis evolves to cumulative destruction of cardiac fibers and marked reparative fibrosis[41, 42].

Apart from the possible ancillary role of neuronal depopulaton and microvascular derangements as mechanisms of CCM, evidence gathered from pathophysiological studies in animal models and in humans is consistent with two prevailing hypotheses to explain the pathogenesis of CCD : 1) *T. cruzi* infection induces immune responses which are targeted at self-tissues and are independent of the persistence of the parasite, so called autoimmune hypothesis[43, 44, 45, 46, 47]; and 2) the persistence of the parasite at specific sites in tissues of the infected host results in chronic inflammatory reactions, the parasite persistence hypothesis[48, 49, 50, 51].

Finding parasites in cardiac tissues from patients in the chronic stages of CD had been very difficult using classical histological techniques. This has been taken as evidence that parasites were not involved in the progressive nature of myocardial damage seen in CCD[52], [53]. However, persistent serologic positivity is found in virtually all patients with end-organ disease even with small numbers of parasites recovered from blood cultures[54]. Moreover, the introduction of more sensitive methods for parasite detection, such as polymerase chain reaction (PCR), in-situ hybridization, and immunoperoxidase techniques, has provided indisputable evidence of parasite persistence in tissues obtained from patients with CCD, topographically related to inflammatory foci[48, 49, 50, 55]. Of note, *T. cruzi* DNA was consistently detected by PCR in heart specimens from patients with CCM but not in the heart tissues from seropositive cadavers without evidence of CCM[48]. Patients with *T. cruzi* DNA detected by PCR also have increased CD8+ cell numbers, and are at a higher risk of progression of cardiomyopathy[56]. Furthermore, from experimental reports using trypanocidal therapy, there is evidence of microbiologic cure and a halt in the progression of the disease, with regression of cardiac inflammation and fibrosis[57, 58]

Collectively these findings support the hypothesis that direct parasitic damage indeed plays a role in the progression of CCM, and lend further support to the notion that antiparasitic therapy in the chronic stages of CD may arrest the progression of disease.

1.8 Antiparasitic Therapy for CD

Clinical experience has been almost exclusively assessed in the acute and indeterminate phases. Experience with nifurtimox (Lampit™, Bayer 2503, Leverkusen, Germany, currently not on the market) and benznidazole (Rochagan™, Roche 7-1051, Sao Paulo, Brazil) was acquired primarily in Argentina and Brazil in the mid 1960s and 1970s[59, 60, 61, 62]. Long-term retrospective observational studies have been the rule and only a handful of randomized clinical trials (RCT's)

have been carried out primarily in children and subjects in the indeterminate phase, with outcomes usually been based on clearance of parasitemia and disappearance of antibodies [9,63, 64].

1.8.1 Acute Phase

Antiparasitic therapy is currently recommended in all acute phase patients, irrespective of the mechanism of transmission, including congenital transmission and accidental infection. A cure (serology and parasitologic negativation) rate of 60 to 80% has been attained in most studies. Very few studies evaluated the effect of antiparasitic therapy on the prevalence of chronic cardiac and/or digestive alterations after therapy in the acute stages. Rassi et al.[65] treated 43 patients in the acute phase of Chagas´ disease with benznidazole or nifurtimox and followed them for several years. The appearance of chronic manifestations was higher in the "non cured" patients (36%) when compared to the "cured" group of patients (7%).

1.8.2 Chronic Phase

Among the several observational trials evaluating the effect of trypanocidal therapy on parasitologic and serologic tests, some have described ECG changes and clinical progression of cardiac disease[66,67,68,69,70]. The results of such studies are discordant, due to differences in populations, methods of evaluation, therapeutic schemes, duration of follow up, cure evaluation criteria, and interpretation of results.

Macedo et al.[66] (1987) studied 171 adults with chronic Chagas disease (103 received nifurtimox or benznidazole, 68 placebo) and followed them for up to 7 years; they reported progression of ECG changes in 6 of treated against 8 placebo patients (5.8 vs. 11.8%, difference not significant).

Ianni et al.[67] (1993) studied 33 adults in the indeterminate form for 8 years and reported ECG evolution in 13,3% (2 out of 15) patients treated with benznidazole and 0% of the cases that received placebo (n = 18). The small study population and the fact that 1 supraventricular extrasystole was considered as ECG evolution in one patient limit the interpretation of their results.

Miranda et al.[68] (1994) described the clinical, ECG and radiographic evolution of 76 patients in the indeterminate or with mild cardiac or digestive alterations treated with benznidazole, and compared with that of 44 untreated matched patients. After 10 to 16 years of follow up, disease progression was significantly higher in the untreated patients (63.6% versus 10.5%, $p < 0, 001$).

Viotti et al.[69] (1994) also assessed the effects of benznidazole, 5mg/kg/day for 30 days on ECG changes and clinical progression in a non-randomized clinical trial of 201 unselected patients with chronic Chagas`disease. After 131 patients received therapy with benznidazole and the remaining 70 were untreated. By the end of the study (mean follow-up 8 years), a higher percentage of three negative serologic reactions was documented in the treated group compared with the untreated group: 19.1% vs. 6%, respectively. A significant reduction in new ECG changes was reported in the group treated with benznidazole, 4.2% compared to 30% in the untreated group; also, a lower frequency in the deterioration of clinical status 2.1% vs 17% was reported. Treatment was discontinued due to side effects in 12%. The most frequent side effect was a moderate allergic dermatitis (77% of patients with side effects) that disappeared after treatment with antihistaminic drugs. No peripheral neuropathy or significant neutropenia was reported in this observational trial.

Fragata Filho et al.[70] (1995) retrospectively compared 71 chagasic patients (58% with mild and 42% without cardiomyopathy) treated with benznidazole to 49 untreated subjects (51% with mild and 49% without cardiomyopathy). Lower incidence of clinical, ECG or radiologic progression of disease was reported in the treated group (7% vs 14.3%, $p < 0.01$) after 7 to 8 years of follow up.

More recently, a few observational studies reported on the long term effects of etiological treatment with three trypanocidal agents (benznidazole, nifurtimox and allopurinol) on hard clinical outcomes (heart failure and/or all-cause mortality) [71,72,73,74]

Analysis of pooled data from these observational studies involving a total of 2.096 chronic chagasic patients with and without cardiomyopathy and followed for 5 to 14 years was performed by Villar[75] (2002), who showed a non-significant reduction of clinical outcomes in the treated patients (2.3 vs 5.0%, OR = 0.55, 95% CI 0.17-1.80 for all-cause mortality, and 1.2 vs 3.8%, OR = 0.28, 95% CI 0.06-1.43 for appearance of heart failure). The heterogeneity of the results, the small number of events recorded and the methodological concerns inherent to observational studies, are limiting factors precluding any reasonable interpretation of the results from such trials.

Three randomized clinical trials (RCT's) have tested benznidazole in the chronic phase of CD, before development of any overt cardiac disease[76,77,78]. Two of them were conducted in school children from Brazil and Argentina and the remaining trial compared nifurtimox to benznidazole in adults. The two studies on children reported on the development of ECG changes, whereas negative seroconversion was assessed in all three trials as a surrogate for parasitic clearance.

Andrade et al.[76] (1996) randomized 64 school children aged 7 to 12 years to benznidazole 7.5 mg/kg/day, and 65 to placebo for 60 days, and followed them during 3 years The primary outcome for efficacy was the disappearance of specific antibodies (negative seroconversion). At the end of follow-up, 37 (58%) of the benznidazole treated subjects and 3 (5%) of those assigned to placebo were negative for *T. cruzi* antibodies. The efficacy of benznidazole treatment estimated by intention-to-treat analysis was 55.8% (95% CI 40.8-67.0).

Sosa-Estani et al.[77] (1998), also conducted a double-blind randomized trial in school children aged 6 to 12 years and randomized 55 to benznidazole 5mg/kg/day and 51 to matching placebo during 60 days. After 48 months, 62% of the benznidazole treated children became seronegative to *T. cruzi antigens* compared to none of the placebo assigned children. Follow up xenodiagnosis was positive in 4.7% of treated children compared to 51.2% in the placebo treated group.

Regarding clinical outcomes, Andrade et al[76] (1996) reported 5 new cases of complete right bundle branch block during follow-up, 4 of which were in the placebo group. Sosa-Estani et al.[77] documented changes in ECG in 2.5% in the benznidazole group compared to 2.4% in the placebo group during follow up. Of note, changes documented were merely ventricular ectopic beats so that no relevant conclusion could be drawn regarding the effect of benznidazole on progression of cardiomyopathy, because of the limited follow-up in very low risk groups of patients. In these two RCTs testing benznidazole in children adverse effects leading to discontinuation of therapy occurred in 7 of 119 patients treated with benznidazole.

Coura et al.[78] (1997) followed 77 patients for 1 year with the indeterminate form of CD, who were randomized to three different treatments: 26 to benznidazole 5mg/kg/day, 27 to nifurtimox 5/mg/kg/day, and 24 to matching placebo during 30 days, and followed during 1 year. Of the 77 patients randomized 64 (83.1%) completed therapy. Withdrawal from the study due to side effects occurred in 11.5% in the benznidazole group, 29.6% in the nifurtimox group and 8.3% in the placebo group. The primary outcome was parasite clearance assessed by xenodiagnosis, which was positive in only 1.8% of the benznidazole, in 9.6% of nifurtimox, and 34.3% of the placebo group.

Villar et al.[79] conducted a metanalysis of 5 small RCTs, and concluded that antitrypanosomal drugs have a beneficial effect on sero-conversion of patients with chronic CD and no overt cardiac manifestations, with an acceptable tolerance, particularly with benznidazole. Overall, benznidazole reduced the proportion of positive xenodiagnosis in both children and adults by about 80%, and led to a 11-fold increase in the rate of negative seroconversion.

When trypanocidal treatment is used for patients with the late chronic phase, a very slow decrease in antibody titers is observed in those who later show evidence of serological cure. Decline in titers may start after 15 or more years. "Cure" rates (completely negative serology)

between 8% and 26% have been reported in the late chronic phase by investigators who were able to follow this group of patients for such a long period of time (nearly 20 years)[80,81].

1.8.3. Benznidazole; Mechanism of Action, Efficacy and Safety in patients with CD

Benznidazole (N-benzil-2nitro-1imidazole-acetamide) has direct action against both the trypomastigotes and intracellular amastygotes, probably by affecting reductive stress, which involves covalent modification of macromolecules by nitroreduction intermediates. The recent demonstration that *T cruzi* strains which over-express superoxide dismutase have increased sensitivity to benznidazole[82] lends support to this mechanism. The activity of these compounds may be enhanced by selectively interfering with the parasite's redox state. This possibility is also supported by the recent discovery of trypanothione and trypanothione reductase, a unique system which replace glutathione and glutathione reductase in trypanosomatids as the main intracellular thiol-redox system[83]. Although there is an active search for specific trypanothione reductase inhibitors as potential antitrypanosomal drugs[84] none of these new class of enzyme inhibitors have shown significant anti-parasitic activity, either *in vitro* or *in vivo*. Thus, benznidazole remains as the only adequate compound to test in appropriately designed clinical trials.

The efficacy of benznidazole in the chronic stages of CD can only be determined by testing the hypothesis that trypanocidal treatment can impact the evolution of disease, preventing or delaying the development of defined clinical forms, no matter what happens with the results of the serological and parasitological tests in the treated and untreated patients. An investigation designed for this purpose should also clarify the unclear safety profile of treatment with benznidazole.

1.9 Study Justification

CD remains an important public health issue as one of the leading causes of cardiomyopathy and sudden cardiac death in most Latin American countries. Although efforts to interrupt transmission of the disease have been successful in several countries these measures have no effect on carriers and patients with symptomatic disease[2, 11, 85, 86,87,88,89,90].

Based on the hypothesis that CCD may indeed be triggered by persistent parasitic infection it appears plausible that trypanocidal therapy may delay or reduce the progression of CCD. This hypothesis needs to be tested in a randomized clinical trial. Therefore a clinical trial determining the role of a trypanocidal agent such as benznidazole, using clinical outcomes to determine the effects on the progression of cardiomyopathy and mortality in CCD, is urgently needed.

This is the goal of the BENEFIT study which will test the hypothesis that 300mg of benznidazole administered for 40 to 80 days, according to the patient's body weight, is able to favorably impact the clinical evolution of patients with chronic chagas's heart disease, with reduction in clinically relevant endpoints such as morbidity and mortality.

2. STUDY OBJECTIVES

2.1 Primary Objective

- To evaluate by means of a double-blind placebo controlled randomized clinical trial whether the use of antitrypanosomal therapy with benznidazole reduces mortality and progression of Chagas cardiomyopathy.

Hypothesis: In patients with CCC, treatment with benznidazole for 40 to 80 days will reduce progression and worsening of heart disease.

2.2 Secondary Objectives

- To evaluate the response to therapy based on baseline PCR and response at 24 months and end of follow-up
- To evaluate whether benznidazole reverses or halts the deterioration of LV function in patients with CD
- To evaluate whether benznidazole prevents the development of new ECG changes (complete bundle branch block, fascicular block, advanced atrio-ventricular block, atrial fibrillation, etc)
- To evaluate the safety of benznidazole in patients with chronic CD

3. PARTICIPANT ELEGIBILITY

This is a prospective, multicenter, international double blind randomized study evaluating benznidazole versus placebo. Subjects with serological and clinical evidence of cardiac CD will be recruited to determine if benznidazole prevents mortality and progression of CCM.

Participants will be randomly allocated to treatment with 300mg/day of benznidazole or matching placebo, divided in two daily doses, for 40 to 80 days. Enrollment will begin in the end of 2004 and should be complete by February of 2009. All patients will be followed by a minimum of 4 years.

3.1 Inclusion Criteria

PATIENTS MUST BE ≥ 18 AND ≤ 65 YEARS
AND HAVE TWO POSITIVE SEROLOGICAL TESTS FOR CHAGAS DISEASE (INDIRECT IMMUNOFLUORESCENCE, INDIRECT HEMMAGLUTINATION, OR ELISA) and

ANY ONE OR MORE of the following markers of cardiac involvement:

a. Abnormal 12 lead ECG: at least two of the following**:**

i. Complete right bundle branch block
ii. Complete left bundle branch block
iii. Left anterior fascicular block
iv. Left posterior fascicular block
v. Ventricular premature beat
vi. First degree AV block > 220 ms, in the absence of drugs that slow AV conduction
vii. Mobitz type I AV block, in the absence of drugs that slow AV
viii. Sinus bradycardia < 50 bpm or sinus pauses > 3.0s, in the absence of sinus node blocking drugs
ix. Primary ST-T changes
x. Abnormal Q waves
xi. Low voltage of QRS
xii. Atrial fibrillation

OR

- **Abnormal 12 lead ECG**: one of the following

1) Mobitz type II, advanced or third degree AV block

OR

b. increased cardio thoracic ratio > 0.50 at baseline on upright chest X ray

OR

c. Evidence of regional wall motion abnormality (hypokinesis, akinesis or dyskinesis) or reduced global left ventricular systolic function LVEF < 50% (2D-Echo, RNA, LV ventriculography) or incresed left ventricular dyastolic diameter (> 55 mm) on 2D-Echo

OR

d. complex ventricular arrhythmias (multiform > 10/hour, couplets or NSVT) on 24 hour ambulatory ECG monitoring

3.2 Exclusion Criteria

a) NYHA heart failure class IV or decompensated heart failure
b) Evidence of concomitant CAD or other etiology of dilated cardiomyopathy.
c) Previous treatment with antitrypanosomal agents or an accepted indication for antiparasitic therapy (e.g. reactivation of Chagas infection due to immunosuppression by several diseases or treatment with steroids)
d) Inability to comply with follow-up
e) History of severe alcohol abuse within 2 years
f) Known chronic renal insufficiency (serum creatinine > 2.5 mg/dl or 200μmol) or hepatic insufficiency (AST/ALT > 3x normal)
g) Pregnancy or breast feeding
h) Megaesophagus with swallowing impairment
i) History of severe alcohol abuse within 2 years
j) Other severe disease significantly curtailing life expectancy

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Patients living in inadequate housing conditions that may predispose to *t. cruzi* re-infection will not be excluded; instead this condition will be appropriately documented and further analysis will be performed.

4. STUDY DESIGN AND TREATMENTS

4.1 Sample Size Calculation

The sample size calculation for this trial is based on observational data indicating that the five year rate for the time to event of the composite endpoint of death, resuscitated cardiac arrest, development of new heart failure, life threatening non-fatal arrhythmias, thromboembolic phenomena, and need for pacemaker implantation or ICD in the placebo group will be around 30%. Event rate calculations were based on pooled data from longitudinal population studies in patients with CD[35, 36]. These studies indicate a 5% annual mortality with sudden cardiac death being responsible for 60% of the mortality. However, event rates among patienta entered into trials is generally lower so we have assumed a 3% -4% annual mortality. It is expected that the annual morbidity rates will also be similar givingh a composite event rate of 6%-8%/yr.

In order to have 90% power at the 5% significance level (two sided) with an overall drop-out rate of 20%, to detect a 20% risk reduction the trial will require the enrolment of at least 3000 patients. Analysis of the primary and secondary outcomes will be performed according to the intention-to-treat principle.

A sensitivity analysis wil be performed in patients with prior sustained ventricular tachycardia, thromboembolic phenomena or heart failure hospitalization. This analysis will allow that the mentioned events are not included as a primary outcome. Also, in patients with advanced AV block at onset, pacemaker insertion will not be counted as an endpoint.

4.2 Treatment

4.2.1 Study Drug

Study drug supply will be coordinated by the the Brazilian Coordinating Center with support by the PHRI-BENEFIT Project Office. Benznidazole 100 mg tablets and matching benznidazole-placebo tablets will be supplied. Non-study medications such as ACE inhibitors, diuretics, digoxin, beta-blockers, amiodarone, ASA or warfarin, will not be supplied. Patients will be encouraged to remain adherent to concomitant therapy according to current guidelines.

4.2.2 Randomization

Patients recruited following strict inclusion and exclusion criteria will be randomized double-blindly to receive 300mg/day of benznidazole in two daily doses for 40 to 80 days or matching placebo using the same administration daily scheme.

As seen on the Table for the Therapeutic Dosage, in page 5, both groups will be treated for 40 to 80 days, according to a direct relationship with the patient' s weight: thus, a patient weighing 40kg will be treated with 300mg daily for 41 days, weighing 51kg, for 51 days, 65kg = 65 days and so on, up to the maximum of 80kg, when a patient with this last weight will receive 300mg daily for 80 days. Hence, the maximum dose to be received, even for patients weighing more than 80 kg will be, at the end o the treatment period, 24g

Patients will be followed with attention paid to development of side effects including dermatitis, leucopenia, peripheral neuropathy and gastrointestinal symptoms. Potential toxic effects will be scrutinized with clinical and laboratory evaluation, including white cell count and hepatic function tests (AST, ALT, serum creatinine). These evaluations will be conducted at baseline, three weeks and 40 to 90 days after the beginning of treatment.

– Additional data will be collected in subgroups of patients to investigate other hypotheses:

4.2.3 Method of assigning patients to treatment group

The patient identification (ID) and treatment numbers will be provided at randomization by a centralized randomization service located at the Brazilian Coordinating Center. A system similar to that used in the CREATE and HOPE trials conducted by the PHRI will be designed. Alterbatively if all sites have access to a computer with internet access, local randomization may be performed in a secure way. Randomization will be 1:1 with stratification according to centre using a random block size. Subsequent treatment numbers for study drug re-supply will be provided.

4.2.4 Permitted concomitant therapy

Any concomitant therapy, including treatments demonstrated to be effective in the study population is permitted.

4.2.5 Treatment adherence

Study continuation is recommended unless clear contraindications arise, study drug should be continued or only briefly interrupted (≤ less than 1 week). The only clear criteria for permanent discontinuation of benznidazole study medication are: (i) significant leukopenia (< 2,500), (ii) serious gastrointestinal symptoms (iii) severe allergic dermopathy, and (iv) peripheral sensitive neuropathy. In other situations, discontinuation of study drug will be discouraged as much as possible. Symptomatic treatment for mild symptoms (nauseas, vomiting, allergy) is allowed. Study drug accountability, as well as all pre-specified concomitant medications, must be appropriately recorded at each scheduled visit.

5. OUTCOMES

5.1 Primary Outcome

The first occurrence of any of the following clinically significant outcomes:

1. Death
2. Cardiac arrest, requiring defibrillator or cardioversion
3. Documented sustained ventricular tachycardia requiring cardioversion
4. New development of symptomatic congestive heart failure fulfilling at least two of the following: a) signs and symptoms of CHF, b) Chest X-ray findings compatible with CHF, c) need for intravenous therapy, and d) hospital admission due to CHF
5. Pacemaker or implantable cardiac defibrillator indication.
6. Stroke or any other thromboembolic event in patients with no prior thromboembolic phenomena
7. Heart transplant

5.2 Secondary Outcomes

a) Response to therapy based on baseline PCR (+/-) and response at 24 month and end of follow-up

b) New development of any of the following echo changes; segmental wall motion abnormalities, ventricular aneurysm, reduction in LV ejection fraction > 5%, increase in LVDD > 5.0 mm compared with baseline.
c) New 12 lead ECG alterations (complete bundle branch block, fascicular block, advanced atrio-ventricular block, atrial fibrillation, etc).
d) Progression of NYHA functional class by at least one category.

6. FOLLOW-UP

6.1 Follow-up Schedule

The clinical and laboratory follow-up will aim primarily in evaluating treatment safety and detection of side effects. For this purpose the first follow-up visit will be scheduled 11± 2 days after the beginning of treatment, when allergic deramtitis typically occurs. The second follow-up visit will be scheduled 3 weeks ± 3 days after the beginning of treatment aiming at the detection of leucopenia, and a final visit will always be scheduled at the end of the treatment period (40 to 80 days, with tolerance of +10 days). Additional visits will occur 6 months after the end of treatment, and from then on annually until the end of the study.

7. STUDY ORGANIZATION

The PHRI-BENEFIT project office at McMaster University in Hamilton, Canada will coordinate the study. BENEFIT is a collaborative effort between the PHRI, and several Central and South America and investigators interested in Chagas disease from Argentina, Brazil, Colombia, and Venezuela, and will also aggregate Peru and Bolivia, El Salvador. It is expected that 75 centers are necessary to enroll 3000 patients (at least 40 patients per centre).

National coordinators will be responsible for recruitment and implementation of the trial. National coordinators will select the study sites and principal investigators in each country. It is estimated that Argentina and Brazil will enroll 2000 patients (1000 each) and 25 centres are needed in each country. Colombia and Venezuela will enroll another 1000 patients (500 each) with another 25 centres between the two countries. To enroll 3000 patients in 24 months we will need to recruit 125 patients monthly. This will be approximately 2 patients per month per center. These estimates suggest that the enrolment phase for BENEFIT is feasible. Drug distribution and labeling will be coordinated from the Brazilian Coordinating Centre.

7.1 BENEFIT Operations Committee

The Operations Committee will consist of a select group of Steering Committee members chosen for their specific expertise, time availability and experience. This group will be responsible for ensuring that study execution and management are of the highest quality. The Operations Committee will convene regularly by teleconference and/or face-to-face meeting (at least every 2 months) to discuss and report on the ongoing supervision of the study.

7.2 Steering Committee

The Steering Committee is composed of a group of national coordinators who will mostly be cardiologists, since patients with CD are most often cared for by cardiologists. This group will be supplemented with experts in CD, and electrophysiology. The committee has the overall responsibility for producing and conducting a scientifically sound design and ensuring accurate

reporting of the study. In that capacity, the Steering Committee must address and resolve scientific issues encountered during the study. This committee will meet at least twice a year.

All proposed ancillary research investigations on patients enrolled in BENEFIT must be approved by the Steering Committee. The primary scientific publication reporting the study results is the responsibility of the Steering Committee. Collaborating Investigators or members of the various study committees wishing to prepare secondary publications must submit proposals and manuscripts to the Steering Committee for approval. However, the final decision on the contents of all publications will be the responsibility of the BENEFIT Operations Committee.

7.3. Data Safety Monitoring Board

The Data Safety Monitoring Committee Board (DSMB) will include an epidemiologist, 2 prominent cardiologists, as well as a statistician. The DSMB provide on-going review of the safety of all the investigational treatments. To facilitate its responsibilities, the DSMB will have an Associated Statistician who will receive study data directly from the Central Study Database and who will remain independent of the trial management team.

The DSMB Associated Statistician, being unblinded, will not be able to edit/alter any part of the Central Study Database. Routine access to the treatment code will be restricted to the Chairman of the DSMB, except for emergency unblinding on a case-by-case basis.

DSMB Responsibilities

Primary:

1) Regular review of safety data and serious adverse events
2) Formal interim analyses of efficacy data
3) Feedback to the Operations Committee

Secondary:

1) Respond to special requests from regulatory authorities or IRBs
2) Recommendations for protocol amendments
3) Verification of the final analysis of the study will be done by the DSMB Associated Statistician

Safety Review

Recommendation to stop a trial early for safety reasons is, by definition, a qualitative judgement. The DSMB is composed of eminent clinicians and methodologists who are experienced with clinical trials and can be relied upon to exercise good judgment in weighing the potential risks and benefits to patients as data accumulate in this trial.

Safety aspects, and more specifically severe dermatitis and/or neuropathy will be monitored. No formal boundaries will be proposed for safety, but clear, consistent, and persistent evidence of net harm that overwhelms any benefit should be apparent. A recommendation to stop the trial will be based on the pattern of treatment effect across all endpoints, as well as the benefit/risk ratio. Two interim analyses to assess futility are scheduled at approximately 1/2 and 3/4 of the total of anticipated events.

The trial may be stopped for efficacy if a reduction in events by four standard deviations, or a three standard deviation excess occur in the first half of the trial, or if a reduction in events by three standard deviations or a 2-standard deviation excess is detected in the second half of the trial. If the upper limit of the 95% CI for the conditional power for the primary outcome falls below 15%, then, all other things being equal, the DSMB may recommend early termination**.**

7.4. Adjudication Committee

This committee, composed of experts in the relevant fields will review, in a blinded manner; all reported outcome events to provide consistency and validity in the assessment of outcomes. Their decisions will be based on blind clinical data and they will consider the impressions of the clinical investigator. Their decisions will be used for the final analysis.

8. PROJECTED TEMPORAL SCHEDULE FOR THE PROJECT DEVELOPMENT

2002 until November 2003: development and finalization of the research protocol

November 2003 to November 2004: center selection and preparation

November 2004 to February 2010: recruitment

November 2004 to February 2013: clinical follow-up

March 2013 to February 2014: results analysis and publication

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10. APPENDIX – TABLE for number of days of treatment to the patient' s body weight

Weight (Kg)	Treatment Duration (days)
40	40
41	41
42	42
43	43
44	44
45	45
46	46
47	47
48	48
49	49
50	50
51	51
52	52
53	53
54	54
55	55
56	56
57	57
58	58
59	59
60	60
61	61
62	62
63	63
64	64
65	65
66	66
67	67
68	68
69	69
70	70
71	71
72	72
73	73
74	74
75	75
76	76
77	77
78	78
79	79
≥80	80

- **Fixed 300mg daily dose for all patients**
- **1 pill qam and 2 pills qpm (q12 hrs)**
- **Each BZ pill = 100 mg (unscored)**

BENEFIT

HISTORY OF PROTOCOL AND AMENDMENTS

Protocol	**Date**	**Reason for Amendment**
Original Protocol	May 12, 2003	n/a
Amendment #1	June 27, 2005	Pilot Study
Amendment #2	August 29, 2005	Pilot Study: Consent Form modification
Amendment #3	May 30, 2006	Extended upper limit of age inclusion criterion from ≤65 years to ≤75 Years. To revert to original version of the consent form (i.e., without amendment 2 alterations).
Amendment #4	Feb 02, 2009	Change of Study Medication presentation and adminsitration schedule. From scored to non-scored tablet. Heart transplant added to primary outcome composite.

STATISTICAL ANALYSIS PLAN

THE BENEFIT Trial

BENznidazole **E**valuation **F**or **I**nterrupting **T**rypanosomiasis

Final Version 1.0
Date: June 13, 2015

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1. LIST OF ABBREVIATIONS

Abbreviation	Full Word
AE	Adverse Event
AF	Atrial Fibrillation
BENEFIT	Benznidazole Evaluation for Interrupting American Trypanosomiasis
BNZ	Benznidazole
CCC	Chronic Chagas' Cardiomyopathy
CD	Chagas Disease
CI	Confidence Interval
CNS	Central Nervous System
CRT-D	Cardiac Resynchronization Therapy Defibrillator
ECG	Electrocardiogram
GEE	Generalized Estimating Equation
HR	Hazard Ratio
ICD	Implantable Cardioverter Defibrillator
ITT	**Intention-To-Treat**
IV	Intravenous
LVEDD	LV end-diastolic diameter
LVEF	Left Ventricular Ejection Fraction
MedDRA	Medical Dictionary for Regulatory Activities
OR	Odds Ratio
PCR	Polymerase Chain Reaction

PHRI	Population Health Research Institute
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
VT	Ventricular Tachycardia

2. INTRODUCTION

This statistical analysis plan (SAP) provides a comprehensive and detailed description of the statistical methods to be used to realize the analysis of the "Benznidazole Evaluation for Interrupting American Trypanosomiasis (BENEFIT) Trial" described in the clinical study proto col (dated February 2, 2009). The objective of a Statistical Analysis Plan is to ensure the maximum credibility of all study findings by means of a pre-specified data analysis. This plan may be revised during the study to accommodate protocol amendments and/or to make changes to adapt to unexpected issues in study execution and/or data that affect planned analyses. All changes will be made by individuals who are blinded to the outcomes by treatment group. The SAP is a working document that will be amended as the trial progresses. The final version of the SAP will be signed off prior to database lock

The BENEFIT trial is a multicenter, prospective, randomized, double-blind, placebo-controlled clinical trial of 2,855 patients with chronic Chagas' cardiomyopathy(CCC) in Latin America. The role of persistent low grade *T. cruzi* infection in the progression of CCC remains a matter of discussion and the efficacy and safety of Benznidazole in this setting has not been tested in a large-scale multicentre clinical trial. Similarly outcome measures to determine efficacy of treatment are limited by the long time serologic Chagas markers take to become negative.

In BENEFIT, Chagasic patients aged ≥18 years and ≤75 years, are eligible if, in addition to having any combination of at least 2 positive serologic tests for CD (indirect immunofluorescence, indirect hemagglutination, or ELISA), have evidence of cardiomyopathy based on the criteria outlined in Table I. Consenting patients who fulfill the eligibility criteria are randomized to receive benznidazole(BNZ) (5 mg/kg per day) or matched placebo for 60 days (randomized from November 2004 to December 2008 with 1543 patients) or a maximum daily dose of 300mg for 60 days if weight was 60kg,, 70 days if weight was 70kg, and so on, to a maximum of 80 days(randomized from January 2009 to October 2011 with 1312 patients). (The reason for this was related with drug supply). The primary outcome is the composite of death; resuscitated cardiac arrest; sustained ventricular tachycardia(VT); new insertion of pacemaker or cardiac defibrillator;

cardiac transplantation; and development of new heart failure, stroke, or systemic or pulmonary thromboembolic events. The average follow-up time will be 5.5 years, the sample size is 2753 patients pts with and the trial has a 90% power to detect a 26.5% relative risk reduction, assuming a yearly event rate of 8% in the control group of the composite primary outcome.

3. STUDY HYPOTHESIS/OBJECTIVES

3.1 Primary objective

To determine whether BNZ reduces the composite primary outcome (death; resuscitated cardiac arrest; sustained VT; new insertion of pacemaker or ICD/CRT; cardiac transplantation; and development of new heart failure, stroke, or systemic thromboembolic events), compared to placebo in patients with CCC.

We will address the primary objective through the following hypotheses:

Ho: $HR_{BNZ/Placebo} = 1$

Ha: $HR_{BNZ/Placebo} \neq 1$

The null hypothesis (Ho) can be rejected if the two-sided p-value is less than 0.05. We will consider BNZ is superior to placebo if the upper bound of the two-sided 95% confidence interval (CI) around $HR_{BNZ/Placebo}$ is less than 1.

3.2 Secondary objectives

The secondary objectives are the following:

- To determine the effects of BNZ compared to placebo regarding negativization of PCR in patients with chronic CCC after 60-80 days of treatment and at 24 months and end of follow-up.
- To evaluate whether BNZ prevents new development of any of the following echo changes; segmental wall motion abnormalities, ventricular aneurysm, reduction in LV ejection fraction > 5%, increase in LVEDD > 5.0 mm compared with baseline.
- To evaluate whether BNZ prevents new 12 lead ECG alterations (complete bundle branch block, fascicular block, advanced atrio-ventricular block, atrial fibrillation(AF), etc).
- To evaluate whether BNZ prevents the progression of NYHA functional class by at least one category.
- To evaluate the safety, tolerability and adherence to BNZ in patients with CCC.

4. POPULATIONS TO BE ANALYZED

4.1 Intention-to-treat Population

All patients who are randomized are included in the analysis according to the treatment groups to which they were randomized, irrespective of whether or not the patient actually received study drug, the patient's compliance with the study protocol or duration of the trial participation. All analyses of primary and secondary endpoints, as well as baseline summaries, subgroup analyses and all analyses related to safety will be based on the intention-to-treat(ITT) principle.
Sensitivity analyses for the primary and secondary outcomes will be performed based on the following two sub population A and B.

4.2 On-Treatment Population A

Population on treatment A (1543 Patients randomized from November 2004 to December 2008) is defined as:

Patient randomized to BNZ or placebo 5mg/kg/day for 60 days.

4.3 On-Treatment Population B

Population on treatment B(1312 Patients randomized from January 2009 to October 2011)

is defined as:

Patients randomized to BNZ total daily dose of 300 mg during 40 to 80 days depending on the patient's total weight); max dose 24 g for 80 Kg.

5. BASELINE CHARACTERISTICS

Summary statistics for demographic data and baseline characteristics using ITT population will be displayed separately by the BNZ and placebo.

For categorical variables, summaries will consist of counts and percentages, with p-values comparing groups from chi-square tests. Note Fisher exact tests will be substituted for chi-square tests where expected counts are <5 per group.

For continuous variables, summaries will consist of means and standard deviations (SDs) with p-values comparing groups from t-tests.

For non-normally distributed variables including time duration variables which are not likely to be normally distributed, summaries will consist of median and percentiles 25^{th}, 75th, with p-values comparing groups from Wilcoxon rank sum tests.

Demographic Characteristics include age, gender, weight, height, heart rate and blood pressure, country. Data for the background characteristics will be collected for education, smoking lifestyle,

alcohol use, employment status, occupation, and housing conditions. Baseline characteristics will include the following:

- Medical history: NYHA function class, previous admission due to heart failure, resuscitated cardiac arrest, sustained VT, internal cardiac defibrillator, atrial fibrillation, pacemaker, stroke/TIA, pulmonary embolism, systemic embolism, syncope, HIV, leukemia or lymphoma, tuberculosis, cancer
- Use of medications: Loop diuretic, Spironolactone, other diuretics, ACE-I, ARB, digoxin, aspirin, beta-blocker, amiodarone, other anti-arrhythmic, oral anticoagulants, antiplatelets
- Baseline ECG results: Normal/abnormal, electrocardiograms
- Chest X-ray: Cardio thoracic ratio > 0.59, signs of pulmonary congestion
- Lab results: WBC count, hemoglobin, platelet count, ALT/TGP, AST/TGO, creatinine
- 2D echocardiogram: LV ejection fraction, LV end-diastolic diameter, LV percent fractional shortening, LV thrombus regional wall-motion abnormalities, aneurysm
- Socio-demographics: Income, education, employment status, occupation, country
- Current housing conditions

6. COMPLIANCE

Patient compliance will be categorized as good (>75%), medium (50-75%), or poor (<50% of taking study medication) and presented by treatment group. The frequency of subjects experiencing study drug interruption, the reasons for the interruption will be summarized by treatment group. The proportion of patients who were lost will be reported.

7. STUDY FOLLOW-UP TIME AND MISSING DATA

7.1 Study follow-up time

Overall follow-up for the BENEFIT Trial will be in average 5.5 years. Active follow-up will be planned during the intervention period that lasts between 40 to 80 days. During this period safety, tolerability and adherence visits are programmed at 11 and 21 days and at the end of therapy (40 to 80 days). The next visit will be at 6 months for the detection of clinical outcomes. Yearly follow-up visits are programmed until study termination at a mean time of 5.5 years. The duration of the follow-up will be summarized by treatment group.

All efforts will be made to collect information related to the clinical outcomes for those participants lost to follow-up. In case of written withdrawal of consent for follow-up visits, and unless otherwise stated by the patient in the informed consent form, investigators will be encouraged to get information from the general practitioner, any other physician or other medical-care provider, or from patient relatives in order to follow the medical status of the patients (especially when they withdrew their consent after having experienced an adverse or serious adverse events). In case of

no contact and missing information on the efficacy assessment, the patient will be censored on the last day of available contact during the study.

For a single event like death the date of event will be defined as the date reported on event CRF. The date of events with potential multiple episodes such as cardiac arrest or stroke will use the date of the first episode. For composite outcomes, the date of the first occurring component will be used as the date of the event. The date of final follow-up will be defined as the last date among all dates of visits/contact and dates of events reported on the event report forms or any laboratory test dates.

In a time-to-event analysis, the survival time will be calculated as the date of event minus date of randomization plus 1 for a patient with an event. For those without an event, the survival time will be the date of final follow-up minus date of randomization plus 1.

7.2 Missing data

Missing data will be vigorously pursued with the quality assurance process. Centres will be requested to collect as much information as possible. In general missing values will be treated as missing, unless otherwise specified. Unknown data will be coded as such in the database and classified as such on any analysis. No attempt will be made to impute missing values and only observed values will be used in the analyses. Depending on the assessment, analyses may not include all patients in an analysis population, because certain patients in the intended population may have missing data.

Patient mortality and morbidity will be determined for all participants regardless of compliance with study medication or visit schedule. For patients who die prior to follow-up evaluation of LV function or ECG, and worse case rank will be assigned to that patient and a secondary non-parametric analysis will be done to examine the sensitivity to these missing data.

8. EFFICACY ANALYSIS

8.1 Definition

8.1.1 Primary Outcome

The primary efficacy outcome is defined as the first occurrence of any of the following clinically significant outcomes at follow-up in patients with CCC

i) Death
ii) Cardiac arrest, requiring defibrillation or cardioversion
iii) Documented spontaneous sustained ventricular tachycardia requiring or not electrical or pharmacological cardioversion

iv) New or worsening symptomatic congestive heart failure with at least two predetermined criteria (signs and symptoms of CHF, Chest X-Ray with fondings compatible with CHF, need for intravenous therapy, or new initiation of diuretics, hospital admission due to HF)
v) Pacemaker or implantable cardiac defibrillator/CRT implantation
vi) Heart transplant
vii) Stroke or systemic embolism (including pulmonary embolism)
viii)

Efficacy variables will be recorded at baseline and after termination of the 80 day course of therapy. Similarly, efficacy parameters will be recorded at month 6, year 1 to 9 and final visits. Investigators will be instructed to complete CRFs and inform of any outcome event within 2 days of their knowledge, or at specified study visit dates. Analysis of efficacy variables will be performed at the different times of collection of these variables as well as at the end of follow-up.

8.1.2 Secondary Outcome

The secondary efficacy outcomes are the following separate individual events:

- Negativization rate of PCR based on baseline and follow-up qualitative PCR result between patients receiving benznidazole compared to placebo.
- Negativization of *T. cruzi* parasite load detected by competitive PCR: Parasitic load is expressed as the amount of *T. cruzi*/ml of blood between patients receiving benznidazole compared to placebo.
- New development of any of the following echo changes; segmental wall motion abnormalities, ventricular aneurysm, reduction in LV ejection fraction > 5%, increase in LVDD > 5.0 mm compared with baseline.
- New 12 lead ECG alterations (complete bundle branch block, fascicular block, advanced atrio-ventricular block, atrial fibrillation, etc).
- Progression of NYHA functional class by at least one category

Other analysis will include regional variation of CCC progression, primary and secondary outcomes in PCR [+] and PCR [-] subjects at baseline.
T. cruzi I vs *T.cruzi II* primary and secondary outcomes as described above.

8.2 Analysis Method

All cardiovascular outcome assessments will be reviewed by an Event Adjudication Committee (EAC), who will be kept blinded to the treatment assignment of participants. All statistical analyses of these outcomes will be based upon adjudicated outcomes, where applicable. In case that an adjudicated result is not available, the clinical investigator's assessment will be accepted. A two-sided significance level of 0.05 will be used for overall analysis. No adjustments for multiplicity will be made for multiple efficacy endpoints.

8.2.1. Analysis of primary outcome

The primary efficacy outcome (composite of death; resuscitated cardiac arrest; sustained ventricular tachycardia; new insertion of pacemaker or ICD/CRT; cardiac transplantation; and development of new heart failure, stroke, or systemic thromboembolic events) will be analyzed using a time-to-event approach, counting the first occurrence of any component of the composite outcome. Censoring will occur if the patient is lost to follow-up or reaches the end of the follow-up period without experiencing the primary outcome. Patients who prematurely discontinue their follow-up visits prior to the development of primary composite outcomes will be censored as of their last completed follow-up visit. The treatment groups will be compared on each secondary efficacy outcome. Cumulative incidence curves over time for each treatment group will be provided using the product limit estimation method and compared using a logrank test. Treatment comparison based on Cox proportional hazard model will be reported as hazard ratio (HR) and 95% confidence interval (CI). The proportionality assumption of the Cox regression model will be assessed graphically and with the use of Schoenfeld residuals, or by including a time-treatment interaction term in the Cox model (time log transformed). The number and incidence of each component of the composite primary outcome will be summarized in each treatment group. For the subgroup analysis (see section 10), test for interaction will be based on Cox regression by including an interaction term with treatment in the model.

8.2.2. Analysis of secondary outcomes

The proportion of negativization of *T. cruzi* PCR detection either at the end of treatment and/or the end of follow-up at 2 years will be analyzed for all participants using Chi-square test. This analysis will also include only those who were positive PCR test to *T. cruzi* at baseline to compare the proportions of the negativization of *T. cruzi* PCR in each treatment group. Logistic regression model will also be employed to incorporate the potential confounding factors. Frequencies and percentages per arm, an Odds Ratio(OR) measuring the treatment effect and its 95% CI will also be reported. With the repeated measurements of PCR at baseline, after termination of the therapy (i.e., 40-80 days), at 2 years and at the end of follow-up, a longitudinal data analysis technique known as a generalized estimating equation (GEE) will be used to compare the differences in changes of the proportion of negativization of *T. cruzi* over time between BNZ and placebo groups. A within-subject correlation structure for the repeated measurement will be carefully chosen before carrying out a GEE analysis by examining the correlation structure of observed data.

The comparisons for new development of echo changes, new 12 lead ECG alterations, and the progression of NYHA functional class by at least one category will be performed using a chi-square test for categorical variables. The treatment effect will be estimated using a logistic regression including treatment group as a covariate. OR and 95% CI will be reported. Multiple logistic regression will be performed in order to incorporate the potential confounding factors. For continuous variables such as the echo changes, the non-parametric test will be used to compare the treatment effect.

9. SAFETY

9.1. Safety Variables

Adverse events (AEs) during the active therapy phase recorded during follow-up visits at 11 and 21 days and at the end of therapy (40 to 80 days) will be closely monitored and reported.

A serious adverse event (SAE) is defined as any untoward medical occurrence that is observed at any dose:

- Results in death or
- Is life threatening or
- Requires in-patient hospitalization, or prolongation of existing hospitalization or
- Results in persistent or significant disability/incapacity or
- Is a medically important event

All adverse events in each treatment group regardless of seriousness or relationship to study drug are to be reported. Whenever possible, symptoms should be grouped as single syndrome or diagnosis.

9.2. Safety Analysis

All randomized patients to BNZ/Placebo will be included in the analysis. The AEs and SAEs will be counted on the date of first dose administered and up to 30 days after administration of the last dose of treatment assigned. Safety will be summarized by tabulations of AEs and SAEs for each treatment group. The analysis of safety will be mainly descriptive. For all safety analyses, the day of the first dose of study medication will be assumed to have occurred on the same day as randomization unless there is a record indicating that study medication was not used immediately after randomization. The separate summaries of adverse events and serious adverse events will be provided by the period of observation, i.e., whether the (serious) adverse event was observed while a patient was on the study medication or after the termination of the study drug use.

All adverse events will be classified on the basis of Medical Dictionary for Regulatory Activities (MedDRA) terminology and summarized for each treatment. Each adverse event will be recorded with a system organ class and a preferred term. In reporting incidence, each subject will be counted only once within a system organ class or a preferred term, and counts will be provided for each treatment group. Percentages will be calculated using the number of randomized patients in each treatment group. For events of particular interest, summary statistics (n, mean, standard deviation, median and range) may be calculated with the time from randomization to onset and the duration

of events. The list of adverse events and serious adverse events will be provided by patient with details of the events.

10. SUBGROUP ANALYSIS

Analysis for consistency of treatment effects in prespecified subgroups will be explored with respect to the time to event primary composite outcome by Cox regression model and the proportion of PCR negative and negativization of *T. cruzi* by logistic regression model. The models will incorporate terms for treatment, the covariate and the treatment-by covariate interaction in order to examine the consistency of results on the treatment differences. The interaction P-values will be presented. The number of patients with outcomes, estimated odds ratio or hazard ratio, and associated 95% confidence intervals will be calculated within each of the subgroups generated by these analyses.

Selected outcomes (primary outcome, the proportion of PCR negative and negativization of *T. cruzi)* will be compared between the following pre-specified groups:

a. Patients with a baseline [+] PCR

b. Patients with baseline [-] PCR

c. *T. cruzi* genotype (*T. cruzi I vs T. cruzi II*) and response to BNZ based on PCR negativization rate and parasite load reduction

d. Gender (male vs female)

e. Patients on amiodarone

f. Patients on spironolactone

g. Country

h. age group (> median vs <= median)

i. baseline ECG alterations

j. LV function and LV end diastolic diameter

As the number of these subgroup variables may be large, the probability of observing at least one statistically significant result may be high. It is assumed that any subject who is non-compliant with benznidazole treatment will, at that point in time, assume a risk of an event that is equal to the risk of a subject in the placebo group.

11. SENSITIVITY ANALYSIS

Other than the sensitivity analyses specified in section 4, a sensitivity analysis for the primary outcome will be performed in patients with prior sustained ventricular tachycardia, thromboembolic phenomena or heart failure hospitalization. This analysis will allow that the mentioned events are not included as a primary outcome. Also, in patients with advanced AV block at onset, pacemaker insertion will not be counted as an endpoint.

12. ADHERENCE TO THE PROTOCOL

Protocol violations are any deviations from the procedures, which include, but are not limited to, the ingestion of disallowed/incorrect medications or non-compliance with study medications or visit procedures and schedules. No patients are to be discontinued from the study due to protocol violations. However, every attempt should be made to ensure that as few protocol violations occur as possible. Further, at each visit, the investigator is to counsel patients, in particular those that are non-compliant, on the importance of taking study medication as directed.

Summary statistics (counts and % of patients) for reported protocol deviations will be presented as the total number of deviations and deviations within each category.

Table I. Inclusion criteria

≥1 of the following (A through E):

A. Abnormal electrocardiogram (at least 2 of the following):
   1. Right bundle-branch block
   2. Left bundle-branch block
   3. Left anterior fascicular block
   4. Left posterior fascicular block
   5. Ventricular premature beats
   6. First degree AV block >220 milliseconds, in absence of drugs slowing AV conduction
   7. Mobitz type I AV block, in absence of drugs slowing AV conduction
   8. Sinus bradycardia <50 beat/min or sinus pauses >3.0 s, in absence of sinus node blocking drugs
   9. Primary ST-T changes
   10. Abnormal Q waves
   11. Low voltage QRS
   12. Atrial fibrillation

B. Abnormal ECG (one of the following):
   1. Mobitz type II, advanced or third degree AV block
   2. Cardiac pacemaker or implanted automatic defibrillator

C. Increased cardiothoracic ratio (>.50)

D. Complex ventricular arrhythmias (multiform >10/h, couplets or NSVT) on 24-h ECG monitoring

E. Evidence of regional wall motion abnormality or reduced (<50%) global LV systolic function (2D Echo, RNA, contrast ventriculography) or increased LV end-diastolic diameter (>55 mm) on 2D Echo

AV, Atrioventricular; *NSVT*, non-sustained ventricular tachycardia.

13. APPROVAL

Version #	1.0
Version Date	2015-06-13

By signing the below, I designate my approval of the above-named version of the BENEFIT Statistical Analysis Plan on behalf of all named authors.

Name	Dr. Salim Yusuf
Role	Chair BENEFIT Steering Committee
Signature	
Date (yyyy/mm/dd)	

By signing the below, I designate my approval of the above-named version of the BENEFIT Statistical Analysis Plan on behalf of all named authors.

Name	Dr. Carlos Morillo
Role	Principal Investigator
Signature	
Date (yyyy/mm/dd)	

By signing the below, I designate my approval of the above-named version of the BENEFIT Statistical Analysis Plan on behalf of all named authors.

Name	Peggy Gao
Role	**Senior Biostatistician, Statistics, PHRI**
Signature	Peggy Gao
Date (yyyy/mm/dd)	2015/06/15