CENTER FOR DRUG EVALUATION AND RESEARCH

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CLINICAL REVIEW(S)

Clinical Review
Maria Allende, M.D.
NDA 209570
(b) (4) CHEMO Benznidazole for Treatment of Chagas Disease

CLINICAL REVIEW

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Reviewer Name(s)	Maria Allende, M.D.
Review Completion Date	
Established Name	Benznidazole
(Proposed) Trade Name	(b) (4)
Applicant	CHEMO Research SL
Formulation(s)	Oral tablets containing 100 mg or 12.5 mg of benznidazole
Dosing Regimen	
Applicant Proposed Indication(s)/Population(s)	Treatment of Chagas disease (American Trypanosomiasis)
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	Treatment of Chagas disease (American Trypanosomiasis)

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Glossary

AC	advisory committee
AE	adverse event
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DMC	data monitoring committee
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
ICH	International Conference on Harmonization
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application

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NME	new molecular entity
OCS	Office of Computational Science
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee
SOC	standard of care
TEAE	treatment emergent adverse event

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1 Executive Summary

1.1. Product Introduction

Benznidazole (BNZ) is an antiprotozoal agent, derived from N-benzyl 2-nitroimidazole.

It belongs to the nitroimidazole drug class and it is used to treat Chagas disease (American trypanosomiasis). Benznidazole came into medical use in 1972 in Latin America. It is on the World Health Organization's List of Essential Medicines for treatment of Chagas disease.

Treatment of Chagas disease is the Applicant's proposed indication, administered orally in doses of 5 to (b) (4) mg/kg/day in two daily doses (b) (4) 12 hours, for 60 days.

The proposed proprietary name is (b) (4) however, it was not accepted. Discussions are ongoing at this time regarding the product name. The Applicant has developed the product as a tablet for oral use:

- 100 mg BNZ (b) (4) scored tablet

- 12.5 mg BNZ tablet

The 12.5 mg BNZ tablets may be (b) (4) made into a slurry formulation with water for immediate administration for patients with smaller body weight (i.e., pediatric patients).

Benznidazole is considered a new molecular entity (NME). It has not been approved in the United States for any indication. Benznidazole, however, manufactured by Roche, and then by LAFEPE and ELEA, has been marketed in 21 endemic countries since the 1970's.

1.2. Conclusions on the Substantial Evidence of Effectiveness

I recommend that benznidazole tablets be approved for treatment of acute and chronic indeterminate Chagas disease without established cardiomyopathy. The data reviewed provide substantial evidence of effectiveness and strongly suggest that benznidazole treatment should not be limited to certain age groups and should be approved for all pediatric populations and adults with acute and chronic indeterminate Chagas disease. The applicant submitted datasets from two adequate and well-controlled trials in pediatric populations showing superiority based on conversions to negative serology by unconventional assays using the F29 and AT antigens, and superiority of benznidazole in quantitative reduction of conventional assays. Conversion of serology to negative on an unconventional F29 or AT assay and significant reductions in serological titers (without complete conversion of serology to negative) on conventional assays are expected, surrogate endpoints reasonably likely to predict meaningful clinical benefit.

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In addition, the Applicant submitted patient-level data from an adequate and well controlled long term follow-up study in the adult population, measuring clinical outcomes of cardiac Chagas disease in adults without heart failure. This trial demonstrated effectiveness of benznidazole treatment on four meaningful clinical endpoints: delayed progression into a more severe cardiac stage (Kuschnir categories), reduction of new ECG changes characteristic of Chagas disease, improved survival (time to death) and decreased mortality rate. In addition, this adult trial demonstrated that favorable clinical outcomes are associated with a conversion of serology to negative. A persistent decrease of the serological titers over several years is reasonably likely to predict serological conversion to negative. This adult study is supported by results from other 3 independent prospective cohorts and one retrospective-prospective study, which also showed evidence of meaningful clinical benefit (delayed progression of disease, decreased incidence of new ECG changes).

The two pediatric and the adult studies are mutually supportive: the pediatric studies help understand the drug effect on significant reduction of conventional serology titers and conversion to negative of unconventional assays (immune responses to specific trypomastigote antigens F-29 and AT-ELISA), which are reasonably likely to predict future conversion to negative of conventional assays. Thus, an accelerated approval pathway may be appropriate. A postmarketing confirmation of clinical benefit could be shown by a prospective serological evaluation in a pediatric population to achieve reversion to negative on conventional serology, which is the endpoint of clinical benefit, in a reasonable time frame of 5-10 years. It is also reasonable to consider accelerated approval in children ages 0 to 6 years, adolescent and adult populations as well, based on the successful results of the two pediatric trials. Conversion of serology to negative was associated to benznidazole treatment in both pediatric and adult studies. In adult studies, conversion of serology to negative was associated with lower incidence of new ECG changes and delayed progression of cardiac disease.

The four clinical endpoints, regardless of serology, are evidence of effectiveness. Mortality and increased survival time without disease progression provide the strongest objective evidence.

The three trials are consistent in their outcomes and mutually supportive and, together with preclinical efficacy in three animal models (biological plausibility) and other clinical data from at least 4 independently conducted long-term controlled studies; make the argument of *"totality of evidence".* Based on this mutually supportive evidence, a Subpart H approval would be supported for children, adolescents and adults with Chagas disease without evidence of established cardiomyopathy. A confirmatory trial in children to verify the serological negativization with conventional assays could be a single arm with historical controls; with a combination of various serological and clinical endpoints (new specific ECG changes for

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example). A post-marketing study is recommended for adults, to collect additional safety data with the to-be-marketed product, and serological endpoints with FDA approved assays.

The limitation of the 3 prospective supportive studies is that, because of their design, and small size, the therapeutic effect cannot be accurately estimated or predicted. In addition, because of the characteristics of the disease, trial results may not be generalizable to all geographical areas, given the variability of the parasite lineage, the unknown and unmeasurable parasitic tissue burden and unknown organ damage already present at the start of treatment (focal myocardial fibrosis and impaired conduction system) produced by the parasite-host immune interactions. The parasite can rarely be isolated from the blood in the indeterminate phase of the disease, and the amastigotes in the heart tissue are sparse and inaccessible for tissue biopsies, which carry considerable risks. This heterogeneity and immeasurable uncertainties, unique to this disease, are also a limitation that affects the conduct and interpretation of clinical trials, which need to have a follow-up of at least 15 years to observe clinical endpoints, and recruit a large number of patients from several regions. These are important feasibility challenges. An additional trial, randomized and placebo controlled with clinical endpoints relevant to Chagas cardiac disease would have been desirable to further boost the totality of the efficacy findings. However, I do not believe this is absolutely necessary to make a decision of approval at this time. No drugs are approved for treatment in the United States, and only one other drug exists for treatment, available only through a CDC protocol. No other drugs were found to be effective in the over 100 years since the disease was discovered in 1909. Chagas disease is a neglected tropical disease with an important, large unmet medical need. Drug shortages have been frequent and severe in the last decade, worldwide. Immunocompromised patients have mortality rates close to 90% when Chagas disease reactivates, and treatment in these situations could provide survival benefit, as shown in numerous case reports and case series. All the evidence collectively indicates that the earlier the treatment, the greater the benefit.

Considering all these factors, and the totality of the evidence of efficacy, I believe we should approve benznidazole based on the principles of broadest flexibility outlined in 21 CFR 312.80, that *"physicians and patients are generally willing to accept greater risks or side effects from drugs that treat life-threatening and severely-debilitating illnesses"* and accept with some uncertainty of the magnitude and predictability of the therapeutic effect, but knowing that in some proportion of patients, it will provide meaningful clinical benefit, most importantly, progression free survival, increased time to death and a lower mortality for benznidazole-treated patients. The regulatory decision risk of denying a benefit in mortality and progression-free disease for a proportion of patients is, in my opinion, greater than the potential risks from a 60-day course of benznidazole treatment.

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1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Benznidazole is a nitroimidazole derivative with activity against *Trypanosoma cruzi* trypomastigotes and amastigotes. Its indication is for treatment of Chagas disease in children, (b) (4) in oral doses of 5 to 8 mg/kg/day for 60 days. In three adequate and well controlled trials, it has demonstrated effectiveness in achieving serological cure, reducing disease progression and mortality, and increasing the time until death. Chagas disease is caused by infection with the protozoan parasite *Trypanosoma cruzi*; the major manifestations are Chagas cardiomyopathy and gastrointestinal disease. Severe heart disease and death occur in about 25% of those infected, up to several decades after infection. Special populations at higher risk for severe disease and mortality are those who are immunosuppressed, such as HIV and cancer patients receiving chemotherapy. Most persons acquire this infection through contact with vector bugs carrying *T. cruzi* in endemic areas of Latin America. Infection can also be acquired by congenital, transfusion, transplantation, and foodborne transmission. An estimated 300,000 persons with Chagas disease live in the United States, but most infections go undetected. An estimated 63–315 number of newborns acquire *T. cruzi* infection congenitally in the United States every year but most infections go undetected and untreated. Based on these estimates, chagasic cardiomyopathy, which can be prevented through early treatment, affects approximately 30,000–45,000 persons in the United States. Most persons complete therapy with benznidazole without serious adverse reactions. Children have fewer adverse reactions than adults. Benznidazole causes cutaneous rashes and hypersensitivity dermatitis, which are the most common adverse reactions, frequently causing drug discontinuations. Other toxicities include peripheral neuropathy and polyneuritis, abdominal pain, decreased appetite, nausea, vomiting and diarrhea, increased liver enzymes, eosinophilia, neutropenia, thrombocytopenia and lymphopenia. Most of the adverse reactions are mild, rarely severe. They are recognized through clinical monitoring and resolved with temporary or permanent drug discontinuations, although in rare cases, it may take several months until full recovery. No deaths have been causally associated with benznidazole. In all cases, the severity of the underlying disease and other concomitant medications has prevented an assessment of causality. Potential risks are from data from animal studies, where carcinogenicity, genotoxicity, fetal toxicity and decreased male fertility were observed. None of these risks have been reported in humans in over 40 years of use outside the United States. Considering the serious and life-threatening nature of Chagas disease, and the demonstrated effectiveness of benznidazole treatment with a single course of therapy at doses of 5 to 8 mg/kg/day for a 60 days duration, the risks of therapy outweigh the benefit of treatment. The approval of benznidazole will address a substantial unmet medical need, since it will be the first drug approved for treatment of Chagas disease in the United States.

No REMS is recommended, since adverse reactions characteristics and management are appropriately addressed through labeling. At this time the review team is discussing the following postmarketing requirements and commitments:

Postmarketing Requirements

- Confirmatory study required as a condition of Subpart H approval: We are proposing an (b) (4), single-arm study (b) (4) (b) (4) PK (b) (4) (b) (4)
- ADME/mass balance study in humans
- Male fertility study in rats (agreed to in the End of Phase 2 meeting on April 27, 2016)

(b) (4)

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Chagas disease is a serious and life threatening disease with 20 to 30% risk of severe cardiac disease manifested as sudden death, severe arrhythmias and heart failure, mostly in young adults. Most cases in the United States are not detected. Chagas is a disease spread by contact with feces of an infected insect (triatomine) carrying a parasite called Trypanosoma cruzi. • The disease is estimated to affect around 6-7 million people worldwide, primarily in rural areas in Latin America. In the United States, the disease is estimated to affect more than 300,000 people, most of whom acquired the disease in foreign countries. • There are two phases of Chagas disease: the acute phase and the	Chagas disease is a serious and neglected tropical disease with potentially life-threatening complications (such as sudden death and heart failure) that can have a significant impact on patients' quality of life. Diagnoses of Chagas disease are likely to increase with increased awareness and knowledge about the disease and blood bank donor screening now done routinely.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	chronic phase. The acute phase is often asymptomatic and can last a few weeks/months after infection. The chronic phase may last decades after infection, and patients may develop more significant symptoms, including problems with swallowing, digestion, constipation or abdominal pain, and heart failure. • Blood screening tests have been approved by FDA in recent years • Chagas disease can have significant physical and emotional impact on patients' quality of life.	
Current Treatment Options	• There are no current treatment options. No drug is approved for treatment of Chagas disease. In the U.S. nifurtimox or benznidazole are provided for treatment through the CDC through single-patient IND applications. Some patients tolerate only one of those two drugs. Several drug shortages have occurred during the last decades, jeopardizing treatment in the United States	There is a significant unmet need for FDA-approved therapies for all patients with Chagas disease. Benznidazole and nifurtimox shortages threaten the availability of treatments for Chagas disease through the CDC protocols.
Benefit	• Two randomized controlled studies in children showed significant reduction of conventional serology titers and negativization of unconventional assays (immune responses to specific trypomastigote antigens F-29 and AT-ELISA), which are reasonably likely to predict clinical benefit. In one adequate and well controlled trial in adult Chagas disease patients, who were assigned to either benznidazole at 5mg/kg/day for 30 day-treatment (n=283) or to remain untreated (n=283), the percentage of patients who changed from a lower to a more advanced cardiac disease stage (Kuschnir group) or cardiac death was lower in the benznidazole group (4%, 12/283) compared to	The data submitted met the evidentiary criteria for approval.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	the untreated group (14%, 41/283) (difference of -10% with 95% confidence interval [-15.2, -5.3], p<0.0001). Three times as many patients in the untreated group (n=45) experienced new ECG abnormalities compared to the benznidazole group (n=15) which was a statistically significant difference (p<0.0001). Benznidazole treatment showed reduced mortality. Significantly fewer patients in the treated group died as compared with untreated controls (3/283, 1.1% vs 12/283, 4.2%, respectively, p 0.03). There was a difference in overall survival probability associated with treatment group (p<0.0097) with those in the untreated group having the lowest survival probability and those in the benznidazole group having the greatest survival probability.	
Risk	• The three submitted studies considered for review and approval included a total of 157 subjects in the pediatric population ages 2 to 12 years and 283 adult patients aged 30 to 50 years who were treated with the benznidazole product manufactured by Roche, for which the Applicant has an adequate bridge. • The most common adverse reactions are allergic dermatitis, mediated by hypersensitivity mechanisms, most commonly rash, usually with eosinophilia, are the most frequently observed manifestation and are the most common cause of treatment discontinuations in children and adults. Severe cutaneous manifestations include erythroderma, toxic epidermal necrolysis (TEN), Stevens Johnson syndrome and	The safety information provided supports the use of benznidazole for the treatment of Chagas disease. Benznidazole adverse reactions are well known, in most cases mild, and even if severe, they are manageable with monitoring and are reversible with temporary or permanent treatment discontinuation. Uncertainties remaining are the potential for carcinogenicity, fetal toxicity, which would preclude its use in pregnancy, and potential decrease on male fertility. To address this risk,

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	acute granulomatous exanthematous pustulosis (AGEP). Symptoms resolve with temporary or permanent discontinuation of treatment. Sometimes corticosteroids are needed to help symptoms resolve. • Gastrointestinal disorders are the second most common toxicity, most commonly abdominal pain, nausea, decreased appetite, vomiting and diarrhea. These are also reversible with temporary or permanent treatment discontinuation. • Peripheral neuropathy and polyneuropathy are infrequent but can be severe and may take several months to resolve. • Bone marrow depression, most commonly neutropenia, lymphopenia and anemia. In rare cases, they may be severe. They resolve with treatment interruption or discontinuation. • Elevation of transaminases and alkaline phosphatase, usually mild, are observed during treatment. They revert to normal after treatment interruption or discontinuation. • Rare adverse reactions include ageusia, migratory arthritis, pain syndrome, sometimes requiring several months to resolve, and have been described in the published literature. • Carcinogenicity in mice and rabbits, embryotoxicity in rats and decreased fertility in male rats was observed in animal studies. These have not been reported in humans, however, they have not been formally evaluated in controlled clinical trials.	a male fertility study in rats will be conducted (agreed to in the End of Phase 2 meeting on April 27, 2016)

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk Management	• Labeling will contain the following Warnings and Precautions: • Potential for Carcinogenicity and Genotoxicity • Embryo-Fetal Toxicity • Cutaneous Hypersensitivity Reactions • Central and Peripheral Nervous System • Bone marrow depression	The label will include drug class related warnings (nitroimidazoles). Use in pregnancy will not be recommended, except in acute disease cases in which the benefit may outweigh the risks of treatment. DRISK and DAIP agreed that REMS is not considered necessary. The proposed labeling and routine reporting/pharmacovigilance are sufficient to mitigate any risks and preserve benefits of benznidazole in the treatment of Chagas disease. No Medication Guide will be issued.

2 Therapeutic Context

2.1. Analysis of Condition

American Trypanosomiasis or Chagas disease

Natural history

Chagas disease or American trypanosomiasis is a parasitic disease caused by a flagellated protozoan of the order *Kinetoplastida* and *Trypanosomatidae* family which cause African and American trypanosomiasis, named *Trypanosoma cruzi*. The disease is endemic to tropical and subtropical regions of the Americas from the southern United States to southern Argentina. It is present mostly in poor, rural areas of Mexico, Central America, and South America. Chronic Chagas disease is a major health problem in many Latin American countries. In these rural regions the disease is most commonly transmitted by an insect vector, member of the *Triatominae* family, subfamily *Reduvidae*, *Triatoma infestans*. *T. cruzi* is carried in the gut of blood-sucking triatomine insects; transmission occurs when, after taking a blood meal, the insect defecates and parasites contained in insect feces enter through the bite site or intact mucous membranes. This parasite has several evolutive forms, two of them occur in humans: the circulating trypomastigote, and the intracellular amastigote, which divides by binary asexual reproduction. In the insect vector, the parasite form undergoes one change: from epimastigote to trypomastigotes in the hindgut. Trypomastigotes are the infective form of the parasite, present in the feces of the insect, which are deposited after they bite to suck blood. Trypomastigotes enter the host cells where they differentiate into amastigotes. Trypomastigotes are able to infect a variety of tissues. Amastigotes, the tissue form of the parasite, divide intracellularly by binary fission and differentiate into trypomastigotes by growing flagellae. Trypomastigotes are then released into the circulation. Trypomastigotes do not replicate and they infect a variety of organ tissues, penetrating muscle cells (cardiac, smooth, and skeletal), neurons, lymph nodes, liver, and spleen, where they persist in the form of amastigotes. Any nucleated cell may be parasitized by amastigotes. The cell cycle is around 14 hours (doubling time), but differences between strains have been found. After 5 to 9 divisions (4 to 7 days), new trypomastigotes (up to 512) disrupt the cell wall of the parasitized cell and circulate or penetrate another nucleated cell (Rassi and Luquetti, 2003)[1]. As long as there are amastigote nests in tissues, cycles of parasitemia, usually asymptomatic, will continue to occur, usually at low grade and intermittently. Parasitemia may be higher and symptomatic if immune suppression occurs in an infected individual. This will be discussed again in the description of the disease phases, under the "Special features of *T. cruzi* infection" heading.

Routes of infection and epidemiological characteristics

[1] Raasi, A., and A. O. Luquetti. "Specific treatment for Trypanosoma cruzi infection (Chagas disease)." *American trypanosomiasis* (2003): 117-125.

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The most common form of infection in endemic countries is vectorial. The cycle of infection is maintained in nature when the insect vector becomes infected from taking a blood meal containing trypomastigotes from an infected animal (domestic and wild mammals, such as dogs, cats, guinea pigs, rodents, marsupials and armadillos) or human. Vector-borne transmission occurs only in North, Central and South America; transmission can also occur through blood transfusion, organ transplant, congenitally, orally and accidentally (Rassi, Lancet 2010[2] and Bern, NEJM 2015[3]).
The oral route of infection due to contaminated sugar cane used in fruit juices has been reported in several outbreaks involving tourists to endemic areas. It is also a common route of infection in certain areas, such as the Amazon and north parts of Brazil. Even though this route of infection is overall rare, however, it has been associated with severe myocarditis and meningoencephalitis, with high mortality (Shikanai-Yasuda, CID 2012[4]). Congenital transmission varies according to the geographical area, and maternal factors such as parasitemia, ranging from 0.7%-18.2% of children from infected mothers (Carlier, Mem Osw Cruz 2015[5], Howard BJOG 2014[6], Hermann, JID 2004[7]). Congenital and blood and tissue transmission are the most common modes of transmission in non-endemic urban areas, and in non-endemic countries, and infection may be transmitted through more than one generation. In the United States, one case of congenital transmission has been reported. However, since the infection is asymptomatic and there is no mandatory screening for it, the actual prevalence in the US might be underestimated. In the United States, the first congenital transmission case was reported in 2010, in Virginia, from a Bolivian mother (MMWR 2010)[8].

Congenital transmission has also been reported in Europe. In Spain, a cohort of 59 Bolivian mothers gave birth to a total of 65 children, of whom 13.5% were infected[9]. Among a cohort of 72 Latin American women who gave birth in Switzerland, Bolivian women (N=30) had the highest prevalence of infection (16.5%), and two cases of congenital transmission reported from Switzerland were born

[2] Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. Lancet 2010; 375:1388 – 1402.
[3] Bern C, Chagas disease N Engl J Med 2015;373:456-66.
[4] Shikanai-Yasuda MA, Carvalho NB. Oral transmission of Chagas disease. Clin Infect Dis 2012;54:845-52.
[5] Carlier Y, Sosa-Estani S, Luquetti AO, Buekens P. Congenital Chagas disease: an update. Mem Inst Oswaldo Cruz **2015**; 110:363–8.
[6] Howard EJ, Xiong X, Carlier Y, Sosa-Estani S, Buekens P. Frequency of the congenital transmission of *Trypanosoma cruzi*: a systematic review and meta-analysis. BJOG 2014; 121:22–33.
[7] Hermann E, Truyens C, Alonso-Vega C, et al. Congenital transmission of *Trypanosoma cruzi* is associated with maternal enhanced parasitemia and decreased production of interferon- gamma in response to parasite antigens. J Infect Dis 2004; 189:1274–81.

[8] Congenital Transmission of Chagas – Virginia, 2010 Centers for Disease Control and Prevention (CDC).. MMWR Morb Mortal Wkly Rep. 2012 Jul 6;61(26):477-9.

[9] Laura Murcia, Bartolomé Carrilero, M. Jose Munoz-Davila, M. Carmen Thomas, Manuel C. López, and Manuel Segovia, Risk Factors and Primary Prevention of Congenital Chagas Disease in a Nonendemic Country Clinical Infectious Diseases 2013;56(4):496–502

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from Bolivian mothers (Jackson 2009)[10].

Immunocompromised hosts (mainly HIV co-infected and transplant patients) are at higher risk for severe infection, due to reactivated disease. Transplantation of the kidney or liver from an infected donor resulted in transmission in 18 – 19 and 29%, respectively[11,12]. Approximately 20% of HIV-*Trypanosoma cruzi* infected patients experience reactivation; manifestations include meningoencephalitis, single brain lesion of identical presentation as that of toxoplasmosis and/or acute myocarditis. Limited data suggest that the rate of congenital *T. cruzi* transmission is higher for HIV-coinfected women, even in the absence of reactivation, than for immunocompetent mothers (Bern, 2012)[13].

Special features of *T. cruzi* infection

The infection has two definite phases: acute and chronic. Once established, the infection lasts for life, independent of organ damage[1]. Spontaneous cures have rarely been reported, and they have not been reported to occur in a short period of time. Solid evidence for spontaneous cure is scarce. This will be discussed later in a separate subsection "Evidence of spontaneous cure of Chagas disease". The infection has two characteristics: the presence of the parasite and a strong humoral response against it, mainly of IgG antibodies. Both characteristics have a different meaning and expression. Circulating parasites are the hallmark of the acute phase, but are scarce in the chronic phase. Antibodies during the acute phase are not useful for diagnosis, but as the chronic phase sets in, antibodies became the hallmark of this phase, because they are nearly always present, in high titers. So, the constant feature of the chronic phase is the presence of antibodies against *T. cruzi.* If an infected individual is successfully treated during the acute or the chronic phase, parasites should disappear as well as those antibodies that were generated by the antigenic stimulus from the parasite or parasite antigens, and formerly present. The antibody responses are a marker of parasitic tissue burden. The amastigotes in the tissues are the source of intermittent cycles of parasitemia, usually asymptomatic, during the indeterminate and chronic phase. As long as there are amastigotes in tissues, parasitemia may continue to occur. This concept is extremely important in order to understand the basis of the clinical follow up of etiologically treated patients, and the length of

[10] Jackson Y, Myers C, Diana A, et al. Congenital Transmission of Chagas Disease in Latin American Immigrants in Switzerland Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 15, No. 4, April 2009

[11] Riarte, A., et al. "Chagas' disease in patients with kidney transplants: 7 years of experience, 1989–1996." *Clinical infectious diseases* 29.3 (1999): 561-567.

[12] Chin-Hong, P. V., et al. "Screening and treatment of Chagas disease in organ transplant recipients in the United States: recommendations from the Chagas in transplant working group." *American journal of transplantation* 11.4 (2011): 672-680.

[13] Bern, C Chagas disease in the immunocompromised host Curr Opin Infect Dis. 2012 Aug;25(4):450-7. doi: 10.1097/QCO.0b013e328354f179

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follow-up needed to observe the disappearance of antibodies. The time to seronegativization is proportional to the time lapse since infection.

Evidence of Spontaneous Cure of Chagas disease

Spontaneous reversion of serology to negative has been observed in prospective and controlled clinical trials in a small number of cases. The percentages observed in these trials range from 0 to 6% (Sosa Estani 1998, De Andrade 1996, Viotti 1994, Gallerano 2000, Streiger 2004, Fabbro 2007, Viotti 2006) in a total of more than 1,100 untreated subjects from these studies. Spontaneous seroconversion to negative is a rarity.

A comprehensive literature search including human and animal data and using the terms "spontaneous"[All Fields] AND cure[All Fields] AND ("Chagas disease"[MeSH Terms] OR ("Chagas"[All Fields] AND "disease"[All Fields]) OR "Chagas disease"[All Fields]) in MEDLINE and EMBASE databases yielded a total of 7 publications. None of these were randomized clinical trials. Two articles were from animal studies, one was a human case series, one was a long term follow-up cohort of 380 untreated patients, and three others were single case reports.

One case series (Zeledon, 1998)[14] reports 6 adult patients from Costa Rica who had been diagnosed with acute Chagas disease, confirmed with clinical and parasitological diagnosis, including serological tests. In 3 of the 6 patients the conventional serological results became negative after 32, 34 and 39 years from the time of the original diagnosis, respectively. The 3 patients had a follow-up of 6 years after first becoming seronegative (1981-1986). The work-up included ECG, ergometry, chest X-ray, manometry of the esophagus, xenodiagnoses and conventional serological tests, plus lytic antibodies. One of them had an incomplete right bundle branch block. None of the 3 patients had presented heart disease, esophageal or colonic disease during the initial acute disease or throughout the follow-up. The three other patients had persistently positive serological results throughout the follow-up period as described in the majority of patients in Latin America.

Patients with spontaneous and post-benznidazole cures were analyzed and compared at one center in Buenos Aires, Argentina (Bertocchi, 2013)[15]. The initial cohort included 925 patients: 533 women and 392 men; 545 were treated with benznidazole and 380 were non-treated patients.

From 107 patients with parasitological cure, 82 had received treatment (77%) and 25 became spontaneously seronegative (23%). Forty-six (43%) and 61 (57%) patients had two and three negative serological tests, respectively. No differences in clinical groups, ECG, echocardiogram and heart disease progression were found in patients who became negative spontaneously or post-treatment.

[14] Zeledon R, Dias JC, Brilla-Salazar A, de Rezende, JM, Vargas LG, Urbina A*: Does spontaneous cure of Chagas disease exist? Exp Parasitol. 1986 Jun;61(3):284-93.*

[15] Bertocchia G, Vigliano C, Lococo B, Pettia M and Viotti R*: Clinical characteristics and outcome of 107 adult patients with chronic Chagas disease and parasitological cure criteria. Trans R Soc Trop Med Hyg, 2013 Jun;107(6):372-6.*

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The time until reversion of serology to negative averaged between 8 to 9 years, similar in treated and non-treated. New ECG changes and changes to a more severe Kuschnir group were observed in 5% and 10% of all 107 patients, without significant differences among treated and untreated patients.

Two case reports were described in patients originally diagnosed with acute disease acquired in childhood, both from endemic areas. One is the case of a 5 year old girl from Minas Gerais, Brazil, who was diagnosed in 1944. She presented a positive thick blood smear, but never again showed serological and/or parasitological evidence of *Trypanosoma cruzi* infection, on several occasions. This patient never received any specific treatment and had remained completely asymptomatic, with normal findings from clinical, electrocardiographic, X-ray and echocardiographic examinations until more than 50 years of follow-up (Dias, 2008)[16]. The other case report (Francolino, 2003)[17] was described in Uruguay. An 87-year-old man who had had a typical acute phase of *Trypanosoma cruzi* infection in 1947 and never received specific treatment against the disease, when examined in 1998 revealed several completely negative parasitological and serological tests, including traditional serology, PCR and flow cytometry. In the clinical work-up in 1998 he presented a complete right bundle branch block, no heart failure and some esophageal retention of contrast seen in the X-ray after swallowing. The authors concluded that the clinical data suggested the possibility of a benign evolution of Chagas' disease, but the basic findings (slight cardiac and esophageal impairment) could also be due to the advanced age of the patient.

In animals, an apparent spontaneous cure was not definitively confirmed in mice (Magalhães, 1994)[18] and has been described in 1 out of 44 opossums (Fernandes 1994)[19] as well, as a rare occurrence, in case reports, and possibly depending on the type of *T. cruzi* strain.

> ***Reviewer's comment:*** *Spontaneous cures of Chagas disease, as defined by the goal standard established by WHO of seroreversion of conventional serology, are very rare and observed over the course of several years or decades (ranging from 7 to more than 30 years). Whether spontaneous or post-treatment, patients with complete seronegativization have a very low risk for cardiac events from Chagas disease. Of note, a few of the patients who became spontaneously seronegative had some signs of the disease such as right bundle branch block and a few experienced clinical changes to a more severe Kuschnir category. It is not possible to definitively conclude that they are completely cured and free of further risk. It is also possible*

[16] Dias E, Martin-Filho OA, Vitelli-Avelar D, Correia D, Lages E, Prata A: *Further evidence of spontaneous cure in human Chagas disease. Rev Soc Bras Med Trop. 2008 Sep-Oct;41(5):505-6*.
[17] Francolino SS, Antunez AF, Talice R, Rosa R, Zelanikio J, de Rezende JM, Romanha AJ, Dias JC: *New evidence of spontaneous cure in human Chagas disease, Rev Soc Bras Med Trop. 2003 Jan-Feb;36(1):103-7.*
[18] Magalhães JB, Andrade SG, *Investigation on the possibility of spontaneous cure of mice infected with different strains of Trypanosoma cruzi, Rev Inst Med Trop Sao Paulo 1994 Nov-Dec;36(6):481-4.*
[19] Fernandes AJ, Luz ZM, Vitor RW, Diotaiuti L, Chiari E, *Possibility of spontaneous cure in opossums*, *Rev Inst Med Trop Sao Paulo. 1994 Sep-Oct;36(5):471-3.*

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that they may represent a few cases of false negative tests because of borderline low titers and very low parasitic burden associated with a better outcome.

Acute phase

After infection of any type, an initial acute phase occurs, usually asymptomatic and lasting 2-4 months, characterized by high parasitemia, observed by several direct methods (microhematocrit, Strout method, quantitative buffy coat, thick blood smear, Giemsa stain). Untreated patients with Chagas's disease of any age (newborns to adults), may later develop irreversible damage to the heart or gastrointestinal tract or experience reactivation of acute disease and related complications (acute myocarditis and encephalitis) as a result of immunosuppression.
Xenodiagnosis was the gold standard of diagnosis in the past; however, because of its complexity, the need for specially trained personnel and the time that it takes to obtain the results, it is rarely used in the diagnosis currently. Molecular diagnoses such as PCR are also used along with direct parasitological methods for early diagnosis and monitoring in the acute phase, when transfusion or transplant transmission or reactivation in an immunocompromised host are suspected and for congenital Chagas disease when antibodies of maternal origin are still circulating, along with other direct methods. Laboratory exposures can also be monitored using PCR. At CDC molecular detection of *T. cruzi* DNA is performed using a combination of three real-time PCR assays (https://www.cdc.gov/dpdx/trypanosomiasisAmerican/index.html).

Rarely, symptoms can be observed in the acute vector-transmitted disease (palpebral edema or Romaña sign, fever, fatigue); however, when they are present, they are highly relevant for the diagnosis. Overall, in 5-10% of the acute infection cases present severe myocarditis and encephalitis with high morbidity and mortality, but in most cases and without treatment parasitemia resolves spontaneously within 90 days and infection progresses to a chronic stage, which is asymptomatic in two thirds of those infected. This asymptomatic stage that follows infection is called the chronic indeterminate phase, characterized by positive serological responses to trypanosomal antigens. Infection is thought to persist for a lifetime, with persistently positive serology. Spontaneous cures are very rare, anecdotal. In prospective, controlled trials, spontaneous cures (defined as negative seroconversion in all three of conventional serological assays) have been reported in 0 to 6% of patients, occurring usually after several years of follow-up.

Chronic Indeterminate (asymptomatic) Chagas disease

The persistent seropositivity without signs or symptoms of disease is the longest phase of Chagas disease, called chronic indeterminate ("silent") disease, which may last a lifetime. In 20-30% of chronic indeterminate cases, after one of two decades, the infection progresses into cardiac and/or gastrointestinal disease. The chronic phase is characterized by positive serological tests that detect IgG antibodies against lysates of whole parasite (epimastigotes) antigens. These assays are called "conventional serological assays". Complement fixation (CFT) was the first conventional serological

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test used (Guerreiro and Machado, 1913)[20], indirect hemagglutination assay (IHA), immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA or EIA) followed. Several types of these assays are commercially available in endemic countries. Conventional serological assays detect a wide range of antigens found in all types of *T. cruzi.* Persistence of positive conventional assays for several years or decades and is thought to be due to persistent antigen stimulation present in macrophages and dendritic cells even after the parasite has been eliminated. This has been observed in treated and cured mice, and it may well happen in humans (Krettli 2009)[21]. The standardization of these various tests and protocols was made possible by a multicenter study involving several laboratories in different countries (Camargo et al. Bull Pan Am Health Org 1986)[22] and other studies with commercially available tests (Leiby, 2000[23]; Gadelha et al. 2003)[24].
At least two of three conventional serological assays: indirect hemagglutination assay (IHA), immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA or EIA) are recommended to confirm the diagnosis. Used in combination, their reported sensitivity and specificity are between 95 to 99%. In 95% of sera, concordant results are obtained with all three tests (WHO 2002)[25]. Three international standardization studies (Camargo, 1986)[26] were carried out in the 1980s at reference laboratories in Brazil, Argentina and the US (CDC). During the third study, four additional laboratories in Bolivia, Chile, Colombia and Panama participated in the standardization program. Standards are set using a panel of sera from infected and uninfected patients. There is no perfect uniform standard across the regions; however, improved agreement was obtained with these reference panels when uniform standards were followed. Validation of conventional assays to confirm parasite presence and lineage in infected patients in the indeterminate and chronic phases is rarely possible, because parasitemia is very low and trypanosomes are seldom recovered from these

[20] Guerreiro, Cesar, and Astrogildo Machado. "Da reação de Bordet e Gengou na moléstia de Carlos Chagas como elemento diagnóstico." *Brasil med* 27 (1913): 225-226.

[21] Krettli, Antoniana Ursine. "The utility of anti-trypomastigote lytic antibodies for determining cure of Trypanosoma cruzi infections in treated patients: an overview and perspectives." *Memórias do Instituto Oswaldo Cruz* 104 (2009): 142-151.

[22] Camargo, Mario E., et al. "Three years of collaboration on the standardization of Chagas' disease serodiagnosis in the Americas: an appraisal." (1986).

[23] Leiby, David A., et al. "Serologic testing for Trypanosoma cruzi: comparison of radioimmunoprecipitation assay with commercially available indirect immunofluorescence assay, indirect hemagglutination assay, and enzyme-linked immunosorbent assay kits." *Journal of clinical microbiology* 38.2 (2000): 639-642.

[24] Gadelha, Andrea Ático Monteiro, et al. "Chagas' disease diagnosis: comparative analysis of recombinant ELISA with conventional ELISA and the haemagglutination test." *Vox sanguinis* 85.3 (2003): 165-170.

[25] WHO Expert Committee. Control of Chagas disease. Brasilia, Brazil: World Health Organization, 2002:1 – 109.

[26] Camargo ME, Segura EL, Kagan IG, Pacheco-Souza JM, Carvalheiro JR, Yanovsky JF, Guimaraes MCS 1986. Three years of collaboration on the standardization of Chagas disease serodiagnosis in the Americas: an appraisal. *Bull Pan Am Health Organ 20*: 233-244.

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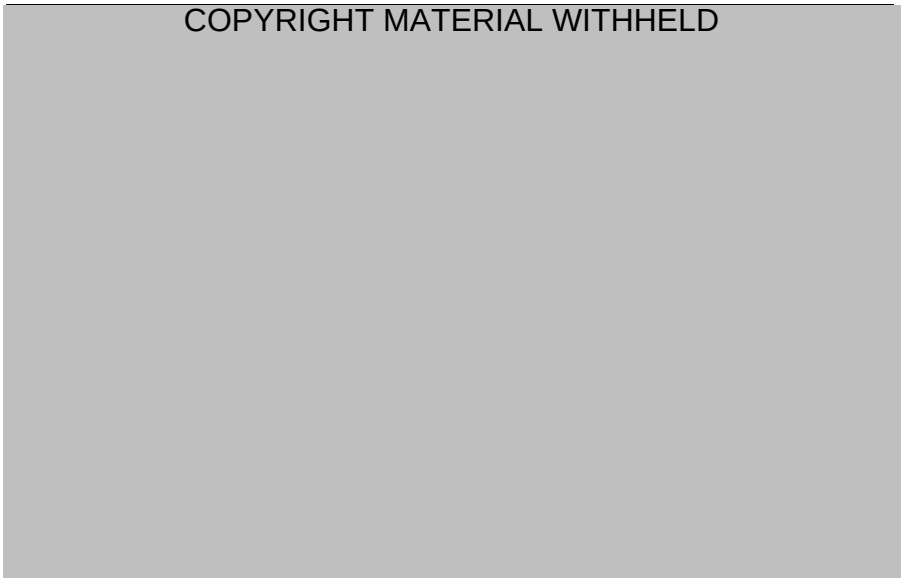
patients. The conventional serological tests are the standard against which newer assays are evaluated.

Some of these assays are FDA cleared for diagnosis or for screening of blood to be used for transfusions. The Center for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend diagnosis of chronic disease by testing with at least two and/or three different conventional serological assays (Table 1).



Serological methods are the gold standard for diagnosis of the chronic phase. PCR sensitivity in chronic *T. cruzi* infection is highly variable, depending on specimen volume, population characteristics, primers and methods. For example, in a population of symptomatic chronic Chagas disease patients, the PCR sensitivity was 57.1% (Schijman, 2011)[27]. For this reason, negative results by PCR cannot provide conclusive evidence for absence of infection or cure (Sosa Estani, 2009[28]; Bern 2015[29]). It is not among the recommended tests for screening blood donors for Chagas disease (FDA guidance 2010)[30].

[27] Schijman, Alejandro G., et al. "International study to evaluate PCR methods for detection of Trypanosoma cruzi DNA in blood samples from Chagas disease patients." *PLoS neglected tropical diseases* 5.1 (2011): e931.

[28] Sosa-Estani, Sergio, Rodolfo Viotti, and Elsa Leonor Segura. "Therapy, diagnosis and prognosis of chronic Chagas disease: insight gained in Argentina." *Memórias do Instituto Oswaldo Cruz* 104 (2009): 167-180.

[29] Bern, Caryn. "Chagas' disease." *New England Journal of Medicine* 373.5 (2015): 456-466.

[30] Guidance for Industry: Use of Serological Tests to Reduce the Risk of Transmission of *Trypanosoma cruzi* Infection in Whole Blood and Blood Components Intended for Transfusion, December 2010. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformatio

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Pathophysiologic characteristics of Chagas disease

Using very sensitive immunohistochemical techniques and the in situ polymerase chain reaction amplification methods, parasitic antigens have been found relatively recently in the hearts of patients with chronic Chagas disease. In addition, an association between the presence of *T. cruzi* antigens and the intensity of the inflammatory process was observed[31], suggesting a direct participation of the parasite in the genesis of chronic Chagas myocarditis. It is possible that even low-grade persistent parasitic presence serves as a continuous antigenic stimulus, and that both *T. cruzi* –induced inflammation and an autoimmune response may play important roles in the pathogenesis of Chagas heart disease. Autonomic denervation is another typical finding and explains the digestive alterations (megaesophagus and megacolon). Parasympathetic denervation in the heart could predispose patients to arrhythmias and sudden death.

The chronic cardiac form of Chagas disease is characterized by a focal inflammatory process composed of lympho-mononuclear cells that produce progressive destruction of cardiac fibers and marked reactive and reparative fibrosis affecting multiple areas of the myocardium. The focal myocardial fibrosis predisposes to cardiac dilation and failure, and leads to formation of narrowed-necked left ventricular apical aneurysms, a hallmark of Chagas heart disease.

The parasympathetic cardiac nerves and the conduction system are preferentially involved, producing intraventricular and atrioventricular (AV node) blocks, sinus node dysfunction and ventricular arrhythmias. The right bundle and the left anterior fascicle are most frequently affected, and are usually the first manifestation of myocardial damage discovered in asymptomatic patients. Bradyarrhythmias are also prevalent in Chagas' heart disease and among them, sick sinus syndrome and second and third degree AV blocks are the most common. Not infrequently, ventricular tachyarrhythmia and AV conduction abnormalities coexist in the same patient. Another typical feature of Chagas heart disease is sudden death, which is caused by ventricular fibrillation (Figure 1) in the vast majority of cases. It can occur as the initial clinical manifestation of the disease. Bradyarrhythmia, thromboembolic phenomena, and, in exceptional cases, the rupture of the apical aneurysm, are other possible causes of sudden death[32].

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n/Guidances/Blood/ucm235855.htm.

[31] Bellotti G, Bocchi EA, De Moraes AV, Higuchi ML, Barbero- Marcial M, Sosa E, Esteves-Filho A, Kalil R, Weiss R, Jatene A, Pileggi F: In vivo detection of *Trypanosoma cruzi* antigens in hearts of patients with chronic Chagas' heart disease. *Am Heart J* 1996; 131:301-307

[32] Rassi, Anis, and William C. Little. "Chagas' heart disease." *Clinical cardiology* 23.12 (2000): 883-889.

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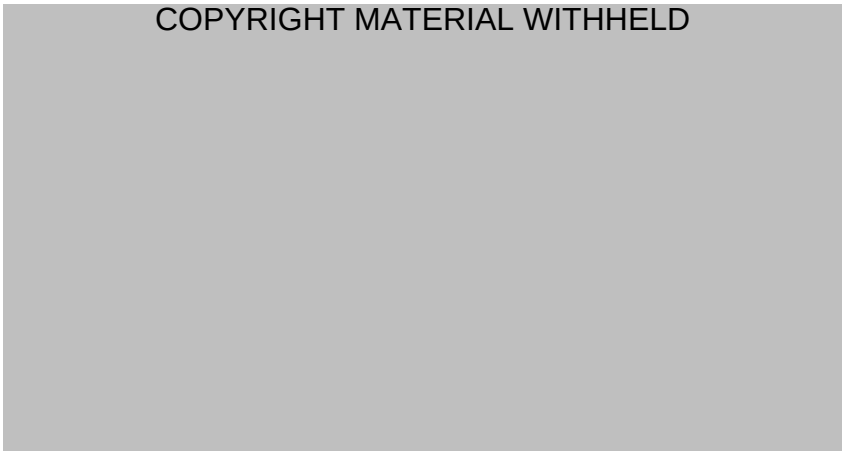

Figure 1: Diagram of the pathophysiology of Chagas heart disease

(Reproduced from Rassi, Anis, and William C. Little. "Chagas' heart disease." Clinical cardiology 23.12 (2000): 883-889

Reviewer's comment: *The parasympathetic denervation of the conduction system and focal myocardial fibrosis mechanisms can cause severe arrhythmias (usually ventricular fibrillation) that cause sudden death, the most common cause of death in Chagas disease. Sudden death can occur without ventricular dysfunction, and even without prior symptoms or signs of established cardiac dilatation and/or failure. Since they may be asymptomatic, patients may not have previous knowledge of established myocardial damage.*

Clinical manifestations of Chagas disease

Cardiac involvement is the most frequent and serious manifestation of chronic Chagas disease. Chagas cardiac disease is manifested by arrhythmias, sudden death, congestive heart failure, and thromboembolic disease, with mortality higher than 50% within 5 years. Sudden death may occur without prior manifestations of cardiac disease and is the most common cause of death in Chagas patients, even in the indeterminate phase of the disease. In approximately 10% of the infected, gastrointestinal disease develops, presented as achalasia or megacolon, due to a weakening of the gastrointestinal wall and impairment of its motility, produced by the infection. Megacolon is manifested by prolonged constipation, and may lead to fecaloma, volvulus and bowel ischemia. This type of disease manifestation is more common in certain geographic areas, such as Central Brazil. The parasite lineage, the route of infection and the amount of exposure may determine the various clinical manifestations of chronic Chagas disease. According to a WHO report from 2002, there are an estimated 18 million infected people living in the Americas, of whom 5.4 million will progress to severe cardiac disease and 900,000 to megaformations (megacolon or achalasia). Although the United States has established enzootic cycles across the southern states, with infected vectors and mammalian hosts such as raccoons, opossums, wood rats, and domestic dogs, the majority of infected U.S. residents are Latin American immigrants who were infected in their home countries.
It is currently estimated that approximately 300,000 infected immigrants are living in the United States. Based on the number of births to Latin American-born women, *T cruzi* prevalence in their home countries, and a conservative estimate of 1%–5% vertical transmission, an estimated 63–315

infected infants are born each year in the United States (Bern C, CID 2009)[33] .
Disease progression from the indeterminate to chronic stages has generally been associated with persistently positive serological tests. Chagas cardiac disease is characterized by patients who had remained seropositive, usually in all serological assays tested. In prospectively long-term followed non-randomized cohorts of treated and untreated patients, a progressive decrease in titers over time or reversion to negative has been a distinct characteristic observed in treated patients but not in those with untreated disease. In four of those prospective non-randomized cohorts, clinical and serological outcomes were also reported, showing less frequent ECG changes and slower disease progression in the treated cohorts as compared with respective untreated controls (Sosa Estani et al, 2009)[34]. Parasite persistence in the myocardium has been associated with increased inflammation and conduction abnormalities and it is currently thought to be the most important mechanism of pathogenesis, rather than others also implied, such as inflammatory immune response resulting in fibrosis, and autonomic dysfunction and local denervation. Early electrocardiographic manifestations of cardiac involvement described as characteristic of Chagas cardiac disease, include right bundle branch block (RBBB) and left anterior hemiblock (LAHB). These two findings (RBBB and LAHB) are very typical, and in patients from an endemic area they are the hallmark of Chagas cardiac disease. Atrioventricular (AV) blocks also occur as conduction system abnormalities progress. Serological tests are usually positive in patients with characteristic heart conduction abnormalities. Ventricular arrhythmias are common, and may cause sudden death even without preceding cardiac symptoms. Intramural ventricular thrombus formation and apical aneurism of the left ventricle are common and characteristic late manifestations of the disease. Heart failure is often a late manifestation of Chagas heart disease. It is usually biventricular with a predominance of right-sided failure (peripheral edema, hepatomegaly, and ascites more prominent than pulmonary congestion) at advanced stages. These stages of cardiac Chagas disease are the bases for the Kuschnir criteria, a classification used in most clinical trials which divides the progression of cardiac disease into four groups: 0 (positive serology only), I (abnormal electrocardiogram [ECG], II (radiologic heart enlargement), and III (overt signs of heart failure). Heart failure of chagasic etiology is associated with higher mortality than heart failure from other causes. Sudden death is the main cause of death in patients with Chagas heart disease, accounting for nearly two- thirds of all deaths, which can occur early or late in the course of the disease, and it is caused by ventricular fibrillation in the vast majority of cases, followed by refractory heart failure (25–30%), and thromboembolism (10–15%). Two risk scores have been validated to predict prognosis of Chagas cardiac disease[35; 16]. Left ventricular dysfunction, evidence of cardiomegaly on radiography and ventricular arrhythmias are significant predictive factors for

[33] Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis 2009;49(5):e52-e54.

[34] Sosa Estani S, Viotti R and Segura E, Therapy, diagnosis and prognosis of Chagas disease: insight gained in Argentina, Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 104(Suppl. I): 167-180, 2009

[35] Rodolfo Viotti, Carlos Vigliano, Bruno Lococo, Marcos Petti, Graciela Bertocchi, María G. Álvarez, and Alejandro Armenti; Clinical Predictors of Chronic Chagasic Myocarditis Progression, Rev Esp Cardiol. 2005;58(9):1037-44

progression of disease and mortality. Survival rates in patients with heart failure (NYHA Class III or IV) were less than 30% at 5 years. Sudden death occurred in 62.3% of the patients who died in a prospectively followed cohort (Rassi NEJM 2006).[36]

Congenital infection

Because trypanosomal infection is life-long, transmission can be observed for more than one generation when transmitted vertically. Most congenital infections are asymptomatic or cause non-specific signs, requiring laboratory screening for detection. A small proportion of congenital infections cause severe morbidity with hepatosplenomegaly, anemia, meningoencephalitis and/or myocarditis with cardio-respiratory insufficiency, with an associated high mortality. Infected infants are presumed to carry the same 20–30% lifetime risk of cardiac or gastrointestinal disease as other infected individuals (C. Bern 2011)[37].

Diagnosis of Chagas disease

Parasitological methods, including identification of trypomastigotes in blood by microscopy, are most effective during acute infections and congenital disease. PCR has been used in the diagnosis of acute infections as an earlier marker of disease reactivation in transplant patients and in early diagnosis of congenital disease, when maternal antibodies preclude the use of serological methods for diagnosis. In congenital disease, conventional serologic testing is recommended to confirm a diagnosis after 9 months of age, when transferred maternal antibodies have disappeared.
Circulating parasite levels decrease rapidly within a few months and are undetectable by most methods during the indeterminate and chronic phase. Direct parasitological methods, including PCR, have lower sensitivity in the chronic phase, therefore a negative direct parasitological test does not rule out the diagnosis in this disease stage.

Diagnosis of chronic (indeterminate or symptomatic) Chagas disease is made by serological tests for antibodies to the parasite. A single test may not be sufficiently sensitive and specific to make the diagnosis in all cases. For this reason, the standard approach recommended by CDC and WHO is to apply two or more tests that use different techniques and/or that detect antibodies to different antigens. Two commonly used techniques are enzyme-linked immunosorbent assay (ELISA) and immunofluorescent antibody test (IFA). To increase accuracy of diagnosis, careful consideration of the patient's history to identify possible risks for infection may be helpful. In the case of discordant serological results with ELISA and IFA, the CDC uses a trypomastigote excreted-secreted antigens (TESA blot) of *T. cruzi*, an immunoblot test developed in-house, not commercially available or FDA-cleared, to confirm the diagnosis.

[36] Rassi A, Development and Validation of a Risk Score for Predicting Death in Chagas' disease N Engl J Med 2006;355:799-808.

[37] Bern, Caryn, Diana L. Martin, and Robert H. Gilman. "Acute and Congenital Chagas disease." *Advances in Parasitology, 75,* p.19-47 (2011).

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In December 2006, the U.S. Food and Drug Administration approved the first screening test for blood donations; a second screening test made by another manufacturer was approved in 2010. From 2010 on, three tests for diagnosis have also been approved.
Blood donor screening tests are not appropriate for clinical diagnostic purposes and additional testing is indicated. The current status of tests for screening and for diagnosis is the following *(this information comes from FDA website, and is consistent with the current 2015 Red Book, or Report of the Committee on Infectious Diseases of the American Academy of Pediatrics*):

For screening:

There are two EIA kits approved by the U.S. Food and Drug Administration (FDA) for blood and organ donor screening in the United States, the Ortho T. cruzi EIA test system, v. 3.0 (Ortho Clinical Diagnostics, Raritan, NJ, USA; approved December 2006) and Abbott Prism Chagas (Abbott Laboratories, Abbott Park, IL, USA; approved May 2010).

For diagnosis:

There are three FDA-approved or -cleared EIAs for clinical diagnosis of Chagas: (b) (4) (b) (4) the Hemagen Chagas Kit (Hemagen Diagnostics, Inc., Columbia, MD, USA) and Chagatest EIA Recombinante v. 3.0 (Laboratorios Weiner, Rosario, Argentina).

<u>Serological tests and clinical outcomes</u>
Three conventional tests are widely used: indirect hemagglutination (IHA), indirect immunofluorescence (IFA) and ELISA. Each of these tests has a sensitivity and specificity of approximately 99%. In 95% of sera, concordant results are obtained among these three tests (WHO 2002). Many of them are commercially available in the endemic countries, for the detection of IgG antibodies *T. cruzi*. Most of the commercially available assays use lysates of the epimastigote form of the parasite grown in liquid culture. More recently assays using recombinant antigens also have been developed[38]. In Latin American countries, where other parasites such as *Leishmania* spp. and *Trypanosoma rangeli* are found, false-positive results by cross reactivity of some epitopes have been reported (Saldaña & Sousa 1996[39], Caballero et al. 2007[40]). Caballero et al reported that when the cases of *Leishmania* were excluded, the specificity increased from 95.57% to 100%.
The performance characteristics and standards for each test vary across regions and countries. A quality control program and a national reference center exist in Argentina and Brazil. In clinical

[38] Otani M et al, WHO comparative evaluation of serologic assays for Chagas disease TRANSFUSION 2009;49:1076-1082.
[39] Saldaña A and Sousa E, *Trypanosoma rangeli* and *Trypanosoma cruzi*: Cross- reaction among their Immunogenic Components Mem Inst Oswaldo Cruz, Rio de janeiro, Vol. 91(1): 81-82, jan./Feb. 1996

[40] Caballero et al., Evaluation of Serological Tests To Identify *Trypanosoma cruzi* Infection in Humans and Determine Cross-Reactivity with *Trypanosoma rangeli* and *Leishmania* spp. Clin Vaccine Immunol August 2007 vol. 14 no. 8 1045-1049

practice, they are used in combination and in multiple serial time-points. Their clinical interpretation is based on the observation of two or three types of tests performed usually at quarterly or yearly intervals along several years. The concordance between tests results over multiple time-points and years of follow-up is used in clinical practice to establish a diagnosis of definitive cure if there is a consistent trend of decreasing antibody titers and a final outcome of reversion to negative is observed in three tests. The goal standard of clinical cure is still today the seronegativization of all tests. There is currently no other reliable earlier marker of cure. Due to the long-lasting maintenance of circulating antibodies, it is difficult to use serology as a marker for cure of the disease even after the successful treatment of *T. cruzi* infection. Reversion of serology to negative takes several years, up to decades (Raasi and Luquetti, 2003)[41]. The time it takes to observe seronegativity after treatment depends on the age of the patient (as a surrogate of time from infection). In adults (ages 17 to 46 years) treated with benznidazole, the earliest time until negative seroconversion was 11 years, and for that to take place in 32% of the population who received this medication, 25 years post-treatment was required (Fabbro 2007)[42]. The time to reversion of serology to negative and the rate at which it occurs in patients treated and not treated are fairly consistent among all long-term follow-up studies done in different geographical regions.

The persistence of three reactive conventional serological tests over 10 years or more of follow-up is related to a greater likelihood of progression of heart disease than the likelihood of heart disease progression associated with patients who have a partial or complete reversion of serology to negative. This usually happens after treatment of the underlying infection but, in a few cases, in about 6% of patients, has happened spontaneously (Viotti et al. 1994[43], Viotti et al. 2006[44], Gallerano & Sosa 2000[45], Fabbro et al. 2007[46]).

[41] Raasi, A., and A. O. Luquetti. "Specific treatment for Trypanosoma cruzi infection (Chagas disease)." *American trypanosomiasis* (2003): 117-125.

[42] Fabbro, Diana L., et al. "Trypanocide treatment among adults with chronic Chagas disease living in Santa Fe city (Argentina), over a mean follow-up of 21 years: parasitological, serological and clinical evolution." *Revista da Sociedade Brasileira de Medicina Tropical* 40.1 (2007): 1-10.

[43] Viotti, Rodolfo, et al. "Treatment of chronic Chagas' disease with benznidazole: clinical and serologic evolution of patients with long-term follow-up." *American heart journal* 127.1 (1994): 151-162.

[44] Viotti, Rodolfo, et al. "Long-Term Cardiac Outcomes of Treating Chronic Chagas Disease with Benznidazole versus No Treatment, A Nonrandomized TrialTreatment of Chronic Chagas Heart Disease." *Annals of internal medicine* 144.10 (2006): 724-734.

[45] Gallerano, Rafael R., and Raúl R. Sosa. "Estudio de intervención en la evolución natural de la enfermedad de Chagas. Evaluación del tratamiento antiparasitario específico. Estudio retrospectivo-prospectivo de terapéutica antiparasitaria." *Rev. Fac. Cienc. Méd.(Córdoba)* 57.2 (2000): 135-162.

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A decrease in the level of antibody titers seems to precede reversion of serology to negative, and has the same meaning from a clinical point of view for medical management, indicating an evolution towards cure, although both require long-term serological follow-up. The time at which a reduction in titers and a seroconversion to negative can be observed depends on two factors: the age of the patients and the time lapsed from infection, although it is possible that the first one may be a surrogate for the second one. The parasite lineage or other geographical differences have been hypothesized to affect the time to negative seroconversion. For example in Central America, studies in children under 13 years of age (Escriba, 2009)[47] have shown a shorter time to negative seroconversion (88% observed 18 months after treatment with benznidazole) than those reported in another study including regions of Bolivia (Yun, 2009)[48]. In Bolivia, negative seroconversions were observed in 5.4% of children by up to 60 months in Entre Ríos; and 0% at an average of 18 months in Sucre.

Four long term prospective, non-randomized studies in adult treated and untreated patients, with an average long-term follow-up ranging from 6 to 21 years, have shown correlations between serological and clinical outcomes (Viotti's studies (1994[49] and 2006[44]), Gallerano 2000[45] and Fabbro 2007[46]). These studies have the limitations of being open-label and not properly randomized (alternate allocation of treatment); however, the allocation in most cases followed an alternate order and was not subject to clinical criteria in mostly asymptomatic patients, the endpoints measured are objective (serological outcomes, ECG and X-rays) and the patients' baseline characteristics were comparable in both groups, with balanced losses to follow-up in both arms. Serology was performed in conditions blinded to treatment allocation. The patients came from the same geographic area and were, in the majority of the cases, asymptomatic at baseline and within the same age range. All these characteristics suggest that the risk for potential biases is not so high as to absolutely preclude an interpretation of study outcomes. The table below summarizes the outcomes of four prospective, non-randomized controlled trials.

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[46] Fabbro, Diana L., et al. "Trypanocide treatment among adults with chronic Chagas disease living in Santa Fe city (Argentina), over a mean follow-up of 21 years: parasitological, serological and clinical evolution." *Revista da Sociedade Brasileira de Medicina Tropical* 40.1 (2007): 1-10.

[47] Escribà, Josep M., et al. "Treatment and seroconversion in a cohort of children suffering from recent chronic Chagas infection in Yoro, Honduras." *Memórias do Instituto Oswaldo Cruz* 104.7 (2009): 986-991.

[48] Yun, Oliver, et al. "Feasibility, drug safety, and effectiveness of etiological treatment programs for Chagas disease in Honduras, Guatemala, and Bolivia: 10-year experience of Médecins Sans Frontières." *PLoS neglected tropical diseases* 3.7 (2009): e488.

[49] Viotti, Rodolfo, et al. "Treatment of chronic Chagas' disease with benznidazole: clinical and serologic evolution of patients with long-term follow-up." *American heart journal* 127.1 (1994): 151-162.

Table 2 Prospective, non-randomized controlled studies of adult patients with Chagas disease that include clinical and serological outcomes

	Study Type	N (arms)	Population	Dosing regimen	Endpoints	Length of Follow-up
Viotti 1994	Prospective, controlled: Benznidazole vs Untreated controls	201 (130/71)	Mean age 46 years (treated) and 47.7 years (untreated). Range 9-73 years old.	5 mg/kg/day for 30 days	Conventional serology and clinical outcomes	Mean 8 years
Gallerano 2000*	Prospective, controlled: allopurinol, benznidazole and nifurtimox vs untreated	535/668 (535 treated: 309, 130*, 96)	>18 y.o. (mean age 36.6 years)	4-8 mg/kg/d for 45-60 days	Conventional serology, xenodiagnosis and clinical outcomes	Mean 74 months
Viotti 2006	Prospective, controlled	566 (283/283)	30-50 y.o. (mean age 39)	5 mg/kg/d for 30 days	Conventional serology and clinical outcomes	Median 9.8 years
Fabbro 2007*	Prospective, controlled	111 (54/57) – 27* Bnz	17-46 y.o.	Benz 5mg/kg/d for 30 days^{o}	Conventional serology, xenodiagnosis and clinical outcomes	Mean 21.8 years

*: Gallerano and Fabbro studies had additional treatment arms (nifurtimox, allopurinol). The N for the benznidazole cohort in these studies was 130 and 27, respectively. º: In accordance to local public health guidelines at the time, half the dose was given during the first week of treatment.

Table 3 Clinical and serological outcomes in prospective, non-randomized controlled studies in adults with Chagas disease

			New ECG changes		Serology reversion to negative		Heart disease progression	
Author	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Viotti 1994	131	70	7/131 (5.3%)	16/70 (22.8%)	21/110 (19.1%)	3/50 (6%)	3/131 (2.3%%)	8/70 (11.4%)
Gallerano (2000)	535 (130bz)	668	5/130 (3.8%)	113/668 (16.9%)	3/130 (3.9%)	0/668 (0)	5/130 (3.8%)	113/668 (16.9%)
Viotti 2006	283	283	15/283 (5%)	45/283 (15%)	32/218 (15%)	12/212 (6%)	12/283 (4%)	41/283 (14%)
Fabbro (2007)	54 (27 bz)	57	2/54 (2/27 bz) (33%)	9/57 (15.7%)	20/54 (9/27 bz) (33%)	0/57 (0)	2/54 (3.7%)	9/57 (15.7%)
Total	**1003**	**1078**						

In the Viotti 2006 study, 566 patients ages 30-50 years and without heart failure (283 benznidazole recipients and 283 untreated) were prospectively followed. Fewer treated patients had progression of disease defined as progression to heart disease or death (12 of 283 or 4% vs. 40 of 283 or 14%, adjusted hazard ratio of 0.24 [95% CI 0.10-0.59]; p=0.002). Conversion to negative results on serologic testing was more frequent in treated patients than in untreated patients (32 of 218 or 15% vs. 12 of

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212 or 6%; adjusted hazard ratio 2.1 [95% CI 1.06-4.06] p=0.034). In the Fabbro study, 2/54 (3.7%) of the treated and 9/57 (15.8%) of the untreated patients showed electrocardiographic disturbances attributable to Chagas cardiomyopathy, with a statistically significant difference (p<0.05). Additional studies in the acute phase, including congenital disease, have shown that serological positivity persists in untreated patients. The correlation of clinical outcomes, though, comes from observational studies in adult treated and untreated patients followed for 9-11 years. This is due to the fact that cardiac disease occurs after decades from infection and only in 25-30% of the patients, even though the infection is present for life. Mortality in patients with cardiac lesion from Chagas disease represents 1.5% of infected patients (Sosa Estani 2006)[50].

Serological titers during follow-up of pediatric and young adult cohorts

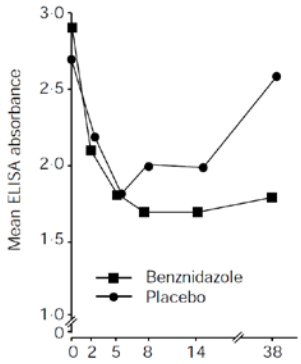
The table below summarizes the main prospective and controlled studies that show the natural history of serological responses in treated vs. untreated pediatric and young adult patients over time.

Publication	Study Population	Treatment regimen	Serological follow-up	Serological outcomes	Remarks
De Andrade The Lancet 1996 (Brazil)	130 children aged 7-12 years, randomized to benznidazole (n=64) or placebo (n=65)	Benznidazole 5 mg/kg/day for 60 days	Conventional serology (ELISA, IFA, IHA) and purified glycoconjugate ELISA test: AT ELISA, at 3, 6, 12 and 36 months.	AT ELISA negativization in 37/64 (58%) treated patients vs. 3/65 (5%) of placebo group (ITT population). GMT by IFA five-fold lower in benznidazole group at 36 months	Significant decrease in titers at 6 months post-treatment, mean estimates of two groups
Sosa Estani Am J Trop Med Hyg 1998 (Argentina)	101 children aged 6-12 years, randomized to benznidazole (n=51) or placebo (n=50) followed for 48 months	Benznidazole 5 mg/kg/day for 60 days	Conventional serology (ELISA, IHA, IFA) and Recombinant ELISA (F29 EIA) at 3,6, 12, 18, 24, and 48 months	Conventional serology: 11/3% seronegativization at 48 months. By recombinant F29 EIA serology: 37.5% at 6 months and 62.1% at 48 months. Placebo= 0 negativization	Significant decrease in titers observed at 6 months post-treatment by analysis of variance or Kruskal-Wallis test. Placebo titers unchanged.
Fabbro	111 patients 17-46	Prospective,	Serological and	Reversion of	Titers

[50] Sosa-Estani, Sergio, and Elsa Leonor Segura. "Etiological treatment in patients infected by Trypanosoma cruzi: experiences in Argentina." *Current opinion in infectious diseases* 19.6 (2006): 583-587.

Rev Soc Bras Med Tropical 2007	y.o. (54 treated with benznidazole or nifurtimox and 57 untreated) mean follow-up of 21 years.	randomly assigned treatment or placebo	clinical follow-up	serology to negative: NF = 55%, BZ = 45%, Placebo (N=57) = 0%, no decrease in titers.	unchanged in placebo group
Streiger Rev Soc Bras Med 2004	95 non-vectorial cases 1-14 y.o. from urban low endemicity area, followed up for up to 24 years of age, 68% born from infected mothers.	Prospective, open label, treatment given to parasitologically positive (71 treated/24 untreated).	Serological (conventional serology) and clinical follow-up	Serology remained positive in all untreated patients, without changes in titers. Treated patients: 75% negativization for ≤4 years and 43% for ≥ 9 years. Time to negativization: 3.6 years if treatment was started between 1-6 years of age and 8 years if started between ages 7-14 years.	More cases with parasitemia in treatment arm, EKG changes more frequent in untreated group at the end of treatment.

Of note, all these studies show a decrease of antibody titers in treated patients, preceding reversion of positive serology to negative, and persistent seropositive outcomes, usually with stable or small fluctuations in titers in untreated patients, and without a clearly descending trend. The two randomized, placebo controlled studies with benznidazole in children ages 6 to 12 (chronic indeterminate phase) show decreasing titers as soon as 6 months after treatment, with an opposite curve direction between treated and untreated patients after that time. This is illustrated in the figure below, from the De Andrade study. In this study, the analysis of titers over time indicated a consistent decrease in antibody concentrations in the benznidazole group and an initial decrease followed by a progressive increase in the placebo group (figure below). A significant difference between the groups was apparent 6 months after completion of treatment, when the 95% CI of the mean estimates of the two groups did not overlap. At the end of follow-up, children who received benznidazole had five-fold lower geometric mean titers by indirect immunofluorescence than placebo-treated children (196 [147–256] *vs* 1068 [809–1408] p<0·00001). Similar findings were reported in Sosa Estani's study.



New electrocardiographic changes and serologic titers

A trend in a correlation of clinical outcomes with serological titers was observed in the Viotti study. When analyzed according to serologic titers, new electrographic changes were found in 6 of 49 (12.2%) and 8 of 33 (24.2%) ($p > 0.10$, non-significant) treated and untreated patients with high titers, respectively. On the other hand, in low titer patients, the percentage who presented new electrocardiographic changes was significantly lower among those treated with benznidazole than among untreated patients (5% [2 of 401] vs 28.5% [4 of 141], $p < 0.05$)(Viotti, 1994)[43]. As previously mentioned, new electrocardiographic changes were not observed in treated or untreated patients with nonreactive serologic tests. In another long term follow-up study, none of the infected participants who developed new electrocardiographic changes (2 of 54 treated and 9 of 57 untreated) had reversion of the serology to negative throughout the follow-up period of more than 15 years (Fabbro 2007)[46].

Serological evolution and PCR measurements in children and adults

Studies comparing serological outcomes and PCR outcomes have been conducted using various PCR assays and conventional serology tests. The PCR tests used have not been quantitative or properly standardized until recently by a validation study (against stocks of *T. cruzi* and serology) sponsored by WHO (Schijman el al. 2011). The information available with the validated quantitative PCR tested in a multicenter trial of 26 centers worldwide comes from treated pediatric patients only. No information is available from untreated pediatric patients. The correlation of PCR values and serological outcomes is not linear or clear. PCR sensitivity is highest in the acute phase (90-100%) and decreases proportionately with time of infection and age (60-70%). Correlations of PCR with clinical outcomes from treated and untreated adult patients are only available in adult patients from the BENEFIT trial[51]. This was a prospective, multicenter, randomized study involving 2854 patients with Chagas cardiomyopathy who received benznidazole or placebo for up to 80 days and were followed for a

[51] Morillo, Carlos A., et al. "Randomized trial of benznidazole for chronic Chagas' cardiomyopathy." *New England Journal of Medicine* 373.14 (2015): 1295-1306.

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mean of 5.4 years. At baseline, 74.4% of participants were in the Class I category of the NYHA, with a mean ejection fraction of 55%. The primary outcome in the time-to-event analysis was the first event of any of the components of the composite outcome of death, resuscitated cardiac arrest, sustained ventricular tachycardia, insertion of a pacemaker or implantable cardioverter–defibrillator, cardiac transplantation, new heart failure, stroke, or other thromboembolic event. The rates of conversion to negative PCR results (PCR conversion) were 66.2% in the benznidazole group and 33.5% in the placebo group at the end of treatment, 55.4% and 35.3%, respectively, at 2 years, and 46.7% and 33.1%, respectively, at 5 years or more (P<0.001 for all comparisons). The effect of treatment on PCR conversion varied according to geographic location and the time of measurement (at 2 years and at 5 years after treatment). However, the rates of PCR conversion did not correspond to effects on clinical outcome (P = 0.16 for interaction). The reason why the conversion to negative PCR did not predict better clinical outcomes in this adult population with cardiac disease is not yet fully understood, however, the results from this study suggest that treatment with benznidazole may be less effective in patients with established cardiomyopathy. Unfortunately, no serological evaluations were collected and reported from this trial, and there are no other currently available data to allow for a comparison of parallel serological and PCR responses in relation to clinical outcomes.

The evidence suggests that PCR is a marker of parasitemia, which is low-grade and intermittent in the indeterminate and chronic phases; serological outcomes are thought to be a marker of whole tissue burden, which has been associated with disease progression in long-term follow-up studies. Parasitological methods to detect parasitemia, such as the xenodiagnoses and PCR, have a lower sensitivity than serological assays to detect the presence of infection in the indeterminate and chronic stages. Because of their low sensitivity, PCR and xenodiagnoses cannot rule out infection and have not been used in screening blood supplies in endemic or non-endemic countries. Epidemiological surveillance programs have shown that serological assays are more sensitive than xenodiagnoses to detect infection in the indeterminate and chronic stages of the disease. The current role PCR has in clinical practice is to provide an earlier diagnosis in acute infection, such as reactivation of disease in transplant and HIV patients, in early congenital disease when the maternal antibodies may confound a diagnosis, to assist in cases of discordant serology, and to monitor treatment response over time. Since there is no historical untreated control information in the pediatric population (acute and chronic indeterminate phases) available with PCR no margin could be established to construct an endpoint to compare effectiveness for an assessment of treatment response. The follow-up period from the pediatric studies conducted so far is not long enough (maximum of 3 years available) to make any correlations to clinical outcomes or to reversion of serological responses to negative in the pediatric and adult indeterminate or chronic stages of the disease. In the acute and congenital Chagas disease, as mentioned above, PCR may have a role in early diagnosis and monitoring of treatment efficacy until negative serology is achieved.

Clinical criteria of cure

Disease progression has been associated with persistent positive serological tests, usually with high, not significantly changing titers over time. In prospectively followed non-randomized cohorts of treated and untreated patients, a decrease in titers or reversion to negative has not been observed in

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untreated disease. Therefore, the widely accepted criteria for cure ("gold standard"), recommended by WHO and national regulatory authorities in endemic countries remains to be the reversion of serology to negative, confirmed with at least two serological tests (ELISA plus either IFA or IHA). The reversion of serology to negative takes years or decades to be observed, largely depending on the time occurred after infection. It is thought that a parasitic cure may occur at an earlier time;

Lytic antibodies and recombinant ELISA assays as an earlier marker of cure

Lytic antibodies are detected with a complement-mediated lysis test utilizing live trypomastigotes from tissue cultures. These lytic antibodies, detected by the complement-mediated lysis (CoML) test, are produced after infection and not after immunization with fixed parasites. Analyses in experimental models have corroborated this hypothesis. The lysis of the trypomastigotes is thought to represent an immune response to only the live forms of the parasite (Porcel 1996[52], Krettli 1982[53], Galvao 1993[54]). The lytic antibodies are present in high titers in advanced Chagas disease, and not in uninfected subjects (Krettli and Brenner, 1982)[55]. The epitope of the lytic antibodies is a glycoprotein of high molecular mass expressed only in the trypomastigotes form (Norris 1989)[56]. A recombinant antigen has been developed, and an ELISA assay based on a recombinant glycopeptide (AT ELISA) was developed and used in a randomized controlled study in children (De Andrade study)[57].

In a cohort of 101 untreated and 82 treated chagasic patients followed for 15 years (Galvao 1993)[58], the lytic antibodies were positive in 100% of patients with positive conventional serology and were the first to become negative while the conventional serology tests (IFA) remained positive. The time

[52] Porcel, Betina M., et al. "Trypanosoma rangeliandTrypanosoma cruzi: Molecular Characterization of Genes Encoding Putative Calcium-Binding Proteins, Highly Conserved in Trypanosomatids." *Experimental parasitology* 84.3 (1996): 387-399.

[53] Krettli, Antoniana U., J. Romeu Cançado, and Zigman Brener. "Effect of specific chemotherapy on the levels of lytic antibodies in Chagas's disease." *Transactions of the Royal Society of Tropical Medicine and Hygiene* 76.3 (1982): 334-340.

[54] Galvão, L. M. C., et al. "Lytic antibody titre as a means of assessing cure after treatment of Chagas disease: a 10 years follow-up study." *Transactions of the Royal Society of Tropical Medicine and Hygiene* 87.2 (1993): 220-223.

[55] Krettli, A. U., and Z. Brener. "Resistance against Trypanosoma cruzi associated to anti-living trypomastigote antibodies." *The Journal of Immunology* 128.5 (1982): 2009-2012.

[56] Norris, K. A., G. Harth, and M. So. "Purification of a Trypanosoma cruzi membrane glycoprotein which elicits lytic antibodies." *Infection and immunity* 57.8 (1989): 2372-2377.

[57] de Andrade, Ana Lucia S. Sgambatti, et al. "Randomised trial of efficacy of benznidazole in treatment of early Trypanosoma cruzi infection." *The Lancet* 348.9039 (1996): 1407-1413.

[58] Galvão, L. M. C., et al. "Lytic antibody titre as a means of assessing cure after treatment of Chagas disease: a 10 years follow-up study." *Transactions of the Royal Society of Tropical Medicine and Hygiene* 87.2 (1993): 220-223.

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for negativization of lytic antibodies varied among patients, from 6 months to 4 years. Treated patients who had reversion of the conventional serological tests to negative during the 15 year follow-up had become seronegative for lytic antibodies several years earlier. The persistence of positive conventional serology over decades is thought to be triggered by immunological memory (B clones) for much longer time after the infection is controlled, similarly to what is observed in chronic diseases such as syphilis. Using a more specific antigen, the lytic antibody assays are thought to reflect a specific response to the presence of live parasites and their disappearance reflects that of live infective trypomastigotes in the host.

ELISA assays using recombinant specific trypanosomal antigens have been developed and used in two randomized controlled studies in children followed up as well with conventional serological assays. Correlation of seropositivity at baseline was observed with the recombinant and conventional serological assays in children from both studies. A retrospective study (Fabbro *et al*, 2013)[59] compared the time at which negative seroconversion was detected by conventional serology (CS) and by the ELISA-F29 test on a cohort of chronic chagasic patients treated with nifurtimox or benznidazole. A retrospective study was performed using preserved serum from 66 asymptomatic chagasic adults under clinical supervision, and bi-annual serological examinations over a mean follow-up of 23 years. Twenty nine patients received trypanocide treatment (benznidazole treated, N=13) and 37 remained untreated. The average time for negative seroconversion was 14.5 ± 5.7 years for ELISA-F29 and 22 ± 4.9 years for CS.

These differences were statistically significant (p = 0.0004, Student's t-test). In the untreated patients group, no negative seroconversion was observed in any of the tests (CS and ELISA-F29).
Based on these limited data, F-29 ELISA appears to have concordance with conventional ELISA outcomes and seems to be an earlier marker of future negative seroconversion observed by conventional ELISA assays.

<u>Chagas disease in the US</u>
There is a growing concern of underrecognized infections in the US due to global migration from endemic countries. PAHO estimates 300,000 to 1 million infected subjects currently living in the US. Conservative estimates (10-15%) of Chagas cardiomyopathy would lead to 30,000-45,000 cases per year in the U.S. Conservative estimate of mother to child transmission (1-5%) would lead to 63-315 cases per year in the U.S.
Several blood banks have started screening for Chagas disease following the FDA approval of diagnostic screening tests in 2010. Serologically confirmed positive donors were seen at a rate of 1 in

[59] Fabbro, Diana, et al. "Evaluation of the ELISA-F29 test as an early marker of therapeutic efficacy in adults with chronic Chagas disease." *Revista do Instituto de Medicina Tropical de São Paulo* 55.3 (2013): 167-172.

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28,000 donations screened (Bern C, et al. 2008)[60]. Treatment for Chagas disease is an unmet medical need in the US.

Vector-transmitted disease in the United States

Triatomines and *T. cruzi* are not new to the United States. The parasite *T. cruzi* was identified in a *Triatoma protracta* in California in 1916 (Kofoid et al., 1916)[61]. At least 24 U.S. species of wildlife mammals in the U.S. have been identified and documented to have been infected with *T. cruzi*, including woodrats, raccoons, and opossums. Domestic dogs have also been found to carry the infection and cardiomyopathy in dogs has been described in Texas by veterinarians. Reports of infections in vectors and reservoir hosts in the U.S. demonstrate the continued existence of a zoonotic cycle of *T. cruzi* in the southern half of the United States for at least 150 years.
The first reports of human autochthonous infection with *T. cruzi* were two separate cases of infants from Texas. From then, and over the following 34 years, cases have been reported from California, Texas and Louisiana. Two studies conducted in collaboration with blood collection agencies, designed to exclude positive donors whose suspected infection had been acquired outside the U.S. or congenitally, detected 21 cases of likely autochthonous T. cruzi infection, bringing the total number of documented infections acquired in the United States to 28 during 1955-2015 (Cantey et al., 2012[62]; Garcia MN et all, 2015[63]). Case report: evidence of autochthonous Chagas disease in south-eastern Texas. *Am J Trop Med Hyg* 92: 325–330).

2.2. Analysis of Current Treatment Options

In the U.S. there are currently no drugs or biological products currently approved to treat Chagas disease. In the U.S. two drugs are available for treatment: nifurtimox and benznidazole. These are obtained through the CDC under two respective single-patient IND applications.

Nifurtimox, a nitrofuran derivative, and benznidazole, a nitroimidazole, have been approved in 21 endemic countries in Latin America since the 1960s and 1970s, respectively. Benznidazole and nifurtimox are treatments included in the WHO Essential Medicines List to treat Chagas disease. Local

[60] Bern, Caryn, et al. "Chagas disease and the US blood supply." *Current opinion in infectious diseases* 21.5 (2008): 476-482.

[61] Kofoid, Charles Atwood, and I. R. E. N. E. McCULLOCH. "On Trypanosoma triatomae, a New Flagellate from a Hemipteran Bug from the Nests of the Wood Rat Neotoma fuscipes." *Univ. California, Publicat. Zool.* 16: 113-126 (1916).

[62] Cantey, Paul T., et al. "The United States Trypanosoma cruzi Infection Study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors." *Transfusion* 52.9 (2012): 1922-1930.

[63] Garcia, Melissa N., et al. "Evidence of autochthonous Chagas disease in southeastern Texas." *The American journal of tropical medicine and hygiene* 92.2 (2015): 325-330.

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guidelines in endemic countries, as well as those from WHO and CDC recommend antitrypanosomal treatment with either nifurtimox or benznidazole for all cases of acute, congenital, and reactivated infection (this latter one mainly organ transplant recipients and AIDS patients), for all children with infection, and for patients up to 18 years of age with chronic indeterminate disease. There is consensus from endemic countries authorities, WHO and CDC that drug treatment should generally be offered to adults aged 19–50 years without advanced Chagas heart disease, and is optional for those older than 50 years because benefit has not been proven in this population.

Other drugs studied for treatment of Chagas disease

Besides nifurtimox, benznidazole is the only other approved drug in endemic countries. Additional therapies studied in clinical trials include allopurinol, itraconazole, posaconazole, and ravuconazole. Early phase research has included other nitroimidazoles, such as fexinidazole, also triazoles derivatives such as Tak-187, a cysteine protease inhibitor, K777, and an antiarrhythmic, amiodarone[64]. These drugs are not currently in an advanced phase of development towards marketing approval in the US or elsewhere.

Current CDC dose recommendations are shown in the table below (from the CDC website):

Drug	Age group	Dosage and duration
Benznidazole	< 12 years	5-7.5 mg/kg per day orally in 2 divided doses for 60 days
	12 years or older	5-7 mg/kg per day orally in 2 divided doses for 60 days
Nifurtimox	≤ 10 years	15-20 mg/kg per day orally in 3 or 4 divided doses for 90 days
	11-16 years	12.5-15 mg/kg per day orally in 3 or 4 divided doses for 90 days
	17 years or older	8-10 mg/kg per day orally in 3 or 4 divided doses for 90 days

WHO dose recommendations for benznidazole are guided by body weight as follows: in patients with a weight of up to 40 kg, the dose of benznidazole is 7.5 mg/kg daily. In patients with body weight over 40 kg, the recommended dose is 5 mg/kg daily. In all cases, the recommended treatment duration is 60 days.

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

[64] Clayton, Julie. "Chagas disease: pushing through the pipeline." *Nature* 465.n7301_supp (2010): S12-S15.

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Benznidazole is a new molecular entity (NME), not currently or ever marketed in the United States. Neither the drug nor any of its components has been marketed or developed in the United States for any indication before. Benznidazole has been available in the United States since the 1990s through compassionate use under an IND held by the CDC.

3.2. Summary of Presubmission/Submission Regulatory Activity

A Pre-IND (PIND) type B Meeting Request was submitted by CHEMO Research (CHEMO) on

June 18, 2013, to discuss a drug development program of benznidazole tablets for the treatment of Chagas disease. PIND number 118976 was assigned on July 9, 2013. The Division of Anti-Infective Products (DAIP) granted a September 17, 2013, meeting on July 15, 2013.

CHEMO submitted a meeting package on July 30, 2013. The proposed indications were the following:

"Treatment of Chagas Disease (b) (4), (b) (4) Trypanosoma cruzi (b) (4) in children (b) (4) (b) (4)

The proposed dose regimen was 5 - (b) (4) mg/kg/day, divided in two daily doses, (b) (4) 60 days.

The sponsor presented a development plan consisting of a planned clinical study in 3-18 year old children and adolescents, along with additional preclinical and clinical information from published literature to address the requirements to support a 505(b)(2) NDA for Benznidazole for treatment of Chagas disease. The meeting objective was to obtain agreement from the Division regarding their proposed plan for their planned NDA submission. The preclinical information submitted consisted of a summary of published toxicology studies of single and repeated doses of benznidazole administered to rats, dogs and rabbits. Genetic and carcinogenic toxicity studies were also summarized. All the preclinical and clinical published data was obtained with the benznidazole product manufactured by Roche, which is no longer commercially available. Roche had transferred its technology to a laboratory owned by the Brazilian government, called LAFEPE, in 2003. LAFEPE's production of benznidazole was currently in shortage due to the lack of API. No CMC information was provided in this first briefing package submitted.

<u>Summary of preclinical studies findings submitted by sponsor</u>

Single oral dose toxicity studies in rats have established that BNZ causes ultrastructural changes in the adrenal cortex, colon, esophagus, ovaries and testis. Chronic repeated dosing to dogs has characterized the neurotoxicity of high oral doses of BNZ. Toxicities are reversible with the exception of some neuronal changes induced by high BNZ doses in dogs (Scharer, 1972) and (Flores-Vieira et al., 1997b).

Numerous genetic toxicity studies have been conducted with BNZ *in vitro* and *in vivo* and in bacterial

and mammalian systems. The weight of evidence indicates that BNZ treatment induces genetic toxicity.

The carcinogenic potential of BNZ has been tested in mice and in rabbits receiving the compound by intraperitoneal (i.p.) injection. BNZ treatment in both species resulted in an increase in lymphomas.

Ultrastructural changes to the reproductive system (Sertoli cells, spermatozoa and ovaries) occur in rats after a high single oral dose of BNZ (100 mg/kg). BNZ has been shown to cross the placenta in pregnant rats and covalently bind to fetal tissues. However, embryo fetal development studies with orally dosed BNZ conducted in rats and in rabbits have not shown BNZ to be embryotoxic or teratogenic. Pharmacokinetics (PK) studies were proposed to be conducted in the planned clinical study. In addition, a summary of published data was submitted in the briefing package.

In the proposed clinical study, approximately 100 patients were going to be enrolled in a single-arm trial, because the use of placebo control was not ethically acceptable in this population in endemic countries where treatment is approved. Comparisons were to be made with historical controls. The primary endpoint was serial negative quantitative PCR results (3 negative PCR results from 3 samples to be collected over 7 days) as a measure of parasitological cure at end of treatment (60 days). The duration of follow-up was going to be 12 months after treatment initiation.

On September 13, 2013, DAIP issued Preliminary Meeting Comments to the questions contained in meeting package, which were discussed at the meeting.

Summary and conclusions of the September 13, 2013 meeting

Pharmacology/Toxicology: The Division stated that the single- and repeated-dose toxicology studies described in the literature and conducted by CHEMO Research appeared to be sufficient. In addition, the Segment I, II, and III reproductive and developmental toxicology studies that were described in the literature, completed by CHEMO Research, or in progress by CHEMO Research at the time, appeared to be sufficient, provided that the final report review was acceptable and supportive.

Clinical Pharmacology: The Division recommended that a mass balance /ADME study be performed in healthy subjects to determine the disposition and major route(s) of excretion/elimination of benznidazole. The results of such a study would help determine whether dose adjustments were needed in patients with renal and/or hepatic impairment. , Information about in vitro metabolism and hERG activity should be provided early in development.

Clinical: The Division recommended the sponsor to characterize the safety profile of benznidazole with a safety database of at least 300 patients at the to-be-marketed dose and duration of therapy. We recommended to obtain and submit with the NDA the source data for the 2 placebo controlled trials (de Andrade, 1996 and Sosa-Estani, 1998), including full study reports, protocols, and electronic datasets, if at all possible. The Division recommended to also include in the study children 0 to 3 year olds and to use serological and also direct parasitological endpoints (Strout, microhematocrit, direct observation). The Division requested additional data regarding the PCR characteristics and methodology and its clinical significance regarding clinical benefit. Of particular concern was the fact that a negative PCR, an experimental assay, was of unclear interpretation, and was not an endpoint used in any of the two randomized controlled studies. The Division also requested details on the methodology and performance characteristics of serological assays (ELISA, IHA and IFA) to be used in the clinical study and also those used in the studies published in the literature. If they were approved by FDA, the brochures needed to be submitted. If not approved, all assays characteristics should be provided.

Meeting agreements

CHEMO indicated that they would provide Segment II and Segment III reproductive studies prior to NDA filing. The Division stated this was acceptable. The Division informed CHEMO that a juvenile animal study would not be needed at the time to support their plan to include very young children in clinical trials. The Division stated CHEMO would need to submit a Thorough QT study, but if they believed one was not needed then CHEMO would need to submit their justification for this rationale. The Division would seek input from the internal QT-IRT staff to assess whether this justification was acceptable.
CHEMO stated they would use PK bridging to assess the comparability of the CHEMO product to the one used in the literature and wanted to know if this would be acceptable. FDA stated this would be fine, but requested as much detail as possible should be submitted, emphasizing that the dosing conditions would need to be the same.

Regulatory-relevant events occurring during 2015

The FDA held a Patient-Focused Drug Development Workshop on Chagas disease on April 28, 2015. At this workshop, patients living with Chagas disease in the United States answered questions and provided examples of how the disease affects their daily lives and what would be the improvements they would like to see regarding the management of the disease diagnosis and treatment. In the

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afternoon session, the workshop focused on scientific aspects of the disease with three lecture presentations and a discussion by a panel of experts which included national and international scientists. This session focused on the currently available knowledge of all aspects of the disease in all its stages, including natural history, diagnosis in each clinical stage, outcomes of treated and untreated disease in different stages, treatment availability in the US and potential regulatory pathways for new drugs to treat Chagas disease.

On August 20, 2015, the FDA added Chagas disease and neurocysticercosis to the list of tropical diseases set forth in the Food, Drug and Cosmetic Act, which are eligible for priority review vouchers (Federal Register/Vol. 80, No. 161, August 20, 2015). The Patient-Focused Drug Development Workshop and the inclusion of Chagas disease in the tropical diseases list of the FD&C Act brought up increased awareness of the need for drug development for Chagas disease and started public discussions on ways to address this unmet medical need. For more information and access to meeting materials and presentations go to: http://wayback.archive-it.org/7993/20170112011649/http://www.fda.gov/Drugs/NewsEvents/ucm420130.htm
Also available is the Voice of the Patient report, prepared to fulfill the objective of the Patient-Focused Drug Development. This is a commitment under the fifth authorization of the Prescription Drug User Fee Act (PDUFA V) that aims to more systematically gather patients' perspectives on their condition and available therapies to treat their condition. For the Voice of the Patient report on Chagas disease please go to:
https://www.fda.gov/ForIndustry/UserFees/PrescriptionDrugUserFee/ucm368342.htm

April 27, 2016 End of Phase 2 Meeting

CHEMO requested this meeting on February 26, 2016 to discuss the ongoing development program and the potential for a 505(b)(2) NDA submission for the treatment of Chagas disease. CHEMO proposed in this meeting's briefing package an indication of:

"Benznidazole for the treatment of Chagas disease"
Dosage Form: scored round tablet
Strength: 100 mg and 12.5 mg
Route of Administration: oral use

Dosing Regimen: (b) (4)
(b) (4)

CHEMO had conducted an in vitro hERG assay, of which results were made available in the briefing package. Benznidazole showed a concentration-dependent inhibition of hERG tail current in stably transfected HEK-293 cells up to a concentration of 100 µM with an average inhibition of 17.0 ±2.7% at this concentration. As the concentration of 100 µM was much higher (approximately 9 fold) than the maximal anticipated therapeutic plasma concentration, this small inhibition was considered not biologically relevant.

CHEMO informed the Division that a CMC meeting had been scheduled for May 2016, with the Office of Pharmaceutical Quality (OPQ). CHEMO stated that they planned to market 12.5 mg and 100 mg tablets. CHEMO stated they would utilize two drug substance providers (b) (4) (b) (4) and noted that the to-be-marketed product would be the Liconsa product. CHEMO confirmed that they were going to include results of the pharmacokinetic bridging studies for both the 12.5 mg and 100 mg tablet formulations. The 12.5 mg tablet would be for pediatric patients, scored in half to ease administration in slurry prepared with water. CHEMO indicated that they had PK data from a study in patients 2 to 12 years old (Altcheh study) done with the LAFEPE product. The Division requested CHEMO to present data to guide dosing in patients 0 to 6 years of age, and to lay out exactly what PK data they have for each age range in the next meeting package. The Division recommended CHEMO to further evaluate the in vivo drug-drug interaction potential of benznidazole after obtaining results from the planned in vitro assessments of the metabolic profiling and inhibitory/inductive effect of benznidazole on the major CP enzymes and transporters. The Division strongly encouraged CHEMO to obtain further data and/or information on the effect of hepatic or renal impairment on the PK of benznidazole. In relation to that, the Division reminded CHEMO of the previous request to conduct a mass balance/ADME study in healthy subjects to determine the disposition and major route(s) of excretion of benznidazole. This study would help determine whether dose adjustment is needed in patients with renal or hepatic toxicity.

The Division inquired if CHEMO had any information on neonates, including natural history studies of pediatric populations from 0 to 6 years of age. The Division requested data on untreated patients who had acquired congenital disease. The Division recommended CHEMO to look for such data to demonstrate the natural evolution of the infection in children who had acquired it congenitally. Data on persistent serologically positive outcomes over time in this population would be useful to understand untreated disease and the effects of treatment on its natural course.

The Division also requested CHEMO to provide information regarding a potential for development of resistance and data on activity against different lineages of *Trypanosoma cruzi*.

<u>CMC Type B End of Phase 2 meeting</u>

A Type B CMC meeting was scheduled for May 16, 2016. However, after the Sponsor received satisfactory responses to the preliminary questions submitted, the meeting was cancelled.
The key issues and recommendations were as follows:

- **Drug substance manufacturers**: For the CMC information for benznidazole, CHEMO proposed to refer to a Drug Master File (DMF) held by the proposed commercial manufacturer, (b) (4) (b) (4). Information in the (b) (4) DMF, the manufacturer used in the product development, should be sufficient to support the NDA, provided that no review issues are found. The Agency requested CHEMO to submit, at the IND or NDA stage, more details on the manufacturing process and the formation and elimination of genotoxic impurities. CHEMO

proposed that the drug substance specification would be based on ICH guidance (i.e. (b) (4). The Agency stated that the~~se~~ proposed specification appeared reasonable; however, final determination would be a review issue.

- **Equivalence of the two drug substance sources**: The Agency recommended that, in addition to the physicochemical characterization of the drug substance from both suppliers and comparison of their impurity profiles, CHEMO provide release and three-month stability data for the drug products manufactured using the two drug substance sources to allow evaluation of equivalence of the different sources of the drug substance. In addition, the Agency recommended that CHEMO provide comparative dissolution profile data for drug product batches manufactured using drug substance batches from each drug substance supplier using an optimal and discriminating dissolution method.

- **Benznidazole Tablets, 12.5 mg and 100 mg**: The Agency requested CHEMO to provide disintegration time limits in the drug product specifications of Benznidazole tablets, 12.5 mg and 100 mg. The Agency agreed to the proposed dissolution test provided by CHEMO in the meeting package and provided a list of general recommendations regarding the dissolution information/data that should be provided in the NDA submission as follows:

  1) **Dissolution method development report**: The report should include the following information with regard to each active ingredient.
  a. Solubility data for the drug substance over the physiologic pH range.
  b. Detailed description of the dissolution test parameters (i.e., equipment/apparatus, type and volume of media, agitation/rotation speed, pH, temperature, etc.). Include a narrative of why these parameters were selected and how the test conditions were optimized (e.g., sink conditions, stability considerations). If applicable, the type and the amount of surfactant added to the dissolution medium, and/or the use of strength dependent dissolution methods should be justified. The dissolution –time profile should be complete and cover at least (b) (4)% of drug release of the label amount or whenever a plateau (i.e., no increase over 3 consecutive time-points) is reached; initial sampling time points typically include 10, 15, 20, 30, 45, 60, 90 and 120 min. At least twelve samples should be used per testing variable.
  c. Data to support the discriminating ability of the selected dissolution method. In general, the testing conducted to demonstrate the discriminating ability of the selected dissolution method should compare the dissolution profiles of the reference (target) product vs. the test (variant) products that are intentionally manufactured with meaningful variations for the most relevant critical manufacturing variables (i.e., ± 10-20% change to the specification-ranges of these variables). If available, submit data showing that the selected dissolution method is able to reject batches that are not bioequivalent to the reference (target) product.
  d. A list of the critical material attributes (CMA) and critical process parameters (CPP) affecting dissolution.
  e. Summary figures and tables showing mean and %RSD cumulative amount of drug released at each sampling time point, and if applicable, f_2 (profile similarity) values.

f. Validation data for the dissolution method (i.e., method robustness, etc.) and analytical method (precision, accuracy, linearity, stability, etc.).
g. A detailed justification of the proposed dissolution acceptance criteria.

2) **Dissolution Acceptance Criteria**: For the selection of the dissolution acceptance criteria for the proposed drug product, the following points should be considered.
a. In setting the dissolution acceptance criteria of the product (i.e., specification sampling time point and specification value), use the dissolution profile data of the pivotal PK and clinical batches, i.e., based on USP Stage 2 dissolution testing (n = 12) of the batches at the time of manufacture and during long-term storage for the duration of the trial(s). In addition, the dissolution profiles of the primary (registration) and supportive stability batches during long-term storage should be considered.
b. The specification time point should be where Q= (b) (4)% drug dissolution occurs. However, for a slowly dissolving product, specifications at two time points may be appropriate. The first time point should be selected during the initial dissolution phase (e.g., 15-30 minutes where about (b) (4)% dissolution occurs) and the second time point should be where Q = (b) (4)% dissolution occurs.

3) **Supporting Data**: The following detailed experimental data should be submitted to support the dissolution method development and setting of acceptance criteria:
a. As much individual vessel data as possible in the narrative portion of the report, particularly regarding investigation of selection of equipment, media, agitation speed, etc.
b. Analysis datasets in ".xpt" format, and their define files. The dataset should contain individual vessel data for all sampling time points.
c. Batch release and stability dissolution data presented graphically. The plot(s) of individual vessel data for the clinical and stability batches should include data at release, time zero stability time point, and over the duration of stability testing under long-term storage conditions.

- **Stability evaluation data required in the NDA submission**: The Agency informed CHEMO that the NDA at the time of submission should include at least 12-months long-term and 6-months accelerated stability data for three registration batches. Data from stability studies should be provided on at least three primary batches of the drug product. The primary batches should be of the same formulation and packaged in the same container closure system as proposed for marketing. The manufacturing process used for primary batches should simulate that to be applied to production batches and should provide product of the same quality and meeting the same specification as that intended for marketing. Two of the three batches should be at least pilot scale batches and the third one can be smaller, if justified. Where possible, batches of the drug product should be manufactured by using different batches of the drug substance. For detailed guidance, the Agency recommended CHEMO to refer to ICH Q1A(R2), Stability Testing of New Drug Substances and Drug Products.

(b) (4)

Timeline of Important Regulatory Milestones:

- July 15, 2016, a 505(b)(2) NDA submission was planned.
- Orphan drug designation: 04/04/2014, Designation #14-4252
- Priority review designation: 2/23/2017
- Fast track designation: n/a since an IND was not submitted
- Pre-NDA meeting: 09/30/2016

Pre-NDA September 30, 2016, agreements were:

Clinical
The therapeutic effect needs to be demonstrated with a comparison of outcomes of treated patients to the outcomes of those with untreated disease. Data that correlate clinical outcomes to assay results will be critical.

Adults
If, in the studies conducted in the adult population, such as the Molina study of benznidazole vs. posaconazole and the Morillo study (BENEFIT), some serology data were collected as a secondary endpoint, it would be helpful for the review. In the NDA please provide a justification to link serological responses and clinical outcomes.

Children
Data on natural history of untreated disease in neonates and children ages 0 to 6 years old will be essential (b) (4) in that age group. The therapeutic effect needs to be demonstrated with a comparison of outcomes of treated patients to the outcomes of those with untreated disease. If you believe that efficacy in children under 6 years of age can be extrapolated from a finding of efficacy in older children, you will need to show that the course of the disease (persistent serological positivity, clinical phases of the disease) and the effects of the drug are sufficiently similar in both groups.

Specific recommendations regarding clinical data submission:

It is highly desirable that you provide as much source information as possible from studies that correlate clinical outcomes with PCR assays or serological responses in children and/or adults. For example, source data from the Fabbro 2007, Streiger 2004, and the Viotti 2006 studies could be

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helpful to support a bridge between treatment, serological responses and clinical outcomes. These studies provide data on untreated patients, and will help to understand outcomes in untreated disease. As discussed previously, data on natural history of untreated disease in neonates and children ages 0 to 6 years old will be essential (b) (4) in that age group. The therapeutic effect needs to be demonstrated with a comparison of outcomes of treated patients to the outcomes of those with untreated disease. If you believe that efficacy in children younger than 6 years of age can be extrapolated from a finding of efficacy in older children, you will need to show that the course of the disease (persistent serological positivity, clinical phases of the disease) and the effects of the drug are sufficiently similar in both groups.

Clinical Pharmacology

Based on the results of human Ether-a-go-go Related Gene (hERG) testing, observations of electrocardiogram (ECG) tracings, and the lack of cardiac toxicity reports in clinical use of BNZ, a tQT study was not conducted. Chemo Research submitted information at the 27 Apr2016 End-of-Phase 2 meeting. The Division requested a QT Interdisciplinary Review Team (IRT) on 8 July 2016, to determine the need for a tQT study. Taking into account the data submitted and the recommendations from the IRT team, the Division agreed that the likelihood of benznidazole to significantly prolong QTc interval was low and determined that a TQT study was not needed for the planned NDA.

CMC

The Agency stated that CHEMO should submit a list of all facilities and their role in the NDA. The Agency noted that CHEMO needed to bridge the two sources of API: (b) (4) and to address other CMC recommendations provided in communications from May 11 and May 26, 2016. The Agency asked about the source of the benznidazole product which is now commercialized in Spain. CHEMO clarified that the product currently distributed in Spain is manufactured by ELEA (Argentina). Testing and release of product is done in (b) (4). Laboratory Liconsa in Spain is only responsible for the (b) (4). The Agency requested complete and current physical addresses of facilities. CHEMO stated they could provide this information.

Major components of the application were expected to be submitted with the original application and are not subject to agreement for late submission.
The Agency agreed to the submission of patient source data from three additional publications (Fabbro, Streiger, Viotti) which could be accepted no later than 2 months after the NDA receipt date.

Statistics

For this NDA, it was agreed that Chemo Research would submit Clinical Data Interchange Standards Consortium (CDISC) compliant Study Data Tabulation Model (SDTM) data and Analysis Data Model (ADaM) data for the studies with complete raw data and data documents. For the studies with

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insufficient information for CDISC data conversion, the raw data would be converted as SAS datasets, and ADaM like analysis datasets would be generated for statistical analysis. SAS transport files (xpt), define.xml or define.pdf and reviewer guides would be included for each of the studies. The Division recommended CHEMO to ensure that all SAS datasets were submitted in English, and suggested to consider submitting the SAS datasets and a sample of SDTM and ADaM datasets for at least the four controlled trials approximately a month prior to the NDA submission so that a preliminary assessment of the quality of the data can be made.

An Integrated Summary of Efficacy (ISE) and Integrated Summary of Safety (ISS) were planned. Integration of safety was expected however pooling of efficacy parameters would not be probably feasible due to the results from different dosages/populations/results. In this case an ISE was planned but data presentation was expected to be summarized tabulations (where feasible) in a side-by-side review.

NDA submission: December 29, 2016

Pursuant to Section 527 of the Food, Drug and Cosmetic Act (FDC Act), Chemo Research, S.L. (Chemo) claimed seven (7) years of marketing exclusivity from the date of approval of (b) (4) (benznidazole) Tablets, indicated for the treatment Chagas disease. Chemo certified that this application was seeking approval for a drug which had orphan drug designation. In addition, pursuant to 21 C.F.R. § 314.50(j), Chemo claims five (5) years of marketing exclusivity for (b) (4) (benznidazole) Tablets under 21 C.F.R. § 314.108(b)(2), the regulation implementing FDC Act § 505(c)(3)(E)(ii). In accordance with 21 C.F.R. § 314.50(j)(3), Chemo made the following assertion:

"To the best of Chemo's knowledge, based on a reasonable investigation, (b) (4) (benznidazole) Tablets contains an active moiety (benznidazole) that has not previously been approved by the Food and Drug Administration under FDC Act § 505(b)".

3.3. Foreign Regulatory Actions and Marketing History

In the early 1970s, Hoffman La-Roche (Roche) developed benznidazole for the treatment of Chagas disease. Roche obtained the registration of benznidazole in Brazil, Argentina, Bolivia, Uruguay, Peru and Nicaragua in the 1970s as Radanil®, Ragonil® or Rochagan®, which are products for oral administration formulated in 100 mg and 50 mg uncoated scored tablets. In 2003, Roche decided to donate all commercial rights and transfer the technology to manufacture BNZ to the Brazilian government as a generic version of Roche's product, and, at the same time, Roche also withdrew its registration.

The product was granted a marketing authorization by the Brazilian Drug Regulatory Authority (ANVISA) in November 2006. Since that time, Laboratorio Farmacéutico de Pemambuco (LAFEPE), a Brazilian government manufacturing facility, has provided benznidazole to patients in Latin America and also to the United States under an IND held by the CDC.

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Benznidazole (Abarax®), 100 mg and 50 mg tablets for oral administration, manufactured by Laboratorio ELEA, Buenos Aires, Argentina, has been approved in the following countries during recent years:

- Argentina, approved on January 16, 2012
- Bolivia, approved on May 29, 2013
- Paraguay, approved on January 9, 2013
- Chile, approved on June 14, 2014

Abarax® is also available in Spain through the procedure "*access to medicines in exceptional situations*" as a "*foreign medicine"* for the treatment of Chagas disease. Up to March 1, 2015, there have been a total of 3,407 treatments distributed in Spain, according to the sponsor's investigators brochure.

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

The Office of Scientific Investigations is planning to conduct a GMP inspection of the facility where the human bioequivalence study was done. The inspection is pending at this time.

Regarding the inspection of source documentation of clinical studies, the available data include spreadsheets marked as "final" provided by the investigators. CHEMO converted the final spreadsheets to conform to CDISC/CDISC-like datasets. The tables and listing were derived from the converted CDISC/CDISC-like datasets.

The trial master file (TMF) held by Chemo Research contains all of the correspondence

(e.g. email) with each investigator. The TMF also includes everything that the investigator provided e.g. copy of spreadsheets, clarification of data field names. The TMF also includes documentation when requested information was not available, such as IRB and ICF.

The TMF is maintained at Chemo Research in Madrid, Spain. OSI is planning an inspection of the TMF; this visit is pending at this time. The major aims of the inspections are to verify the sponsor's claims about the source and nature of the original NDA data.

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4.2. Product Quality

Benznidazole is an antiprotozoal agent of the nitroimidazole group derived from N-benzyl 2-nitroimidazole. Benznidazole drug substance is yellowish practically crystalline powder insoluble in water, sparingly soluble in acetone, and slightly soluble in methanol and ethanol. The chemistry manufacturing and controls information for benznidazole drug substance has been provided via a reference to DMF (b) (4) held by (b) (4). DMF (b) (4) has been reviewed in support of this NDA and was found to be acceptable (review dated May 24, 2017, by the CMC team in Panorama).

The drug product, benznidazole tablets, is supplied in two strengths, 100 mg and 12.5 mg. Benznidazole tablets, 100 mg, are round white tablets about 10 mm diameter and scored twice in cross on both side and debossed with an "E" on one side on each split portion. Benznidazole tablets, 12.5 mg, are round, white tablets about 5 mm and debossed with and "E" on one side. The 12.5 mg tablets can be may be (b) (4) made into slurry for administration for pediatric patients who cannot swallow an intact tablet. The inactive ingredients include pregelatinized corn starch, monohydrate lactose, sodium croscarmellose, microcrystalline cellulose, magnesium stearate, (b) (4) (b) (4) All the excipients used in the manufacture of benznidazole tablets, 12.5 and 100 mg, meet the USP/NF specifications.

Comparative data were provided in the NDA for benznidazole drug substance manufactured by (b) (4) (used to make clinical supplies) and benznidazole drug substance manufactured by (b) (4) (the proposed commercial manufacturer) used to make drug product commercial batches. Based on the evaluation of these data, the two drug substances sourced from (b) (4) sites were found comparable. Stability data from the DMF holder support a retest period of (b) (4) for benznidazole drug substance manufactured at (b) (4) (b) (4) and stored at (b) (4)

During the review, several revisions to the proposed drug product specification were recommended by reviewers such a revision of the proposed acceptance criterion for dissolution (as discussed below), shortening of the proposed disintegration time, inclusion of the test for (b) (4) content and inclusion of content uniformity (b) (4) in the uniformity of dosage forms. In addition, the listing of impurities was revised to follow nomenclature recommended by the ICH Q3B guidance. The drug product specification, as revised by the CMC team, was found to be adequate. The analytical methods are described in reasonable detail and adequate validation data have been provided. The assay and chromatographic purity analytical procedures are currently under evaluation by the FDA laboratory.

4.3. Clinical Microbiology

Mechanism of action

Although the mechanism of action is not fully understood, it is known that benznidazole is reduced by a type I nitroreductase (NTR) enzyme of *T. cruzi*, which is O_2 insensitive. The metabolites released may promote damage to macromolecules such as DNA. *T. cruzi* is susceptible to the cell damage induced by these metabolites because enzymes scavenging free radicals are absent or have very low activities in the parasite.

In mammalian cells, however, benznidazole is metabolized by reduction of the nitro group to an amino group by a type II NTR, an O_2-sensitive enzyme. The resulting production of H_2O_2 and superoxide anions may cause oxidative cell damage. This mechanism is thought to be responsible for benznidazole side effects in humans. Some authors have postulated that a reduced activity of this enzyme in young children may explain the reduced toxicity observed in children as compared to adults (Viotti 2009)[65]. Benznidazole has parasiticidal activity against both trypomastigotes and amastigotes in cell cultures (Maya 2007)[66].

In vitro and in vivo susceptibility and parasite diversity

At present, *T. cruzi* is partitioned into six discrete typing units (DTUs), TcI–TcVI. Besides the geographic distribution of these DTUs in the sylvatic and domestic cycles, there are important differences in resistance or susceptibility to benznidazole. These have been described in laboratory experimentation, or in clinical studies, among different strains of the parasite. The distinct geographic distributions of the six *T. cruzi* subtypes, termed TcI-TcVI, are illustrated in the Figure below.

[65] Viotti, Rodolfo, et al. "Side effects of benznidazole as treatment in chronic Chagas disease: fears and realities." *Expert review of anti-infective therapy* 7.2 (2009): 157-163.

[66] Maya, Juan Diego, et al. "Mode of action of natural and synthetic drugs against Trypanosoma cruzi and their interaction with the mammalian host." *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 146.4 (2007): 601-620.

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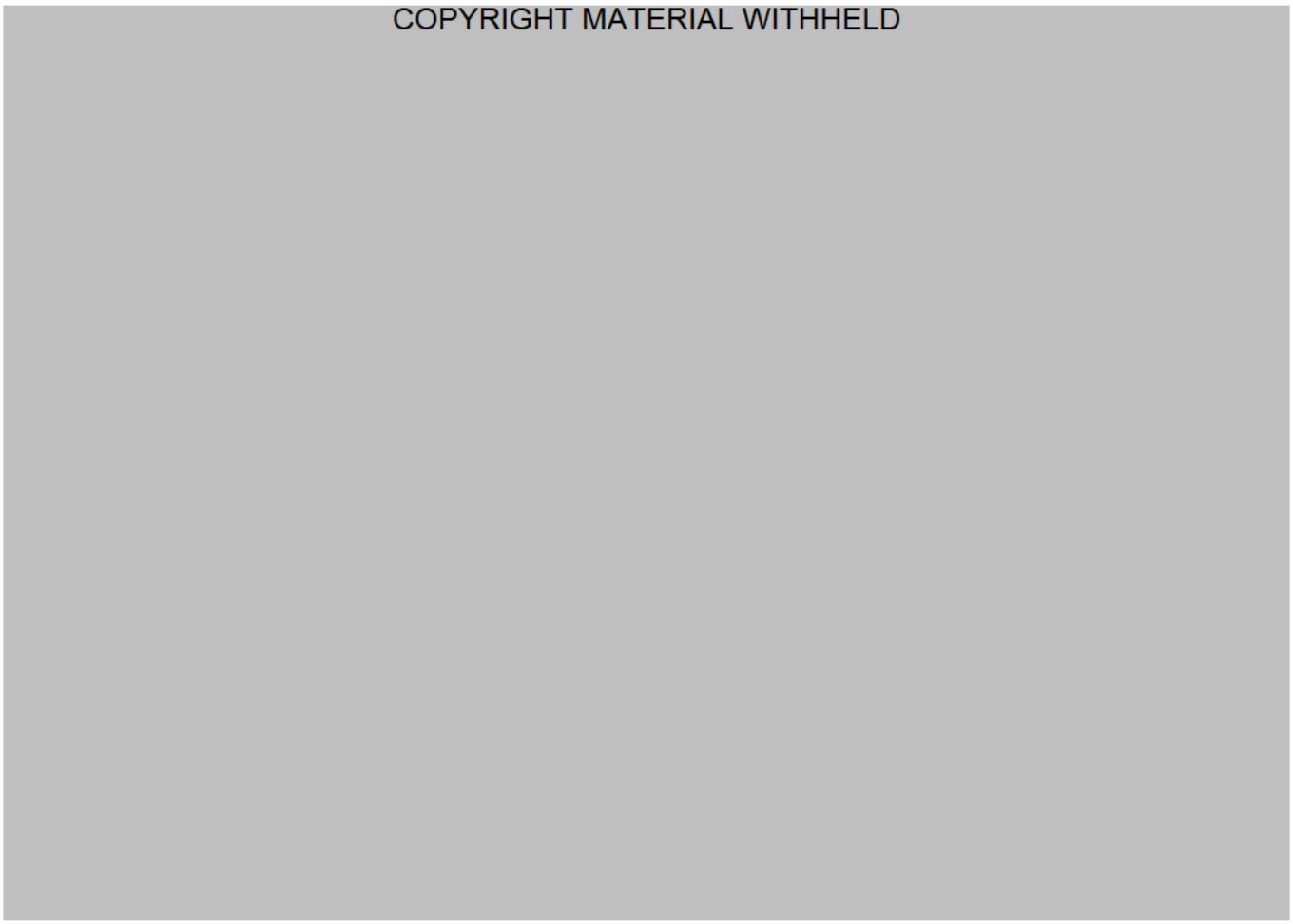

Figure 2:Approximate geographical distribution of T. cruzi DTUs in domestic and silvatic transmission cycles. *Source:* (Zingales, 2012)[22]

TcI, TcII, TcV and TcVI are the main agents of human Chagas disease in the Americas, and all are capable of causing cardiomyopathies, however, only DTUs TcII, TcV and TcVI have been so far associated to chronic digestive syndromes (Zingales, 2012)[67]. In addition, the route of infection may be different in certain regions and may cause different clinical presentations. For example, in the Amazon Basin, the oral route is the most frequent form of transmission, and acute Chagas disease manifestations are more frequently observed.

In the Southern Cone region, where *T. infestans* is the main vector, TcII, TcV and TcVI are the main causes of Chagas disease. TcII predominates in eastern and central Brazil, TcV in Argentina, Bolivia, and Paraguay, and TcVI in the Gran Chaco (forest region of western Bolivia, Paraguay, northern

[67] Zingales, Bianca, et al. "The revised Trypanosoma cruzi subspecific nomenclature: rationale, epidemiological relevance and research applications." *Infection, Genetics and Evolution* 12.2 (2012): 240-253.

Argentina and south of Brazil). Finally, Leiby and colleagues[68] suggest that different strains of *T. cruzi* may pose varying risks of transmission, especially regarding blood products and congenital transmission. Most of the immigrant population in Spain where a number of cases of transfusion transmission have been documented is derived from South America where *T. cruzi* (Tc) Lineages II to VI predominate, whereas the immigrant population in the United States, where the rate of transmission is low, is more likely to hail from Mexico and Central America where Tc Lineage I is found. The authors report a significantly higher rate of hemoculture positivity with Tc Lineage II to VI (11 of 24 [45.8%]) versus Tc Lineage I (2 of 90 [2.2%]). Higher levels of parasitemia with Tc Lineages II to VI may explain the higher rates of transmission in countries where immigration from South America predominates. A study conducted by the Centro de Transfusión de Cruz Roja Española in Madrid provides further support for this hypothesis: 15 of 49 (30.6%) blood donors found to be *T. cruzi* antibody positive were also positive by hemoculture, with parasite levels between 1 and 10 parasites/mL. These donors were all born in South America and most were from Bolivia. Also, in Bolivia, the rate of congenital transmission is also higher than in Brazil, and the predominance of lineage V may help explain this difference. Among United States donors, the American Red Cross reviewed a sample of presumptive parasite lineages and compared to hemoculture results. Authors described that only two of 157 (1.3%) TcI versus 13 of 38 (34.2%) TcII/TcV/TcVI non-US donors were parasitemic; three of 44 (6.8%) US donors were TcV or TcVI[69]. This is consistent with what was found in Spain as well.

Regarding microbiological susceptibility, time kill studies suggest that the activity of benznidazole may be concentration and time dependent. The 50% inhibitory concentrations (IC_{50}) against the epimastigotes or the amastigotes of the laboratory strains belonging to different DTUs as well as the clinical isolates were ≤19.5 μg/mL. As is the case with other parasitic and fungal infections, there are no standardized methods to test drug susceptibility of the isolates and the clinical relevance of *in vitro* sensitivity testing is difficult to generalize, given the variability of parasitic tissue burden and its distribution and the variable pharmacokinetics of benznidazole among patients. It is not possible to measure the tissue burden since heart tissue biopsies carry high risk and are unreliable to quantify parasitic load because of the sparse nature of the amastigotes in tissue.

Activity in animal models of T. cruzi

Chagas disease induced experimentally in dogs has been shown to resemble the human disease in all its phases (WHO bulletin 1991). The disease in dogs is not fatal, and it reproduces the stages of cardiac disease. However, the differentiation between the chronic-intermediate (silent phase) and the chronic phase with manifestations of cardiomyopathy is difficult to make in dogs models. In dogs

[68] Leiby DA, Nguyen L, Procter C, Townsend L, Stramer SL. *Impact of Trypanosoma cruzi phylogenetic lineage on transfusion transmission in the United States [abstract]. Vox Sang 2011;99 (Suppl 1):64*
[69] Leiby, David A., et al. "Frequency of Trypanosoma cruzi parasitemia among infected blood donors with a potential association between parasite lineage and transfusion transmission." *Transfusion* (2017).

infected with the Berenice-78 strain (sensitive to benznidazole) it was shown that benznidazole treatment in the acute phase (20–30 days after infection) was able to cure 75–100% of animals, reducing tissue damage and preventing electrocardiographic alterations (Caldas et al., 2013[70]). In one study (Guedes, 2002)[71], 3 month-old mongrel dogs were infected with *Trypanosoma cruzi* strains of different susceptibilities to benznidazole and treated with the same therapeutic scheme as used for human chagasic disease. The treatment with benznidazole was able to prevent death and induced parasitological cure in 62.5% (acute phase, treated 10-22 days after infection) and 38.7% (chronic recent phase, treated 100 days after infection) of the tested animals. However, in another study, when benznidazole treatment of chronic *T. cruzi* infection in 4 month-old mongrel dogs was initiated 120 days after infection, treatment was efficient in reducing systolic cardiac function alterations, but it was unable to prevent the long-term development of cardiomegaly, including an increase in the left atrial volume (Santos, 2012)[72]. The antibody titers in infected dogs treated with benznidazole were lower than in untreated dogs against both epimastigote and trypomastigote antigens of the Y strain; the decrease in antibody titers was more pronounced in dogs that were parasitologically cured. Lytic antibodies, measured by complement mediated lysis (CoML) of the trypomastigotes of the Y strain of *T. cruzi* were also reduced. There was a difference in antibody response in animals infected with the Y or BE-78 strain of *T. cruzi*. Despite the variability, treatment with benznidazole reduced IgG, IgG1 and IgG2 levels during the 6 month observation period.

Chagas disease also occurs naturally in dogs, and its course has remarkable similarities to the disease in humans. A study to assess frequency, geographic distribution, and clinical spectrum of Chagas disease in domestic dogs from Texas was conducted[73]. Serology, histopathology, and clinical case records from multiple institutions for the time period 1993–2007 were analyzed. A total of 537 serologically and/or histopathologically confirmed cases were documented. Increased seropositivity was observed during the follow-up period, with an overall rate of 20.3%. Dogs less than 1 year of age accounted for approximately half of all acute deaths (without previous manifestations of illness). In non-acute death cases, duration of apparent illness ranged from 1 day to 6 weeks. In cases with clinical descriptions, cardiomegaly and ascites were the most frequent findings. The most consistent microscopic finding was myocarditis in 97.9% of the histopathologically confirmed cases, and in T. cruzi was found in 81.7% of the heart tissues. A total of 83% of the cases had serologically confirmed

[70] Caldas, Ivo Santana, et al. "Myocardial scars correlate with electrocardiographic changes in chronic Trypanosoma cruzi infection for dogs treated with Benznidazole." *Tropical Medicine & International Health* 18.1 (2013): 75-84.

[71] da Matta Guedes, Paulo Marcos, et al. "The dog as model for chemotherapy of the Chagas' disease." *Acta tropica* 84.1 (2002): 9-17.

[72] Santos, Fabiane M., et al. "Cardiomyopathy prognosis after benznidazole treatment in chronic canine Chagas' disease." *Journal of antimicrobial chemotherapy* 67.8 (2012): 1987-1995.

[73] Kjos, S. A., et al. "Distribution and characterization of canine Chagas disease in Texas." *Veterinary parasitology* 152.3 (2008): 249-256.

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diagnosis. No information on treatment was available from this study.

In smaller laboratory animals the course of the disease varies widely, depending upon the host and parasite strains used, the route of inoculation, and the size of the inoculum. The activity of benznidazole was measured in mice, rabbits, and dogs infected with trypomastigotes of approximately 64 strains/clones (a majority of the strains were tested in mice) of *T. cruzi*. Several aspects of *T. cruzi* infection in these animal models mimic human disease. Like humans, parasitemia develops after incubation and is followed by a latent phase. The hearts of benznidazole-treated mice had decreased parasitism and myocarditis compared to the hearts of untreated chagasic mice. The levels of antibodies against *T. cruzi* antigens were lower in the sera from benznidazole-treated mice than in the sera from untreated mice[74].

In rabbits infected with the Ernestina strain of *T. cruzi*, treatment with benznidazole during the acute phase was effective in reducing the duration of parasitemia to 1 month, while the infected controls had persisting parasitemia for more than 5 months and suppressed the cell-mediated immunity to related and unrelated antigens in the treated animals (Teixeira, 1990[75])

Reviewer's comment: *The efficacy of benznidazole has been demonstrated in vitro and in vivo in several acute and chronic experimental animal models of Chagas disease in different species, including dogs, mice and rabbits. The animal studies show a similar correlation of outcomes of cure and seronegativization and of decreased parasite burden with decreased serological titers (measured by conventional serological assays), to the ones described in human studies.*

A correlation of outcomes of cure by seronegativization and of decreased parasite burden by decreased serological titers (measured by conventional serological assays) in animal studies, was similar to what is described in human studies.

Dr. Shukal Bala, Ph.D., DAIP, Microbiology Reviewer and by Dr. Noel Gerald, Ph.D., Division of Microbiology Devices, OIR, CDRH, with a team from CDRH have reviewed the characteristics and performance of the serological assays used as primary endpoints in the Sosa Estani and De Andrade. In these studies serological responses were measured with conventional assays (ELISA, IFA and IHA) and another ELISA testing recombinant parasite antigens, specifically the F-29 ELISA assay and the A&T ELISA assay (chemiluminescence antibodies assay).

Dr. Noel Gerald concluded that "it is not clear that a precise estimate of the efficacy of treatment

[74] Garcia, Simone, et al. "Treatment with benznidazole during the chronic phase of experimental Chagas' disease decreases cardiac alterations." *Antimicrobial agents and chemotherapy* 49.4 (2005): 1521-1528.

[75] Teixeira, Antônio RL, et al. "Chagas' disease: lymphoma growth in rabbits treated with Benznidazole." *The American Journal of Tropical Medicine and Hygiene* 43.2 (1990): 146-158.

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effect could be calculated from these serological tests alone and they should not be considered primary endpoints upon which to make a regulatory decision." He states that details of the methods used and data supporting performance characteristics of the assays in the laboratory where testing of clinical specimens was performed was limited; some of the deficiencies include lack of information on positive and negative controls (calibrators) used, the reproducibility, as well as absence of data to support the cut-offs used to characterize patients as seropositive or seronegative. A consensus on the definition of a clinical meaningful decrease (such as 2-fold, 4-fold decrease, as is the case in other infectious diseases) is lacking for Chagas disease, therefore his advice is to only consider clinical endpoints for approval.

I agree there is a lack of consensus on a specific quantification of a titer decrease in Chagas disease. However, I disagree that we cannot clinically interpret the outcomes of serological test results. These assays are interpreted in the context of the concordance of responses of at least two of three different types of tests (IFA, IHA or ELISA). The diagnosis is established by that concordance and the clinical follow-up is done with multiple serial measurements on more than one type of test, over the years. The multiple measurements with consistent trends, decreasing or increasing, indicate a clear differentiation towards a cure or a treatment failure. The concordance of serological outcomes in two of three assays enhances the sensitivity and specificity of each individual assay. This is because of the regional diversity of the parasite and the diversity of the immune response to recognize different epitopes in varying degrees according to the antigen presentation provided by each the three assays. The in vivo cycle of development of an indeterminate number of intracellular nests of amastigotes in tissues and their burst into blood trypomastigotes is not a synchronous process, and this also affects the serological response.

The use of conventional serological assays in combination is the formal recommendation from WHO. There is considerable experience with these assays in Latin America for over 40 years.

The serological assays for the two studies in children, and the Viotti study in adults, were performed at reference centers in Brazil and Argentina, which are regional centers of the WHO, and for which there is a quality assurance program established since 1986.

We may not have the specific technical details available now more than 20 years after the studies were conducted. However, the conventional assays used in the three studies (Sosa Estani, De Andrade and Viotti) were done at national reference centers and had been validated and standardized at different stages in multinational studies (Camargo, 1986[76]) coordinated and sponsored by WHO and PAHO in collaboration with Dr. Franklin Neva, NIH and Dr. Irving Kagan, from CDC. These conventional serological assays have been the standard from which newer commercial assays have been developed

[76] Camargo, Mario E., et al. "Three years of collaboration on the standardization of Chagas' disease serodiagnosis in the Americas: an appraisal." (1986).

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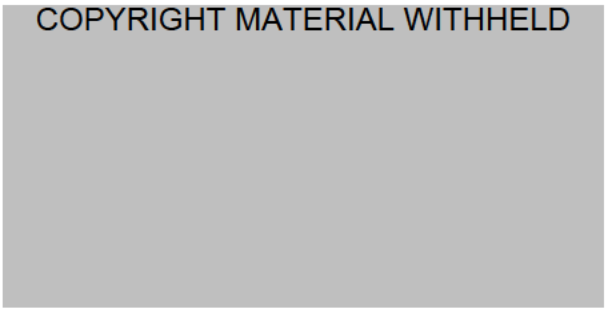
and compared to. With the years, some changes to the methodology of the assays have been made to improve their efficiency, automatization and performance; however, used in combination, they are still the gold standard of diagnosis and cure of Chagas disease. The conventional serological assays are generally in agreement. For example, the graph below shows the similarity among the findings of three serologic study assays: IIF (indirect immunofluorescence), ELISA (enzyme-linked immunosorbant assay) and IHA (indirect hemagglutination assay) in the study of Galvão, 2003[77].

Figure 3: ROC curve of IIF titers, IHA titers, and ELISA index for the identification of children treated with benznidazole after three years of follow-up

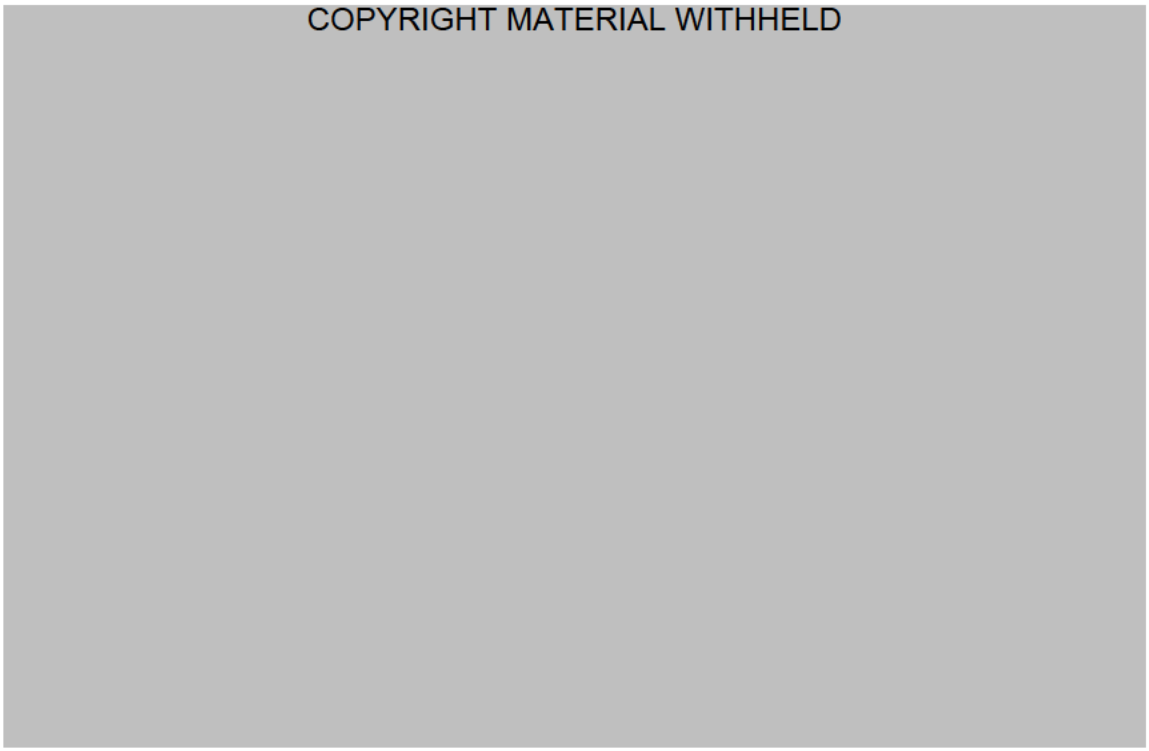


Source: Galvão, 2003[93]

There was no significant difference in the area under the receiver operating characteristic (ROC) curves of the three assays (Figure 3). In this study (De Andrade study), a decline in the serologic titer over time post-treatment was observed in the benznidazole treated but not in the untreated children. At least 6 prospective and controlled studies with long term follow-up in children and adults show serological reduction in treated but not in untreated patients. The rate of reduction observed is consistent among them and has been characterized as age-dependent. If treated at an older age, the serological negativization occurs much later than if treated at a young age. The Figure below shows the percent of patients who were nonreactive Appears this way stamp? (seronegative) at short and longer times post treatment if they were treated as children <5 years of age, as children ≥5 years of age or as adults.

[77] Galvão, Lúcia MC, et al. "PCR assay for monitoring Trypanosoma cruzi parasitemia in childhood after specific chemotherapy." *Journal of clinical microbiology* 41.11 (2003): 5066-5070.

Figure 4: Decrease in the seronegativization measured post treatment as a function of the age at which a Chagas patient is treated (Sosa Estani, Viotti and Segura 2009[78])



4.4. Nonclinical Pharmacology/Toxicology

The following is a summary of the major preclinical findings associated with benznidazole, identified by Dr. James Wild, Ph.D., Pharmacology and Toxicology reviewer for this NDA.

Genotoxicity and Carcinogenicity: Benznidazole has been shown to be mutagenic in Ames assays and to increase the frequency of sister chromatid exchange and micronuclei *in vitro* in human cells, and *in vivo* in mice, but also produce negative clastogenic results in other chromosome aberration and micronucleus studies in mice and rats. However, in chagasic children, the incidence of micronucleated lymphocytes and chromosome aberrations both significantly increased approximately two fold after benznidazole treatment. The weight of evidence from the published studies suggests a genotoxic potential for benznidazole in humans.

Benznidazole has been reported to be carcinogenic in animals dramatically increasing the incidence of lymphomas in rabbits. These results and the genotoxicity results suggest a carcinogenicity potential for benznidazole, but the actual potential in humans has not been established.

[78] Sosa-Estani, Sergio, Rodolfo Viotti, and Elsa Leonor Segura. "Therapy, diagnosis and prognosis of chronic Chagas disease: insight gained in Argentina." *Memórias do Instituto Oswaldo Cruz* 104 (2009): 167-180.

Reproductive toxicity: A clinically relevant toxicity associated with benznidazole treatment was dose-dependent testicular atrophy and inhibition or arrest of spermatogenesis in testes and epididymides in rats. The toxicity was characterized by tubular atrophy, peritubular interstitial edema, and accumulation of syncytial/degenerate sperm in seminiferous tubules and epididymides. This finding is especially of interest because of the questions that remain about the reversibility of the testicular and fertility effects, the relevance of the finding to humans, and if relevant to humans, what effects might be expected in developing children. Perhaps tellingly, testicular and fertility adverse effects have not been reported in clinical studies with benznidazole in adults and children with Chagas disease. Benznidazole did not reduce the pregnancy rates in a female fertility study in rats, but findings associated with embryo patency did occur with significant decreases in live embryos associated with increased postimplantation loss in pregnant females administered the high dose of 150 mg/kg. This finding was consistent with similar findings in the rat embryo-fetal study with a high dose of 150 mg/kg/day and in the rat pre-postnatal study with a high dose of 75 mg/kg/day indicating benznidazole-related impairment of female reproduction at the level of embryo patency following implantation.

In a rat embryo-fetal study, a significant finding was increased total, external, visceral, and skeletal malformations in pregnant females treated with the high-dose of 150 mg/kg/day benznidazole compared to vehicle-control dams. Teratogenicity was not evident in the rabbit embryo-fetal study, perhaps due to the lower dose range (2.5 to 25 mg/kg/day) used for this study. In both rats and rabbits, pregnant females experienced pronounced weight reductions that were benznidazole dose-dependent indicating a consistent potential to produce maternal toxicity.

In the pre-postnatal study in rats, embryo implantation and patency were also affected in first generation dams born to mothers treated with benznidazole during gestation suggesting the potential for generational effects. Cesarean section findings in the F1 dams included: higher pre-implantation loss and reduced numbers of corpora lutea, implantations, and live embryos. Other developmental toxicity results in the pre-postnatal study included: reduced mean body weights for F1 males at PND 21, and reduced testicular size and impaired spermatogenesis and mating in 5% of the mid- and high-dose F1 males that were selected for mating. However, the testicular effects were not widespread and did not alter the mean fertility results or the mean results for testicular sperm counts in F1 males or mean motility and progression data for epididymal sperm in F1 males.

Neurotoxicity: Another serious nonclinical toxicity of benznidazole associated with chronic repeated dosing is severe neurotoxicity in dogs including seizure contractions, opisthotonos, and nystagmus proceeding to death. However similar findings were not observed in other test species, including in rabbits, guinea pigs, rats, or mice. Also, similar neurological toxicity has not been reported for humans clinically treated with benznidazole, with clinical neurological findings limited to a low incidence of peripheral neuropathy.

Hepatic, Renal and Endocrine toxicities: Other dose-dependent toxicity findings reported in the 26-week toxicology study in rats included reversible centrilobular hepatocellular hypertrophy,

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eosinophilic droplets and karyomegaly in kidney tubular epithelial cells, vacuolation in the pars distalis in the pituitary gland, increased incidence of extramedullary hematopoiesis and hemosiderin deposits in the spleen, and minimal follicular hypertrophy of the thyroid gland. It is not clear that these toxicities are of concern for clinical administration of benznidazole because related adverse events in humans have not been reported.

Pharmacological characteristics and potential for drug interactions: Benznidazole was shown to only minimally inhibit hERG potassium channels *in vitro* at a concentration higher than the expected plasma concentration associated with clinical dosing.

In pharmacokinetic studies, benznidazole did not accumulate with repeated dosing in rats, and distributed widely in tissues including placenta and fetuses in pregnant rats and testes in male rats. The plasma $t_{1/2}$ was approximately 1.5 hours in mice, 2-2.5 hours in rats, 4-5 hours in sheep, and 9-11 hours in dogs. Plasma protein binding in test species was approximately 50% and approximately 10% of the administered benznidazole dose was excreted in urine in rats. Benznidazole metabolized poorly in hepatic microsomes from test species and humans and there were no human specific metabolites. A primary metabolic pathway is nitroreduction via catalysis with benznidazole nitroreductases, and the reactive metabolites covalently bind to tissues often with correlating toxicity.

Pharmacological/Toxicological Reviewer's Conclusions: Overall, although most of the nonclinical toxicology data do not strongly support the safe use of the highest recommended clinical dose of benznidazole, the safe clinical use of benznidazole is supported by its extensive history of use in Latin America, and fairly extensive clinical safety data.

Questions remain regarding specific toxicities including toxicity to male reproductive organs, possible inhibition of male fertility and teratogenicity in pregnant females. Because of these questions, further clinical study is recommended postmarketing including a registry recording effects on male fertility and for female pregnancy outcomes.

4.5. Clinical Pharmacology

In this NME 505(b)(2) submission, the Applicant has provided publications and subject level data from previously completed placebo-controlled trials, analyses of pro- and retrospective observational studies, reviews of benznidazole use in the treatment of Chagas disease, and published case study reports. In these studies, various benznidazole formulations were evaluated including the Hoffman-LaRoche formulation: Radanil®. However, neither of the two to-be-marketed formulations (i.e., 100 mg and 12.5 mg tablets) was used in clinical trials from which the supportive evidence of effectiveness is based upon. To support an exposure bridge between the to-be-marketed formulations and Radanil formulation, the Applicant conducted two clinical pharmacology studies in healthy volunteers. The primary focus of the Clinical Pharmacology review is the evaluation of the provided exposure bridge between the to-be-marketed formulations and Radanil formulation. In addition, while there is exposure data available in pediatrics 2 – 12 years, the pediatric efficacy study included only pediatrics 6 – 12 years. Upon request of the Review team, the clinical pharmacology team utilized the available

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information to determine dosing for pediatrics 2 – 6 years. Given the limited information regarding the exposure-response efficacy and safety relationships of benznidazole treatment in Chagas disease, the aim of the additional analyses was to identify a benznidazole dosage regimen that is expected to provide matching BNZ systemic exposures to the exposures in pediatrics 6 –12 years. Based on the findings, the team recommends a weight-based dosing regimen for pediatric patients (2-12 years of age) (b) (4)

The Office of Clinical Pharmacology has reviewed the information provided by the Applicant in NDA 209570 and considers the information to be acceptable from a clinical pharmacology perspective.

The Clinical Pharmacology review team considered that this application is approvable from a clinical pharmacology perspective, provided that (1) an agreement is reached between the Applicant and the Agency on the Post-Marketing Requirements and Commitments listed in this review, (2) an agreement is reached on the dosing regimen, and 3) acceptable findings from the pending clinical site inspection report for Studies LPR1747-101 and LPR1747-102.

4.5.1. **Mechanism of Action**

The trypanocidal mechanism of action of benznidazole is not fully elucidated, however, it is known to depend on an activation step mediated by the action of a parasite type I nitroreductase (NTR). This enzyme is absent in mammalian cells, and is present in a subset of protozoan parasites, including trypanosomes. The role of this enzyme in drug activation has been genetically and biochemically described. It catalyzes the 2-electron reduction of nitroheterocyclic compounds within the parasite, producing toxic metabolites without significant generation of superoxide (Wilkinson 2011)[79]. Benznidazole acts through the formation of free radicals and electrophilic metabolites that are generated when its nitro group is reduced to an amino group by the action of nitroreductases (Maya et al[80]., 2007; Wilkinson et al.,2008[81]). In contrast to nifurtimox, which also generates O2 and H2O2 after the nitro group is reduced, BZ reduction does not produce reactive oxygen species (Docampo and Moreno, 1984[82]; Moreno et al., 1982[83]). It is hypothesized that the trypanocidal effect of BZ is

[79] Wilkinson S, Bot C, Kelly J and Hall B *: Trypanocidal Activity of Nitroaromatic Prodrugs: Current Treatments and Future Perspectives, Current Topics in Medicinal Chemistry, 2011, 11, 2072-2084*

[80] Maya, Juan Diego, et al*. "Mode of action of natural and synthetic drugs against Trypanosoma cruzi and their interaction with the mammalian host." Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 146.4 (2007): 601-620.

[81] Wilkinson, Shane R., et al. *"A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes." Proceedings of the National Academy of Sciences* 105.13 (2008): 5022-5027.

[82] Docampo, Roberto, and Silvia NJ Moreno*. "Free-radical intermediates in the antiparasitic action of drugs and phagocytic cells." Free radicals in biology* 6 (1984): 243-288.

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caused by covalent binding of its reduced metabolites to macromolecules of the parasite (Maya et al., 2004). Nevertheless, it is also reported that BZ induces oxidative stress within the parasite (Pedrosa et al., 2001[84]).

4.5.2. **Pharmacodynamics**

To date, no pharmacokinetic/pharmacodynamic relationship or target exposure values, other than keeping benznidazole trough concentrations within or above what is generally considered the *in vitro* trypanosomicidal range (3 to 6 mg/liter)[85]. In many cases, there is no correlation between the *in vitro* susceptibility of a given *T. cruzi* strain to benznidazole and the efficacy of antiparasitic treatment with these drugs in experimental animals or human patients. This lack of correlation is multifactorial, due to parasite differences and host factors, which may contribute to the known variability of the outcome of etiological treatment. Several investigators have described the appearance of peripheral neuropathy as when total benznidazole doses exceed 18 grams, i.e., in courses that last more than 30 days[86]. In adult studies in which treatment did not exceed 30 days, peripheral neuropathy has not been reported[87]. A relationship between plasmatic benznidazole concentrations and adverse drug reactions was not observed in a small series of 6 adult patients with Chagas disease in Argentina (Fernandez, 2016[88]) and also in another series of 54 cases of adult Chagas disease in Spain (Pinazo, 2013)[89].

4.5.3. **Pharmacokinetics**

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[83] Moreno, Silvia NJ, et al. *"Different behaviors of benznidazole as free radical generator with mammalian and Trypanosoma cruzi microsomal preparations." Archives of biochemistry and biophysics* 218.2 (1982): 585-591.

[84] Pedrosa, Rozangela C., et al. *"Time-dependent oxidative stress caused by benznidazole." Redox Report* 6.4 (2001): 265-270.

[85] Richle, R. W., and J. Raaflaub. *"Difference of effective antitrypanosomal dosages of benznidazole in mice and man. Chemotherapeutic and pharmacokinetic results." Acta tropica* 37.3 (1980): 257-261.

[86] CanÇado, J. Romeu. "Long term evaluation of etiological treatment of Chagas disease with benznidazole." *Revista do Instituto de Medicina Tropical de Sao Paulo* 44.1 (2002): 29-37.

[87] Viotti, Rodolfo, et al. "Side effects of benznidazole as treatment in chronic Chagas disease: fears and realities." *Expert review of anti-infective therapy* 7.2 (2009): 157-163.

[88] Fernández, Marisa Liliana, et al. *"Pharmacokinetic and pharmacodynamic responses in adult patients with Chagas disease treated with a new formulation of benznidazole." Memórias do Instituto Oswaldo Cruz* AHEAD (2016): 0-0.

[89] Pinazo, María-Jesús, et al. *"Benznidazole-related adverse drug reactions and their relationship to serum drug concentrations in patients with chronic Chagas disease." Antimicrobial agents and chemotherapy* 57.1 (2013): 390-395.

The Applicant has provided a cross-study comparison report that compared the BNZ exposure between clinical trial formulation Radanil® (100 mg benznidazole tablet, from Roche) and the to-be-marketed 100 mg benznidazole tablet manufactured by the Applicant. The Clinical Pharmacology review team has concluded that the data presented by the Applicant is adequate to support a bridge to the formulation from Roche, with which the studies selected by this reviewer to support safety and efficacy in children and adults were conducted. (b) (4)

(b) (4)

The following is a summary of the clinical pharmacokinetics of benznidazole. This information comes from studies with benznidazole (from CHEMO) or benznidazole manufactured by Roche (Radanil), for which the Applicant has demonstrated adequate comparability:

Absorption: Absolute bioavailability has not been determined. The observed mean maximum concentration (T_{max}) following a single dose of (b) (4) 100 mg tablet in healthy subjects ranged between 2 - 4 hours. No significant food effect was observed on the systemic exposure to benznidazole (from CHEMO) 100 mg tablet.

Distribution: benznidazole plasma protein binding is approximately 44 %. Body weight was identified as significant covariate on apparent volume of distribution (Vd/F). Studies in pregnant rats have shown that benznidazole is able to cross the placenta and bind to fetal tissues (DeToranzo, 1984)[90]. It also crosses the brain blood barrier, and it has been measured in normal brain and spinal fluid. For these properties, it has been studied in the treatment of brain tumors (Roberts, 1984)[91]. It is also found in breast milk. In one study with 12 lactating women (Garcia Bournissen, 2015)[92], treated with a median dose of 5.65 mg/kg/day twice daily, five mothers had adverse drug events (45%), but no adverse drug reactions or any untoward outcomes were observed in the breastfed infants. Median milk benznidazole concentration was 3.8 mg/L (range 0.3–5.9) in milk and 6.26 mg/L (range 0.3–12.6) in plasma. The median benznidazole milk to plasma ratio was 0.52 (range 0.3–2.79). The median

[90] De Toranzo, E. G., M. Masana, and J. A. Castro. "Administration of benznidazole, a chemotherapeutic agent against Chagas disease, to pregnant rats. Covalent binding of reactive metabolites to fetal and maternal proteins." *Archives internationales de pharmacodynamie et de therapie* 272.1 (1984): 17-23.

[91] Roberts, J. T., et al. "A phase I study of the combination of benznidazole and CCNU in man." *International Journal of Radiation Oncology* Biology* Physics* 10.9 (1984): 1745-1748.

[92] García-Bournissen, Facundo, et al. "Limited infant exposure to benznidazole through breast milk during maternal treatment for Chagas disease." *Archives of disease in childhood* 100.1 (2015): 90-94.

relative benznidazole dose received by the infant (assuming a daily breast milk intake of 150 mL/kg/day) was 12.3% of the maternal dose per kg (range 5.5%–17%).

Metabolism & Excretion: The mean elimination half-life of BNZ following a single dose in healthy subjects was about 12 - 13 hours (Raaflaub, 1978)[93]. Metabolic processes of benznidazole, such as its nitroreduction, are known to occur in the liver, by the cytochrome P450 reductase (Castro, 2006)[94]. The multiple dose kinetics of benznidazole (Radanil, Roche) was studied in 8 adult patients with Chagas disease over the course of a 4 week treatment with doses starting at 3 mg/kg/day and progressively increased to 5 and 7 mg/kg/day, in two daily doses with an interval of 12 hours, during the first week of treatment. Measurements were taken from the 10th to the 25th day of treatment. Five additional samples were taken on the last day of treatment, from 12 hours to 60 hours after the last dose, to determine the half-life of the individual patients. The average half-life was 13.75 hours in these patients, similar to that of healthy volunteers after a single dose. The highest individual maximum was 16.4 µg/mL and the lowest minimum was 5.4 µg/mL (Raaflaub, 1979)[95]. Another study was done with benznidazole (ABARAX, ELEA, Argentina) in 39 chronic adult Chagas disease patients (Soy, 2015)[96] treated with 2.5 mg/kg/every 12 hours for 60 days. In this study, an elimination half-life was reported to be as long as 36 hours. Data from simulations revealed that a dose of 2.5 mg/kg/12 h might lead to overexposure in most patients. A lower dose (2.5 mg/kg/24 h) was able to achieve trough BNZ plasma concentrations within the accepted therapeutic range of 3 to 6 mg/liter. Benznidazole is eliminated in the urine in unaltered form, in variable proportions.

Based on a pharmacokinetic study in children ages 2 to 12 (Altcheh, 2011)[97], body weight was identified as significant covariate on apparent clearance (CL/F). Lower body weight appears to be related to increased clearance. Results from the mass-balance/ADME (absorption, distribution, metabolism, elimination) study conducted by the Applicant are pending. The Applicant plans to submit this information by 4Q 2017 – 1Q 2018. This study will provide data on the pharmacokinetic profile of benznidazole.

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[93] Raaflaub, J., and W. H. Ziegler. "Single-dose pharmacokinetics of the trypanosomicide benznidazole in man." *Arzneimittel-Forschung* 29.10 (1978): 1611-1614.

[94] Castro, José A., María Montalto deMecca, and Laura C. Bartel. "Toxic side effects of drugs used to treat Chagas' disease (American trypanosomiasis)." *Human & experimental toxicology* 25.8 (2006): 471-479.

[95] Raaflaub, J. "Multiple-dose kinetics of the trypanosomicide benznidazole in man." *Arzneimittel-Forschung* 30.12 (1979): 2192-2194.

[96] Soy, D., et al. "Population pharmacokinetics of benznidazole in adult patients with Chagas disease." *Antimicrobial agents and chemotherapy* 59.6 (2015): 3342-3349.

[97] Altcheh, Jaime, et al. *"Adverse events after the use of benznidazole in infants and children with Chagas disease." Pediatrics* 127.1 (2011): e212-e218.

Medical Reviewer's comment: *Studies with Radanil® (Roche), for which the Applicant has demonstrated an adequate bridge, showed similar half-life in healthy adults and in chronic patients. Some studies suggest that in adults, a lower dose of 2.5 mg/kg/day may achieve similar plasma concentrations as those observed with the 5 mg/kg/day dose currently used in adults and children. There is variability of the pharmacokinetics of benznidazole reported in different populations, and it is multifactorial. This could be due to the patients' weights, the different formulations, and the methods used to measure them, and other unknown individual metabolic factors. Because there are geographical differences in the strains susceptibility, and it is usually not possible to isolate the parasite from patients (in particular from those in indeterminate and chronic stages), the clinical effectiveness of benznidazole cannot be reliably measured or estimated in vitro. In addition, there are no standardized methods to assess susceptibility of strains in vitro. For more details on susceptibility testing, please refer to the Clinical Microbiology review by Dr. Shukal Bala, Ph.D.*

The Clinical Pharmacology review team is awaiting more data from the ADME study being conducted by the Applicant to make conclusions and recommendations on the dosing schedule of benznidazole (b) (4).

4.6. Devices and Companion Diagnostic Issues

This section is not applicable to this review.

Consumer Study Reviews

This section is not applicable, since no patient self-selection or human factors studies were considered necessary for the evaluation of benznidazole efficacy or safety in the postmarketing setting.

5 Sources of Clinical Data and Review Strategy

5.1. Table of Clinical Studies

The review of safety and efficacy will be based on the two randomized controlled studies (Sosa Estani 1998 and De Andrade 1996) conducted in children ages 6 to 12 years, as previously communicated to the sponsor. These studies are based on serological endpoints, and the product used is the benznidazole from Roche, for which the sponsor has presented an adequate bioequivalence comparison. The safety review will also be based on these studies, and in the published data available, from other prospective controlled studies in pediatric and adult populations. Data were not pooled due to different study designs and outcome definitions. However, the safety data from the two randomized controlled studies, since they share several similarities, were pooled.

Because the two randomized controlled studies did not observe clinical outcomes in the follow-up period, additional supportive evidence will be reviewed to justify the interpretation of the serological endpoints used in the Sosa Estani and De Andrade studies to determine whether they are reasonably

likely to predict clinical benefit. The serological responses in these studies were measured with two kinds of serological assays: lytic antibodies (F-29 and AT-ELISA) and with conventional serology (ELISA, IFA, IHA). The primary endpoint in these two studies, the negative seroconversion at the end of the study follow-up, was met with measurements of lytic antibodies only. Evidence to demonstrate the significance of these lytic antibody responses as predictive of future negative seroconversion measured by conventional serological tests will be reviewed. In summary, lytic antibodies responses as a surrogate of another surrogate marker, the negative seroconversion by conventional serological assays (which is still the current goal standard of clinical cure), will be reviewed.

The supportive evidence for the negative seroconversion by conventional assays as a surrogate of clinical benefit comes primarily from 4 prospective, controlled, non-randomized trials of adult patients with long term follow-up (Gallerano 2000, Fabbro 2007, Viotti 1994, Viotti 2006), where clinical and serological outcomes have been observed using conventional serological assays (ELISA, IFA, IHA). Since these studies did not collect lytic antibody responses, which was the primary endpoint in the randomized controlled studies in children, additional supportive evidence from preclinical and clinical studies that show a correlation of responses between lytic antibodies and conventional serology, from the available literature (mainly from Fabbro 2013, Krettli 1978, 1982, Krettli and Brenner 1976 and 1982, among others) will be reviewed. The sponsor submitted datasets from one of the four studies (Viotti 2006), on February 24, 2017, which will be reviewed to assess the evidence regarding a correlation between serological endpoints and clinical outcomes, and the possibility of an extrapolation of serological endpoints to other patient populations. This study will also be reviewed to determine whether benznidazole treatment provided clinical benefit in this patient population.

The four additional studies submitted, two in adult populations (DNDi E-1224 and Molina 2014) and two in children (DNDi PEDBZ-01 and Altcheh 2014) had a follow-up of approximately 1 year and their primary endpoint was based on PCR measurements. Even though serological outcomes were also measured in some of them, the short time of follow-up did not allow for an observation of reversion of serology to negative in comparison to a control group in any of the patients. In addition, these 4 studies were conducted with benznidazole from a different manufacturer, LAFEPE, and a pharmacological bridge to that product is not currently available; therefore, they cannot support the indication. Table 4 summarizes the 6 studies submitted in support of the indication of treatment of Chagas disease. The Viotti 2006 study, submitted at a later time, includes patient-level data, and it will be reviewed in support of safety and efficacy, as mentioned above.

Table 4 - Clinical studies submitted by Applicant in support of indication of treatment of Chagas disease – Studies including patient level data to support efficacy and safety

	Sosa Estani 1998	De Andrade 1996	DNDi PEDBZ-01	DNDi-E1224	Molina 2014	Altcheh 2014
Study type	RCT DB PC	RCT DB PC	Open label PK	RCT included placebo arm	RCT Active arm - No	Open label PK

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			uncontrolled		placebo control	uncontrolled
Population	6-12 y.o.	7-12 y.o.	0-12 y.o.	18-45 y.o.	>18	2-12 y.o.
N (arms)	106 (55/51)	130 (65/64)	81	231 (45/46/48/45/47)	79 (26/26/26)	44
Dosing regimen	Benznidazole 5mg/kg/d for 60 days	Benznidazole 7.5 mg/kg/d for 60 days	Benznidazole 7.5 mg/kg/d for 60 days	Ravuconazole-3 dose levels-Benznidazole-Placebo	Benznidazole-Posaconazole HD and LD	Benznidazole 5-8mg/kg/d for 60 days
Endpoints	Conventional serology (3 assays) and F-29 ELISA, xenodiagnosis, EKG	Conventional serology (3 assays) and AT-ELISA, EKG	PK parameters, Conventional serology (2 assays) and PCR	Eradication of parasitemia evaluated with PCR	Eradication of parasitemia evaluated with PCR and serology	PK parameters, Conventional serology (2 assays) and PCR
Length of Follow-up	4 years	3 years	10 weeks	12 months	10 months	10 weeks

5.2. Review Strategy

Each individual study submitted, and those found from our independent literature search will be reviewed. Data were not pooled due to different study designs and outcome definitions.
Maria Allende, M.D., performed the safety review and, together with the Statistical team, performed the efficacy review. The Applicant initially submitted 6 key studies which include patient-level data as the basis to support the safety and efficacy of benznidazole in adults and children (please see table below). In addition to these, the Applicant submitted on February 24, 2017, additional patient-level data from one prospective and controlled study in adult patients with Chagas disease, the Viotti study, which includes clinical outcomes on treated and untreated patients.

Summary of study designs submitted by Applicant in support of treatment of Chagas indication – Studies including patient level data

	Sosa Estani 1998	De Andrade 1996	DNDi PEDBZ-01	DNDi-E1224	Molina 2014	Altcheh 2014
Study type	RCT DB PC	RCT DB PC	Open label PK uncontrolled	RCT included placebo arm	RCT Active arm - No placebo control	Open label PK uncontrolled
Population	6-12 y.o.	7-12 y.o.	0-12 y.o.	18-45 y.o.	>18	2-12 y.o.
N (arms)	106 (55/51)	130 (65/64)	81	231 (45/46/48/45/47)	79 (26/26/26)	38

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Dosing regimen	Benznidazole 5mg/kg/d for 60 days	Benznidazole 7.5 mg/kg/d for 60 days	Benznidazole 7.5 mg/kg/d for 60 days	Ravuconazole-3 dose levels-Benznidazole-Placebo	Benznidazole-Posaconazole HD and LD	Benznidazole 5-8mg/kg/d for 60 days
Endpoints	Conventional serology (3 assays) and F-29 ELISA, xenodiagnosis , EKG	Conventional serology (3 assays) and AT-ELISA, EKG	PK parameters, Conventional serology (2 assays) and PCR	Eradication of parasitemia evaluated with PCR	Eradication of parasitemia evaluated with PCR and serology	PK parameters, Conventional serology (2 assays) and PCR
Length of Follow-up	4 years	3 years	10 weeks	12 months	10 months	10 weeks

The review of safety will include all 4studies mentioned above (Sosa Estani, 1998; De Andrade 1996; Altcheh 2014; Viotti 2006), plus a review of the published with the Radanil (Roche) product. The review of the literature will also include reviewer's searches in addition to the Applicant's submitted materials. The sources for this search include the FAERS database and the WHO safety database accessible through Empirica Study.

The review of efficacy will be primarily focused on prospective, controlled studies with long term follow-up which have reported clinical outcomes. In children, these are shown in Table 2, and are the randomized controlled studies by Sosa Estani and the De Andrade. In adults, there are 4 controlled, prospective, non-randomized studies: Viotti 1994, Gallerano 2000, Viotti 2006 and Fabbro 2007. Other clinical studies will be reviewed to support of the interpretability of study endpoints used in the randomized controlled studies in children (Sosa Estani 2002, Fabbro 2013, TRAENA ASTMH presentation 2013).

6 Review of Relevant Individual Trials Used to Support Efficacy

6.1. Sosa Estani

6.1.1. Study Design

Overview and Objective

The Sosa Estani 1998 study was a randomized, placebo controlled and double-blind trial of benznidazole in children ages 6 to 12 years old. The purpose was to assess the safety and efficacy of benznidazole given orally at 5mg/kg/day in two divided doses for 60 days.

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Trial Design

This study was a double-blind, randomized, clinical field trial carried out in the Province of Salta in northwestern Argentina. The children lived in 14 localities in an area of approximately 200 km2. Sixty-six percent of the children lived in rural areas. The area has been under continuous triatomine surveillance by sanitary agents since 1982 (i.e., they undertook regular insecticidal actions against infestations). This was a condition for the selection of the study area. The investigators assumed that the children's infection with *T. cruzi* would be more than six years old. Subjects with at least two positive results for antibodies to *T. cruzi* by serologic tests, including EIA, IHA and IFA were included.

Children were excluded from the trial for any of the following reasons:

1) Presence of any chronic health condition (such as epilepsy, malnutrition, asthma, severe anemia);

2) Presence of any acute infectious disease;

3) Lack of consent from their parents to participate in the study; and

4) Unstable residence.

The children were matched in each locality by age to receive oral benznidazole, (Radanil®; Roche, Olivos, Argentina) or placebo. Fifty-five children were allocated to the benznidazole group and 51 children to the placebo group. Benznidazole (5 mg/kg/day) was administered to each child for 60 days. The pills were administered by parents, teachers, or nurses from the health services who were specifically trained for such a task.

Informed consent was obtained twice, before screening and before recruitment into the trial. The protocol was reviewed and approved by the Ethical Committee of the Instituto Nacional de Chagas Dr. Mario Fatala Chaben.

Study evaluations: Continuous medical assistance (anamnesis, physical examinations, and electrocardiograms [ECGs]) was provided during the trial. Laboratory tests (red blood cell and leukocyte counts, hematocrit, erythrocyte sedimentation rate, bilirubin, creatinine, aspartate aminotransferase, and alanine aminotransferase levels, and urine tests) were performed at days 21 and 60 after initiation of treatment to assess drug toxicity. Side effects were considered mild if the patients showed some adverse effects but were able to complete the treatment; moderate if treatment had to be suspended because of some side effect; and severe if it was suspended and the patients needed special treatment.

Serology: Repeated serologic tests were carried out immediately before and at 3, 6, 12, 18, 24 and 48 months after the onset of treatment; these included an enzymatic immunoassay (EIA), an indirect hemagglutination assay (IHA), and an indirect immunofluorescence assay (IFA). A new EIA (F29 EIA), using a T. cruzi flagellar calcium-binding protein (F29) as antigen, was used to assess parasitologic cure of patients. The clone encoding the whole protein was subcloned in the pMAL-p2 expression vector

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and expressed in Escherichia coli. Cutoff values to determine seropositivity were as follows:

IHA: ≥1/32 dilution represent positive

IFA: ≥1/32 dilution represent positive

Conventional ELISA: Cut-off OD >0.200

F29 ELISA: Cut-off OD≥0.170

Xenodiagnosis: All children were tested by xenodiagnoses using two boxes with 10 *Triatoma infestans* third or fourth instar nymphs each at the end of the follow-up (at 24 and 48 months visits only).

Study Endpoints

This double blinded, randomized pediatric field trial of benznidazole treatment used conventional serology (EIA, Conventional ELISA) and the F29-ELISA as primary endpoints. Two other conventional serology tests were performed at baseline and throughout the follow-up period. Electrocardiograms (ECG) were performed at baseline and throughout the follow-up period to determine incidence of ECG abnormalities in treated and non-treated patients.

Primary efficacy endpoint

The primary efficacy endpoint was the proportion of children seronegative against *T. cruzi* at the end of the 4 year follow-up period through enzymatic immunoassay (EIA/Conventional ELISA) against *T. cruzi.*

The secondary endpoints were:

- Proportion of children seronegative against *T. cruzi* at the end of follow-up period, which are assessed by flagellar calcium-binding protein (F29) (CL-EIA), an indirect hemagglutination assay (IHA), and an indirect immunofluorescence assay (IFA).
- Antibody titers assessed by serologic tests during follow-up period.
- Proportion of children seronegative against *T. cruzi* at the end of follow-up period through the xenodiagnosis assay.
- The safety endpoint was the incidence of AEs.

Statistical Analysis Plan

Analysis Populations

Intent-to-treat (ITT) population was defined as all randomized subjects who at least once had been exposed to the drug/placebo in the study.

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Per-protocol (PP) population was defined as all randomized subjects who had been exposed to the drug/placebo in the study and had at least one endpoint measurement at end of treatment or during follow-up visits.

Safety population was defined as all enrolled subjects who at least once had been exposed to the drug/placebo in the study.

Data handling

Summaries for continuous variables included the descriptive statistics for number of subjects (n), mean (arithmetic and geometric when appropriate), standard deviation (SD), minimum (min), median, and maximum (max). Summaries for categorical (discrete) variables included the descriptive statistics frequency and percentage.

Primary Endpoint Analysis

The Intent-to-treat (ITT) population will be used to analyze the primary endpoint, and the Per-protocol population will be used for the primary endpoint as a sensitivity analysis. All endpoints listed below will be presented. A p-value on the proportion difference will be calculated using a chi-square test if all expected counts are greater than 5; otherwise, Fisher's exact test will be used. The ITT population will be used for all secondary endpoints.

- Proportion of children seronegative against T. cruzi during follow-up period assessed by an enzyme immunoassay (EIA).
- Proportion of children seronegative against T. cruzi, at the end of 4 year follow-up period through enzymatic immunoassay against T. cruzi flagellar calcium-binding protein (CL- EIA), including timepoints baseline, and follow-up 6, 12, 24 and 48 months.
- Proportion of children seronegative against T. cruzi during follow-up period assessed by an indirect hemagglutination assay (IHA).
- Proportion of children seronegative against T. cruzi during follow-up period assessed by an indirect immunofluorescence assay (IFA)
- Proportion of children seronegative against T. cruzi at the end of follow-up period through the xenodiagnosis assay.

Protocol Amendments
There were no amendments to this study protocol.

Data Quality and Integrity: Sponsor's Assurance

The sponsor has stated that this trial was approved and monitored by an independent Institutional Review Board. Study participants were consented twice, first at screening and then at randomization.

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The sponsor also states that the trial was sponsored and audited twice by the TDR, WHO, however a copy of the corresponding audits certificates was not available at this time.
Laboratory standardization and quality assurance procedures in the document “Benznidazole NDA: serological and molecular testing of efficacy endpoints in the clinical studies”, submitted in module 5.3.5.1. Along with this document, the sponsor has submitted the Laboratory Manual- Diagnosis Of Parasitic Diseases- Instituto Nacional De Parasitologia "Dr. MarioFatala Chaben" [“Dr. Mario Fatala Chaben” National Parasitology Institute] (Original in Spanish and English translation provided).

6.1.2. Study Results

Compliance with Good Clinical Practices

The Applicant states that the study has complied in accordance with good clinical practice (GCP).

Financial Disclosure

The Applicant has adequately disclosed financial interests/arrangements according to 21 CFR Part 54.

Patient Disposition
A total of 106 patients were randomized, of which 55 were allocated to benznidazole treatment (5mg/kg/day for 60 days) and 51 to placebo. A total of 101 patients (92.5%) completed more than 30 days of therapy (51 in the placebo group and 50 in the benznidazole group) and had serological outcomes. A total of 103 participants completed the study follow-up (50 in the placebo and 53 in the benznidazole groups, respectively). A total of 5 of 55 patients (9.0%) did not complete 60 days of treatment due to treatment related adverse reactions in the benznidazole group (source: Table 14.3.1.1.2.1, Module 5). The patients who did not complete the study follow-up were 1 in the placebo group (2%) and 2 in the benznidazole group (3.6%). The reasons for discontinuation are not available. However, a review of these subjects suggests that adverse events could have been the reason. The disposition of all randomized subjects is shown in the table below.

Table 5: Disposition of all randomized patients - Sosa Estani

Treatment Discontinuation	Benznidazole (N = 55)	Placebo (N =51)
Completed treatment, n (%)		
Yes	50 (90.9)	51 (100)
No	5 (9.1)	0 (0.0)

Source: sponsor’s provided patient-level data, ADSL

Protocol Violations/Deviations

No protocol violations or deviations were reported.

Table of Demographic Characteristics

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This study was conducted in Argentina. Age and sex were provided, but not race or ethnicity. The race/ethnicity categories used in the US studies do not apply to this study population. Most of the population of Argentina is white.

Table 6: Demographic characteristics of the ITT population - Sosa Estani study

Patient characteristics[1]		Placebo	Benznidazole
N randomized		51	55
Age (in years)	Mean	10	9.7
	Median	10	10
	Maximum	14	14
Age Groups N(%)	Children ages 6 years to <12	39 (76.5%)	40 (72.7%)
	Adolescents, 12 years or older	12 (23.5%)	15 (27.3%)
Sex	Male	25 (49%)	25 (45.5%)
	Female	26 (51%)	30 (54.5%)
Body weight (kg)	Mean	31.6	32.3
	Std Dev	9.01	9.19
	Minimum	16	20
	Median	30	30.5
	Maximum	54	61

[1] Data on race and/or ethnicity were not collected. All patients were from Argentina.
Source: Extracted from Table 14.1.2.2, provided by the sponsor in Module 5.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

None of the participants were receiving other concomitant medications.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

The information available on treatment compliance is described above in Patient Disposition. No other details are available.

Efficacy Results – Primary Endpoint

The primary endpoint as stated in the statistical analysis plan was negative seroconversion at the end of the 4 year follow-up period using EIA/Conventional ELISA. The publication based on the study focused on the chemiluminescent enzymatic immunoassay (F29) in the evaluation of seroconversion at end of follow-up. In this review, we will consider the outcomes of all four serological tests and xenodiagnoses to interpret the therapeutic effect of benznidazole. Results from the efficacy endpoint measured with Chemiluminiscent Enzymatic Immunoassay F-29 and conventional ELISA assay are shown in the tables that follow (Table 9 through Table 14), at baseline and at follow-up visits. At

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baseline, a total of 13 (23.6%) and 12 (23.5%) subjects had negative results for the Chemiluminescent Enzymatic Immunoassay F-29 in the benznidazole and placebo arms, respectively. The subjects who were negative by this F-29 assay were proportional in both arms, and could possibly represent false negatives, because they were positive by the ELISA conventional assay. To better assess the effect of benznidazole on seroconversion and seroreduction, an mITT population was selected, consisting of all participants who were seropositive by all serological assays at baseline. The majority of the patients who were negative pre-treatment remained negative and all subjects with missing data continued to have missing data at the remaining time points. Therefore, our efficacy analysis focused on an mITT population that included patients who were positive at baseline. The primary endpoint was met in the mITT population at end of follow-up (Month 48) in responses measured with the Chemiluminescent Enzymatic Immunoassay F-29. There is a significant difference between treatment groups in the proportions of patients who were seronegative at months 12, 24, and 48 with more patients on benznidazole seroconverting compared to placebo. The treatment effect gets larger over time. A total of 24 out of 40 participants (60%) had negative responses at Month 48 compared with 5 out of 37 participants in the placebo group (13.5%), with a $p<0.01$, calculated using an exact method. The responses with conventional ELISA (or EIA), IHA and IFA did not reach statistical significance at Month 48, although a substantial decrease in optical densities and titers was observed in these responses, from Month 6 and later, reaching statistical significance in the comparison with the placebo group at Month 48. The treatment effect gets larger over time. The mean titer change from baseline and observed in 48 subjects in the benznidazole group was -0.129, [95% CI -0.512, 0.123], and in 49 participants in the placebo group was -0.031 [95% CI -0.226, 0.233]. The difference between treatment and placebo arm (benznidazole mean minus mean of placebo group) was 0.160 [95% CI-0.201, -0.119]. The standard deviation was 10 and 9 in benznidazole and placebo arms, respectively. The p value was <0.01. Even though negative seroconversion responses measured by IFA and IHA were not significantly different between benznidazole and treated patients, there were significant differences between treatment groups in the average change from baseline in serologic titer at months 6, 12, 24, and 48. Participants who achieved negative seroconversion as measured by at least 2 of the conventional serology assays (ELISA conv., IFA and IHA) were observed more frequently in the treated group (n=4) than in placebo (n=2). The table below shows these results. One patient in the treatment group achieved seroconversion in all three conventional serological tests. None in the placebo group achieved three negative conventional serological tests responses.

Table 7: Serological responses by conventional ELISA, IHA and IFA in treated and untreated patients at Month 48 - Sosa Estani

Benznidazole 5mg/kg for 60 Days	Patient ID	ELISA	IFA	IHA
	999-0103	0	0	1
	999-0702	0	0	1
	999-2108	0	0	1
	999-3038	1	1	1
	999-3453	1	1	0

	999-3885	1	0	1
	999-3891	1	1	1
Total N treated group		**4**	**3**	**6**
Placebo	999-1731	1	1	0
	999-4162	1	0	0
Total N untreated group		**2**	**1**	**0**

An analysis was done on the total number of negative seroconversions observed with any of the three conventional assays (ELISA, IFA and IHA) at Month 48 in treated and untreated subjects. Two subjects were excluded from benznidazole arm and one from the placebo arm for missing all assessments (baseline and post-baseline for all conventional serological tests). All other subjects were positive for conventional ELISA at baseline. Note that the percentages and n are slightly different from the individual assay and excluded from the individual assay analysis. Even if these negative seroconversions are not statistically significant, they follow a similar trend, and are numerically higher in treated patients as compared to untreated ones. The individual assays responses with corresponding percentages are also shown. There is an overall moderate concordance of responses with the three assays. For more details, please refer to Dr. Felicia Griffin's statistical review. These serological responses are shown in the Table 8, below.

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Table 8: Subjects with negative seroconversions observed by conventional serological assays at Month 48 - Sosa Estani study

Negative serology at 48 weeks	Benznidazole N = 53	Placebo N = 50	Fisher's Exact Test two-sided p-value
N= negative by one or more of the three assays	7 (13.2)	2 (4.0)	0.162
EIA	4 (7.5)	2 (4.0)	
IHA	6 (11.3)	0 (0.0)	
IFA	3 (5.7)	1 (2.0)	

Since the Chemiluminescent ELISA F-29 is a non-conventional assay that measures specific responses to trypomastigotes antigens, correlations between this assay and the three conventional serology assays, ELISA, IFA and IHA, were explored by the Statistical team. Among the four serologic assays, a moderate correlation is seen between EIA and F29 (r=.515; Pearson). Both IHA (r=0.385; Spearman) and IFA (r=0.282; Spearman) display a weak correlation with F29. Among the four serologic assays, a moderate correlation is seen between EIA and F29 (r=.515; Pearson). Both IHA (r=0.385; Spearman) and IFA (r=0.282; Spearman) display a weak correlation with F29. Xenodiagnosis was only measured at months 24 and 48 and not pre-treatment, so the analysis was only conducted using the ITT population. The percentage of patients classified as negative at the 4 year follow-up was numerically higher in the benznidazole group (36.4%) compared to the placebo group (17.7%). The observed difference in seronegative rate was borderline statistically significant (p-value 0.049). The source of data used to populate these tables presented below are the datasets containing patient-level data submitted by the sponsor and analyzed by the primary Statistical Reviewer, Dr. Felicia Griffin.

Table 9: Chemiluminescent Enzymatic Immunoassay F-29, Pre-treatment Values (ITT)

Visit	**Benznidazole (N=55)**	**Placebo (N=51)**	**P-value***
Pretreatment, n (%)			
Negative	13 (23.6)	12 (23.5)	1.000
Positive	40	37	
Missing	2	2	

*Patients with missing values were imputed as positive. The p-value for the difference in seronegative rate (Benznidazole treatment group minus placebo group) for the difference were calculated using an exact method.

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Table 10: Chemiluminescent Immunoassay F-29 - Serologic response at various time points (mITT population)

Visit*	Benznidazole (N=40)	Placebo (N=37)	P-value**
Month 6, n (%)			
Negative	8 (20.0)	3 (8.1)	0.196
Positive	23	29	
Missing	9	5	
Month 12, n (%)			
Negative	10 (25.0)	2 (5.41)	0.026
Positive	22	30	
Missing	8	5	
Month 24, n (%)			
Negative n, %	15 (37.5)	4 (10.8)	0.008
Positive n	17	29	
Missing n	8	4	
Month 48, n (%)			
Negative	24 (60.0)	5 (13.5)	<0.001
Positive	15	29	
Missing	1	4	

*Patients with missing values were imputed as positive** The p-value for the difference in seronegative rate (Benznidazole treatment group minus placebo group) for the difference were calculated using an exact method.

Table 11: Enzymatic Immunoassay (ELISA) Pre-treatment (ITT)

Visit	Benznidazole (N=55)	Placebo (N=51)	P-value*
Pretreatment, n (%)			
Negative	0 (0.0)	0 (0.0)	1.000
Positive	53	50	
Missing	2	1	

***The p-value for the difference in seronegative rates (Benznidazole treatment group minus placebo group) was calculated using an exact method**

Table 12: Enzymatic Immunoassay (ELISA) in follow-up visits (ITT)

Visit*	Benznidazole (N=53)	Placebo (N=50)	P-value**
Month 3, n (%)			
Negative	5 (9.4)	0 (0.0)	0.057
Positive	44	44	
Missing	4	6	

Month 6, n (%)			
Negative	4 (7.6)	0 (0.0)	0.118
Positive	44	39	
Missing	5	11	
Month 12, n (%)			
Negative	5 (9.4)	1 (2.0)	0.206
Positive	47	46	
Missing	1	3	
Month 18, n (%)			
Negative	5 (9.4)	1 (2.0)	0.206
Positive	45	47	
Missing	3	2	
Month 24, n (%)			
Negative	7 (13.2)	1 (2.0)	0.061
Positive	43	48	
Missing	3	1	
Month 48, n (%)			
Negative	4 (7.5)	2 (4.0)	0.68
Positive	44	42	
Missing	5	6	

*Patients with missing values were imputed as positive** The p-value for the difference in seronegative rate (Benznidazole treatment group minus placebo group) for the difference were calculated using an exact method.

Table 13: Pre-Treatment Serologic Titers with Enzymatic Immunoassay (ELISA)

Visit	Titer	Benznidazole (N=55)	Placebo (N=51)	Difference* (95%CI)**	P-value**
Pre-treatment	Mean (SD) Range Missing	0.465 (0.10) [0.226, 0.690] 2	0.474 (0.09) [0.298, 0.723] 1	-0.009 (-0.047, 0.029)	0.635

*Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded.

Table 14: Serologic Titers with Enzymatic Immunoassay (ELISA) in follow-up visits

Visit	Titer	Benznidazole (N=53)	Placebo (N=50)	Difference* (95%CI)**	P-value**
Baseline	Mean (SD) Range Missing	0.465 (.10) [0.226, 0.690] 0	0.474 (0.10) [0.298, 0.723] 0	-0.009 (-0.047, 0.029)	0.636
Change from Baseline					

Month 3	Mean (SD) Range Missing	-0.074 (.10) [-0.432, 0.045] 4	0.013 (.06) [-0.108, 0.173] 6	-0.0874 (-0.122, -0.053)	<.0001
Month 6	Mean (SD) Range Missing	-0.101 (0.09) [-0.044, 0.046] 5	0.004 [-0.084, 0.159] 11	-0.105 (-0.138, -0.072)	<.0001
Month 12	Mean (SD) Range Missing	-0.103 (0.09) [-0.453, 0.114] 1	0.006 (.05) [-0.130, 0.150] 3	-0.109 (-0.139, -0.080)	<.0001
Month 18	Mean (SD) Range Missing	-0.113 (.10) [-0.491, 0.092] 3	-0.012 [-0.184, 0.079] 2	-0.101 (-0.135, -0.068)	<.0001
Month 24	Mean (SD) Range Missing	-0.143 (.09) [-0.459, 0.090] 3	0.004 (.05) [-0.134, 0.145] 1	-0.147 (-0.176, -0.118)	<.0001
Month 48	Mean (SD) Range Missing	-0.129 (.10) [-0.512, 0.123] 5	-0.031 (0.09) [-0.226, 0.233] 6	-0.160 (-0.201, -0.119)	<.0001

*Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded

Data Quality and Integrity – Reviewers' Assessment

This study was conducted at local schools and the records are not available on site. Some records were available and kept by the Sponsor. A full review of source data was not possible, given the time lapse from the study conduction. Please refer to the OSI review for more details.

Efficacy Results – Secondary and other relevant endpoints

Relevant secondary endpoints were the responses to other conventional assays (IFA and IHA). The rates of conversion to negative were not statistically significant in benznidazole treated subjects as compared with placebo recipients, although they were numerically higher in benznidazole treated subjects. These responses are shown in the tables below: Table 16, Table 17 and Table 18 show conversions to negative and titers changes from baseline with IFA. Table 19, Table 20, Table 21, and Table 22 show responses and titers changes from baseline with IHA. With IFA and IHA, just like it was observed with Chemiluminescent ELISA and conventional ELISA described previously, there were significant differences between treatment groups in the average change from baseline in serologic titer at all time points. In addition, xenodiagnoses results from samples taken at Month 24 and at

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Month 48 are shown in Table 23. No baseline results are available, since these measurements were only done at Month 24 and at Month 48. There was good concordance between the results of the positive serological tests and positive findings by xenodiagnosis at Month 24 and at Month 48. For more details on this concordance with serological results, please see Table 14 in the review of Dr. Shukal Bala, Microbiology reviewer.

In the ITT analysis, 20 out of 55 and 9 out of 51 subjects in the treatment and placebo groups, respectively, had negative xenodiagnoses at Month 48. The percentage of patients classified as negative at the 4 year follow-up was numerically higher in the benznidazole group (36.4%) compared to the placebo group (17.7%). The observed difference in seronegative rate was borderline statistically significant (p-value 0.049).

Table 15: Pre-Treatment serological values with Immunofluorescence Assay (IFA) (ITT)

Visit*	Benznidazole (N=55)	Placebo (N=51)	P-value**
Pretreatment, n (%)			
Negative	0 (0.0)	2 (3.92)	0.2291
Positive	53	48	
Missing	2	1	

*Patients with missing values were imputed as positive ** The p-value for the difference in seronegative rates (Benznidazole treatment group minus placebo group) was calculated using an exact method

Table 16: Serological responses with Immunofluorescence Assay (IFA) at follow-up visits

Visit*	Benznidazole (N=53)	Placebo (N=48)	P-value**
Month 3, n (%)			
Negative	5 (9.4)	0	0.057
Positive	44	43	
Missing	4	5	
Month 6, n (%)			
Negative	3 (5.67)	1 (2.01)	0.619
Positive	45	36	
Missing	5	11	
Month 12, n (%)			
Negative	7 (13.2)	0 (0.0)	0.132
Positive	45	45	
Missing	1	3	
Month 18, n (%)			
Negative	6 (11.3)	1	0.115
Positive	44	45	
Missing	3	2	

Month 24, n (%)			
Negative Positive Missing	9 (17.00) 40 3	3 (6.3) 44 1	0.128
Month 48, n (%)			
Negative Positive Missing	3 (5.66) 45 5	0 (0.0) 42 6	0.244

*Patients with missing values were imputed as positive** The p-value for the difference in seronegative rate (Benznidazole treatment group minus placebo group) for the difference were calculated using an exact method

Table 17: Pre-treatment titers with Immunofluorescence (IFA) (ITT)

Visit	Titer	Benznidazole (N=55)	Placebo (N=51)	Difference* (95%CI)	P-value**
Pre-treatment	Mean (SD) Range Missing	4.85 (0.79) [3.47, 6.24] 2	4.7 (0.84) [2.77, 6.24] 1	0.15 (-0.17, 0.47)	0.3630

*Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded. The values are presented in log-transform.

Table 18: Titers during follow-up with Immunofluorescence (IFA) (mITT)

Visit	Titer	Benznidazole (N=53)	Placebo (N=48)	Difference* (95%CI)**	P-value**
Pre-treatment	Mean (SD) Range Missing	4.85 (0.79) [3.47, 6.24] 0	4.78 (0.76) [3.5, 6.24] 0	0.07 (-0.24, 0.38)	0.5000
Change from Baseline					
Month 3	Mean (SD) Range Missing	-.59 (0.75) [-3.47, 0.69] 4	-0.26 (0.76) [-1.39, 1.39] 5	-0.34 (-0.65, -0.02)	0.1362
Month 6	Mean (SD) Range Missing	-0.78 (1.08) [-4.85, 1.39] 5	-0.24 (1.28) [-2.08, 2.07] 11	-0.54 (-1.05, -0.03)	0.0361
Month 12	Mean (SD) Range Missing	-0.84 (0.82) [-3.47, 0.69] 1	-0.28 (0.87) [-1.39, 2.08] 3	-0.56 (-.90, -0.22)	0.0395
Month 18	Mean (SD) Range Missing	-0.89 (1.11) [-4.16, 1.39] 3	-0.21 (0.92) [-2.78, 2.08] 2	-0.68 (-1.09, -0.26)	0.1087
Month 24	Mean (SD) Range Missing	-1.19 (1.37) [-6.23, 0.69] 3	-0.25 (0.92) [-2.78, 2.07] 1	-0.94 (-1.41 -0.47)	0.0010

Month 48	Mean (SD) Range Missing	-0.61 (1.49) [-6.24, 2.08] 5	0.31 (0.91) [-1.39, 2.08] 6	-0.92 (-1.45, -0.39)	<.0001

*Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded. The values are presented in log-transform. P-value determined from a model of results at various time points with baseline and treatment as covariates

Table 19: Pre-Treatment Serological Responses with Indirect Hemagglutination Assay (IHA) (ITT)

Visit*	Benznidazole (N=55)	Placebo (N=51)	P-value**
Pretreatment, n (%)			
Negative	1 (1.82)	0 (0.0)	1.000
Positive	52	50	
Missing	2	1	

*Patients with missing values were imputed as positive. ** The p-value for the difference in seronegative rates (Benznidazole treatment group minus placebo group) was calculated using an exact method.

Table 20: Serological Responses with Indirect Hemagglutination Assay (IHA) in follow-up visits

Visit*	Benznidazole (N=52)	Placebo (N=50)	P-value**
Month 3, n (%)			
Negative	5 (9.6)	0 (0.0)	0.057
Positive	43	44	
Missing	4	6	
Month 6, n (%)			
Negative	4 (7.7)	0 (0.0)	0.118
Positive	43	39	
Missing	5	11	
Month 12, n (%)			
Negative	5 (9.6)	0 (0.0)	0.057
Positive	46	47	
Missing	1	3	
Month 18, n (%)			
Negative	6 (11.5)	0 (0.0)	0.015
Positive	43	48	
Missing	3	2	
Month 24, n (%)			
Negative	4 (7.7)	0 (0.0)	0.118
Positive	45	48	
Missing	3	1	
Month 48, n (%)			
Negative	5 (9.6)	0 (0.0)	0.057
Positive	42	44	
Missing	5	6	

*Patients with missing values were imputed as positive** The p-value for the difference in seronegative rate (Benznidazole treatment group minus placebo group) for the difference were calculated using an exact method.

Table 21: Pre-Treatment titers using Indirect Hemagglutination (IHA) (ITT)

Visit	Titer	Benznidazole (N=55)	Placebo (N=51)	Difference* (95%CI)**	P-value**
Pre-treatment	Mean (SD) Range Missing	5.59 (1.21) [0, 6.93] 2	5.59 (0.82) [3.47, 6.93] 1	-0.002 (-0.40, 0.40)	0.4224

***Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded. The values are presented in log-transform**

Table 22: Serological titers during follow-up using Indirect Hemagglutination (IHA) (mITT)

Visit	Titer	Benznidazole (N=52)	Placebo (N=50)	Difference* (95%CI)	P-value**
Baseline	Mean (SD) Range Missing	5.69 (0.93) [3.47, 6.93] 0	5.59 (0.82) [3.47, 6.93] 0	0.11 (-0.24, 0.45)	0.3488
Change from Baseline					
Month 3	Mean (SD) Range Missing	-0.64 (1.41) [-6.24, 0.69] 4	0.13 (0.62) [-1.39, 2.08] 6	-0.78 (-1.23, -0.32)	0.0115
Month 6	Mean (SD) Range Missing	-0.80 (1.39) [-6.24, 0.69] 5	-0.04 (0.75) [-1.39, 2.08] 11	-0.77 (-1.25, -0.27)	0.0256
Month 12	Mean (SD) Range Missing	-0.87 (1.23) [-6.24, 0.69] 1	0.04 (0.67) [-2.77, 1.39] 3	-0.91 (-1.34, -0.49)	<.0001
Month 18	Mean (SD) Range Missing	-1.10 (1.46) [-6.24, 0.69] 3	0.00 (0.62) [-1.39, 1.39] 2	-1.10 (-1.56, -0.65)	<.0001
Month 24	Mean (SD) Range Missing	-0.92 (1.10) [-4.85, 0.69] 3	-0.18 (0.81) [-2.08, 1.39] 1	-0.74 (-1.12, -0.35)	0.0079
Month 48	Mean (SD) Range Missing	-1.50 (1.18) [-4.85, 0.69] 5	-0.42 (0.97) [-3.47, 1.39] 6	-1.08 (-1.53 -0.63)	<.0001

*Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded. The values are presented in log-transform. P-value determined from a model of results at various time points with baseline and treatment as covariates

Table 23: Xenodiagnosis outcomes at Month 24 and Month 48 (ITT)

Visit*	Benznidazole (N=55)	Placebo (N=51)	P-value**
Month 24			
Negative	23 (41.8%)	13 (25.5%)	0.101
Positive	27	36	
Missing	5	2	
Month 48			
Negative	20 (36.4%)	9 (17.7%)	0.049
Positive	28	35	
Missing	7	7	

*Patients with missing values were imputed as positive. ** The p-values for the differences in negative rates (Benznidazole treatment group minus placebo group) were calculated using an exact method.

Dose/Dose Response

Only one dose was evaluated in this trial.

Persistence of Responses over Time

Results from 46 benznidazole recipients who were all positive at baseline and also at Month 48 are available from a publication of a follow-up study (Sosa Estani 2002)[98]. Persistence of responses was evaluated at a 9 years (Month 108) post-randomization evaluation, using F-29 ELISA, and AT-ELISA (ELISA to detect lytic antibodies, also used in the De Andrade study) and conventional serological assays (IFA, conventional ELISA and IHA). Negative serological responses were observed in 76.9% of the participants at 9 years follow-up. From the 46 seropositive subjects, 17 of them were 5 to 9 years old and 29 of them were 10 to 14 years old. Reversion of serology to negative as measured by both F-29 ELISA and AT ELISA and IFA using conventional serology (IFA and conventional ELISA), was observed in 9 (31%) of the 29 subjects ages 10 to 14 years, and in 2 (11.8%) of 17 subjects ages 5 to 9 years. The total number of seroconversions was 11 of 46 treated subjects (23.9%) at 9 years. Xenodiagnosis was performed in 44 of the 46 participants and was positive in 4.3% of them. None of the seronegative subjects had positive xenodiagnosis tests.

Persistence of Effect

The effect is measured by serological endpoints only. The study follow-up of the treated and untreated patients up to 9 years showed persistent seronegative responses and an increase in the

[98] Sosa-Estani, Sergio, et al. "Evolución serológica a largo plazo en ninos infectados por Trypanosoma cruzi que cursan fase clınica indeterminada, tratados con Benznidazol." *Proceedings of the 2do Simposio Internacional de Enfermedad de Chagas en Internet*. Federación Argentina de Cardiología, 2002.

Reference ID: 4134724

proportion of participants with negative seroconversion as measured by at least two conventional serological assays, a total of 11/46 (23.91%) participants, had negative seroconversion observed at 9 years[99]. The length of the follow-up needed to observe clinical outcomes could be as long as 25 years.

Additional Analyses Conducted on the Individual Trial

Please refer to the Statistical Review by Dr. Felicia Griffin for additional details.

6.2. De Andrade

6.2.1. Study Design

Overview and Objective

This was a randomized, double-blind, placebo-controlled trial to evaluate the safety and efficacy of oral benznidazole administered at 7.5 mg/kg/day for 60 days in children ages 7 to 12 from a rural area of Brazil with endemic Chagas' disease.

Trial Design

Between 1991 and 1995, a total of 2434 school children aged 7 to 12 years attending 60 village schools in the study area in Central Brazil were screened for antibodies to *T. cruzi* by indirect immunofluorescence (IFA), indirect hemagglutination (IHA) and ELISA. The criteria for a child to be classified as seropositive and included in the trial were a reciprocal titer on indirect immunofluorescence of 40 or more, an ELISA index of 1·2 or more, and a reciprocal titer on indirect hemagglutination of 16 or more. An additional nonconventional assay, a chemiluminescent enzyme-linked immunosorbent assay with purified trypomastigote mucin antigen (A&T CL-ELISA, called AT-ELISA for simplification), designed to detect specific purified trypanosomal antigens from cell-cultured trypomastigotes of the Y strain was also used. Serum samples were diluted 1 in 2000 and the titers were expressed as the ratio of the luminometer reading to the cut-off value (defined as ten times the negative control mean minus the background control mean for each plate). Positive serum samples gave ratios greater than 1.0. The various serological tests were performed in a blinded fashion regarding treatment allocation and other results at the WHO Reference Laboratory for Chagas disease serology, Federal University of Goiás, Brazil. The AT-ELISA was done at Federal University of Sao Paolo, Brazil.

The subjects with positive results in all three tests were 130, and were randomized in blocks of six children within each school, after stratification for sex and age, to receive benznidazole (N=64) at 7.5

[99] Sosa-Estani, Sergio, et al. "Evolución serológica a largo plazo en ninos infectados por Trypanosoma cruzi que cursan fase clınica indeterminada, tratados con Benznidazol." *Proceedings of the 2do Simposio Internacional de Enfermedad de Chagas en Internet*. Federación Argentina de Cardiología, 2002.

Reference ID: 4134724

mg/kg/day (Roche, Brazil) divided in two daily doses for 60 days or placebo (N=65) which had the same composition as the drug except for the active compound. One subject moved out of the area just after randomization and was not included in the analyses. Treatment was administered by the school teacher at the beginning and at the end of the school day. During the weekends the children were given the exact number of doses needed for self-administration. Treatment compliance was assessed by counting the remaining pills on the next school day. Medical supervision was established through the treatment period to ensure compliance and monitoring of side effects. Clinical and laboratory safety evaluations (12-lead electrocardiograms (ECG) at rest, blood samples for leucocyte counting, and measurement of packed-cell volume, serum aspartate and alanine aminotransferases, urea nitrogen, and creatinine) were performed at baseline and at days 7, 14 and 45 of treatment. Serological evaluations were performed on blood samples drawn on the last day of treatment (day 60), and 3, 6, 12 and 36 months after completion of treatment.

All samples were tested simultaneously by the four serological tests at the end of the trial to avoid observer and process variation. The primary endpoint was the disappearance of specific antibodies (negative seroconversion) by the end of a 3-year follow-up. The secondary endpoint was the reduction of antibody titers (three fold or greater) through repeated serological tests. Insecticidal measures were taken throughout the trial to reduce the risk of infection. All dwellings in the study communities were sprayed with deltamethrin by the Chagas' Disease Control Program, before the trial and every 6 months. A sample of seronegative children in the community (N=175) was tested every 6 months for seroconversion. No incident case was detected during trial follow-up.

Clinical examination and laboratory tests were done for all 130 potential trial participants at baseline. No child was excluded from the trial because of abnormal results.

The primary objective of this study was:

- To evaluate the proportion of children with negative seroconversion of specific antibodies to *T cruzi* assessed by Enzyme immunoassay (Conventional ELISA) between treatment groups.

The secondary objectives of this study were:

- To evaluate the reduction of antibody titers on serological test by EIA, indirect immunofluorescence (IFA), indirect hemagglutination (IHA) and AT- ELISA.

- To evaluate the proportion of children with negative seroconversion of specific antibodies to *T cruzi* assessed by indirect immunofluorescence (IFA), indirect hemagglutination (IHA) and AT-ELISA between treatment groups

- To evaluate the rate of adverse events (AE).

Study Endpoints

Primary Endpoint (according to the Statistical Analysis Plan submitted by the Applicant):

- The primary efficacy end point was the proportion of children with negative seroconversion, test result less or equal to 1.0, assessed by ELISA on year 3.

In the study publication, the primary endpoint was the absence of specific antibodies at the end of 3 years. Secondary endpoint was three-fold or greater seroreduction. As mentioned earlier, we will consider responses to all four serological assays and xenodiagnosis in our review to assess benznidazole's therapeutic effect.

Secondary Endpoints:

- Titers of specific antibodies to *T cruzi* assessed by AT-ELISA, indirect hemagglutination (IHA), indirect immunofluorescence (IFA) and EIA on year 3.

- The proportion of children with negative seroconversion: test result less than 40 assessed by indirect immunofluorescence (IFA), or test results less than 16 assessed indirect hemagglutination (IHA), or test results less than 1.2 assessed by CLEIA on year 3.

- The safety endpoints will be incidence of anemia, leucopenia and skin lesion.

Statistical Analysis Plan

Sample size

The sample size of 129 subjects was estimated to detect a 15-20 % negative seroconversion rate in the benznidazole group, at 5% significance with 90% power.

Analysis Populations

Safety population: all enrolled subjects who received at least one dose of study drug (benznidazole or placebo).

Intent-to-Treat population: all enrolled subjects who were randomized and receive at least one dose of study drug (benznidazole or placebo).

Per-Protocol Population: all enrolled subjects who were randomized and received at least one dose of study drug (benznidazole or placebo) and completed study assessments.

Efficacy and Safety Analyses

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The intent-to-treat (ITT) population was used to analyze the primary endpoint, and the per-protocol population was used for the primary endpoint as a sensitivity analysis. ITT population will be used for all secondary endpoints.

Safety evaluations were based on the incidence of adverse events (AEs) and subjects' clinical laboratory results. Quantitative results (actual values and change from baseline) in clinical laboratory parameters will be summarized for each visit by treatment.

Missing Data

Subjects with missing data at month 36 are considered to not have negative seroconversion.

Protocol Amendments

No amendments were done to this original protocol.

Data Quality and Integrity: Sponsor's Assurance

According to the publication (De Andrade et al., 1996) and confirmed by the Principal Investigator to the Sponsor, the study protocol was ethically and technically reviewed and approved by the Regional Medical Council in accordance with WHO guidelines for biomedical research:

Conselho Regional de Medicina do Estado de Goiás

R. T. 28, 245 - St. Bueno, Goiânia - GO, 74210-040, Brasil

6.2.2. Study Results

Compliance with Good Clinical Practices

According to the publication and confirmed by the Sponsor, the study was conducted in compliance with Good Clinical Practices.

Financial Disclosure

The Sponsor has provided completed form 3454 assuring that it has not any disclosable financial arrangements and interests in relation with this and the other mentioned clinical studies. These forms were submitted in Module 1.3.4

Patient Disposition

A total of 6 patients in the treated group and 11 in the untreated group did not complete the study follow-up. In the study publication, the reasons for study discontinuation are mentioned in one table.

Subjects lost to follow-up were 4 in the benznidazole arm and 7 in the placebo arm. One subject withdrew from the study due to an adverse event; all other discontinuations in both groups are mentioned as caused by various reasons (moved away, mixed tablets, refused blood draws, treatment discontinuation).

Table 24: Disposition of all Randomized Subjects - De Andrade Study

Treatment Discontinuation	Benznidazole (N = 64)	Placebo (N =65)
Completed treatment, n (%)		
Yes	58 (90.6)	54 (83.1)
No	6 (9.4)	11 (16.9)

Protocol Violations/Deviations

No protocol violations were reported.

Table of Demographic Characteristics

Baseline characteristics were balanced between treated and untreated groups, as shown below.

Table 25: Demographics Characteristics - De Andrade study

Demographic characteristic	Benznidazole (N = 64)	Placebo (N = 65)
Gender, n (%)		
Male	38 (59.4)	38 (58.5)
Female	26 (40.6)	27 (41.5)
Age group (years), n (%)		
7 to 9	24 (37.5)	22 (33.9)
10 to 12	40 (62.5)	43 (66.1)
Baseline ECG, n (%)		
Normal	58 (90.6)	58 (89.2)
Abnormal	6 (9.4)	7 (10.7)

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

No other characteristics are available. ECGs at baseline were done. Participants in the Benznidazole group: 2 complete right bundle branch; 1 ectopic rhythm; 1 first-degree atrioventricular block. Placebo group: 7 patients had complete right bundle branch block.

Concomitant medications were not reported in the article or the Applicant patient-level data submitted. In the article, additional baseline characteristics regarding hematological laboratory values and liver enzymes were reported, and appeared comparable among treated and untreated patients. The table from the article is presented below (Table 22). The individual laboratory data was not available in the Applicant's submission, therefore the data presented and summarized in the publication is shown. Treated and untreated groups had comparable baseline values in laboratory tests and baseline ECG characteristics.

Table 26: Additional baseline characteristics of De Andrade study subjects presented in the publication

	Benznidazole (n=64)	**Placebo (n=65)**
Male/female	38/26	38/27
Age group		
7-9 years	24	22
10-12 years	40	43
ECG Abnormalities*	6	7
Mean (SD; range) hematological measures		
Packed-cell volume (%)	36.6 (2.7; 30-42)	37.1 (2.7; 32-48)
Leucocyte count ($x10^9$/L)	8.71 (4.97; 3.80-39.4)	7.86 (2.98; 3.20-17.0)
Neutrophil count ($x10^9$/L)	3.48 (1.95; 0.43-12.2)	3.00 (1.77; 0.96-10.8)
Mean (SD; range) liver function test results		
Serum aspartate aminotransferase (U/L)	19.1 (7.9; 8-38)	19.9 (6.1; 6-38)
Serum alanine aminotransferase (U/L)	15.9 (7.0; 4-37)	16.7 (6.4; 4-30)

*Benznidazole group: 2 complete right bundle branch block; 1 left anterior hemiblock; 1 second-degree atrioventricular block; 1 ectopic rhythm; 1 first-degree atrioventricular block. Placebo group: 7 complete right bundle branch block.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

No information is available.

Efficacy Results - Primary Endpoint

Based on the data submitted in the NDA, 35 patients (54.7%) in the benznidazole group became seronegative with the AT ELISA vs. 3 patients (4.6%) in the placebo group. The results provided below are based on this reviewer's analyses of each serologic endpoint using the data submitted. The efficacy endpoint assessed with AT-ELISA (Chemiluminescent enzymatic immunoassay) was met in the ITT population. Using the data submitted, the percentages of patients who were seronegative at the 3 year follow-up were significantly different between the two treatments. In addition, there was a significant difference between treatment groups at month 36 for the average change from baseline in serologic titers ($p < .0001$). The percentages of patients who were seronegative measured by ELISA conventional assay at the 3 year follow-up were not significantly different between the two treatments with 6.3% in the benznidazole group and 0 in the placebo group. However, there was a

significant difference between treatment groups in the average change from baseline in serologic titer at the 36 month follow-up. Similarly, negative seroconversion responses measured by IFA and IHA are not significantly different in treated and untreated participants, however, there was a significant decrease in serological titers measured by all four serological assays (non-conventional and conventional) throughout the follow-up period and at month 36. A correlation of the responses observed with the four assays was explored by the Statistical team. Both Pearson and Spearman methods were used to evaluate the linear or monotonic relationship between the two variables. Among the four serologic assays, a moderate correlation is seen between ELISA and AT-ELISA (r=0.51; Pearson). Both IHA and IFA display a weak correlation with AT-ELISA. All 4 patients in the benznidazole group that were classified as seronegative at the 3 year follow-up using conventional ELISA were also classified as seronegative using the non-conventional ELISA (AT-ELISA). In addition, of the 3 patients in the benznidazole group who were classified as seronegative at the 3 year follow-up using IFA, 2 were also classified as negative using AT-ELISA. Further, of the 9 patients in the benznidazole group who were classified as seronegative at the 3 year follow-up using IHA, 7 were also classified as negative using AT-ELISA. An analysis was done to assess the number of subjects with negative seroconversions measured by conventional serological assays. A total of 14 subjects had at least one negative seroconversion at Month 36 as measured by any of the three types of assays and no subject in the placebo group had negative seroconversions at the end of follow-up. Even though this analysis identified only 2 subjects with at least two negative seroconversions measured by conventional assays, the trend towards higher number of negative seroconversions measured by at least one conventional assays in the treatment group was significant, which, together with titers reduction suggest a trend towards serological cure, which in the length of follow-up of this study was not possible to observe. This is shown in the table below (Table 23)

Table 27: Negative seroconversions measured by chemiluminescent assay (AT-ELISA) and three conventional serological assays at Month 36 - De Andrade study

Analysis Visit	Assay type	Benznidazole n (%)	Placebo n (%)
Follow-up at Month 36	Chemiluminescent Enzymatic Immunoassay (AT-ELISA)	35 (54.69%)	3 (4.62%)
	Enzyme Immunoassay (ELISA)	4 (6.25%)	0 (0.00%)
	Indirect Hemagglutination Assay (IHA)	9 (14.06%)	0 (0.0%)
	Indirect Immunofluorescence Assay (IFA)	3 (4.69%)	0 (0.0%)
Total N with negative seroconversion at Month 36	**Any one of the three conventional assays (ELISA, IFA, IHA)**	**14 (21.9%)**	**0 (0.00%)***

*Fisher's Exact Test two-sided p-value <0.0001

The tables below show the baseline values and the evolution of serological responses during the study. Table 24 shows the results from the efficacy analysis of serological outcomes at baseline and Month 36 using the AT-ELISA in the ITT population. Using the data submitted, the percentages of patients who were seronegative at the 3 year follow-up were significantly different between the two treatments with 54.7% in the benznidazole group and 4.6% in the placebo group. The average change from baseline in serologic titer at month 36 follow up using AT-ELISA is displayed below in Figure 5, and using conventional ELISA, in Figure 6. The mean and standard deviation of the titers changes from baseline in benznidazole treated and controls at Month 36 are shown in Table 25. There is a significant difference between treatment groups at month 36 for the average titers change from baseline in serologic titers, showing significant titers reductions in benznidazole treated subjects (p <.0001). Negative seroconversions at Month 36 measured by ELISA are shown in Table 26. There were 4 (6.25%) benznidazole treated subjects and 0 (0%) in the control group who presented negative seroconversion at Month 36. This was borderline significant (p=0.0577).

Negative seroconversion responses measured at Month 36 with the other two conventional assays, IFA and IHA, are shown in Table 28, through Table 31.

The negative seroconversions and titers reductions at Month 36 is presented in these tables for IFA and IHA. Negative seroconversions measured by IFA were observed in 3 (4.9%) of benznidazole treated subjects and in 0 of the placebo controls. This did not reach statistical significance, p=0.1192 (Table 28), but statistically significant IFA titers reductions were observed in benznidazole treated subjects as compared to controls (Table 29). Responses measured by IHA, however, showed negative seroconversion in 9 (14.7%) benznidazole treated subjects and in 0 control subjects. This was statistically significant (p=0.0013) (shown in Table 30), as well as the titers reductions measured with IHA, which were statistically significant in benznidazole recipients vs controls (p=0.0001) (Table 31).

As a summary of conventional assays responses we can conclude that all conventional assays responses showed consistent titer reductions at Month 36 in benznidazole treated patients, all of which were statistically significant (p=<0.0001). Negative seroconversions were borderline significant (p=0.0577) with ELISA conventional assay and statistically significant (p=0.0013) measured with IHA responses at Month 36.

Table 28: Chemiluminescent Enzymatic Immunoassay (AT-ELISA) (ITT) - De Andrade

Visit*	Benznidazole (N=64)	Placebo (N=65)	P-value**
Pre-Treatment, n (%)			
Negative	0 (0.0)	0 (0.0)	
Positive	64	65	
Month 36, n (%)			
Negative	35 (54.7)	3 (4.6)	<.0001
Positive	29	62	
Missing	6	11	

Reference ID: 4134724

*Patients with missing values were imputed as positive **The p values for the difference in seronegative rates (Benznidazole treatment group minus Placebo group) was calculated using an exact method

Figure 5: Chemiluminescent Enzymatic Immunoassay (AT-ELISA) at Baseline and Month 36 - De Andrade study

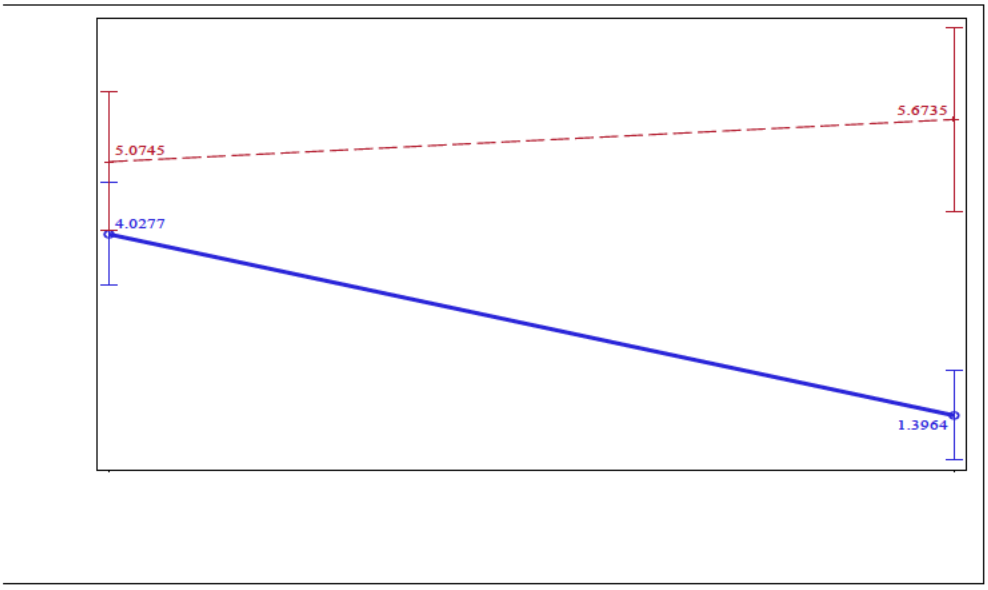
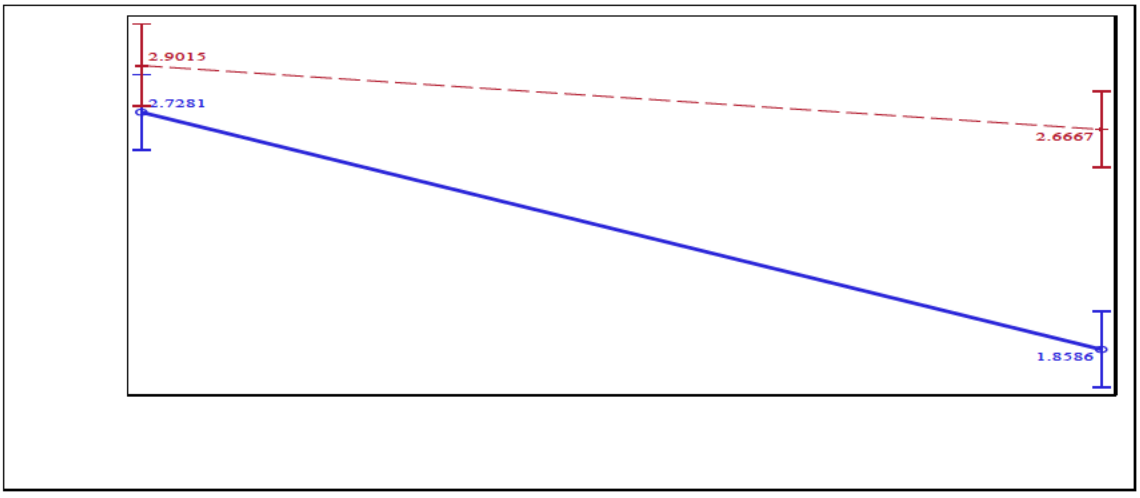


Figure 6: Serological Titers with Enzymatic Immunoassay (ELISA or EIA) at Baseline and Month 36 – De Andrade study



Data Quality and Integrity - Reviewers' Assessment

Limited source data was available to allow for a full evaluation. For more details, please refer to the OSI review.

Efficacy Results – Secondary and other relevant endpoints

Table 29: Chemiluminescent Assay (AT-ELISA) Titers Post-Treatment - De Andrade

Visit	Titer	Benznidazole (N=64)	Placebo (N=65)	Difference* (95%CI)**	P-value**
Baseline	Mean (SD) Range Missing	4.028 (2.95) [1.001, 18. 109]	5.075 (4.04) [1.005, 22.363]	-1.047 (-2.281, 0.188)	0.0959
Change from Baseline					
Month 36	Mean (SD) Range Missing	-2.524 (2.15) [-11.842, 1.763] 6	0.5371 (4.52) [-20.019, 13.894] 11	-3.061 (-4.374, -1.749)	<.0001

*Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded

Table 30: Serological Responses with Enzymatic Immunoassay (ELISA) at Month 36 - De Andrade

Visit*	Benznidazole (N=64)	Placebo (N=65)	P-value**
Pre-Treatment, n (%)			
Negative Positive	0 (0.0) 64	0 (0.0) 65	
Month 36, n (%)			
Negative Positive Missing	4 (6.25) 54 6	0 (0.0) 54 11	0.0577

*Patients with missing values were imputed as positive. ** The p-value for the difference in seronegative rates (Benznidazole treatment group minus placebo group) was calculated using an exact method.

Table 31: Serological Titers Reductions with Enzymatic Immunoassay (ELISA) at Month 36 – De Andrade study

Visit	Titer	Benznidazole (N=64)	Placebo (N=65)	Difference* (95%CI)**	P-value**
Baseline	Mean (SD) Range	2.728 (0.56) [1.200, 4.000]	2.901 (0.61) [1.600, 4.300]	-0.173 (-0.377, 0.030)	0.0945
Change from Baseline					
Month 36	Mean (SD) Range Missing	-0.870 (0.539) [-2.500, 0.600] 6	-0.198 (0.06) [-1.300, 0.800] 11	-0.673 (-0.861, -0.484)	<.0001

*Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded.

Table 32: Serological Responses with Immunofluorescence Assay (IFA) at Month 36 – De Andrade study

Visit*	Benznidazole (N=64)	Placebo (N=65)	P-value**
Pre-Treatment, n (%)			
Negative Positive	0 (0.0) 64	0 (0.0) 65	
Month 36, n (%)			
Negative Positive Missing	3 (4.29) 55 6	0 (0.0) 54 11	0.1192

*Patients with missing values were imputed as positive. ** The p-value for the difference in seronegative rates (Benznidazole treatment group minus placebo group) was calculated using an exact method.

Table 33: Serological Titers Reduction with Indirect Immunofluorescence (IFA) at Month 36 – De Andrade study

Visit	Titer	Benznidazole (N=64)	Placebo (N=65)	Difference* (95%CI)	P-value**
Pre-Treatment	Mean (SD) Range	7.38 (0.75) [5.76, 8.54]	7.40 (0.72) [5.77, 8.54]	-0.18 (-0.27, 0.24)	0.8442
Change from Baseline					
Month 36	Mean (SD) Range Missing	-2.08 (1.06) [-4.9, 0] 6	-0.44 (1.07) [-3.47, 2.07] 11	-1.64 (-2.04, -1.24)	<.0001

*Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded
The values are presented in log-transform.

Table 34: Serological Responses with Immunohemagglutination Assay (IHA) at Month 36 – De Andrade study

Visit*	Benznidazole (N=64)	Placebo (N=65)	P-value**
Pre-Treatment, n (%)			
Negative	0 (0.0)	0 (0.0)	
Positive	64	65	
Month 36, n (%)			
Negative	9 (14.7)	0 (0.0)	0.0013
Positive	49	54	
Missing	6	11	

*Patients with missing values were imputed as positive. ** The p-value for the difference in seronegative rates (Benznidazole treatment group minus placebo group) was calculated using an exact method.

Table 35: Serological Titers Reduction at 36 Months by IHA – De Andrade study

Visit	Titer	Benznidazole (N=64)	Placebo (N=65)	Difference* (95%CI)	P-value**
Baseline	Mean (SD)	5.30 (1.32)	5.60 (1.26)	-0.29	0.7545
	Range	[2.77, 9.01]	[2.77, 9.01]	(-0.74, 0.16)	
Change from Baseline					
Month 36	Mean (SD)	-1.43 (1.19)	-0.15 (1.01)	-1.28	<.0001
	Range	[-5.54, 1.39]	[-2.77, 2.08]	(-1.69, -0.87)	
	Missing	6	11		

*Difference = difference in titers (Benznidazole treatment group minus placebo group) **Patients with missing values were excluded. The values are presented in log-transform

Dose/Dose Response

All subject received the same dose regimen. A dose response was not evaluated for more than one dose.

Persistence of Response over Time

The durability of response was assessed at a 6 year follow-up in another study (De Andrade 2004). In this study, 53 (82.8%) of 64 participants allocated in the treatment group were evaluated and 46 (70.8%) of 65 from the placebo arm completed the follow-up. A total of 33 of the 37 children who had received benznidazole chemotherapy and were seronegative after three years follow-up had remained seronegative after six years, whereas 14 of 21 children shifted from seropositive to seronegative, resulting in a total of 47 cases with negative seroconversion and 6 with seropositive response at 6 years follow-up.

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In the placebo arm, of 52 individuals who were seropositive after three years of follow-up, 32 remained positive after six years, whereas 11 showed seroconversion.

The median A&T CL-ELISA titer for the benznidazole-treated group was 0.151 (negative result), while it was strongly positive (1.997) for the placebo group after the six-year follow-up.

The efficacy of benznidazole was calculated as 100 × (1 - RR), where RR is the ratio of children with negative seroconversion in the benznidazole group to the corresponding number of children in the placebo group. The per-protocol efficacy of benznidazole was 84.7% (95% CI 66.8–92.9) and in the ITT analysis, it was 64.7% (95% CI 50.2–78.7).

Consistency between the decrease of antibody titers and significantly lower *T. cruzi* parasitemia measured by a polymerase chain reaction in the benznidazole group (39.6%) compared with the placebo group (64.2%) was demonstrated three years after treatment in another study done with this same patient population from the De Andrade study (Galvao 2003[100]).

Persistence of Effect

No incident case of ECG abnormality was found in this extended evaluation, although early development of cardiomyopathy (complete right bundle branch block) was detected in five children (four in the placebo group) after three years of treatment. In the 6 year follow-up described in the publication (mentioned above) no additional ECG abnormalities were observed.

Additional Analyses Conducted on the Individual Trial

An additional analysis was performed on the frequency of participants who presented negative seroconversion measured by unconventional and conventional serological assays (ELISA, IFA and IHA) at the end of follow-up (36 months), presented in the previous section. Even though there were only two subjects who were seronegative by both IHA and IFA at Month 36, the number of subjects with a negative seroconversion response in at least one of the three conventional serological assays was 14 in the treated group as compared to 0 in the placebo group. This difference was statistically significant with a Fisher's exact test two-sided p-value of <0.0001, and it shows a trend towards seronegativization, which is present only in the treated group and not in the controls.

No subject had three negative conventional serological tests during the 36 Month follow-up. No negative seroconversions measured by conventional serological assays were observed in the placebo group.

[100] Galvão, Lúcia MC, et al. "PCR assay for monitoring Trypanosoma cruzi parasitemia in childhood after specific chemotherapy." *Journal of clinical microbiology* 41.11 (2003): 5066-5070.

Reference ID: 4134724

At baseline, 6 participants had ECG changes in the treated group and 7 in the untreated group. New ECG changes were observed in 4 untreated subjects and in 1 treated subject by the end of the study follow-up period. No details are provided about the type of changes. A trend towards more frequent changes in the untreated group was observed. These were described in the article as characteristic of Chagas disease (RBBB, HAB, first-degree block). It is likely that they represent changes characteristic of Chagas disease, however, without the actual description the clinical significance of these is uncertain.

6.3. Viotti

This was a prospective, controlled, non-randomized study of 566 adult subjects, ages 30 to 50 years, mean 39 years, diagnosed with Chagas disease with 3 positive results on three different types of conventional serologic tests, without evidence of heart failure, who were assigned to benznidazole treatment administered orally at 5mg/kg/day in two daily doses for 30 days or no treatment. Patients were assigned by using an alternating sequence wherein every other individual enrolled (for example, patients 1, 3, and 5) was assigned to treatment and the alternate individuals (for ex- ample, patients 2, 4, and 6) were assigned to the control group.

The study was done at one single center, the hospital Eva Peron in Buenos Aires, Argentina. The serological tests were performed at the national reference center, Instituto Nacional de Parasitologia Dr. Mario Fatala Chaben. Subjects were followed up clinically for a median of 9.8 years.

6.3.1. Study Design

This study was conducted at the hospital Eva Peron, Buenos Aires, Argentina, between 1984 and 2001. The design, conduct, analysis, and submission of the paper for publication were the sole responsibility of the authors. The study did not have an industry or government sponsor.

Patients were screened during their first hospital visit to determine their eligibility for inclusion in the study. During this period, investigators obtained the patients' medical history, performed a physical examination and clinical laboratory tests, including baseline serologic testing, electrocardiography, chest radiography and echocardiography. A diagnosis of *T. cruzi* infection was made by serologic testing, including complement fixation, indirect hemagglutination, immunofluorescence, or enzyme-linked immuno- sorbent assay, performed at the reference center, Instituto Nacional de Parasitologıa Dr. Mario Fatala Chaben.

Patients were stratified as follows according to the clinical classification of Kuschnir and colleagues (Kuschnir, 1985[101]): group 0, positive results of serologic testing, normal results on electrocardiography and chest radiography and no cardiac enlargement; group I, positive results on

[101] Kuschnir, E., et al. "Evaluation of cardiac function by radioisotopic angiography, in patients with chronic Chagas cardiopathy." *Arquivos brasileiros de cardiologia* 45.4 (1985): 249.

Reference ID: 4134724

serologic testing, abnormal results on electrocardiography, normal results on chest radiography, and no cardiac enlargement; group II, positive results on serologic testing, abnormal results of electrocardiography and chest radiography with cardiac enlargement but no clinical signs of heart failure; group III, positive results of serologic testing, abnormal results on electrocardiography and chest radiography with cardiac enlargement, and clinical signs of heart failure. The serological criterion is included in the Kuschnir category for stratification at baseline.

Kuschnir added this criterion because serological diagnosis enables the early identification of the chagasic patient and the study of the initial forms of cardiopathy before the onset of cardiomegaly and/or cardiac insufficiency. These changes are rare in the other forms of cardiomyopathy in which these early stages of cardiopathy would go unnoticed (this is the explanation given by Kuschnir in his 1985 publication).

The changes in Kuschnir category group during follow-up were based solely on the clinical findings to determine the changes to classify patients only into a more severe group.

The serological changes were described as the secondary outcome (persistence of 3 positive results on serologic evaluation or complete seroconversion) in the last follow-up visit for patients in each group during the long term follow-up. Negative seroconversion was not used to re-classify a patient into a less severe Kuschnir group. The re-classifications of patients into Kuschnir categories were only from a less severe to a more severe group, and only based on clinical events.

Thirty- to 50-year-old patients with 3 positive results on serologic tests for *T. cruzi* infection and no clinical signs of heart failure (Kuschnir groups 0, I, or II) at admission were considered for inclusion in the study. Patients older than 50 years of age were excluded to avoid misinterpretation of electrocardiographic changes; patients younger than 30 years of age were excluded because patients in this age group rarely presented to this center. A total of 772 patients did not fulfill the age inclusion criteria and thus were excluded from the study. Sixty-nine patients with overt heart failure (Kuschnir group III, considered to have irreversible end-stage disease), 81 patients with a history of previous treatment for *T. cruzi* infection (complete, incomplete, or unknown), and 113 patients with only two positive results on serologic tests were excluded from the study. A total of 297 patients with concomitant disorders, such as chronic obstructive pulmonary disease, hypothyroidism and hyperthyroidism, cancer, valvular heart disease, arterial hypertension, congenital heart disease, coronary artery disease, alcoholism, diabetes mellitus, morbid obesity, or other severe systemic diseases, were also excluded. The recruitment period ended in 2001.

Treatment intervention

Eligible patients were assigned to receive either oral, self-administered benznidazole twice per day, at a maximum dosage of 5 mg/kg of body weight per day for 30 consecutive days, or no treatment. Patients were assigned by using an alternating sequence wherein every other individual enrolled (for example, patients 1, 3, and 5) was assigned to treatment and the alternate individuals (for ex- ample, patients 2, 4, and 6) were assigned to the control group. The investigators also included 34 patients

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from a previous study (17 patients assigned to receive treatment and 17 patients assigned to remain untreated) who met the inclusion and exclusion criteria of the current trial.

A pathologist who was not involved in the clinical evaluation assigned patients to treatment and control groups. If a patient withdrew from the study or declined to participate, the same physician maintained the 1:1 ratio by assigning the next eligible patient to the respective group. Physical examination was performed by three participating cardiologists who were not aware of their patients' assignments.

Data Collection and Follow-up

The clinical status of enrolled patients was evaluated by serial electrocardiography, chest radiography, and serologic testing for *T. cruzi* infection. Follow-up visits and results of electrocardiography were recorded every 6, 4, and 3 months in patients in Kuschnir groups 0, I, and II, respectively.

Complete left and right bundle-branch block, left anterior fascicular block, sinus bradycardia less than 50 beats/min, electric inactivation areas, types 2 and 3 atrioventricular block, sustained supraventricular arrhythmias, non-sustained and sustained ventricular tachycardia, and pacemaker implantation were all considered electrocardiographic abnormalities related to Chagas heart disease whereas other abnormalities were considered nonspecific. Chest radiographs were obtained annually for all patients to evaluate heart size and signs of heart failure. A cardiothoracic ratio greater than 0.50 indicated cardiac enlargement. Bidimensional echocardiography was performed according to the guidelines from the American Society of Echocardiography on all patients with cardiac enlargement on chest radiographs. A left ventricular end- diastolic diameter of 57 mm or greater was considered to be left ventricle dilation. The 57-mm limit was determined from the values obtained from healthy adults at the echocardiographic department at our hospital.

Two cardiologists who had access to patient information but were blinded to treatment assignment and to the results of the interim analysis reviewed the electrocardiogram tracings, chest radiographs, and echocardiograms; disagreements were resolved by discussion. Standard criteria were used for electrocardiographic diagnosis of Chagas disease. The cardiothoracic ratio was obtained by using the relation between the transverse diameter of the heart and the transverse diameter of the thorax at the level of the right diaphragmatic cupula. Three serologic tests for *T. cruzi* infection, enzyme-linked immunosorbent assay, indirect hemagglutination, and immunofluorescence tests were used for follow-up, which was done at 3-year intervals.

Patients were informed about possible side effects of benznidazole therapy and were advised to consult the study physician immediately if a symptom occurred. Information on adverse events was obtained on days 10 and 30 after treatment initiation. No data regarding adverse events were collected from control patients. Patients who discontinued treatment because of side effects were included in the intention-to-treat analysis. Follow-up stopped in December 2004.

Outcomes evaluated

The primary outcome was a change from a lower to a more advanced Kuschnir group or cardiac death (change of clinical group). The investigators also examined factors associated with this outcome. Secondary outcomes were the appearance of new abnormalities on electrocardiography, persistence of three positive results on serologic evaluation, or complete negative seroconversion on the last serologic test done for each patient.

Statistical Analysis Plan (as submitted by the Applicant on 02-24-2017)

Sample size: The sample size was calculated on the basis of a previous study (Viotti et al, 1994) with 8 years of follow-up, in which untreated patients had a rate of 17% for change of clinical group. An expected reduction of 40% was established for benznidazole-treated patients.

For an α value of 0.05 and 1-β value of 0.9, the sample size was calculated to be 319 patients per group.

Data Handling: Missing data were not be imputed in listings.

Analysis Populations: Outcome analyses were be performed using the intention-to-treat (ITT) population. ITT Population: all subjects who were assigned to treated and untreated groups.

Outcome analysis: The primary and secondary outcomes were analyzed using Cox proportional hazard model with outcome variable(s) as dependent variable and treatment as independent variable. The hazard ratios with Wald 95% confidence intervals (CIs) for treatment (benznidazole versus no treatment) were displayed. Time of follow-up for study subjects was considered as the time of event or censor.

Subgroup analyses were performed for the primary outcome of change of clinical group. The subgroups will include the followings:

- Gender: Male vs. Female
- Clinical group at admission: 0, I, II
- Electrocardiographic findings at baseline: Normal vs. Abnormal (Including all abnormal findings regardless of related to Chagas disease
- New electrocardiographic abnormalities: Yes vs. No
- Persistence of positive serologic results: Yes, No, Unknown

Multivariate Cox proportional hazards regression analyses were also be used to calculate the hazard ratios with Wald 95% confidence intervals (CIs) for treatment with BZN versus no treatment, adjusted

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for age, sex, clinical group at admission, left ventricular ejection fraction, and left ventricular diastolic diameter (change of clinical group; new electrocardiographic abnormalities) and for age, sex, and clinical group at admission (serologic evaluation including 3 positive results at the end of study and complete seronegative conversion at the end of study). Death was adjusted for left ventricular ejection fraction.

The investigators tested assumptions of the proportional hazards by using log negative log estimated survivor function plot If the log-log survival curves for BZN versus no treatment were not crossed

(parallel), they claimed the proportional hazard assumption holds. Kaplan-Meier curves of cumulative of percentage of patients for each outcome by treatment were provided.

A worst-case sensitivity analysis was applied to address differential withdrawal between treated and untreated groups. The investigators assumed that no patients in the untreated lost to follow-up group and 20% to 40% of patients in the treated lost to follow-up group changed clinical group. Cox proportional hazards regression analyses for the change of clinical group were used to calculate the hazard ratios with Wald 95% CIs for treatment with BZN versus no treatment. Similarly logistic regression for each outcome was performed.

Demographic and baseline characteristics were summarized for all ITT subjects. Tables and listings were provided for all patient-level data.

The above descriptions of the methodology were submitted by the Applicant, and are described in the article. For this review, the analyses done by the Statistical team and by this reviewer are presented and discussed below.

6.3.2. Study Results

The analyses presented are from the patient-level datasets provided by the Applicant.

Compliance with Good Clinical Practices

According to the publication (Viotti et al, 2006) and confirmed by the sponsor with the Lead Principal Investigator, the protocol was reviewed and approved by the Local Institutional Review Committee at the Hospital Eva Peron in Buenos Aires, Argentina.

Financial Disclosures

All materials, equipment, and drugs were provided by Ministerio de Salud, Provincia de Buenos Aires, Argentina (Ministry of Health, Province of Buenos Aires, Argentina) at the local public hospital. No funds or any other type of support was received from any industry sponsor.

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Patient Disposition

A total of 598 patients were assigned to the treated or untreated groups (294 in the benznidazole group and 304 in the untreated group); however, there were 32 patients who withdrew from the study before being administered the treatment or control (11 in the benznidazole group and 21 in the untreated group). Therefore, a total of 283 patients were in the benznidazole group and 283 patients were in the untreated group. The follow-up time was fairly balanced between the two groups with a median number of years of follow-up of 10 years for both groups. Balance seen between these arms helps with the interpretation of the study as there are concerns regarding the study not being randomized. The sample size was calculated based on a previous study with 8 years of follow-up. It was calculated to be 319 patients per group. Interim analyses were conducted every 5 years (a total of two interim analyses were done according to the publication). The plan was to stop the study when significant results were obtained or the planned sample size was reached. No adjustments for multiple testing were planned or conducted.

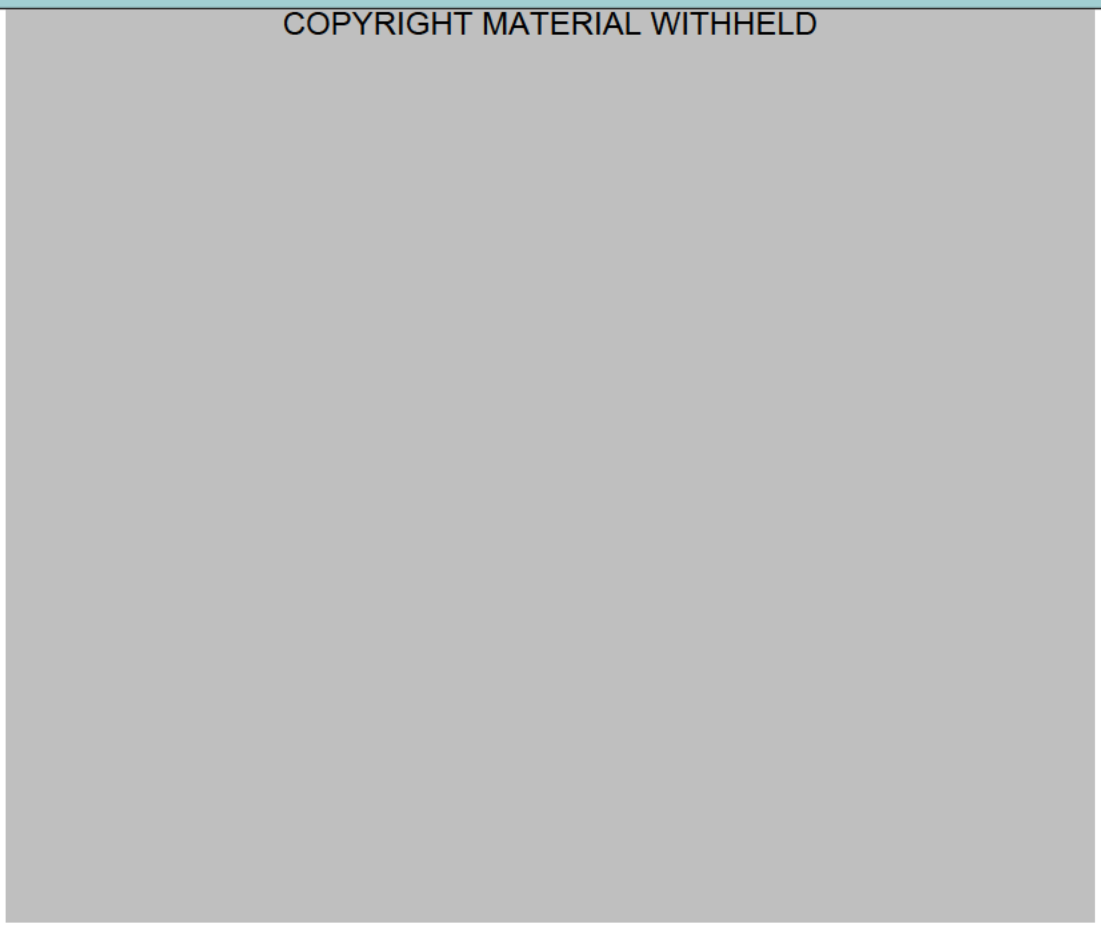
The article states that approximately 20% of patients (54 of 283 in the treated group and 57 of 283 in the untreated group) from both groups were lost to follow-up during the study.

The mean years of follow-up were similar in treated and untreated patients: mean of 11.2 years (SD6.5) and 10.2 years (SD 5.8), in treated and untreated groups, respectively, with similar IQR of 5.8 and 5.4, respectively.

However, it is not clear why some patients were considered lost to follow-up while others were not. In our Statistical team's analyses, the follow-up times of those lost to follow-up were 0.1 to 19.91 years. The follow-up times of those not considered lost to follow-up were 0.43 to 26.11. In the patients who were considered not lost to follow-up the reason for discontinuation is unclear.

The article states that treated patients who were lost to follow-up were more likely to be male. Untreated patients who were lost to follow-up were younger and were more likely to be in Kuschnir group 0 at admission than untreated patients who were not lost to follow-up. The flow diagram from the publication is presented below.

Figure 1. Flow diagram of the screening process, reasons for nonenrollment, and follow-up.



Protocol Violations/Deviations

No protocol violations were reported for this study in the publication or in the sponsor's submitted data.

Table of Demographic Characteristics

The baseline characteristics were balanced in both treated and untreated patients. Most patients were born in rural endemic areas: 246 of 283 (86.9%) in the treated group and 244 of 283 of the untreated group (86.2%), and treated and untreated patients had lived in an endemic area for a mean of 7.32 and 8.13 years, respectively. No information is available on the mode of infection in the study population. Having lived in an endemic area, it is presumed to be of vectorial origin, however, other modes of infection, such as congenital or blood or tissue products, cannot be ruled out since no additional information is available from these patients. The majority of the patients in the treated and untreated groups (63.6% in each group) had normal ECG findings and were asymptomatic for heart disease (42.8% and 45.2%, of treated and untreated patients, respectively) and were younger than 45 years of age at enrollment (mean age of 39.3 and 39.4, in treated and untreated groups, with a

standard deviation of 5.3 and 5.8, respectively). Other characteristics, such as vital signs, ECG findings, echocardiographic findings and medication on admission, were balanced between groups and are presented in detail in the table from the publication, copied below.

Table 36: Baseline Characteristics of All Patients - Viotti 2006 study

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Treatment Compliance

No details were provided on treatment compliance. It is unclear whether how this was evaluated during the study.

Efficacy Results – Primary Endpoint

The comparison of follow-up data vs clinical category at admission (Kuschnir groups

0, I, and II) showed that treated patients were significantly less likely than untreated patients to change clinical group. The percentage of patients who changed from a lower to a more advanced Kuschnir group or cardiac death was lower in the benznidazole group (4%, 12/283) compared to the untreated group (14%, 41/283) (difference of -10% with 95% confidence interval [-15.2, -5.3], $p<0.0001$). The clinical group changed in 6 of 180 treated patients (3.3%) and 13 of 180 untreated patients (7.2%) in Kuschnir group 0, 3 of 73 treated patients (4.1%) and 14 of 75 untreated patients (18.7%) in Kuschnir group I, and 3 of 30 treated patients (10%) and 13 of 28 untreated patients (46.4%) in Kuschnir group II. This information is represented in the table below. A Kaplan-Meier graph is presented after the table.

Table 37: Change in Kuschnir Groups or Cardiac Death - Viotti 2006 study

Benznidazole	**New Kuschnir Group**				
Baseline Kuschnir Group	**0**	**1**	**2**	**3**	**Death**
0 (n=180)		5 (2.8%)	1 (0.6%)	0 (0)	0 (0)
1 (n=73)			2 (2.7%)	0 (0)	1 (1.4%)
2 (n=30)				1 (3.3%)	2 (6.7%)
Untreated	**New Kuschnir Group**				
Baseline Kuschnir Group	**0**	**1**	**2**	**3**	**Death**
0 (n=180)		11 (6.1%)	2 (1.1%)	0 (0)	0 (0)
1 (n=75)			7 (9.3%)	5 (6.7%)	2 (2.7%)
2 (n=28)				7 (25.0%)	7 (25.0%)

Figure 7: Kaplan-Meier curve of the cumulative percentage of patients who changed clinical group based on their benznidazole treatment or non-treatment (from publication)

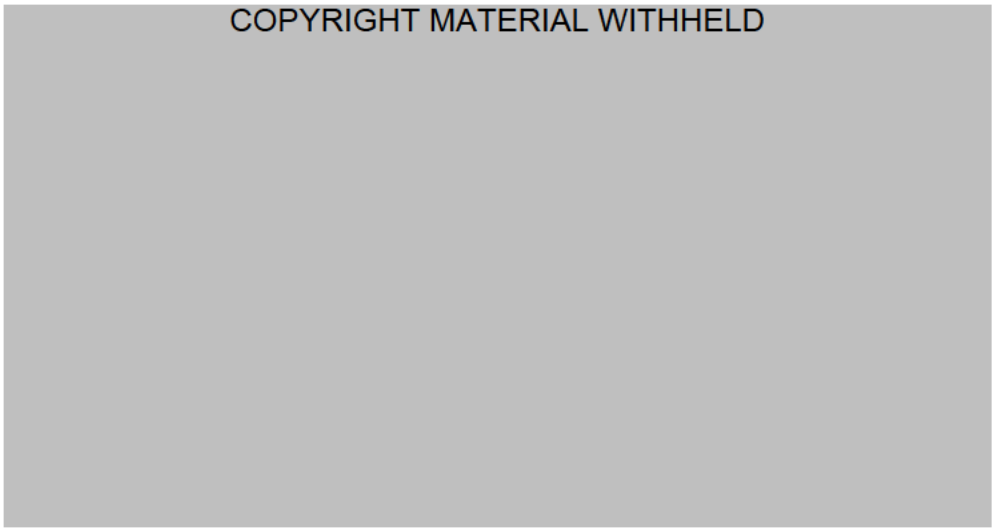

Figure 9. Kaplan–Meier curves of the cumulative percentage of patients who changed clinical group based on their BNZ treatment or non-treatment.[46]

Secondary Outcomes

Three times as many patients in the untreated group (n=45) experienced new ECG abnormalities compared to the benznidazole group (n=15) which was a statistically significant difference. Significant differences in favor of benznidazole were also found for the proportion of patients with three negative serologic tests and mortality. Only a few patients who seroconverted experienced a new ECG abnormality (3.1% in the benznidazole group and 0% in the untreated group) compared to those patients who did not seroconvert (7.0% in the benznidazole group and 17% in the untreated group).

Complete seronegative conversion was more frequent in treated patients than in untreated patients (32 of 218 treated patients [15%] vs. 12 of 212 untreated patients [6%]; adjusted HR, 2.1 [CI, 1.06 to 4.06]; p = 0.034). Complete seronegative status was achieved at a median of 11.7 years (25% to 75% interquartile range, 5.9 to 15.7 years).

Changes of clinical group were observed more frequently in patients with 3 persistent positive results on serologic tests than in those with other serologic outcomes (33 of 307 patients [10.7%] vs. 3 of 123 patients [2.4%]; HR, 4.88 [CI, 1.48 to 16.05]; p = 0.009).

None of the patients who achieved complete negative results on serologic testing changed clinical group during the follow-up, regardless of treatment. The mortality rate during the follow-up period was lower in the treated group (3 of 283 patients [1.1%]) than in the untreated group (12 of 283 patients [4.2%]). The frequency of death paralleled the patients' Kuschnir grouping: 2 patients (13.3%) were in Kuschnir group I, 5 patients (33.3%) were in group II, and 8 patients (53.3%) were in group III. Thus, in this study, negative seroconversion of Chagas serology paralleled improvement in clinical cardiac outcome, and fewer deaths (heart failure and sudden death) occurred in the treated patient group. The primary and secondary outcomes are presented in the tables that follow.

Table 38: Seroconversion to negative - Viotti 2006 study

Results	**Benznidazole (N=283) n(%)**	**Untreated (N=283) n(%)**	**Difference (95% CI)**
Seroconversion	32 (11.3)	12 (4.2)	7.0 (2.7, 11.4)
No seroconversion	184 (65)	196 (69.3)	
Missing	64 (22.6)	63 (22.3)	
Death	3 (1.1)	12 (4.2)	

Table 39: Seroconversion to negative based on Baseline Kuschnir Group (ITT)- Viotti 2006 study

Benznidazole, N= 283	**Baseline Kuschnir Group**		
Baseline Kuschnir Group	**0 (n= 180)**	**1 (n=73)**	**2 (n=30)**
Conversion, n	19 (10.6%)	11 (15.1%)	2 (6.7%)
No Conversion, n	116	48	22
Missing, n	45	14	6
Untreated, N= 283	**Baseline Kuschnir Group**		
Baseline Kuschnir Group	**0 (n=180)**	**1 (n=75)**	**2 (n=28)**
Conversion, n	9 (5%)	3 (4%)	0
No Conversion, n	125	58	17
Missing, n	46	14	11

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Table 40: Additional clinical and serological outcomes - Viotti 2006 study

Event	Benznidazole, N= 283	Untreated, N= 283	P-value
Positive Results on Three Serologic Tests	130 (59.6)	177 (83.5)	<0.0001
New ECG Abnormalities	15 (5.3)	45 (15.9)	<0.0001
Mortality	3 (1.1)	12 (4.2)	0.033

Note: All subjects who seroconverted were alive and had no new ECG abnormalities except for one patient who was seronegative in the Benznidazole group and experienced a new ECG abnormality.

Mortality in treated and untreated patients

A total of 15 patients died during the study follow-up, 3 in the treated group and 12 in the untreated group. The patient-level data listings provided by the sponsor show that sudden death was the most common cause of death overall, occurring in 9 of 15 (60%) patients , followed by heart failure and/or a combination of both. In the treatment group, the cause of death observed in three patients were attributed to sudden death and heart failure in one, heart failure in another and unknown in one patient. In the untreated group, 8 of 12 (66.6%) deaths were sudden deaths, three were caused by heart failure and in one patient the cause was unknown. In the benznidazole group, two of the patients who died were in Kuschnir Group 2, and one was in Group 1. In the untreated group, 4 patients were in Group 1, and 8 were in Group 2. In 4 of the untreated patients who died, the last serological control had shown three persistently positive tests. There were no deaths among those who seroconverted.

No dates of deaths were provided, however, the individual length of follow-up for each patient was provided up to the time of death in listing 16.2.6.4.

Time of follow-up until death for treated and untreated patients who died during the study is presented in the table below. The observed predominance of women is consistent in both groups, and it is consistent with the sex distribution of patients during study follow-up, comparable in both groups. The statistical team assessed whether the predicted mortality was different for the two groups using a rank test for censored survival data. There was a difference in overall survival probability associated with treatment group ($p<0.0097$) with those in the untreated group having the lowest survival probability and those in the benznidazole group having the greatest survival probability.

Table 41: Time until death and demographics in treated and untreated patients - Viotti 2006 study

Length of follow-up until death occurrence	Benznidazole treated (N=3)	Untreated Patients (N=12)
Mean (years)	13.57	6.23
Median (years)	12.36	4.61
Range	9 to 12.36	2.95 to 18.68
Mean age at death	55.6	47.2
Sex distribution	2 females, 1 male	8 females, 4 males

Conclusions:

The Viotti study enrolled adult subjects between the ages of 30 and 49 who were not currently experiencing heart failure and most of them (63%) did not present ECG changes characteristic of early Chagas cardiac disease. Subjects were followed for a variable length of time ranging from .10 to 27.02 years. Benznidazole showed a significant effect in preventing patients from advancing to more severe Kuschnir groups. This is reflected also in an increase in the time until death and lower mortality rate observed in the treated patients as compared to untreated controls. There were also significant benefits seen with benznidazole in the proportion of positive results on three serologic tests, new ECG abnormalities, and mortality compared to the untreated group. There are some drawbacks to this trial. It was not randomized; instead, the assignment to groups was made using alternating sequence according to the publication. However, the two arms did appear balanced in baseline characteristics. It was an unblinded study, however, the study endpoints were objective (ECG changes, chest x-rays, serological measurements and mortality). Two cardiologists external to the trial discussed the adjudication to Kuschnir groups without knowledge of the treatment assignment. The strengths of this study are several: the selection criteria, excluding other causes of cardiac disease and similar exposure to vector mediated disease, the length of the follow-up, and the use of both clinical and serological follow-up at regular intervals. The serological follow-up was performed at the same national reference center throughout the study. Even though bias cannot be ruled out, these characteristics of the study design and disease history (most patients are asymptomatic and free of heart failure in early stages) lessen the concern for a major bias in this trial. The results are still interpretable, with some caveats about the precise magnitude, given the non-randomized design. The replacement of subjects who declined to participate after treatment assignments is another limitation. However, given the well balanced baseline characteristics in both groups, and the difficulty to accurately predict clinical outcomes at baseline because of the nature of the disease history, in which signs and symptoms are minimal at early stages, this is not likely to have produced a major bias in the results. The lack of granular data that would allow for the assessment of the timing of

Reference ID: 4134724

outcomes is another limitation, however, the individual length of follow-up is provided for each patient. The authors mention that the follow-up was carried out until events were reported and assessed. It is likely, but not confirmed, that these individual lengths of follow-up represent the time until clinical outcomes of disease progression and death occurred. The 20% lost to follow-up as reported in the article is unclear since these patients had similar lengths of follow-up as those "not lost to follow-up" and contributed to endpoints and were known to be alive until the time of the last individual length of follow-up reported. Because of the variability of the disease in different geographical areas, it is difficult to conclude on the generalizability of this study results to all patients with Chagas disease in other areas.

This is the most rigorous of all prospective studies conducted in adult Chagas disease patients. Its findings are consistent with what is known about the pathophysiology of the disease and its progression to cardiac related morbidity and death previously described in humans and also in animal models. Benznidazole treatment showed therapeutic benefit demonstrated in a delayed progression of disease, new ECG changes, survival time and mortality. The therapeutic effect was associated with an increased in the number of negative seroconversions, which in normal hosts represent the lack of or highly reduced antigenic stimulation by the parasite. Therefore, this is thought to represent absent or markedly reduced parasitic tissue burden. The lack of a reliable method to demonstrate parasitic cure is a limitation in the evaluation of the efficacy of treatment in Chagas disease. Another limitation of the disease nature is the impossibility of accurately determining when cardiac tissue damage is present, and when it is irreversible. As evidenced by histopathological findings in the hearts of patients in different Kuschnir stages[102], the presence of myocardial fibrosis is more frequent in advanced stages, however, it is variable among patients and can also be observed at early stages. This may limit the cure rate of some patients, even at an early stage, and it may not be predicted.

6.4. Efficacy data available from other published prospective, controlled trials

In addition to the Viotti 2006 study, for which the sponsor submitted patient-level data, there are three other prospective and controlled, non-randomized studies of benznidazole conducted in adult patients which report clinical outcomes in long term follow-up (Viotti 1994[103], Gallerano 2000[104],

[102] Schijman, Alejandro G., et al. "Trypanosoma cruzi DNA in cardiac lesions of Argentinean patients with end-stage chronic Chagas heart disease." *The American journal of tropical medicine and hygiene* 70.2 (2004): 210-220.
[103] Viotti R, Vigliano C, Armenti H, Segura E.*: Treatment of chronic Chagas' disease with benznidazole: clinical and serological evolution of the patients with long term follow-up. Am Heart J. 1994 Jan;127(1):151-62.*
[104] Gallerano R, Sosa R, *Study of intervention in natural evolution of Chagas's disease, Evaluation of specific anti-parasitic treatment. Rev Fac Cien Med Univ Nac Cordoba. 2000;57(2):135-62*

Reference ID: 4134724

Fabbro 2007[105]). These three studies were conducted by independent investigators in three different regions of Argentina, who worked in public institutions taking care of Chagas patients and were not sponsored by any private industry or institution. The trial design and support was their own, performed as part of their standard patient care follow-up at their institutions. A fourth study includes prospective-retrospective data and was done in Brazil. Its findings are also going to be presented and discussed. It was also conducted by independent investigators and not financed by private industry.

The table below describes the study design of the four studies (including Viotti 2006 described in the previous section, with patient-level data). At the time these trials were planned and conducted (late 1980s and early 1990s), treatment of Chagas disease in the chronic indeterminate and chronic established phases was not generally recommended. Experimental studies with nifurtimox started in 1965[106] and with benznidazole, in 1973[107]. There was no data about the efficacy of treatment of chronic indeterminate disease, and the available data from animal models and some controlled human studies suggested that treatment could only be effective in acute disease. Tolerability of benznidazole in adults was known to be lower than in children; therefore, treatment of chronic indeterminate disease was optional and rarely prescribed because of unknown effectiveness to assess risk benefit. Treatment of chronic cardiomyopathy was not recommended because it was thought to be ineffective at this late stage.

Table 42: Four prospective, controlled, non-randomized clinical trials of benznidazole in adult patients with Chagas disease reporting clinical outcomes

	Study Type	N (arms) Treated/Untreated	Population	Dosing regimen	Endpoints	Length of Follow-up
Viotti 1994	Prospective, controlled: Benznidazole vs Untreated controls	201 (130/71)	30-30 y.o. (mean age 46)	5 mg/kg/day for 30 days	Conventional serology and clinical outcomes	Mean 8 years
Gallerano 2000*	Prospective, controlled: allopurinol, benznidazole and nifurtimox vs untreated	535/668 (535 treated: 309, Benz 130*, 96)	>18 y.o. (mean age 36.6 years)	4-8 mg/kg/d for 45-60 days	Conventional serology, xenodiagnosis and clinical outcomes	Mean 74 months

[105] Fabbro D, Streiger M, Arias E, Bizal M, del Barco M, Amicone N: *Trypanocide treatment among adults with chronic Chagas' disease living in Santa Fe City (Argentina), over a mean follow-up of 21 years: parasitological, serological and clinical evolution.* Rev Soc Bras Med Trop. 2007 Jan-Feb;40(1):1-10.
[106] Ferreira, H. de O. "Tratamento da doença de Chagas (fase aguda) com Bayer 2502." *Revista do Instituto de Medicina Tropical de São Paulo* 9 (1967): 343-345.
[107] Richle, R. "Chemotherapy of experimental acute Chagas disease in mice: beneficial effect of Ro-71051 on parasitemia and tissue parasitism." *Le Progres Medical* 101.282 (1973): 117.

Reference ID: 4134724

Viotti 2006	Prospective, controlled	566 (283/283)	30-50 y.o. (mean age 39)	5 mg/kg/d for 30 days	Conventional serology and clinical outcomes	Median 9.8 years
Fabbro 2007*	Prospective, controlled	111 (54/57) – 27* Bnz	17-46 y.o.	5mg/kg/d for 30 days^{o}	Conventional serology, xenodiagnosis and clinical outcomes	Mean 20.6 years

*: Gallerano and Fabbro studies had additional treatment arms (nifurtimox, allopurinol). The N for the benznidazole cohort in these studies was 130 and 27, respectively. ^{o}: In accordance to local public health guidelines at the time, half the dose was given during the first week of treatment.

Table 43: Clinical and serological outcomes of four prospective, controlled, non-randomized trials of adult patients with Chagas disease

Publication	**Treatment Arms, N**		**New ECG changes**		**Heart disease progression**		**Serology reversion to negative**	
Author/year	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Viotti 1994	131	70	7/131 (5.3%)	16/70 (22.8%)	8/70 (2.3%)	3/131 (11%)	21/110 (19.1%)	3/50 (6%)
Gallerano 2000	535 (130benz)	668	5/130 (3.8%)	113/668 (16.9%)	5/130 (3.8%)	113/668 (16.9%)	3/130* (3.9%)	0/668 (0)
Viotti 2006	283	283	15/283 (5%)	45/283 (15%)	12/283 (4%)	41/283 (14%)	32/218 (15%)	12/212 (6%)
Fabbro2007	54 (27 benz)	57	2/54 (3.7%) (2/27 benz)	9/57 (15.7%)	2/54 (3.7%)	9/57 (15.7%)	20/54* (37%) (9/27 benz)	0/57 (0)
Total	**1003**	**1078**						

*Gallerano and Fabbro report declining titers in treatment arms in treated patients, even in those who did not become seronegative by the end of follow-up. Fabbro did not describe how heart disease progression was defined. These two studies had additional treatment arms (allopurinol and nifurtimox in the Gallerano study, and nifurtimox in the Fabbro study). The outcome for benznidazole is indicated in parentheses" (benz)" for these studies.

Viotti et al. (1994)[103]**:** This was a prospective, non-randomized, untreated-controlled study with 201 adult patients, mean age 46 years, monitored for 8 years, classified at entry by Kuschnir categories of severity. There was observed ECG evolution in 7/131 cases (5.3%) treated with benznidazole (5 mg/kg/day for 30 days) and 16/70 (22.8%) in the control group. In patients older than 50 years of age, the ECG alterations occurred in 3/36 (8.3%) of the treated cases and 7/40 (17.5%) of the untreated ones; differences were not statistically significant in this group. For those under 50 years old, such alteration occurred in 4/95 (4.2%) and 9/30 (30%), respectively, for treated and untreated patients, and were significantly different ($p<0.001$). No patients with three negative serologic reactions presented new ECG changes. Treated patients who still presented three positive serologic reactions developed fewer new ECG changes than untreated patients (2.1% and 29.4%, $p<0.01$). Changes to a more severe clinical group were observed in 3 of 131 (2.3%) and 8 of 70 (11%) of treated and untreated patients, respectively. Two patients died during the follow-up, one treated and one

untreated. After 8 years of follow-up, 68% of the untreated patients presented positive serology as compared with 48.2% of those in the benznidazole-treated group ($p<0.02$). These study findings were the bases for the design of the second, larger study (Viotti 2006).

Fabbro et al. (2007)[105]: The efficacy of treatment with nifurtimox and/or benznidazole among adults with chronic Chagas disease with no previous electrocardiographic disturbances was evaluated over a mean follow-up of 21 years, by means of conventional serology, xenodiagnosis, clinical examination, electrocardiograms and chest X-ray. One hundred and eleven patients, between 17 and 46 years old, were studied: 54 underwent treatment (nifurtimox 27, benznidazole 27) and 57 remained untreated (control group). Xenodiagnosis was performed on 65% of them: 36/38 of the treated and 9/34 of the untreated patients had previous positive xenodiagnosis. Post-treatment, 133 xenodiagnoses were performed on 41 patients, all resulting negative. In the control group, 29 xenodiagnoses were performed on 14 patients; 2 resulted positive. Sera stored during the follow-up were simultaneously analyzed through conventional serology tests (IHA; direct agglutination with 2-mercaptoethanol [DA-2ME]; IIF). The serological evolution in the treated group was: a) 37% underwent negative seroconversion (nifurtimox 11, benznidazole 9); b) 27.8% decreased titers (nifurtimox 9, benznidazole 6), 9 showed inconclusive final serology (nifurtimox 7, benznidazole 2); c) 35.2% remained positive with constant titers (nifurtimox 7; benznidazole 12). The control group conserved the initial antibody levels during the follow-up. In the clinical evolution, 2/54 (3.7%) of the treated and 9/57 (15.8%) of the untreated patients showed electrocardiographic disturbances attributable to Chagas myocardiopathy, with a statistically relevant difference ($p<0.05$). Treatment caused parasitological cure as measured by conversion of serological responses to negative in at least 37% of the chronically infected adults and a protective effect on their clinical evolution. In this study, 20% of children seroconverted by about 4 years, whereas it took approximately 18 years for adults to reach the same milestone. The highest probability of seroconverting in children was about 65% (see Figure 8), whereas in adults it was only about 37% (see Figure 9).Thus, children are likely to seroconvert more rapidly than are adults, and children are almost twice as likely to seroconvert than are adults, showing higher cure rates by seronegativization.

Figure 8: Probability of seroconversion in children treated and untreated, over prolonged follow-up

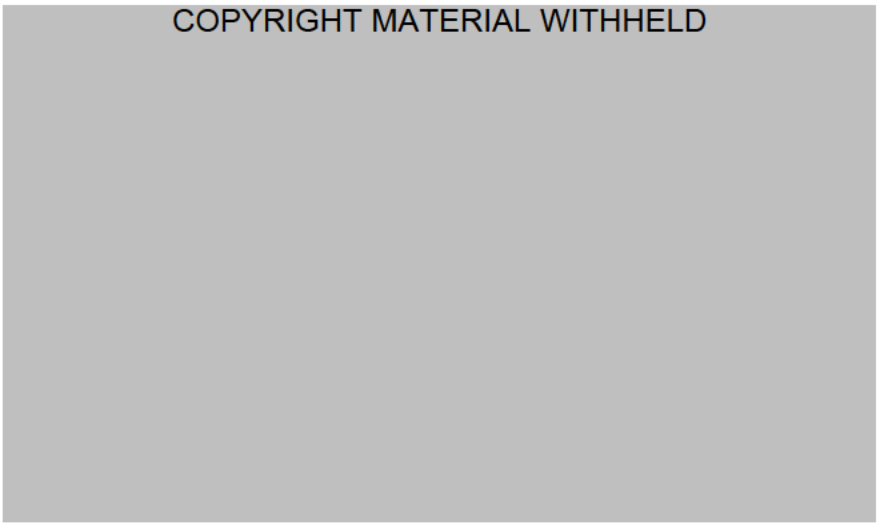
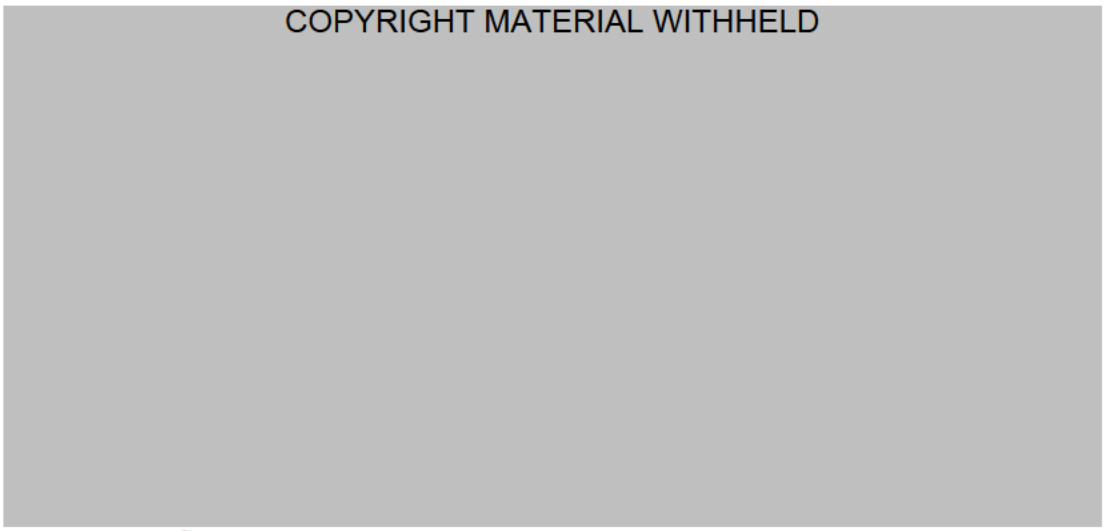


Figure 9: Probability of negative seroconversion in adults patients with chronic Chagas disease treated with benznidazole or nifurtimox or untreated, over time



Source: Fabbro, 2007[71]

Streiger (2004)[108] In another study conducted in 95 Chagas infected children ages 1 to 14, of whom 24 were untreated (Streiger, 2004), 53 benznidazole treated children at a dose of 5 mg/kg/day for 30 days, had a statistically significantly greater probability of achieving serologic negativization than did the untreated children ($p < 0.05$). All of the 24 untreated children remained seropositive throughout

[108] Streiger, Mirtha L., et al. "Longitudinal study and specific chemotherapy in children with chronic Chagas' disease, residing in a low endemicity area of Argentina." *Revista da Sociedade Brasileira de Medicina Tropical* 37.5 (2004): 365-375.

the follow-up period ranging from 8 to 24 years. In the children that were treated, the percentage of conversion to seronegative diminishes with the age at which treatment is administered from 75% at ≤4 years to 43% at ≥9 years. These results indicate the importance of the diagnosis and early treatment since the benefit is greater, the lower the age, as was also suggested in other studies. No clinical outcomes were observed in the follow-up of this study, in treated or untreated patients.

Gallerano et al. (2000)[109]: This study included 1203 chronic Chagas patients who remained untreated (668) or were given one of three drug treatments (535) (benznidazole, N=130), nifurtimox or allopurinol). Benznidazole doses ranged from 4 to 8 mg/kg/day for 45 to 60 days. Several differences characterized the two groups, suggesting a possible bias to treat patients with signs of higher parasitic tissue burden, who had none or few ECG changes. For example, the treated group was younger (mean age of 29.6 years vs 36.6 years in treated and untreated groups, respectively) and only 72 (14.2%) had an abnormal ECG. However, 225 (33.7%) of 668 untreated patients had abnormal ECG at baseline. In the benznidazole group, 7.7% of patients had abnormal ECG at baseline. Only 112 of the 130 benznidazole treated patients had xenodiagnosis prior to treatment, 17 of them showing positive (15.2%). Xenodiagnosis immediately after treatment was negative in 88.2% of these 17 patients. At the end of the follow-up period, with a mean of 1.5 XD per patient, XD was negative in 76.4% of them. The mean duration of follow-up in months (from first to last appointment) in the benznidazole group was 80.4 months (SD = +- 54 months). A total of 3.8% of patients presented evidence of ECG progression during the follow-up period.

Spontaneous conversion of serology responses to negative by conventional serology was not observed in any of the untreated patients, whereas 5 of 130 (3.9%) benznidazole patients reverted to negative. A benefit in mortality was also observed in treated vs untreated patients (pooled treated vs untreated patients). The mortality is presented for the whole group of treated patients (benznidazole, nifurtimox and allopurinol). One patient died in the benznidazole group, from causes other than cardiac or Chagas disease, according to the authors.

[109] Gallerano, Rafael R., and Raúl R. Sosa. "Estudio de intervención en la evolución natural de la enfermedad de Chagas. Evaluación del tratamiento antiparasitario específico. Estudio retrospectivo-prospectivo de terapéutica antiparasitaria." *Rev. Fac. Cienc. Méd.(Córdoba)* 57.2 (2000): 135-162.

Reference ID: 4134724

Figure 10: Comparison of the progression of cardiomyopathy in 1203 Chagas patients in long term follow-up (Gallerano, 2000)

No details are available regarding losses of follow-up, confounding factors and other missing data by treatment group. The length of follow-up was more prolonged in treated patients.

An important observation from this trial comes from the large size (n=668) of untreated patients under prolonged clinical follow-up including serial ECG and chest X-rays.

Evidence of ECG progressive changes were found in 113 of 668 (16.9%) untreated patients. A total of 29 patients (5.5%) died during the course of the disease, and the authors report an attributed mortality to Chagas disease of 4.3%. Cardiac deterioration progressed more rapidly once abnormal ECG findings had been observed than when such findings were not present. Spontaneous negativization in conventional serology was not observed in any of the untreated patients. The rate of 3.8% of seronegativization in benznidazole treated patients at approximately 5 years of follow-up is consistent with the rate observed in the Viotti (11%) and Fabbro (33.7%) studies at approximately 10 and 20 years of follow-up, respectively.

Fragata Filho et al. (1995)[110] This author reported, in a study with 120 chronic Chagas patients, 71 of whom were treated, 30 (42%) of whom were in the indeterminate phase and 41 (58%) presented mild signs of cardiac involvement, with follow-up for 7.19 ± 5.36 years, new ECG changes in 7% for benznidazole-treated cases (n = 71) and in 14.3% for the untreated group (n = 49). Two out of 71 (3.8%) treated patients had negative seroconversion. Baseline characteristics are summarized only for the treated patients: mean age 36.7 ±9.1, 49 (69%) were male, 55 (77%) had normal chest X-ray and 30 (42%) had normal ECG. The authors report that 60 patients (84%) apparently had a favorable reversion of their condition, however no description of the findings to confirm this observation are mentioned. Five patients deteriorated (7%) and in three the xenodiagnoses became positive. One patient died from causes other than Chagas disease. The evaluation was retrospective and the authors did not report many details on the selection criteria, although they describe their institution's exclusion criteria for treatment: severe cardiac disease, severe medical condition that compromises

[110] Fragata Filho, Abílio Augusto, Marco Aurélio Dias da Silva, and Elias Boainain. "Ethiological treatment of acute and chronic Chagas' heart disease." *Sao Paulo Medical Journal* 113.2 (1995): 867-872.

Reference ID: 4134724

patient's outlook, age older than 50, residence in area of high endemicity and malnutrition. Presumably, these were applied to select the patients for treatment. No baseline characteristics of untreated patients are described. Potential confounding factors are not discussed. Even with these study limitations, the prolonged follow-up available for these patients shows a decreased rate in the incidence of new ECG changes attributed to Chagas disease in treated vs. untreated patients (7% vs 14.3% respectively). The magnitude of this decrease may represent an overestimation if the baseline characteristics or confounding factors were imbalanced; however, this information is not available.

Fragata Filho et al (2016)[111]: This was a prospective-retrospective observational trial done in Brazil. A total of 310 patients who had normal ECGs at the first medical visit performed before 2002 were included.

A total of 267 patients were treated with benznidazole, whereas 47 remained untreated. Treated patients were younger (56.07 years x 68.89 years, p <0.0001), predominantly male (36.90% vs. 21.30%, p: 0.045), had left the endemic area more recently (16.77 years vs 19.65 years, p: 0.012), and 208 (79.08%) maintained normal ECGs, compared to 22 (46.81%) of the non-treated individuals (p <0.0001). There were two or more results of the immunofluorescence test in 171 patients 11 (6.43%) untreated and 160 (93.57%) treated patients. These results remained stable in untreated patients (232.72 ± 104.02 and 254.54 ± 93.41, first and second titers, respectively), whereas in the treated individuals, the titers decreased (144.90 ± 109.80 and 70.25 ± 74.70: p < 0.0001). Conversion to negative of the immunofluorescence titer (< 1/40) occurred in 60 patients treated with benznidazole (37.50%), at an average of 14 years' follow-up, but in none of the untreated individuals. Normal ECGs were maintained by 208 (79.08%) of the BNZ treated patients, compared to 22 (46.81%) of the non-treated individuals (p < 0.0001). The occurrence of ECG abnormalities and relevant clinical events (heart failure, stroke, total mortality, and cardiovascular death) was less prevalent in treated patients (p <0.001, p = 0.022, p = 0.047, respectively). Among the 80 patients who had worsening of the ECGs, eight (10%) died, and among the 230 who maintained normal ECGs, four (1.7%) died (p= 0.002). In the multivariate analysis with the dependent variables being the occurrence of combined events (heart failure, stroke, and total mortality) and five independent variables (treatment with benznidazole, follow-up time, males, white ethnicity, and age), treatment was found to be the only variable associated with protection against events. The results from a logistic regression model for this study are presented below.

[111] Fragata-Filho, Abilio Augusto, et al. "Evaluation of parasiticide treatment with benznidazol in the electrocardiographic, clinical, and serological evolution of Chagas disease." *PLoS neglected tropical diseases* 10.3 (2016): e0004508.

Reference ID: 4134724

Table 44: Multivariate analysis of risk factors for cardiac events in Chagas patients

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SOURCE: Fragetta-Filo, 2016[29]

Although the small number of serologic tests in untreated patients did not allow the correlation of the decrease of these titers with ECG alterations, benznidazole treatment was identified in a multivariate analysis as a significant protective factor against worsening of ECG and death. The authors conclude that the persistence of a normal electrocardiogram (ECG) in Chagas patients provides a similar prognosis to that of a non-diseased population. This study had a selection bias towards treatment of younger patients who had more recently lived in an endemic area, and were more likely to have a normal ECG (79% vs 46.9% of untreated patients). The treated group was much larger (n=267) than the untreated group (n=47). Because of these differences, the demonstrated benefit of benznidazole treatment over clinical outcomes and ECG changes may have been overestimated. The strength of this study is the long term follow-up available for these patients to observe the ECG evolution and a decreased rate of relevant clinical outcomes associated with persistently normal ECG in both treated and untreated patients.

Viotti 2011[112]: The purpose of this study was to evaluate serological responses with a multiplex assay by comparison with those of conventional serology assays; however, it shows the evolution of conventional serology outcomes in treated and untreated patients over 36 months. It was a prospective study of adult patients (older than 21) classified in Group 0 or Group 1 of the Kuschnir category. Patients were followed with serial ECGs and clinical exam. Changes in antibody levels, including seronegative conversion as well as declines in titers, were serially measured in 53 benznidazole-treated and 89 untreated chronic patients in Buenos Aires, Argentina with a median follow-up of 36 months. IHA titers decreased in 22 out of 53 (41%) treated patients and in 8 out of 89 (9%) untreated subjects, p,< 0.001. Mean absorbance by ELISA decreased in 18 out of 53 (34%) treated and in 6 out of 89 (7%) untreated subjects, p,<0.001. Finally, a decline in titers by IFI assays was observed in 18 out of 53 (34%) treated and in 7 out of 89 (8%) untreated subjects, p, <0.001. In treated subjects, the median follow-up period to detect a decline in antibody levels was 27 months

[112] Viotti, Rodolfo, et al. "Impact of aetiological treatment on conventional and multiplex serology in chronic Chagas disease." *PLoS neglected tropical diseases* 5.9 (2011): e1314.

(interquartile range 25 to 75% 16.5–38.5),while a median of 24 months (interquartile range 25 to 75% 21–27.7) was required to identify negative seroconversion on 2 or 3 tests.

The etiological treatment with benznidazole was the only variable that correlated with the decline of titers or seronegative conversion as determined by multivariate analysis.

Decrease of titers (34/53 [64%] treated vs. 19/89 [21%] untreated, p<0.001) and seronegative conversion (21/53, [40%] treated vs. 6/89, [7%] untreated, p<0.001) in at least one conventional serological test were significantly higher in the benznidazole-treated group compare with the untreated group. When not only complete seronegative conversion but also seronegative conversion on 2 tests and the decreases of titers on 2 or 3 tests were considered, the impact of treatment on conventional serology increased from 21% (11/53 subjects) to 45% (24/53 subjects). This study showed a significant decrease in antibody titers and negative seroconversion, in treated patients as compared to untreated ones. None of the treated patients presented new ECG changes during the follow-up period.

Pediatric cohorts with negative seroconversion endpoints:

In a prospective, controlled study conducted in 95 treated[113] (n=64 received benznidazole and n=7 received nifurtimox) and untreated (n=24) children aged 1-14 years, were followed for 8 to 24 years. Regarding *Trypanosoma cruzi* transmission, the studied individuals presented multi- risk factors: vectorial, congenital and blood transfusion.

The treated children had a statistically significantly greater probability of achieving serologic negativization than did the untreated children (p <0.05). Further, children in the 1-6-year age group had an even greater likelihood of benefiting from benznidazole treatment than did children in the 7-14-year age group, as the younger children achieved serologic negativization sooner than did the older children, and the younger children had a greater likelihood of eventually achieving negative seroconversion (~78% vs. ~58%, respectively). The untreated patients did not have any changes in their serology and remained positive throughout the entire follow-up (n=14 of them were followed=up for a period ranging from 8 to 24 years). Among the 71 treated children, the percentage of treated children presenting negative serological results decrease according to the age when treatment was given: 75% became negative when treated at less than 4 years of age and 43% when treated at age 9 years or older.

In addition to the data presented so far, there are data presented on children and adolescents treated in the indeterminate chronic phase in field conditions in projects from the Doctors Without Borders /

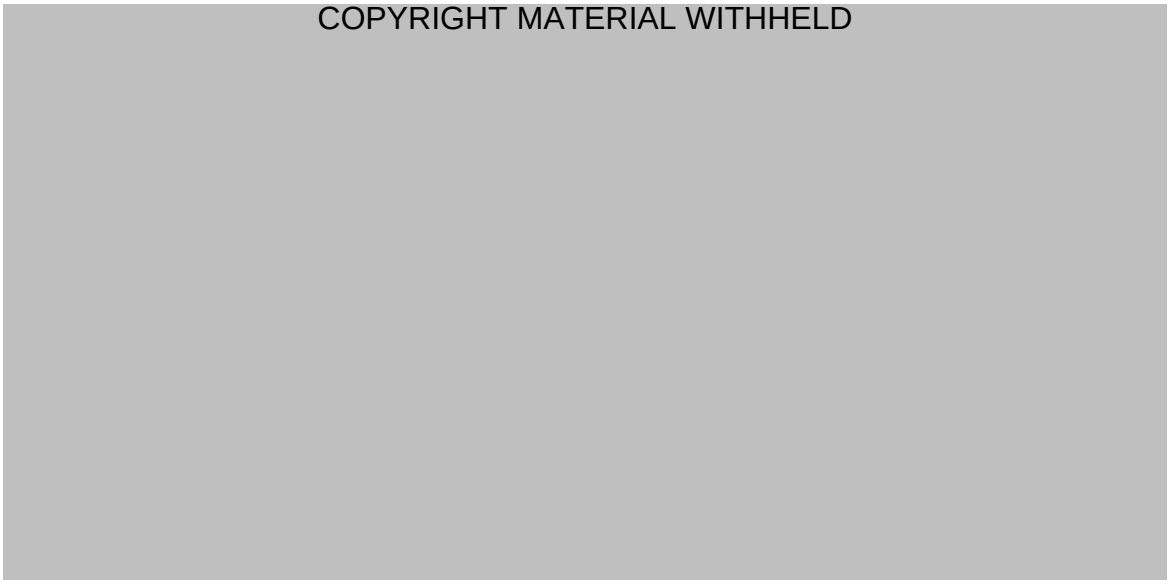
[113] Streiger, Mirtha L., et al. "Longitudinal study and specific chemotherapy in children with chronic Chagas' disease, residing in a low endemicity area of Argentina." *Revista da Sociedade Brasileira de Medicina Tropical* 37.5 (2004): 365-375.

Reference ID: 4134724

Médécins Sans Frontières (MSF) reported by Yun *et al.*, 2009[114]. For 10 years, MSF implemented Chagas disease control programs for Chagas disease at Yoro, Honduras (1999- 2002), Olapa, Guatemala (2003-2006), Entre Ríos, Bolivia (2002 – 2006) and Sucre, Bolivia (2005-2008), focused on the diagnosis and treatment of patients up to 18 years old.

Diagnoses were confirmed by positive results in two different serology tests (conventional ELISA and recombinant IHA, and IIF for indeterminate or discordant findings). Patients testing positive for *T. cruzi* were treated with benznidazole 7.5 mg/kg/day administered two or three times a day for 60 days. The efficacy of the treatment was assessed between 18 and 36 months post-treatment through convention ELISA serology, confirming the negative findings through recombinant ELISA. According to the WHO protocol, patients presenting two non-reactive serology tests (conventional and recombinant ELISA) on the same sample and on the same day were rated as cured.

Table 45: Characteristics and outcomes of the Chagas Disease Control Programs implemented by MSF between 1999 and 2008.



Source: Yun et al. (2009)

There was a marked difference in results among the various programs. At Yoro, the seroconversion rate was 87.1% (202/232) after 18 months of follow-up and 92.7% (215/232) after 36 months, indicating that the treatment was extremely efficacious. In a more detailed analysis of the Program at Yoro, Honduras, Escriba *et al*.69 (2009) showed an overall seroconversion rate at 18 months of 88.2% (95% CI, 84-92.4%), rising to 93.9% (95% CI, 90.8- 97%) at three years. However, this increase was not

[114] Yun, Oliver, et al. "Feasibility, drug safety, and effectiveness of etiological treatment programs for Chagas disease in Honduras, Guatemala, and Bolivia: 10-year experience of Médecins Sans Frontières." *PLoS neglected tropical diseases* 3.7 (2009): e488.

statistically significantly. Of the 229 patients who were monitored for more than 18 months, 85.2% (95% CI, 80.5-89.8%) presented a reaction in the antibody titrations (recombinant ELISA by optical density) of ≥ 75% in relation to the initial values, and 93.4% (95% CI, 90.2-96.7%) after three years. This difference is statistically significant. No material differences were noted by gender or age range in the seroconversion rate and anti *T. cruzi* antibody titers.

At Olopa, the seroconversion rate after 18 months was 58.1% of patients for whom data were available (25.5% of the cohort patients or 18/31), also suggesting that the treatment was efficacious.

The seroconversion rates were lower at the two centers in Bolivia. In Entre Ríos, preliminary findings indicated a seroconversion rate of 5.4% (59/1,101) post-treatment up to 60 months post-treatment, with 950 patients monitored for a period of more than 18 months. The seroconversion rates during the follow-up at 18 to 60 months were higher in the lower age group (24.2% [16/66] among children < 5 years, 4.6% [14/305] in the age group of 5 to 9 years old and 1.9% [12/638] in the 10 to 14 years old age range). At Sucre, none of the 276 patients monitored for a period of 9 to 27 months post-treatment, presented seroconversion.

According to Yun *et al*., these differences in the seroconversion rates may be explained by 1) the delay in negative seroconversion by conventional serology, which in children may take between 5 to 10 years in Latin America; 2) different susceptibility to treatment of the various parasite lineages (predominance of type I *T. cruzi* in Central America type II in South America); 3) potential differences in treatment efficacy as a function of the proximity of the acute phase; and iv) constraints of data analyses due to some variability in age groups and post-treatment follow-up times.

Medical Officer comments on pediatric studies with seroconversion endpoints: *Despite the heterogeneity of the studies presented in terms of objectives, geographic location, age ranges, numbers of children included in these studies, therapeutic schemes used, duration of post-treatment monitoring and the serological tests used, there is clear evidence of the efficacy of benznidazole for treatment of children infected by T. cruzi, evidenced by marked decreased in titers and negative seroconversion in conventional serological assays, which is much less frequently observed in untreated controls. Younger children, particularly those younger than 4 years of age, have the greatest likelihood of presenting negative seroconversion. There is great variability in the percentages of negative seroconversion (5%-75%), possibly due to the differences in parasite lineages, length of follow-up and variability of the tests used. However, children studies show a consistent trend towards higher rates of negativization observed at relatively shorter periods of time as compared with adults.*

Medical Officer comments on studies with clinical outcomes: *The collective evidence from prospective controlled cohorts reporting clinical outcomes consistently shows a decrease of new electrocardiographic changes associated with a decrease in the incidence of ECG changes, cardiac events and deaths in benznidazole treated patients as compared with untreated controls, especially when no abnormal ECG and no signs or symptoms of cardiac disease are present before treatment is started. Conversions of serology responses to negative are more frequent in treated patients, and observed over several years of follow-up. The findings are consistent across regions and in more than*

one country. For example, the rate of conversions to negative was 5% in the Gallerano study at a mean of 5 years, at 11% in the Viotti study at a mean follow-up of 10 years and it was 33.7% in the Fabbro study at a mean of 21 years. These three studies were conducted in three different regions of Argentina (Cordoba, Buenos Aires and Santa Fe). A slightly higher rate of 37% seronegativization at a mean of 14 years was observed in the Fragata Filho study, done in Brazil. Two studies showed a marked increase in both the rate and frequency of conversions of serological responses to negative at younger ages, greatest in children younger than 4 years of age.

The magnitude of the therapeutic effect over cardiac outcomes as compared with that of the untreated controls is difficult to quantify with precision because of the limitations of the non-randomized study designs, some patient selection biases, unknown confounders and lack of details about the missing data. It is possible that the therapeutic effect might have been overestimated. However, given the biological plausibility, and the documentation of parasiticidal effect observed, it is reasonable to conclude that benznidazole is able to provide clinical benefit to some patients, decreasing or preventing the number of new ECG changes and avoiding or slowing the progression of cardiac disease.

The untreated controls (total N=1078 in four prospective controlled cohorts, and N=87 in two retrospective-prospective cohorts) provide a source of data for the natural history of untreated disease, which has remarkable similarities with that of the control groups of the two randomized controlled studies in children. In untreated children or adults, the serological evolution is characterized by a constant persistence of positive titers with no or very few negative seroconversions, and little or no change in serological titers (two studies in adults report titer decreases, Gallerano 2000 and Fabbro 2007). In the two children studies, the length of the follow-up did not allow for the observation of clinical outcomes, however, the small number of new ECG changes observed in the follow-up period occurred more frequently in the untreated control as compared with the treated subjects of one of the two randomized trials (4 vs 1 study subjects, respectively [De Andrade study]). The untreated adult controls in the studies summarized had a higher number of new ECG changes and signs and/or symptoms of cardiac disease over the follow-up period. A benefit in mortality seems to be suggested in the Gallerano study, although the baseline differences may have increased the magnitude of this effect. Importantly, the patients who maintained a normal ECG, untreated or treated, did not develop cardiac disease during the prolonged follow-up of these studies. Benznidazole treated patients were consistently more likely to have a lower incidence of new ECG changes and fewer cardiac events.

6.5. Efficacy in Acute and Congenital Chagas Disease

Benznidazole has been shown to be effective in treating the acute phase of Chagas disease in single cohort studies, with variable follow-up. The published studies available, submitted by the Applicant, are summarized below.

Reference ID: 4134724

Barclay *et al*. (1978)[115] [Paper in Spanish] followed during 18 months 139 cases of acute infection in children (age not disclosed). The diagnosis was carried out by xenodiagnosis and Strout method (concentration followed by microscopic examination). Two different benznidazole regimens were evaluated: i) 5 mg/kg/day during 30 days administered to 32 patients; and ii) increasing doses of BNZ (3 mg/kg/day up to 7.5 - 10 mg/kg/day in the first 12 days and maintained during 30 days administered to 107 patients. The efficacy of dose regimen treatments were evaluated in 86 patients using parasitological test (xenodiagnostic) and serological tests (evolution of the results from complement fixation test [TFC], indirect fluorescence assay (IFA) and indirect hemagglutination (HAI]) which were systematically performed over a period of 18 months. Thirty percent of the patients were lost in the follow-up. At the end of the follow-up (18 months) 88% of the patients showed negativization of xenodiagnosis , 87% (27/ 31) and 91% (41/45) became negative by TFC and IFA, respectively ($p < 0.001$).

Ferreira *et al* (1988)[116] [Paper in Portuguese] In a cohort study conducted by from 1965 to 1985, 17 children (9 months - 18 years) were diagnosed with acute infection after blood transfusion. They were treated with benznidazole orally at a dose of 5 mg/kg/day divided in two doses during 60 days. The follow-up ranged from 6 to 13 years. Seven patients were lost in the follow-up. The total cure rate for BNZ was 70%. Only data from 7 patients treated with BNZ were disclosed. At the end of the follow-up, all these patients showed negativization of serological tests (HAI, IFA and TCF) and no patients showed positive parasitological tests (xenodiagnosis).

Cancado *et al*. (2002)[117] investigated the cure rate after long term evaluation of benznidazole in acute Chagas disease in Brazil. Twenty-one patients were evaluated after a follow-up period of 13 to 21 years. The age of the patients ranged from 6 months to 39 years old. Doses varied from 5 to 30 mg/kg/day, during 19 to 60 days. Quantitative serological reactions included IFA, IHA and ELISA (this last one not in all patients). Serological cure at the end of the follow-up period was observed in 16 of 21 patients (76%). The 16 patients presented negative serological tests results during a period of at least 13 years and therefore were considered cured. The other 5 patients were persistently positive and were considered therapeutic failures.

Oral transmission outbreaks: An outbreak of acute Chagas disease in Brazil was studied by Umezawa et al. (1996). Eighteen patients who were infected from the same source (sugar cane used in acai juice) were diagnosed by ELISA. The age of the patients varied from 8 to 64 years old. All patients

[115] Barclay, CA, et al. "Pharmacological aspects and therapeutic results of benznidazole treatment of chagasic infection." *PRENSA MEDICA ARGENTINA* 65.7 (1978): 239-244.

[116] Ferreira, Humberto de Oliveira. "Tratamento específico na fase aguda da doença de Chagas." *J. pediatr.(Rio J.)* 64.4 (1988): 126-8.

[117] Cançado, J. Romeu. "Long term evaluation of etiological treatment of Chagas disease with benznidazole." *Revista do Instituto de Medicina Tropical de São Paulo* 44.1 (2002): 29-37.

Reference ID: 4134724

received benznidazole in a 7 mg/kg/day schedule for children and 5 mg/kg/day schedule for adults during 60 days. Two additional individuals who acquired Chagas disease by accidental laboratory contamination were treated and included in the analysis. As control groups, patients with chronic Chagas disease and negative sera from blood bank to Chagas disease were included in the tests.

After treatment, all patients showed negative xenodiagnosis. Follow-up consisted on measurement at 54 days after infection and 3 years after the end of therapy.

All treated patients showed an intense decline of antibodies (lgG and lgM) after three years of treatment. However, six patients (30%) maintained positive lgG levels by serological tests, but reactivity was lower than that measured before treatment.

Inglessis *et al*. (1998)[118] evaluated 10 cases of myocarditis in acute Chagas in Venezuela. The patients ranged from 12 to 45 years old and were treated with BNZ at 5 mg/ kg/day during 60 days (80% of the patients) and during 30 days (20% of the patients) . Before treatment, all patients showed positive parasitemia and positive serological tests. Parasitemia became negative at the end of the treatment in all cases (100%), but serology remained positive in 80 % of the cases at 11months (8/10) and in 75 % (3/4) at 5 years of follow-up.

Bastos *et al.* (2010)[119] evaluated the clinical outcomes in two micro-outbreaks in Brazil of acute Chagas disease after contamination of sugar cane juice. Thirteen patients (9 to 61 years old) were treated after diagnosis by serological and parasitological tests, with 300 mg/day during 60 days. Patients in this outbreak had very high incidence of acute *T. cruzi* myocarditis. After treatment, 91.7% of patients showed normalized electrocardiograms readings. After treatment, EKG readings normalized in 91.7% of patients. Ventricular repolarization abnormalities persisted in 50% of the patients, while sinus bradycardia was observed in 18%. The systolic ejection fraction normalized in two out of three patients with initially depressed ventricular function, while pericardial effusion disappeared. Six months after the end of treatment, all patients showed an improvement in the clinical symptoms, as well as a decrease in the number of electrocardiogram and echocardiography abnormalities.

Efficacy in Congenital disease

Several studies with benznidazole have been conducted in congenital disease. None of these include an untreated control group, which is the main limitation, however, they provide evidence of serological and parasitological cure in prolonged follow-up.

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[118] Inglessis, I., et al. "Clinical, parasitological and histopathologic follow-up studies of acute Chagas patients treated with benznidazole." *Archivos del Instituto de Cardiologia de Mexico* 68.5 (1997): 405-410.

[119] Bastos, Claudilson JC, et al. "Clinical outcomes of thirteen patients with acute Chagas disease acquired through oral transmission from two urban outbreaks in northeastern Brazil." *PLoS neglected tropical diseases* 4.6 (2010): e711.

Reference ID: 4134724

A randomized clinical study was conducted by the L'Institut de recherche pour le développement (IRD) (2008-2009) on *T. cruzi* transmission (Chippaux, 2010[120]), comparing the reduction in anti *T. cruzi* antibodies titers among non-infected newborns, with those of newborns with congenital infections treated with two therapeutic benznidazole schemes, in order to determine the serological cure criteria. The other available studies were conducted in situations of pre-natal screening, upon detection of congenital infections among newborns of mothers infected by *T. cruzi,* and following determination of the prevalence of a congenital transmission (Blanco *et al*., 2000[121]; Russomando *et al*., 1998[122]; Salas *et al*., 2007[123]; Torrico *et al*., 2004[124]) or evaluating the PCR assay as a tool for diagnosing and assessing parasitological response to treatment (Schijman *et al*., 2003)[125].

Chippaux et al. 2010[120] In a randomized controlled study conducted by IRD (2008-2009) in Bolivia of 111 newborns with congenital infections diagnosed at birth through direct microscopic observation (microhematocrit [MH]), 58 children were treated from the first day of life with benznidazole 2.5 mg/kg/day administered twice a day for 60 days, and 52 children with benznidazole 7.5 mg/kg/day administered once a day for 30 days. In order to compare the reduction in anti *T. cruzi* antibody titers, two other study arms included 68 newborns with seropositive mothers who did not present parasitemia at birth, and 78 newborns with seronegative mothers. Blood samples were taken at 30 and 60 days and 10 ± 2 months and, with seropositive ELISA testing after ten months, every two months until seroconversion. All parasitology tests (MH) showed negative results before the ninth month. After ten months of follow-up, ELISA was negative for 90.7% of the children treated (98/108) with all the seropositive tests becoming negative after 16 months, indicating a response to treatment. No significant difference was noted in the proportion of children presenting seroconversion after ten months between the groups treated with different therapeutic schemes (p=1.0), nor among the

[120] Chippaux, Jean-Philippe, et al. "Antibody drop in newborns congenitally infected by Trypanosoma cruzi treated with benznidazole." *Tropical Medicine & International Health* 15.1 (2010): 87-93.

[121] Blanco, Sonia B., et al. "Congenital transmission of Trypanosoma cruzi: an operational outline for detecting and treating infected infants in north-western Argentina." *Tropical Medicine & International Health* 5.4 (2000): 293-301.

[122] Russomando, Graciela, et al. "Treatment of congenital Chagas' disease diagnosed and followed up by the polymerase chain reaction." *The American journal of tropical medicine and hygiene* 59.3 (1998): 487-491.

[123] Salas, N. A., et al. "Risk factors and consequences of congenital Chagas disease in Yacuiba, south Bolivia." *Tropical Medicine & International Health* 12.12 (2007): 1498-1505.

[124] Torrico, M. C., et al. "Estimation of the parasitemia in Trypanosoma cruzi human infection: high parasitemias are associated with severe and fatal congenital Chagas disease." *Revista da Sociedade Brasileira de Medicina Tropical* 38 (2004): 58-61.

[125] Schijman, Alejandro G., et al. "Aetiological treatment of congenital Chagas' disease diagnosed and monitored by the polymerase chain reaction." *Journal of Antimicrobial Chemotherapy* 52.3 (2003): 441-449.

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average figures for the antibody titers. This study suggests that a 30-day treatment may be as effective as a 60-day course, since serological cure did not differ in these groups.

Blanco *et al*. (2000)[121] in Argentina, did a study on 32 children born to infected mothers and diagnosed as positive up to six months old through parasitology (MH) and serology tests (IHA, ELISA [IgG and IgM antihuman] and IFA. Of 32 children, 3 were treated with benznidazole, 5 mg/kg per day, two or three times a day for three days; the remaining infants were treated with nifurtimox. The 32 children treated presented negative parasitology (MH) tests when examined between 6 and 24 months after the end of treatment, and 30 of the 32 children presented serological cure, up to 2 years of follow-up. The other two children were lost to follow-up. This study does not provide individual efficacy data on the three children treated with benznidazole.

Russomando *et al*. (1998)[122] This study was conducted in Paraguay. Six children born from infected mothers and diagnosed as positive through direct microscopic observation (MH) and/or hemoculture (Hc), IFA, ELISA and the PCR technique, were treated with benznidazole, 7 mg/kg per day, taken twice a day for 60 days. Treatment began at different times: two babies were treated at birth, and four others began treatment at between 3 months to 22 months after birth. After 24 months of follow-up, all babies presented negative results in the parasitology tests (Hc, MH, PCR) with negative seroconversion by IIF and ELISA. Seroconversion occurred at between two months and eight months after the end of the treatment, suggesting better outcomes for early treatment.

Schijman *et al*. (2003)[125] This study was conducted in Argentina, in 40 of 152 children born to mothers who were seropositive for *T. cruzi*, diagnosed by MH and PCR (50 children between 0 and six months old) and through serology (IHA, ELISA) and PCR (102 children between seven months and 17 years old), were treated with Nx, 10-15 mg/kg/day or benznidazole, 5-8 mg/kg/day in two doses per day for 60 days. The efficacy of the treatment was assessed by age group, and the treated children were monitored for 36 months after the end of the treatment. No differences were observed in clinical and serological results. A total of 100% of children between 0 to 3 months old age (10 children), and 66.7% of children between seven months to two years old (6 children) were considered cured based on the serology test (negative for IgG anti *T. cruzi a*ntibodies), while for the age group more than 3 years old (24 children), cures were documented in only 12.5% of the cases during the study observation period ($P = 0.023$). It was also noted that seroconversion occurred faster in children who began treatment during the first few months of life, indicating the possibility of greater efficacy for early treatment. This is consistent with what was observed in the Streiger 2004 study: higher rates of seroconversion at a shorter amount of time when treatment is initiated in children younger than 4 years of age.

6.6. Efficacy in the Immunosuppressed Host

Chagas disease reactivation occurs in two settings: in organ transplantation and in HIV co-infection. The frequency of reactivation was evaluated in two Brazilian cohorts. It was approximately 20% in HIV co-infected patients in the series of 53 patients followed for a mean of 65.5 months and 15% in the series of 20 patients followed for 35.8 months. CNS reactivation is associated with a case-fatality rate of 79–100%. In reported cases, the median survival post-diagnosis was less than a month. In the

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larger Brazilian cohort, two of 11 patients with reactivation based on microscopic parasitemia were asymptomatic; these two patients were treated with benznidazole and survived to the end of the follow-up period.

Almeida reports that from 100 HIV infected patients with reactivation of *T. cruzi* was a total of 87 (87%) of 100 cases were treated with benznidazole, and in this group the longest survival was observed. The contribution of antiretroviral therapy to the success of benznidazole treatment cannot be ruled out. However, benznidazole is likely to have contributed to a more prolonged survival. A total of 15 case reports and one review of a large series of reactivation of HIV co-infected patients have shown improved survival as compared to untreated patients and high cure rates (up to 100%) of bone marrow and solid organ transplant patients and in HIV co-infected reactivations.

6.7. Efficacy in congenital disease transmission

Two large observational studies have also confirmed that women treated before pregnancy are significantly less likely than untreated women to transmit the infection to their off- spring, which provides additional support for the treatment of girls and non-pregnant women of reproductive age.

An observational study (Fabbro et al 2014)[126] evaluated the efficacy of trypanocidal therapy to prevent congenital Chagas disease and compared the clinical and serological evolution between treated and untreated infected mothers. This was a multicenter, observational study on a cohort of mothers infected with T. cruzi, with and without trypanocidal treatment before pregnancy. Their children were studied to detect congenital infection. Among 354 "chronically infected mother-biological child" pairs, 132 were treated women, of whom 72 were treated with benznidazole at a median dose of 5.16 mg/kg/day for a median time of 45 ±14 days, and 222 were untreated women. Among the children born to untreated women, investigators detected 34 infected with T. cruzi (15.3%), whose only antecedent was maternal infection.

Among the 132 children of previously treated women, no infection with T. cruzi was found (0.0%) ($p<0.05$). Among 117 mothers with clinical and serological follow up, 71 had been treated and 46 were untreated. The women were grouped into three groups. Group A: 25 treated before 15 years of age; Group B: 46 treated at 15 or more years of age; Group C: untreated, average age of 29.2 ± 6.2 years at study entry. Follow-up for Groups A, B and C was 16.3 ± 5.8, 17.5 ± 9.2 and 18.6 ± 8.6 years respectively. Negative seroconversion: Group A, 64.0% (16/25); Group B, 32.6% (15/46); Group C, no seronegativity was observed. Clinical electrocardiographic alterations compatible with chagasic cardiomyopathy: Group A 0.0% (0/25); B 2.2% (1/46) and C 15.2% (7/46). The trypanocidal treatment of women with chronic Chagas infection was effective in preventing the congenital transmission of Trypanosoma cruzi to their children; it had also a protective effect on the women's clinical evolution and serological cure could be demonstrated in many treated women after over 10 years of follow up.

[126] Fabbro DL, Danesi E, Olivera V, Codebó MO, Denner S, Heredia C, et al. (2014) Trypanocide Treatment of Women Infected with *Trypanosoma cruzi* and Its Effect on Preventing Congenital Chagas. PLoS Negl Trop Dis 8(11): e3312.

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Similar findings were reported in a cohort of 394 female patients treated in Buenos Aires[127], Argentina, in which 15 women had pregnancies (16 children) and were included in a follow-up single cohort study. In 14/15 patients (93.3%) BZ treatment was prescribed, with a median dose of 6.2 mg/kg/day b.i.d. (range of 5-7.6 mg/kg/day). One patient (6.6%) received 9.4 mg/kg/day of nifurtimox. Mean duration of treatment was 51 days (19-60 days). No congenital transmission was observed in these children. During post-treatment follow-up, a 34% decrease in antibody titers was observed as measured by conventional ELISA and a 30.5% decrease was measured by IHA compared to the baseline value (i.e., when diagnosis was done). Negative seroconversion was observed in one patient (6.7%). The mother that showed negative seroconversion had been treated at the age of nine years old. The estimated congenital infection rate observed in this region is 4-10%. Although the number of mothers treated in this study is not high, the findings reinforce previously published data suggesting the benefits of treatment in women of childbearing age to prevent congenital transmission, which is an important route of infection.

Benznidazole treatment of infected women of childbearing age not only has the potential to reduce morbidity and mortality caused by heart disease and digestive disorders in those patients, but it can also be an effective strategy to prevent congenital transmission of *T. cruzi*, the main route of transmission of Chagas disease in areas with no vector transmission, such as the United States.

Another longitudinal cohort study evaluated the congenital transmission of Chagas disease (CD) in a nonendemic area, in Spain[128]. The offspring of 59 seropositive pregnant mothers were followed up. Nine newborns were found to have acquired the disease congenitally. Thus, among seropositive mothers, the congenital transmission rate was 13.8% (9 infected newborns of 65 live births). All the infected children were born to Bolivian mothers. A total of 10 of these women had received treatment before pregnancy. No infected infants were detected among 10 mothers who were treated before they became pregnant, compared with 16.4% (9 of 55 live births) among untreated mothers.

Infants and mothers were orally treated with benznidazole, at 10 mg/kg body weight per day in newborns and 100 mg 3 times a day in women for 60 days. In cases of congenital transmission, hemoculture and parasite lineage typing were performed. This represents a transmission rate of 13.8% among seropositive mothers (9 infected newborns of 65 total live births). Treatment before pregnancy prevented congenital infection, confirming again the findings of previous studies.

[127] Moscatelli, Guillermo, et al. "Prevention of congenital Chagas through treatment of girls and women of childbearing age." *Memórias do Instituto Oswaldo Cruz* 110.4 (2015): 507-509.

[128] Murcia, Laura, et al. "Risk factors and primary prevention of congenital Chagas disease in a nonendemic country." *Clinical Infectious Diseases* 56.4 (2012): 496-502.

7 Integrated Review of Effectiveness

7.1. Assessment of Efficacy Across Trials

7.1.1. Primary Endpoints

The serological primary endpoints of randomized controlled studies, as measured by non-conventional and conventional serological assays show a consistently higher rate of conversions of serological responses to negative and reduction in titers in both studies. In adult studies, negative seroconversions by conventional serological assays have been associated with better clinical outcomes related to cardiac disease progression and death. A decrease in titers has been observed preceding negative seroconversion in long-term prospectively followed cohorts.

7.1.2. Secondary and Other Endpoints

Similar trends have been observed in secondary endpoints of serological titers reduction and conversions of serology responses to negative in both children and adults. Incidence of new ECG abnormalities have been lower in frequency in children from randomized controlled trials in extended follow-up up to 9 years and also in adult trials.

7.1.3. Subpopulations

There are no trials conducted in renal or hepatic disease. No information is available from any of these or other subpopulations

7.1.4. Dose and Dose-Response

There were no formal studies of dose response. The initial dose and duration were selected based on a mouse model in which absence of parasites was demonstrated only after 60 days of treatment (Brener, 1961). Several trials in adults have used 30 days of treatment due to some evidence (Cancado, 2002) that a total dose exceeding 18 g was associated with neurological side effects, most frequently peripheral neuropathy, which has been reversible though in some cases it takes up to 10 months to resolve after discontinuation of therapy. On randomized study in children with congenital disease showed no differences in the rate of conversions of serology to negative after 1 year when comparing 30 vs 60 days of treatment (Chippaux, 2010)[120].

7.1.5. Onset, Duration, and Durability of Efficacy Effects

The evaluation of efficacy of benznidazole treatment takes several years and is proportional to the age of the patient. In young children serological cures can be observed within 3-7 years depending on the age. In adults, it usually takes decades. Once a pattern of decreasing serological titers over time has been observed, no changes in the opposite direction, (i.e., progressively increasing titers over time) have been described in two randomized controlled studies in children and in at least four long

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term prospective and controlled trials. Progressive increases in titers could indicate a reinfection, since serological responses do not confer effective immunity to new infections. Treatment interruption due to adverse events, occurring more frequently in adults in rates of 12 to 18%, could decrease its efficacy. Some limited amount of data is available from a study in patients who did not complete treatment. In a cohort of 81 adult patients[129] with three positive tests for *Trypanosoma cruzi* infection and serological monitoring following incomplete treatment with benznidazole for a median of 10 days, 20 percent of these patients (16/81) met the criteria for serological cure, suggesting that it may be possible for some patients to obtain some benefit from a shorter treatment. In another small retrospective cohort with 10 year follow-up, in which 30% of the patients did not complete treatment (Lauria Pires, 2000)[130], benznidazole treatment did not apparently prevent new ECG changes. However, the baseline ECG characteristics of these patients were not described in the publication, and the study is a retrospective review.

7.2. Additional Efficacy Considerations

7.2.1. Considerations on Benefit in the Postmarket Setting

The trials supporting the efficacy of benznidazole were conducted in endemic countries, and it is presumed that most of the patients had acquired the disease through vector transmission. In non-endemic countries, the most common routes of transmission are congenital and through blood products and organs transplantation. Less common forms would be vector transmission and laboratory accidents. However, no major differences are expected in the response to treatment according to these forms of transmission. The majority of the persons that will receive treatment in the US are immigrants who acquired the disease in their endemic countries. The same geographic variability in the rates of efficacy is expected in this population, just as it is in their countries of origin.

Although an estimated 300,000 persons with Chagas disease live in the United States, little is known about the burden of chagasic heart disease. There is no required reporting of Chagas disease nationwide or frequent routine work-up for this diagnosis; therefore the only estimates come from published case reports and requests for treatment that the CDC receives. An undetermined number of people in the US get diagnosed through blood screening when attempting to donate blood. A few others have acquired the disease through vector transmission, which is not expected to be a common route.

It is not known how often congenital or vector-borne transmission of *T. cruzi* occurs in the United States, although it is known that infected mothers and infected vector bugs are found in this country.

[129] Alvarez, M. G., et al. "Seronegative conversion after incomplete benznidazole treatment in chronic Chagas disease." *Transactions of the Royal Society of Tropical Medicine and Hygiene* 106.10 (2012): 636-638.

[130] Lauria-Pires, Liana, et al. "Progressive chronic Chagas heart disease ten years after treatment with anti-Trypanosoma cruzi nitroderivatives." *The American journal of tropical medicine and hygiene* 63.3 (2000): 111-118.

Approximately 63–315 babies acquire *T. cruzi* infection congenitally in the United States every year but most infections go undetected and untreated. If routine screening is implemented in specific populations in the near future, it is expected that women of childbearing age will be treated.

Based on these estimates of total number of infected people in the US, chagasic cardiomyopathy, which can be prevented through early treatment, affects approximately 30,000–45,000 persons in the United States. Screening programs directed towards immigrants of endemic countries would increase the number of treated patients in the young adult population.

With the blood bank screening for Chagas disease in blood donors, implemented during the last decade, it is expected that progressively more cases will be diagnosed and treated.

7.2.2. Other Relevant Benefits

This section does not apply to benznidazole.

7.3. Integrated Assessment of Effectiveness

Integrated Assessment of Effectiveness

The submitted evidence in support of the effectiveness of benznidazole has met the statutory evidentiary standard of substantial evidence of effectiveness. Evidence consisting of three adequate and well controlled clinical studies, one conducted in adults and two in children, for which the Applicant submitted patient-level data, supported by publications of similar studies reporting clinical outcomes, and additional in vitro and in vivo studies demonstrating parasiticidal activity of benznidazole allows me to conclude that benznidazole is effective in treating Chagas disease, reducing the progression of cardiac disease, reducing its mortality rate and improving survival.

The evidence of efficacy in children in the intermediate phase is based on two adequate, randomized and placebo controlled clinical studies, of a total of 130 (De Andrade study) and 106 (Sosa Estani study) participants, respectively, diagnosed with three positive serological conventional assays for Chagas disease (IHA, IFA, ELISA), done in children ages 6 to 12 years. A total of 55 children were randomized to treatment with benznidazole orally at 5 mg/kg/day for 60 days in one study and 64 in the other study received 7.5 mg/kg/day for 60 days, respectively. The primary efficacy endpoint was the proportion of children seronegative against *T. cruzi* at the end of the 4 year follow-up period through enzymatic immunoassay (EIA/Conventional ELISA) against *T. cruzi* in the Sosa Estani study. The primary efficacy endpoint in the De Andrade study was the proportion of children with negative seroconversion, assessed by ELISA at 3 years follow-up. Both studies used, in addition to conventional ELISA, another ELISA test as a secondary endpoint, targeted to specific trypanosomal antigens, the F-29 and the AT-ELISA, in the Sosa Estani and the De Andrade studies, respectively. The F29 and AT antigens represent antigens from the flagellum of the epimastigotes and trypomastigotes, respectively, of *T. cruzi* and may be a reflection of antibody response to live trypanosomes. Analyses in experimental models have corroborated this hypothesis. Prolonged follow-up of treated patients in

small cohorts have shown that the negative seroconversion of the F-29 and AT ELISA precede the negative seroconversion of conventional serological assays, which are the gold standard of cure. Both the Sosa Estani and the De Andrade studies showed that benznidazole treatment for 60 days decreases the proportion of subjects that remain seropositive as well as decreasing the antibody titers against *T. cruzi* as measured by the ELISA conventional assay, especially against the recombinant AT and F-29 antigens within 3 and 4 years after treatment, respectively.

In the De Andrade study, 4 of 64 (6.25%) treated subjects and 0 of 65 (0%) subjects in the placebo group had negative seroconversion by the conventional ELISA (p=0.0577), and 35 of 64 (54.7%) subjects and 3 of 65 (4.6%) subjects had negative seroconversion measured by the AT ELISA (p=<.0001). There was a significant decrease in serological titers of the conventional ELISA at the 36 month follow-up (mean difference -0.673 95% CI [-0.861, -0.484], p=<.0001.

In the Sosa Estani study, 24 of 40 (60.0%) and 5 of 37 (13.5%) had negative seroconversion by F-29 ELISA at the end of the 48 month follow-up, p=<0.001. A significant reduction of titers was observed in the conventional ELISA (mean difference -1.60 95% CI [-0.201, -0.119].

At the end of the follow-up period, a higher number of subjects with negative seroconversion was observed in the treated group as compared with the placebo group at the end of the follow-up period. In the Sosa Estani, 7 of 53 (13.2%) treated and 2 of 50 (4%) placebo subjects had negative seroconversion by any of the three conventional assay and 14 of 64 and 0 of 60 subjects in the De Andrade study had negative seroconversion by any of the three conventional serological tests (p=<0.0001). The significant decrease in titers and the higher number of negative seroconversions by conventional tests are evidence of a reasonably likely evolution towards a cure, since a progressive trend of decreasing titers and conversions of serology to negative was observed throughout the follow-up period, in only one direction.

Electrocardiographical (ECG) changes characteristic of Chagas cardiomyopathy were evaluated in these two studies. At baseline, 6 of 64 and 7 of 65 patients had electrocardiographic changes at baseline in the De Andrade study. At the end of the follow-up period, ECG readings showed one (1·7%) incident case of complete right bund le branch block in the benznidazole group and four (6·9%) in the placebo group (p=0·36). Even though the incidence of new ECG changes did not reach statistical significance, a trend towards more ECG changes in the placebo group was noted.

In the Sosa Estani study, baseline ECG examinations showed some abnormalities characteristic of Chagas disease (left anterior hemiblock or right bundle branch block level II) in 5.0% of the benznidazole treated group and 4.8% of the placebo group ($P > 0.05$). After 48 months, changes in the ECGs were detected in 2.5% (1 of 40) of the benznidazole treated group and in 2.4% (1 of 41) of the treated group ($P > 0.05$). The small number of cases precludes any conclusion regarding incident ECG changes in the Sosa Estani study.

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Additional follow-up published for the Sosa Estani and De Andrade studies

After 9 years (108 months) follow-up, 77% of the treated children from the Sosa Estani study presented negative conventional serology (ELISA, IFA), 88.2% were in the 5-9 year age bracket and 69% in the age bracket between 10 and 14 years of age. The durability of response was assessed at a 6 year follow-up in another study (De Andrade 2004). In this study, 53 (82.8%) of 64 participants allocated in the treatment group were evaluated and 46 (70.8%) of 65 from the placebo arm completed the follow-up 72 months post-randomization. A total of 33 of the 37 children who had received benznidazole chemotherapy and were seronegative after three years follow-up had remained seronegative after six years, whereas 14 of 21 children shifted from seropositive to seronegative, resulting in a total of 47 cases with negative seroconversion and 6 with seropositive response at 6 years follow-up.

In the placebo arm, of 52 individuals who were seropositive after three years of follow-up, 32 remained positive after six years, whereas 11 showed seroconversion.

The median A-T ELISA value for the benznidazole-treated group was 0.151 (negative result), while it was strongly positive (1.997) for the placebo group after the six-year follow-up.

The efficacy of benznidazole was calculated as 100 × (1 - RR), where RR is the ratio of children with negative seroconversion in the benznidazole group to the corresponding number of children in the placebo group. The per-protocol efficacy of benznidazole was 84.7% (95% CI 66.8–92.9) and in the ITT analysis, it was 64.7% (95% CI 50.2–78.7).

Consistency between the decrease of antibody titers and significantly lower *T. cruzi* parasitemia measured by a polymerase chain reaction in the benznidazole group (39.6%) compared with the placebo group (64.2%) was demonstrated three years after treatment in another study done with this same patient population from the De Andrade study (Galvao 2003).

Evidence of activity in the chronic phase

The direct evidence of clinical benefit is based on one adequate and well controlled study in adult patients, ages 18 to 50 years, treated with oral benznidazole at 5 mg/kg/day for 30 days. The primary outcome of this prospective, non-randomized, well controlled study was a change from a lower to a more advanced Kuschnir group or cardiac death (change of clinical group to a more severe one). Secondary outcomes were the appearance of new abnormalities on electrocardiography, persistence of 3 positive results on serologic evaluation, or complete negative seroconversion on the last serologic test done for each patient. Fewer patients in the treated group progressed to a more severe clinical Kuschnir group (12 of 283 patients [4.2%]) compared with those in the untreated group (41 of 283 patients [14.4%]; adjusted HR, 0.24 [95% CI, 0.10 to 0.59]; p = 0.002). The clinical group changed in 6 of 180 treated patients (3.3%) and 13 of 180 untreated patients (7.2%) in Kuschnir group 0, 3 of 73 treated patients (4.1%) and 14 of 75 untreated patients (18.7%) in Kuschnir group I, and 3 of 30 treated patients (10%) and 14 of 28 untreated patients (50%) in Kuschnir group II. These outcomes

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show that the progression to a more severe Kuschnir group was lower for the treated subjects across all of the Kuschnir categories of severity.

The mortality rate during the follow-up period was lower in the treated group (3 of 283 patients [1.1%]) than in the untreated group (12 of 283 patients [4.2%]). Sudden death was the most common cause of death overall, occurring in 9 of 15 (60%) patients, followed by heart failure and/or a combination of both. The frequency of death paralleled the patients' Kuschnir grouping: 2 patients (13.3%) were in Kuschnir group I, 5 patients (33.3%) were in group II, and 8 patients (53.3%) were in group III.

Benznidazole-treated patients had a mean of 13.57 years until death and in untreated patients, mean time until death was 6.23 years. This was a significant difference in survival. Baseline age and sex distribution were comparable.

Complete seronegative conversion was more frequent in treated patients than in untreated patients (32 of 218 treated patients [15%] vs. 12 of 212 untreated patients [6%]; adjusted HR, 2.1 [CI, 1.06 to 4.06]; p = 0.034).

None of the patients who achieved complete negative results on serologic testing changed clinical group during the follow-up, regardless of treatment. Thus, this study showed that conversion to negative of Chagas conventional serology paralleled improvement in clinical cardiac outcome, and significantly fewer deaths (heart failure and sudden death) occurred in the treated group.

In addition to the Viotti 2006 study, for which the sponsor submitted patient-level data, other three published, prospective and controlled, non-randomized studies of benznidazole conducted in adult patients without heart failure, which report clinical outcomes in long term follow-up (Viotti 1994[131], Gallerano 2000[132], Fabbro 2007[133]). Even though some details are not available from the publications, because of the baseline comparability of treated and untreated patients and similar, prolonged follow-up evaluations, these trials provide supportive evidence of incidence of clinical events in treated and untreated patients over more than a decade of follow-up. They showed a decreased incidence of EKG changes associated with Chagas cardiomyopathy and a decrease of progression to more severe Kuschnir categories in the treatment group. The baseline characteristics were comparable in treated and untreated groups, with similar length of follow-up in treated and untreated groups. A summary of the clinical outcomes from these studies is shown in the table below.

[131] Viotti R, Vigliano C, Armenti H, Segura E.*: Treatment of chronic Chagas' disease with benznidazole: clinical and serological evolution of the patients with long term follow-up. Am Heart J. 1994 Jan;127(1):151-62.*
[132] Gallerano R, Sosa R, *Study of intervention in natural evolution of Chagas's disease, Evaluation of specific anti-parasitic treatment. Rev Fac Cien Med Univ Nac Cordoba. 2000;57(2):135-62*
[133] Fabbro D, Streiger M, Arias E, Bizal M, del Barco M, Amicone N: *Trypanocide treatment among adults with chronic Chagas' disease living in Santa Fe City (Argentina), over a mean follow-up of 21 years: parasitological, serological and clinical evolution.* Rev Soc Bras Med Trop. 2007 Jan-Feb;40(1):1-10.

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Publication	Treatment Arms, N		New ECG changes		Heart disease progression		Serology reversion to negative	
Author/year	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Viotti 1994	131	70	7/131 (5.3%)	16/70 (22.8%)	8/70 (2.3%)	3/131 (11%)	21/110 (19.1%)	3/50 (6%)
Gallerano 2000	535 (130benz)	668	5/130 (3.8%)	113/668 (16.9%)	5/130 (3.8%)	113/668 (16.9%)	3/130* (3.9%)	0/668 (0)
Viotti 2006	283	283	15/283 (5%)	45/283 (15%)	12/283 (4%)	41/283 (14%)	32/218 (15%)	12/212 (6%)
Fabbro2007	54 (27 benz)	57	2/54 (3.7%) (2/27 benz)	9/57 (15.7%)	2/54 (3.7%)	9/57 (15.7%)	20/54* (37%) (9/27 benz)	0/57 (0)
Total	**1003**	**1078**						

In addition, Fragata Filho et al. (1995) reported, in a study with 120 chronic patients from Brazil with follow-up for 7-8 years, ECG evolution in 7% for Bz-treated cases (n = 71) and 14.3% for the untreated group (n = 49).

All these studies also show that seronegativization and reduction of titers (seroreduction reported in two of the four studies) are associated with reduced heart disease progression.

The complete conversion to negative of conventional serology has also been observed in mice and dogs. Overall, the studies suggest that treatment with benznidazole is effective in decreasing parasitic load in blood and tissues including heart, improving survival and decreasing antibody response to *T. cruzi* antigens. The efficacy of benznidazole has been demonstrated in dogs, mice and rabbits, which reproduce the human disease, in acute and chronic Chagas disease models. In summary, the consistency of the level of therapeutic efficacy observed for benznidazole, measured by more than one type of conventional assays, and the consistent correlation of negative seroconversion with better clinical outcomes in these studies supports the efficacy of benznidazole in the treatment of chronic Chagas disease. Serological conversion to negative as measured by conventional assays is the endpoint reported in several uncontrolled, open-label and long term follow-up cohorts. The outcomes reported in these studies provide additional evidence of consistent treatment effect across different populations.

Evidence against efficacy in adults with chronic disease

This was a prospective, multicenter, randomized study (BENEFIT)[134] involving 2854 patients with Chagas' cardiomyopathy who received benznidazole or placebo for up to 80 days and were followed

[134]Morillo, Carlos A., et al. "Randomized trial of benznidazole for chronic Chagas' cardiomyopathy." *New England Journal of Medicine* 373.14 (2015): 1295-1306.

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for a mean of 5.4 years. At baseline, 74.4% of participants were in the Class I category of the NYHA, with a mean ejection fraction of 55%. The primary outcome in the time-to-event analysis was the first event of any of the components of the composite outcome of death, resuscitated cardiac arrest, sustained ventricular tachycardia, insertion of a pacemaker or implantable cardioverter–defibrillator, cardiac transplantation, new heart failure, stroke, or other thromboembolic event. The rates of conversion to negative PCR results (PCR conversion) were 66.2% in the benznidazole group and 33.5% in the placebo group at the end of treatment, 55.4% and 35.3%, respectively, at 2 years, and 46.7% and 33.1%, respectively, at 5 years or more (P<0.001 for all comparisons). The effect of treatment on PCR conversion varied according to geographic location and the time of measurement (a decrease at 2 years, followed by a subsequent increase 5 years after treatment). The rates of PCR conversion did not correspond to effects on clinical outcome (P = 0.16 for interaction). Unfortunately, no serological evaluations were collected and reported from this trial, and there are no other currently available data to allow for a comparison of parallel serological and PCR responses in relation to clinical outcomes.

Several important differences in the baseline characteristics of the BENEFIT study patients were noted when compared with those of the Viotti study. The Viotti study subjects had a mean age of 39 years and two-thirds had normal cardiac function at baseline. In contrast, the BENEFIT trial study patients had a mean age of 55 years, all had cardiac damage on the basis of electrocardiographic abnormalities, and nearly one-half had decreased ejection fraction at baseline, indicating ventricular dysfunction. These results suggest that older age and established cardiac dysfunction limit the efficacy of benznidazole treatment, for whom it should probably not be indicated. Patients who had a left ventricular ejection fraction of less than 40% accounted for 63% of the primary outcome events in both the benznidazole group and the placebo group.

A table comparing baseline characteristics in both studies, illustrating important differences in baseline evidence of cardiac disease is presented blow.

Table 46: Baseline characteristics of patients enrolled in Viotti and Morillo's BENEFIT trials

Baseline characteristics – ITT Population, treated patients	Viotti 2006	Morillo 2015
N	283	1431
Age (mean)	39.3	55.4
Mean left ventricular ejection fraction	66.6%	54.4%
< 40%	6/566 (1.1%)	389/2247 (17.3%)

Normal ECG	136 (48.1%)	96 (6.7%)
Atrial Fibrillation	3/566 (0.5%)	197/2854 (6.9%)
Sinus bradycardia < 50 beats/min	14/566 (2.5%)	320/2683 (11.2%)
NYHA Class I or II	27/566 (4.8%)	2780/2852 (97.5%)
NYHA Class III	0 (0%)	39 (2.7%)
Implantable pacemaker	6 (1.1%)	205 (14.3%)
Receiving antiarrhythmic medication	46 (8.1%)	284 (19.8%)

Although this study was a large trial involving patients with established Chagas cardiomyopathy, it is possible that it could have missed small differences in risk (e.g., a relative risk reduction of 10%). The 95% confidence intervals in this study analyses rule out a relative risk reduction of 20%, which is smaller than the difference that the study was designed to detect. The outcomes do not show a trend towards the opposite direction, favoring placebo. It is still possible that a 10% benefit might have been undetected, and if present, this could mean some clinical benefit for a proportion of the large population of patients with Chagas disease and no other treatment option.

Evidence of effectiveness in acute, reactivated and congenital disease

The totality of the evidence from animal and human studies shows that treatment with benznidazole is most effective when administered early in the course of the infection, when parasitemia is highest and the seeding of tissues is occurring. A shortening of the duration of parasitemia in the early phase reduced the total tissue burden in animal models of mice, rabbits and dogs. A total of 272 patients with acute Chagas disease from Brazil, Argentina and Venezuela, ages 0 to 64 years, treated with benznidazole in doses of 5 to 10 mg/kg/day in single cohorts followed for a variable range of time up to 21 years showed cure rates measured by conventional serological methods of 66.7% to 100%. This decrease was also observed in parasitemia observed by direct parasitological methods used by most studies, showing a decrease in the rate of and a shortened duration of it in treated patients. Data on electrocardiographical findings were available for 3 studies, the efficacy rates ranging from 0 to 50.0%, with a mean weighted percentage of 36.4 (95%CI 16-56). Echocardiographic findings were available in 2 studies, the improvement/efficacy a mean weighted percentage of 41.7 (95%CI 14-70). These independent single cohorts, from three countries, although small, show that benznidazole treatment is able to prevent cardiac injury from trypanosomes.

Reactivation of Chagas disease in HIV and transplant patients

HIV infected and transplant patients infected with *T. cruzi* are at risk of reactivation of Chagas disease. Approximately 20% of HIV–*T. cruzi* infected patients experience reactivation; manifestations include meningoencephalitis and/or myocarditis. Central nervous system reactivation is associated with a case-fatality rate of 79–100%. In the larger Brazilian cohort, two of 11 patients with reactivation were treated with benznidazole and survived to the end of the follow-up period. Although the evidence is based on case reports and case series, the high fatality rate of reactivation of Chagas disease and the known parasiticidal activity of benznidazole support its use in acute reactivated disease.

Limited data suggest that the rate of congenital *T. cruzi* transmission is higher for HIV-coinfected women, even in the absence of reactivation, than for immunocompetent mothers (Sartori *et al*, 2007 and Scapellato *et al*, 1995). Infants co- infected with HIV and *T. cruzi* may also be more likely to have symptoms, especially neurologic symptoms. Case reports show serological cures and improved survival, including a case of an HIV-infected mother treated while pregnant, with successful outcome reported for her and her baby (Bissio *et al*, 2013)

Acute Chagas disease

Serological negativization responses occurred in more than 90% of children with congenital infections treated during the first year of life.

Cançado *et al*. (2002) monitored a group of 21 patients with acute infections between 7 months and 60 years old, for a period of 13 to 21 years, treated with benznidazole between 1974 and 1982, with therapeutic schemes varying from 5 to 10 mg/kg/day, taken two or three times a day, for periods varying between 30 and 60 days. During follow-up period of 13 years, 16 (76%) of the patients presented negative serology tests (IHA, IIF and ELISA) and were defined as cured. The other five patients presented persistently positive tests, indicating treatment failure.

Ferreira *et al* (1988) reports a 100% seroconversion observed at 15 years of follow-up in 17 children 2 to 18 years of age.

Congenital disease

In a randomized controlled study conducted by IRD (2008-2009) in Bolivia of 111 newborns with congenital infections diagnosed at birth through direct microscopic observation (microhematocrit [MH]), 58 children were treated from the first day of life with benznidazole 2.5 mg/kg/day administered twice a day for 60 days, and 52 children with benznidazole 7.5 mg/kg/day administered once a day for 30 days. In order to compare the reduction in anti *T. cruzi* antibody titers, two other study arms included 68 newborns with seropositive mothers who did not present parasitemia at birth, and 78 newborns with seronegative mothers. Blood samples were taken at 30 and 60 days and 10 ± 2 months and, with seropositive ELISA testing after ten months, every two months until seroconversion. All parasitology tests (MH) showed negative results before the ninth month. After ten

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months of follow-up, ELISA was negative for 90.7% of the children treated (98/108) with all the seropositive tests becoming negative after 16 months, indicating a response to treatment. No significant difference was noted in the proportion of children presenting seroconversion after ten months between the groups treated with different therapeutic schemes (p=1.0), nor among the average figures for the antibody titers.

In the study conducted by Blanco *et al*. (2000) in Argentina, 32 children born to infected mothers and diagnosed as positive up to six months old through parasitology (MH) and serology tests (IHA, ELISA [IgG and IgM antihuman] and IIF in case of IgG serology findings) were treated orally with Nx at 10 mg/kg per day, two or three times a day for 60 days (29 children) or with benznidazole, 5 mg/kg per day, two or three times a day for three days (3 children presenting adverse reactions to Nx). The 32 children treated presented negative parasitology (MH) tests when examined between 6 and 24 months after the end of treatment, and 30 of the 32 children presented seronegative tests, indicating response to treatment of congenital infections, regardless of the medication administered.

In the study conducted by Russomando *et al*. (1998) in Paraguay, six children born from infected mothers and diagnosed as positive through direct microscopic observation (MH) and/or hemoculture (Hc), IIF, ELISA and the PCR technique, were treated with benznidazole, 7 mg/kg per day, taken twice a day for 60 days. Treatment began at different times: two babies were treated at birth, and four others began treatment at between 3 months to 22 months after birth. After 24 months of follow-up, all babies presented negative results in the parasitology tests (Hc, MH, PCR) with negative seroconversion by IIF and ELISA. Seroconversion occurred at between two months and eight months after the end of the treatment, suggesting better outcomes for early treatment.

In the prospective study conducted by Schijman *et al*. (2003) in Argentina, 40 of the 152 children born to mothers seroreactive for *T. cruzi*, diagnosed by MH and PCR (50 children between 0 and six months old) and through serology (IHA, ELISA) and PCR (102 children between seven months and 17 years old), were treated with Nx, 10-15 mg/kg/day or benznidazole, 5-8 mg/kg/day in two doses per day for 60 days. The efficacy of the treatment was assessed by age group, and the treated children were monitored for 36 months after the end of the treatment. No differences were observed in clinical and serological results, nor on the PCR testing between the two treatment groups. 100% of children between 0 to 3 months old age (10 children), and 66.7% of children between seven months to two years old (6 children) were considered cured based on the serology test (negative for IgG anti *T. cruzi a*ntibodies), while for the age group more than 3 years old (24 children), cures were documented in only 12.5% of the cases during the study observation period (*P* = 0.023). It was also noted that seroconversion occurred faster in children who began treatment during the first few months of life, indicating the possibility of greater efficacy for early treatment, despite difficulties in determining cures for patients in the indeterminate phase, as conventional serology remains positive for many years after treatment.

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Additional evidence indicating effectiveness outcomes of public health interest

Observational studies have also confirmed that women treated before pregnancy are significantly less likely than untreated women to transmit the infection to their off- spring, which provides additional support for the treatment of girls and non-pregnant women of reproductive age.

An observational study (Fabbro *et al* 2014) evaluated the efficacy of trypanocidal therapy to prevent congenital Chagas disease and compared the clinical and serological evolution between treated and untreated infected mothers. This was a multicenter, observational study on a cohort of mothers infected with T. cruzi, with and without trypanocidal treatment (N=72 treated with benznidazole) before pregnancy. Their children were studied to detect congenital infection. Among 354 "chronically infected mother-biological child" pairs, 132 were treated women and 222 were untreated women. Among the children born to untreated women, investigators detected 34 infected with T. cruzi (15.3%), whose only antecedent was maternal infection.

Among the 132 children of previously treated women, no infection with T. cruzi was found (0.0%) ($p<0.05$). Among 117 mothers with clinical and serological follow up, 71 had been treated and 46 were untreated. The women were grouped into three groups. Group A: 25 treated before 15 years of age; Group B: 46 treated at 15 or more years of age; Group C: untreated, average age of 29.2 ± 6.2 years at study entry. Follow-up for Groups A, B and C was 16.3 ± 5.8, 17.5 ± 9.2 and 18.6 ± 8.6 years respectively. Negative seroconversion: Group A, 64.0% (16/25); Group B, 32.6% (15/46); Group C, no seronegativity was observed. Clinical electrocardiographic alterations compatible with chagasic cardiomyopathy: Group A 0.0% (0/25); B 2.2% (1/46) and C 15.2% (7/46). The trypanocidal treatment of women with chronic Chagas infection was effective in preventing the congenital transmission of Trypanosoma cruzi to their children; it had also a protective effect on the women's clinical evolution and deparasitation could be demonstrated in many treated women after over 10 years of follow up.

Similar findings were reported in a cohort of 394 female patients treated in Buenos Aires, Argentina, in which 15 women had pregnancies (16 children) and were included in a follow-up single cohort study. No congenital transmission was observed in these children. During post-treatment follow-up, a 34% decrease in antibody titers was observed as measured by conventional ELISA and a 30.5% decrease was measured by IHA compared to the baseline value (i.e., when diagnosis was done). Negative seroconversion was observed in one patient (6.7%). The estimated congenital infection rate observed in this region is 4-10%. The findings of this cohort reinforce previously published data suggesting the benefits of etiological treatment in women of childbearing age to prevent congenital transmission, which is an important route of infection.

Benznidazole treatment of infected women of childbearing age not only has the potential to reduce morbidity and mortality caused by heart disease and digestive disorders in those patients, but it can also be an effective strategy to prevent congenital transmission of *T. cruzi*, the main route of transmission of Chagas disease in areas with no vector transmission, such as the United States.

Another longitudinal cohort study evaluated the congenital transmission of Chagas disease (CD) in a nonendemic area, in Spain[135]. The offspring of 59 seropositive pregnant mothers were followed up. Nine newborns were found to have acquired the disease congenitally. Thus, among seropositive mothers, the congenital transmission rate was 13.8% (9 infected newborns of 65 live births). All the infected children were born to Bolivian mothers. A total of 10 of these women had received treatment before pregnancy. No infected infants were detected among 10 mothers who were treated before they became pregnant, compared with 16.4% (9 of 55 live births) among untreated mothers.

Infants and mothers were orally treated with benznidazole, at 10 mg/kg body weight per day in newborns and 100 mg 3 times a day in women for 60 days. In cases of congenital transmission, hemoculture and parasite lineage typing were performed. This represents a transmission rate of 13.8% among seropositive mothers (9 infected newborns of 65 total live births). Treatment before pregnancy prevented congenital infection, confirming again the findings of previous studies.

Conclusions

The outcomes of multiple clinical studies on the antiparasitic treatment of Chagas disease patients support the benefit of treating the patient as early as possible: the rate of benznidazole-induced serologic negativization decreases as patients age, as do the odds of ultimately achieving negativization. This is further supported by preclinical and clinical evidence on the pathophysiology of the disease, which shows that a decrease and shortening of the duration of parasitemia decreases tissue burden and diminishes the likelihood of inflammation and fibrosis in the heart to a greater extent when the parasiticidal drug is given early in the course of infection. Once the heart damage is established, the effectiveness of benznidazole is much less clear and probably non-existent. Benznidazole therapeutic effect is strongly associated with serological cure (negativization of all serological tests that were positive at diagnosis).

The three adequate and well controlled trials submitted by the Applicant in support of clinical effectiveness of benznidazole, supported by preclinical efficacy and findings from additional long-term follow-up cohorts, constitute substantial evidence of clinical benefit for children and adult populations. To confirm the predictability of the surrogate serological endpoints, based on responses to recombinant antigens, used in the two randomized controlled studies in children (Sosa Estani and De Andrade), and to provide further evidence of comparability of older versions of conventional assays used in these studies with newer serological methods approved by FDA, a Subpart H expedited approval is recommended for children ages 2-12 years. (b) (4)

[135] Murcia, Laura, et al. "Risk factors and primary prevention of congenital Chagas disease in a nonendemic country." *Clinical Infectious Diseases* 56.4 (2012): 496-502.

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Treatment of Chagas disease in acute and reactivated disease is recommended, considering the totality of the evidence of parasiticidal action and the context of high mortality of untreated disease in this setting (8 to 35% mortality in oral outbreaks and up to 90% in reactivated disease in immunosuppressed patients). (b) (4) Chagas disease is a neglected tropical disease with no currently approved treatment. It is a serious life-threatening disease associated with increased mortality in young patients, and especially in immunocompromised patients. It represents a serious unmet medical need for a seriously neglected tropical disease. Because there is no method to distinguish and predicting which patients will be affected with cardiac disease in a long term follow-up of up to several decades, and the difficulties in establishing the presence of heart disease (and the reversibility of it) at an early stage, I believe treatment should be recommended in all cases as early as possible, since parasiticidal treatment may be life-saving for many, decreasing the blood and tissue burden. The uncertainty about true efficacy is given by the inherent variability of the disease in several regions, and several unknown factors that are not able to confirm in most cases (amount of exposure, route/s and time of infection and host response factors influencing the disease course). These limitations may not be overcome because of the nature of the disease and the inability to quantify tissue burden in a systematic way across populations. Randomized controlled trials with untreated control groups are not ethically feasible since drugs are approved for use in endemic countries, where most patients reside. A clinical trial design including clinical endpoints would require several decades of follow-up. Considering these feasibility hindrances, and the evidence of benznidazole parasiticidal activity and demonstrated clinical benefit on the progression of disease and mortality, (b) (4)

8 Review of Safety

8.1. Safety Review Approach

The review of safety will include primarily the two randomized and controlled studies in children and the supportive data from adult and children studies done with the Radanil product (Altcheh and Viotti 2006). The latter two (Altcheh and the Viotti studies), provide supportive evidence even though the data are not compared to a control arm. The Altcheh study was an uncontrolled cohort (n=38), designed to explore pharmacokinetics endpoints, and the Viotti study, even though it was controlled, monitored adverse events only in the treatment arm, and the database contain only information about frequency of adverse events, which are briefly discussed in the publication, and in another published comprehensive safety review of 1047 patients with long term follow-up. Since benznidazole has been available for more than four decades, the review of published literature on safety data, mainly from cohorts with a comparator and adequate follow-up data, and single cohorts with prospective data collection will be reviewed and summarized. Despite the variability of dose and

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treatment regimens and some lack of details regarding the methodology of safety evaluations, the high number of publications with long term follow-up and the consistent pattern observed in the safety profile described, provide a source from which to derive useful prescribing information. The safety profile described in publications is also consistent with what is known about benznidazole drug class, the nitroimidazoles.

The DNDi adult studies were reviewed, even though the product used in these two studies used the LAFEPE benznidazole product. These studies are small and only one of them has a control arm. Their review focused in the presence and consistency of findings described in the other studies, and any other potentially safety signal. Therefore, they provide additional support to safety conclusions and will contribute to the knowledge of the overall safety profile of benznidazole.

8.2. Review of the Safety Database

8.2.1. Overall Exposure

Benznidazole has been approved since 1972 and has been in clinical use since then for treatment of Chagas disease in all endemic countries in Latin America. The adverse events profile is described in the literature. Randomized controlled trials are few, and most of the information comes from prospective controlled or single cohorts followed for several years. The information from published studies in the literature comes from trials done with the Roche (Radanil or Rochagan) product. The Applicant has provided data comparing their product to the Roche product, however, an adequate comparison with the benznidazole manufactured by LAFEPE has not been submitted. The exposure to the drug product manufactured by the Applicant is limited to 36 (19 males, 17 females) healthy subjects from two bioequivalence and pharmacokinetics studies.

The Applicant has submitted datasets derived from 6 clinical trials. Four of these trials (DNDiPK , Altcheh, Sosa Estani and De Andrade) were conducted in children, ages 0 to 14 years. Two of these trials, randomized and controlled conducted with the benznidazole manufactured by Roche, include a total of 119 exposed subjects. These are the main source of safety data in the pediatric population. An additional uncontrolled PK and safety study in children with the benznidazole product manufactured by Roche included 38 exposed children ages 2 to 12 years. A total of 157 subjects in the pediatric population ages 2 to 12 years were exposed to the benznidazole manufactured by Roche, for which the Applicant has an adequate bridge.

Including the trial done with the LAFEPE product, the DNDPK BNZ study (N=81), for which the Applicant has not provided an adequate bioequivalence, the total number of subjects exposed to benznidazole is 238.

Reference ID: 4134724

Of the adult trials, the Applicant submitted one randomized placebo-controlled trial, the DNDi1224 study, where a total of 47 subjects were exposed to benznidazole, and one uncontrolled trial, the Molina study, where a total of 26 patients were exposed to benznidazole, both conducted with the benznidazole manufactured by LAFEPE.

After the initial filing, the Applicant submitted an additional controlled study in adults with patient-level data (the Viotti study), totaling 283 adult subjects exposed to benznidazole, manufactured by Roche.

Summarizing the total number of subjects with exposure to benznidazole manufactured by Roche in patient-level datasets provided by the Applicant, there are a total of 157 subjects ages 2 to 14 years, of which 119 subjects are from randomized, placebo controlled trials (Sosa Estani and De Andrade) done with the benznidazole from Roche, and 38 subjects are from one uncontrolled pharmacokinetics and safety study. In the adult safety database there are a total of 283 subjects ages 30 to 50 years exposed to benznidazole manufactured by Roche in a controlled trial (Viotti). A total of 36 subjects (healthy volunteers) were exposed to the benznidazole manufactured by the Applicant (the marketed-to-be product). No other studies using the benznidazole from Roche in adults were submitted with patient-level datasets. The rest of the safety information is described in other controlled studies with long term follow-up in published literature.

Safety data from these trials, prioritizing the findings from controlled trials, and especially those conducted with the product for which the Applicant has presented adequate comparability, will be presented and discussed.

Safety Database for the Individuals exposed to Benznidazole[1] in this development program for the indication under review Total N= 476		
Clinical Trial Groups	New Drug (n= 476)	Placebo (n= 399)
Normal Volunteers[2]	36	0
Controlled trials conducted for this indication[3]	402	399[4]
All other than controlled trials conducted for this indication[5]	38	0

[1] *Benznidazole* means the drug product being considered for approval, which includes data from benznidazole manufactured by Roche® for which the Applicant has demonstrated an adequate bioequivalence.
[2] Benznidazole used is the to-be marketed product
[4] From this total, n=283 are untreated controls who did not contribute safety data in the Viotti study
[3, 4] to be used in product's labeling

Reference ID: 4134724

Importantly, data from published literature, deriving from controlled prospective cohorts and uncontrolled cohorts with prospective follow-up and safety data collection will be reviewed and summarized.

8.2.2. Relevant characteristics of the safety population:

Patient-level data includes those from children ages 2 to 14 years and adult patients, from age 18 to 50 years. All these studies with patient-level data were conducted outside the United States, in Argentina, Brazil and Bolivia, where the disease is endemic. The gender distribution is balanced in these trials. No details on race distribution are provided in these studies. However, differences in race or country are not expected to affect the applicability of the study results to the US population. Furthermore, it is expected that a large proportion of patients who will receive benznidazole treatment in the US will be natives or descendants from Latin America.

In addition to the studies with patient-level data, there are numerous controlled studies and observational uncontrolled cohorts which report safety findings in patients from ages 0 to 50 years, and case reports and case series reporting safety data in individuals up to 73 years of age. Safety data available from controlled and uncontrolled cohorts in the literature are from at least 10 Latin American countries (Argentina, Brazil, Bolivia, Chile, Uruguay, Paraguay, Venezuela, Mexico, Honduras, Guatemala), from observational cohorts from the United States, from Spain, Switzerland, Italy, Sweden and Japan. Some of the published studies have data on individual patients characteristics, others only provide an overall description, without enough information on methodology used in data collection. There are no studies describing the use of benznidazole in patients with renal or liver disease. Other concomitant illnesses or treatments are not described in some publications. Despite the limitations, safety findings reported from different geographic areas by independent investigators are consistent in descriptions of adverse events types and frequencies.

8.2.3. Adequacy of the safety database

The totality of the available safety database, which includes patient-level data and published literature, is adequate to make an assessment of safety of benznidazole for the treatment of children (b) (4) with Chagas disease.

8.3. Adequacy of Applicant's Clinical Safety Assessments

8.3.1. Issues Regarding Data Integrity and Submission Quality

Overall, the Applicant has submitted an adequate number of published studies, which are the framework for the review (b) (4) patient-level data submitted.
The patient-level data submitted have limitations in the description of adverse events with enough

Reference ID: 4134724

details regarding specific characteristics, timing during the study and, in some cases, how resolution was confirmed. The frequency and the system organ class are described in all studies, however, for example, the preferred terms and characteristics of the adverse events in the De Andrade study were not provided. Some of the information lacking in the safety datasets is described in the corresponding publications. Other publications include a safety review of these studies presented with patient-level data. The Applicant's summary of safety does not include a detailed description of the most important adverse events causing withdrawals and/or interferences with daily activities. The safety summary does not adequately prioritize or summarize the safety information according to the studies' design (controlled, uncontrolled) and sample size. The Applicant's review of safety does not summarize the safety data across all studies to provide their own recommendation on monitoring adverse events to convey in the product label. An information request has been submitted and we are waiting for the response. Another deficiency noted in the Applicant's safety review is the lack of details regarding post-marketing data. Benznidazole has been available in endemic countries and the FAERS database contains reports, as well as other sources, for example the WHO database. This information is lacking in the Applicant's submission. There are no descriptions of the number of death reports associated with the use of benznidazole, only a general comment about the impossibility of assessing causality of deaths. No quantification of death reports has been provided. I present the information I found in FAERS and WHO databases, along with literature searches on adverse events associated with withdrawals, need special monitoring and interfere with daily activities (skin rashes, liver function, and neurological adverse events).

8.3.2. Categorization of Adverse Events

The submitted studies were conducted more than two decades ago and the source description of adverse events was not completely available to verify the verbatim term used. These studies were not designed or submitted before for registration purposes. The Applicant has used some source documents kept by the investigators and entered the data into AdAM format, using the MedDRA hierarchy. Despite these limitations, the data are adequate for review. These data are reviewed in the context of the available published literature from endemic countries, as well as from the United States and Europe, which show a good amount of description and similar patterns with respect to the type of adverse events and their relationship to treatment. In addition, the drug class to which benznidazole belongs to, the nitroimidazoles, has been well characterized and provides additional source of information that will be considered in labeling recommendations. In summary, the submitted trials with patient-level data are an important part of the evaluation of safety, within the context of what is known about benznidazole use in the published literature and the safety profile of the drug class it belongs to.

8.3.3. Routine Clinical Tests

Overall, the clinical evaluations in the studies submitted and several of those published in the available literature provide an adequate description, with some variability in the timing of the clinical and laboratory assessments in some studies. In studies submitted and in those published, investigators have monitored for laboratory toxicities throughout the treatment with hematological

Reference ID: 4134724

and chemistries (liver function tests and creatinine). The available information provided allows for an overall assessment, despite some limitations, on the clinical meaningfulness of the safety data evaluations.

8.4. Safety Results

8.4.1. Deaths

No deaths occurred in the two randomized controlled trials in children ages 6 to 12 years. Deaths in the Viotti study, discussed in Section 6, did not have a temporal association with benznidazole treatment. In the other adult studies submitted by the Applicant, no deaths were observed. Chagas disease is associated with significant mortality, and the patient deaths mentioned in some of the publications are not attributed to benznidazole administration but to the disease itself. This is an important confounding factor that limits the assessment of causality. None of the publications reviewed refer to benznidazole as the only factor directly associated to patients death.

A review of FAERS cases was conducted by Dr. Ana Szarfman, M.D., Ph.D., Safety Data Mining Developer and Medical Informatics Analyst, CDER, FDA, using search terms “benznidazole” OR “Abarax”, “Rochagan”, “Radanil” AND “Trypanosomiasis”. This search was performed on May 24, 2017 and revealed a total of 38 cases reported. Narratives were available from these, which included 4 cases published in the literature, one of them also describing findings of a pharmacovigilance study done in Spain. There were several duplicates. An additional consult was requested more recently from OSE and those results are commented below. There were some overlap of cases between these two searches, but the conclusions remain the same.

The pharmacovigilance study identified 4 subjects who were hospitalized with serious skin adverse reactions, classified as DRESS and AGEP. However, the publication provided no details on how the diagnoses were established (no biopsy reports), no clear description of the lesions or the underlying disease or concomitant medications. It is possible that these cases were indeed DRESS and AGEP, however, it is also possible that the symptoms and signs described were caused by, for example, acute Chagas disease. This is not known from the publication.

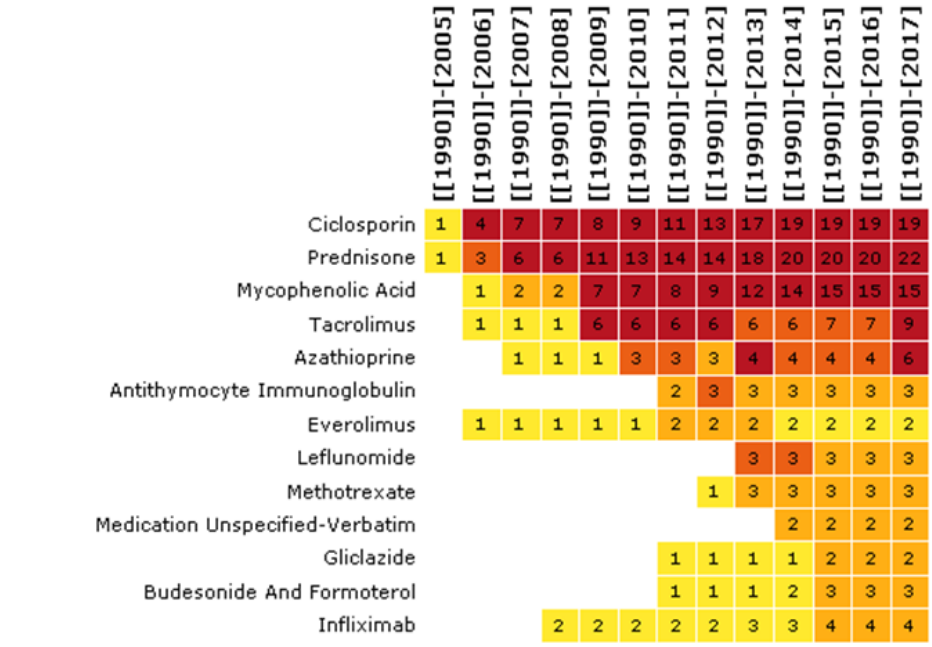
A review of the cases narratives showed that 36 of them were from immunocompromised patients, receiving heart, liver of kidney transplants. In all cases, reactivation of Chagas disease was confirmed. The use of benznidazole was concomitant to several other immunosuppressive medications and antimicrobial agents (cyclosporine, methylprednisolone, sirolimus, basiliximab, mycophenolate mofetil, trimethoprim-sulfamethoxazole,). The two narratives from non-immunocompromised patients described a case of a 6 year old boy with “acute liver failure”, with ascites and hypogammaglobulinemia, which was resolving at the time of the report. Another case was reported in a 39 year old woman who was enrolled in a clinical trial and presented severe nausea and vomiting, skin rash and liver function abnormalities while

treated with Posaconazole and benznidazole.

A total of 19 cases had a fatal outcome. The attributed mortality reported was the underlying severity of the medical condition, graft rejection, multi-organ failure due to sepsis with *Serratia* or *Acinetobacter* confirmed bacteremia (two cases), and cardiogenic shock due to myocarditis, confirmed by autopsy to be caused by T. cruzi. In at least three cases, treatment with benznidazole had reduced the size of a brain lesion and decreased the parasitemia before death. There was no clear pattern of temporal relationship to benznidazole in any of the death cases. In all cases, concomitant medications with known adverse reactions also described for benznidazole (hypersensitivity reactions, rashes, elevation of transaminases), prevented an assessment of causality. The severity of the underlying medical conditions and high mortality in the immunocompromised patients with reactivation of Chagas disease, and the lack of availability of a defined denominator, limits the assessment of the contribution of benznidazole to the causality of these cases.

In the FAERS database, a Safety Data Mining analysis of the PT American Trypanosomiasis' shows the following association with multiple immunosuppressive drugs (Benznidazole does not appear because it was not decoded):

Event=American trypanosomiasis



Notes

Run
- Name: Generic By Year (S)
- ID: 17965
- Created By: Empirica Signal Administrator
- Created Date: 06/18/2017 09:59:11 EDT
- Configuration: FAERS BestRep (S)
- As Of Date: 06/01/2017 00:00:00
- Event Hierarchy: MedDRA 20.0

Selection Criteria
- Dimension: 2
- Pattern: Generic name + PT(American trypanosomiasis)
- Subset: (All)

Note: These data do not, by themselves, demonstrate causal associations; they may serve as a signal for further investigation.

This analysis further reveals the presence of concomitant medications that confound the assessment of the contribution of benznidazole to these patients' outcomes.

We consulted with OSE to provide an independent analysis of cases in FAERS. The analysis was conducted by Dr. Timothy Jancel, PharmD, OSE reviewer and Dr. Kelly Cao, PharmD, OSE team leader. The search strategy was as follows:

FAERS Search Strategy	
Date of Search	June 27, 2017
Time Period Search	No limits
Search Type	FAERS Business Intelligence Solution (FBIS)
Reporter Narrative	Benznidazole, Abarax, Radanil, Rochagan

Regardless of coded PTs, the search aimed to identify AEs that were possibly associated with benznidazole. The FAERS search retrieved 50 reports. After removing duplicate reports (18), those that reported adverse events not associated with benznidazole (19), and adverse events not of interest (3), 10 FAERS cases, involving 17 patients (two cases reported multiple patients), were identified that reported adverse events with the following SOCs: *Blood and lymphatic system disorders*, *Nervous system disorders*, and *Skin and subcutaneous tissue disorders, and Hepatobiliary disorders*. Six of the 10 FAERS reports were literature reports and 3 of the 10 FAERS reports were from a phase 2 study of oral posaconazole and benznidazole for the treatment of asymptomatic chronic CD. The 10 FAERS cases are summarized below:

1) ***Blood and lymphatic system disorders***

FAERS # **6287300 (*version* 1)** Manufacturer Control #2007US000534 Country Brazil
Initial FDA Received Date March 28, 2007
Literature Report : *D'Albuquerque LA1, Gonzalez AM, Filho HL, et al. Liver transplantation from deceased donors serologically positive for Chagas disease. Am J Transplant. 2007;7:680-4.*

Narrative: A 47-year-old male with alpha-1 antitrypsin deficiency received a liver transplant from a deceased donor who was serologically positive for CD. The patient was serologically negative for CD prior to transplant and postoperatively received benznidazole (200 mg every 12 hours), tacrolimus, mycophenolate, and corticosteroids. The first week after transplant, the patient presented with a persistent temperature over 37.5◦C, severe anemia (laboratory values not reported), and pneumonia. In addition, the patient also developed gastroparesis (possibly secondary to sepsis according to the authors) resulting in the discontinuation of benznidazole on

postoperative day eight. The patient was discharged two months after transplant; however, four months later, he had another episode of pneumonia with sepsis. Parasitemia analyses did not show evidence of CD. Thoracoscopy was indicated and a pleural biopsy diagnosed pulmonary tuberculosis. The patient died a short period later despite receiving therapy.

Reviewer Comments: *Limited case details preclude a meaningful causality assessment between anemia and benznidazole. The reported event of severe anemia was confounded by concomitant administration with other drugs labeled for anemia (tacrolimus and mycophenolate) and liver transplantation (post-liver-transplant anemia is reported to be common after liver transplantation and is likely multifactorial).2*

FAERS # 7768590 (*version* 2) Manufacturer Control #PHHY2011CH03731 Country Switzerland
Initial FDA Received Date January 21, 2011
Literature Report: *Jackson Y, Dang T, Schnetzler B, Pascual M, Meylan P. Trypanosoma cruzi fatal reactivation in a heart transplant recipient in Switzerland. J Heart Lung Transplant. 2011;30:484-5.*

Narrative: A 57-year-old female, originally from Brazil, received a heart transplant due to terminal dilated cardiomyopathy of unknown origin. Immunosuppression included induction with thymoglobulin, cyclosporine, mycophenolate, and prednisone. On post-transplant day 22, graft rejection was diagnosed (no parasites were reported on biopsy specimens) with a good response to methylprednisolone. On post-transplant day 33, erythematous subcutaneous nodules appeared on her legs and she was eventually diagnosed with CD reactivation. Despite starting benznidazole on post-transplant day 46, her clinical condition worsened, skin lesions became bullous and necrotic, and hemophagocytic syndrome developed. She died on post-transplant day 53 of multiple organ failure associated with refractory shock and *Serratia marcescens* bacteremia.
Reviewer Comments: *Limited case details preclude a meaningful causality assessment between benznidazole and hemophagocytic syndrome.*

FAERS # 8166223 (*version 1*) Manufacturer Control #PHHY2011CO85580 Country Columbia
Initial FDA Received Date October 3, 2011
Literature Report: Mestra L, Lopez L, Robledo SM, et al. Transfusion-transmitted visceral leishmaniasis caused by Leishmania (Leishmania) mexicana in an immunocompromised patient: a case report. Transfusion. 2011;51:1919-23.

Narrative: A 42-year-old male received a kidney transplant after developing terminal renal failure due to lupus nephritis. After transplant, the patient developed anemia, mycophenolate–associated thrombocytopenia, and leukopenia, for which he received

multiple blood and platelet transfusions. Subsequently, the patient experienced acute graft rejection accompanied by hepatosplenomegaly. On post-transplant day 32, the patient presented with fever and was diagnosed with CD. Treatment with benznidazole was started but was discontinued eight days later because of severe leukopenia and a positive test for cytomegalovirus (CMV). Treatment with mycophenolate was resumed along with administration of ganciclovir, methylprednisolone, and cyclosporine. Nifurtimox treatment was administered and days later the patient was discharged without leukopenia. Two months later, the patient returned to the hospital suffering from delirium, fever, respiratory symptoms, anemia, and bleeding gums. Renal biopsy revealed histiocyte clusters with intracellular microorganisms (*T. cruzi*). Treatment with nifurtimox was started again but discontinued soon afterwards because of mental disturbances attributed to the drug. The patient was hospitalized several times because of repeated episodes with the same clinical features. The patient received further blood transfusions and the immunosuppressive treatment was continued. Several months later, the patient was hospitalized once again due to marked coagulopathy, fever, and pancytopenia.

Reviewer Comments: *Limited case details preclude a meaningful causality assessment between benznidazole and leukopenia. The reported event of leukopenia occurred prior to and during the administration of benznidazole and was confounded by concomitant administration with mycophenolate (labeled for leukopenia) and CMV infection (leukopenia can be associated with the direct effects of CMV replication).3 Pancytopenia was reported several months after benznidazole was discontinued.*

FAERS # 11994492 (*version* 2)
Manufacturer Control # PHHY2015ES168800 Country Spain
Initial FDA Received Date February 3, 2016
Literature Report: Rodriguez-Guardado A, González ML, Rodriguez M, et al. Trypanosoma cruzi infection in a Spanish liver transplant recipient. Clin Microbiol Infect. 2015;21:687.e1-3.

Narrative: A 52-year-old female received a liver transplant due to primary biliary cirrhosis; immunosuppression included prednisone, tacrolimus, and mycophenolate. The carrier status of CD was communicated from the hospital of origin 10 months after transplantation and she was subsequently diagnosed with CD by positive serology. The patient remained asymptomatic and ECG studies, chest x-ray, echocardiography, barium enema, and brain CT were normal. Treatment was initiated with benznidazole (5 mg/kg/day) in ascending doses of 50 mg every two days to reach a full dose with good tolerance. Before starting benznidazole, the patient had a blood count with hemoglobin of 10.1 g/dL, platelets of 123,000/mm3, and leukocytes of 4,600/mm3. On the fourth day of benznidazole treatment, results for parasitemia were negative but the patient developed severe neutropenia (160 neutrophils/mm3), forcing the

suspension of treatment and the introduction of granulocyte colony stimulating factor. After neutrophil levels had recovered to above 1,000 neutrophils/mm3, benznidazole treatment for 60 days was completed with colony-stimulating factor three times weekly until the end of treatment.

Reviewer Comments: *The reported event of neutropenia was confounded by concomitant administration with other drugs labeled for neutropenia (tacrolimus and mycophenolate). Neutropenia has been reported in patients with CD treated with benznidazole.4*

2) ***Nervous system disorders***

FAERS # 9433952 (*version 10*)
Manufacturer Control # ARMERCK-1307ARG016731 Country Argentina
Initial FDA Received Date August 1, 2013

Narrative: A 49-year-old female was enrolled in a study entitled "Phase II Proof-of-Activity Study of Oral Posaconazole in the Treatment of Asymptomatic Chronic Chagas Disease". The patient started posaconazole (400 mg twice daily, oral suspension) and benznidazole (200 mg twice daily) for chronic CD. Approximately seven weeks later, the patient began to feel "pedal distal bilateral anesthesia" that was initially treated with oral vitamin B Complex. Posaconazole and benznidazole were both discontinued eight days after the initial report of the event. The event did not improve and the patient was transferred to a neurologist the following month where a diagnosis of peripheral neuropathy was made and confirmed by electromyogram. The patient suffered some disability during walking and was started on pregabalin. Over the next several months, the pregabalin dose was increased and symptoms improved and a repeat electromyogram showed slight improvement in sensorial values and nerve motor values. At the time of the report, the outcome of peripheral neuropathy was reported as improved, not resolved. The investigator considered the event of peripheral neuropathy as serious, causing disability and medically significant. The investigator considered peripheral neuropathy to be probably related to posaconazole and benznidazole.

Reviewer Comments: *Although posaconazole is not labeled for peripheral neuropathy without concomitant administration with vincristine, reversible and irreversible peripheral neuropathy has been associated with long-term triazole antifungal therapy.5 Benznidazole has been reported to cause dose-dependent peripheral neuropathy (up to 30% of patients), is usually reversible, but complete resolution may require months.6*

3) ***Skin and subcutaneous tissue disorders***

FAERS # **9016321 (*version 2*)** Manufacturer Control #AR-009507513-1301ARG004300 Country Argentina
Initial FDA Received Date January 16, 2013

Narrative: A 31-year-old female was enrolled on a study entitled "Phase II Proof-of-Activity Study of Oral Posaconazole in the Treatment of Asymptomatic Chronic Chagas Disease". The patient started posaconazole (400 mg twice daily, oral suspension) and benznidazole (200 mg twice daily) for chronic CD. One week later, the patient experienced erythroderma grade 4 that began with "generalized erythema and edema that itch and spread throughout the body surface, associated with fever of 39.5 and food vomiting episode". Benznidazole and posaconazole were discontinued and she was treated with diphenhydramine. At the time of the report, the outcome of erythroderma was unknown. The reporter assessed causality as unlikely related to posaconazole and probably related to benznidazole.

Reviewer Comments: *Difficult to assess causality because benznidazole and posaconazole were started and discontinued at the same time. Posaconazole is labeled for allergic reaction, hypersensitivity, rash, and pruritus. Benznidazole has been associated with rash, dermatitis, photosensitivity, exfoliative dermatitis, and dermatitis associated with fever and lymphadenopathy.6*

FAERS # **9060895 (*version 3*)** Manufacturer Control #ARMERCK-1302ARG002488
Country Argentina
Initial FDA Received Date February 12, 2013

Narrative: A 44-year-old female was enrolled on a study entitled "Phase II Proof-of-Activity Study of Oral Posaconazole in the Treatment of Asymptomatic Chronic Chagas Disease". The patient started posaconazole (400 mg twice daily, oral suspension) and benznidazole (200 mg twice daily) for chronic CD; concomitant medications included enalapril. Posaconazole and benznidazole were continued for approximately two months and then discontinued for an unknown reason. Approximately four months after posaconazole and benznidazole were discontinued, the patient experienced photodermatitis like lupus erythematosus that started with dark skin lesions in the shoulder and dorso-lumbar area, and joint pain in the hands and shoulders. The patient was evaluated by a dermatologist and laboratory findings included positive antinuclear antibodies and skin biopsy reported "photodermatitis like lupus erythematosus". Ten months after discontinuing benznidazole and posaconazole, the patient was evaluated by a rheumatologist and was later prescribed hidroquinone. At the time of the report, the outcome of photodermatitis like lupus erythematosus was reported as improved (also reported as not resolved). The reporter considered photodermatitis like lupus

erythematosus to be medically important and possibly related to posaconazole and benznidazole.

Reviewer Comments: *The events occurred approximately four months after posaconazole and benznidazole were discontinued and were confounded by concomitant administration with enalapril (full dates of administration were not reported) which is labeled for the following adverse reactions (skin): exfoliative dermatitis, toxic epidermal necrolysis, Stevens-Johnson syndrome, pemphigus, herpes zoster, erythema multiforme, urticaria, pruritus, alopecia, flushing, diaphoresis, photosensitivity.*

FAERS # 9647910 (*version 1*) Manufacturer Control #ESUCBSA-101466 Country Spain
Initial FDA Received Date October 28, 2013

Narrative: A 6-year-old male with CD received benznidazole (225 mg three times daily) and his past medical history included attention deficit hyperactivity disorder treated with methylphenidate. Thirty-two days after starting benznidazole, the patient developed fever and toxicoderma; methylphenidate was discontinued one day prior to this event for an unknown reason. The patient was treated with an antihistamine and corticosteroids for the toxicoderma which was suspected due to drug hypersensitivity syndrome. After 48 hours of hospitalization, the patient developed small generalized vesicles. The patient had generalized skin peeling with blister in bending zones. A skin biopsy confirmed toxicoderma. The event of toxicoderma was resolving. Two days after benznidazole was discontinued, the patient developed photophobia and acute hepatic failure which eventually resolved 13 days later. The reporter assessed the causality of acute hepatic failure, toxic skin eruption, photophobia, and fever to be related to methylphenidate and benznidazole.

Reviewer Comments: *The events were confounded by concomitant administration with methylphenidate which is labeled for the following: hypersensitivity, rash, urticaria, fever, arthralgia, exfoliative dermatitis, erythema multiforme with histopathological findings of necrotizing vasculitis, and thrombocytopenic purpura. Benznidazole has been*
associated with rash, dermatitis, photosensitivity, exfoliative dermatitis, and dermatitis associated with fever and lymphadenopathy.6

FAERS # 12168878 (*version 2*)
Manufacturer Control # ES-PFIZERINC-2016139733 Country: Spain
Initial FDA Received Date March 10, 2016

Literature Report: Noguerado-Mellado B, Rojas-Pérez-Ezquerra P, Calderón-Moreno M, Morales-Cabeza C, Tornero-Molina P. Allergy to benznidazole: cross-reactivity with other nitroimidazoles. J Allergy Clin Immunol Pract. 2017;5:827-828.

Narrative: The aim of this study was to establish the utility of a patch test to confirm the diagnosis and to identify cross-reactivity between benznidazole and metronidazole. The authors report a series of six females diagnosed with CD who were referred to an allergy department with suspected hypersensitivity reactions to benznidazole. The median age was 33 years (range, 27 – 43 years) and all were born in Bolivia and had been living in Spain for more than 10 years. After a median of 7.5 days of treatment (range, 3 – 15 days), all six patients presented with an itchy maculopapular generalized exanthema that resolved with antihistamines and corticosteroids in 15 – 20 days. Three patients developed desquamation and residual lesions. None of these patients had histories of adverse reactions to metronidazole.

Reviewer Comments: *Limited case details preclude a meaningful causality assessment between benznidazole and the reported clinical diagnosis of maculopapular generalized exanthema in six patients. Benznidazole has been associated with rash, dermatitis, photosensitivity, exfoliative dermatitis, and dermatitis associated with fever and lymphadenopathy.6*

FAERS # 13245254 (*version 1*)
Manufacturer Control # ESJNJFOC-20170205776 Country Spain
Initial FDA Received Date February 17, 2017
Literature Report
Ramírez E, Medrano-Casique N, Tong HY, et al. Eosinophilic drug reactions detected by a prospective pharmacovigilance programme in a tertiary hospital. Br J Clin Pharmacol. 2017;83:400-415.

Narrative: The authors conducted a prospective evaluation of all eosinophilic drug reactions at La Paz University Hospital, a tertiary-care teaching facility in Madrid, Spain. Acute generalized exanthematous pustulosis was detected in one patient receiving benznidazole. In addition, drug reaction with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome was identified in two cases receiving benznidazole; the first case was classified as overlapping with possible Stevens–Johnson syndrome, and the second case was diagnosed as overlapping with possible acute generalized exanthematous pustulosis. Demographics and clinical details for each case were not reported.

Reviewer Comments: *Limited case details preclude a meaningful causality assessment between benznidazole and the reported events. Benznidazole has been associated with rash, dermatitis, photosensitivity, exfoliative dermatitis, and dermatitis associated with fever and lymphadenopathy.6*

Reviewer's comments:

Ten FAERS cases involving 17 patients were identified that reported adverse events with benznidazole and the following SOCs: *Blood and lymphatic system disorders, Nervous system disorders,* and *Skin and subcutaneous tissue disorders*. Overall, the 10 cases either contained limited case details that preclude a meaningful causality assessment or were confounded by concomitant medications.

1 REFERENCES

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5) Maheshwari A1, Mishra R, Thuluvath PJ. Post-liver-transplant anemia: etiology and management. *Liver Transpl*. 2004;10:165-73.
6) Lumbreras C, Manuel O, Len O, et al. Cytomegalovirus infection in solid organ transplant recipients. *Clin Microbiol Infect*. 2014;20 Suppl 7:19-26.
7) Pinazo MJ, Guerrero L, Posada E, et al. Benznidazole-related adverse drug reactions and their relationship to serum drug concentrations in patients with chronic Chagas disease. *Antimicrob Agents Chemother*. 2013;57:390-5.
8) Baxter CG, Marshall A, Roberts M, Felton TW, Denning DW. Peripheral neuropathy in patients on long-term triazole antifungal therapy. *J Antimicrob Chemother*. 2011;66:2136-9.
9) Bern C. Antitrypanosomal therapy for chronic Chagas' disease. *N Engl J Med*. 2011;364:2527-34.

Another search was performed by Dr. Ana Szarfman in the World Health Organization database, the VigiBase, and results are presented below. This report does not contain outcomes or narratives, however, it contains data on frequency of reports submitted throughout several years, with types of adverse events and relative frequency reflected in a sector map that follows an algorithm. This provides information of the frequency and distribution of adverse reactions, and allows for the possibility of exploring any potentially new safety signal. However, the assessment of causality is limited because of the lack of narratives in this database. Therefore, this information is complementary to that of FAERS and may serve as a source for

the post-marketing section of the label as well.

World Health Organization Database: The source of the data for this data mining analysis is VigiBase, the World Health Organization's Adverse Drug Reaction (ADR) database maintained by Uppsala Monitoring Centre (UMC). The MGPS safety data mining outputs were generated by the Empirica Signal Vigibase software.

Several different views of the Safety Data Mining analysis of benznidazole in the data collected since 2003, including the following tables and graphs that display the following information:

1) Progression of the total number of reports for Benznidazole

2) EBGM (EB05, EB95) values and number of reports by Preferred Tem (PT) in the current Vigibase database

3) Progression of the EBGM values (color) and number of cumulative reports for each PT

4) Sector Map display of EBGM values by PT organized by HLT, HLGT, and SOC, showing below the display a list of the top 100 PTs in descending order of EBGM values.

Safety Data Mining (DM):

The Multi-Item Gamma Poisson Shrinker (MGPS) algorithm that is used to perform Safety Data Mining Analyses is being used by the FDA to screen for higher than expected reporting relationships in the huge FAERS database of over 8 million spontaneous adverse event reports collected since 1968. The same algorithm was adopted by the MHRA, several other regulatory agencies, Industry and Academia, and it was recommended to be adopted in two Institute of Medicine (IOM) Reports.

The MGPS systematically identifies and "shrinks" the very common and volatile observed:expected ratios with the small number of events and expectations in huge data resources. This process guards against generating multiple false-positive signals due to multiple independent comparisons. The adjusted value of an observed:expected ratio is denoted as the Empiric Bayes Geometric Mean (EBGM); the lower and upper 95% confidence interval limits of the EBGM are denoted as EB05 and EB95, respectively. A unique advantage of the MGPS is its ability to rapidly and routinely stratify huge numbers of reports based on several confounders such as age, sex, year of report, and other variables (over 2,000 different categories).

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The EBGM values, also known as adjusted relative-reporting ratios are generated simultaneously for the entire FAERS database. The EBGM values provide an estimate of the relative-reporting ratio of any adverse event for a particular drug, relative to all other drugs and adverse events in FAERS or in other pertinent databases. As we described above, MGPS calculates lower and upper 90% confidence limits for the EBGM scores, denoted as EB05 and EB95, respectively. Higher EBGM values for a particular drug-adverse event pair suggests greater disproportionality in the reporting rate between that drug and the adverse event in FAERS compared with all other drugs and adverse events in the database. In general, drug-adverse event pairs with EB05 >2 are more frequently evaluated as potential safety signals, as these are adverse events that are reported at least at twice the expected rate for a particular drug or biologic product.

The results of Safety DM should be viewed as hypothesis-generating and be evaluated in the context of other relevant data. The results cannot establish or refute causal associations between drugs and adverse events. Additionally, the absence of a signal, based on DM algorithms, does not rule out a safety problem. Review of relevant individual adverse event reports is critical to the evaluation.

This algorithm is also being applied to the Uppsala Monitoring System Adverse Event Database from this WHO Collaborative Centre that contains over 14 million adverse event reports submitted by regulatory agencies from multiple countries. This data resource is known as Vigibase.

References:

Szarfman A, Machado SG, O'Neill RT. Use of screening algorithms and computer systems to efficiently signal higher-than-expected combinations of drugs and events in the US FDA's spontaneous reports database. Drug Saf. 2002;25(6):381-92.

Szarfman A, Levine JG, Tonning JM. A new paradigm for analyzing adverse drug events In: Computer Applications in Pharmaceutical Research and Development, Edited by Sean Ekins. ISBN 0-471-73779-8 Copyright © 2006 John Wiley & Sons, Inc.

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Table 47: Benznidazole: Progression of the total number of reports by year since 2003

N	SUBSET
2	1968-2003
2	1968-2005
2	1968-2004
72	1968-2006
87	1968-2007
101	1968-2008
114	1968-2009
125	1968-2010

136	1968-2011
167	1968-2012
196	1968-2013
210	1968-2014
226	1968-2015
244	1968-2017
244	1968-2016

Dimension: 1 Selection Criteria: Generic_Name (Abridged)(ATC5=Benznidazole) Subset: (All)
15 rows Sorted by N

The total number of safety reports since 1968, available in the WHO "vigibase" database, extracted by Data Mining, presented in the table below.

Table 48: Benznidazole: number of reports by Preferred Tem (PT)

PT	N	EB05	EBGM	EB95
Drug reaction with eosinophilia and systemic symptoms	14	32.682	52.128	79.783
Abdominal pain	40	4.995	6.538	8.465
Hypertransaminasaemia	3	1.293	5.635	62.19
Hepatitis	8	2.87	5.565	12.126
Eosinophilia	5	2.072	5.251	22.05
Dermatitis exfoliative	4	1.563	3.993	13.07
Rash pruritic	8	1.966	3.597	6.168
Eyelid oedema	8	1.908	3.49	5.982
Toxic epidermal necrolysis	4	1.428	3.421	7.424
Decreased appetite	13	2.016	3.229	4.963
Unevaluable event	6	1.602	3.22	5.943
Rash	53	2.507	3.156	3.933
Toxic skin eruption	3	1.099	3.068	7.7
Arthralgia	12	1.852	3.026	4.725
Rash generalized	5	1.331	2.856	5.539
Erythema multiforme	4	1.171	2.749	5.707
Musculoskeletal pain	4	1.165	2.735	5.677
Myalgia	8	1.292	2.36	4.034
Headache	28	1.608	2.212	2.983
Hypersensitivity	7	1.072	2.042	3.606
Pruritus	29	1.467	2.006	2.692
Rash erythematous	8	1.061	1.938	3.312

Dimension: 2 Selection Criteria: Generic_Name (Abridged)(ATC5=Benznidazole) + PT Subset: 1968-2017 Where: EB05 > 1.0

Figure 11 : Progression of the EBGM values (color) and number of cumulative reports by PT since 2003

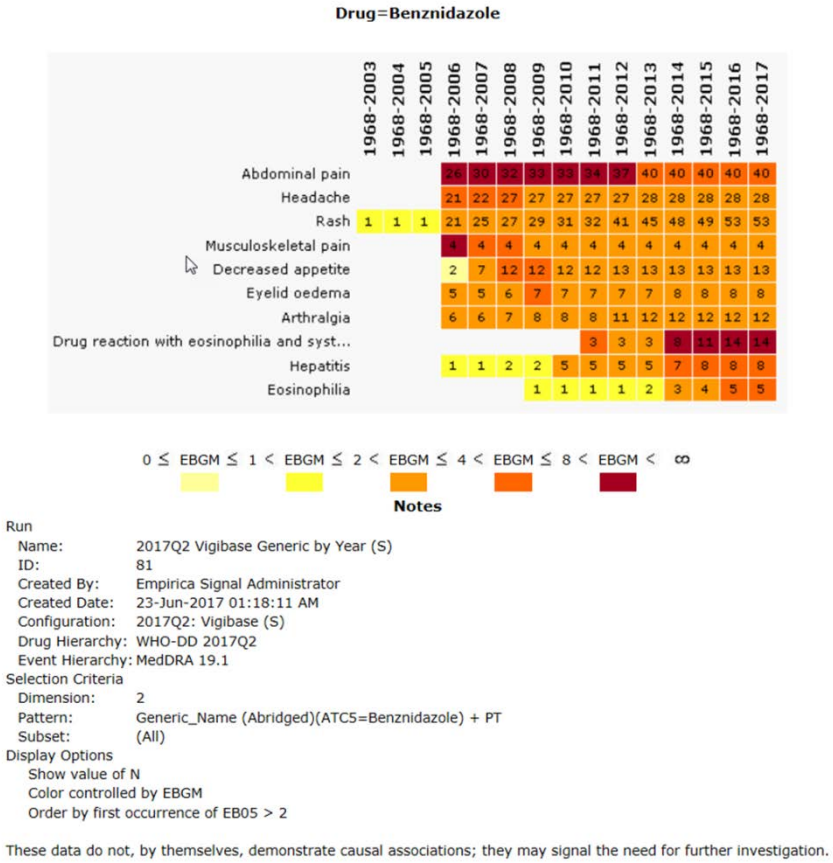

Figure 2: Sector Map display of EBGM values by PT organized by HLT, HLGT, and SOC, showing below the graphic display a list of the top 100 PTs in descending order of EBGM values.

The color, size, position in space, grouping, and ranking of tiles provide a "big picture" overview of the adverse event profile of a drug

Color:
Red corresponds to stronger signals,

Size: A large tile (with a white border) defines each SOC (System Organ Class) in the MedDRA dictionary
The box size for each PT term (preferred adverse event term) is based on the number of serious cases of the term alone in the FAERS database
The size of each PT is stable over displays of different drugs

Position in space: SOCs and PTs are always represented in the same area of the sector map. The position of each SOC and PT is stable over displays of different drugs

Grouping: PTs are grouped by high level term (HLT), high level group (HLGT), and SOC

Ranking:
PTs are ranked below the display in descending order of EBGM values

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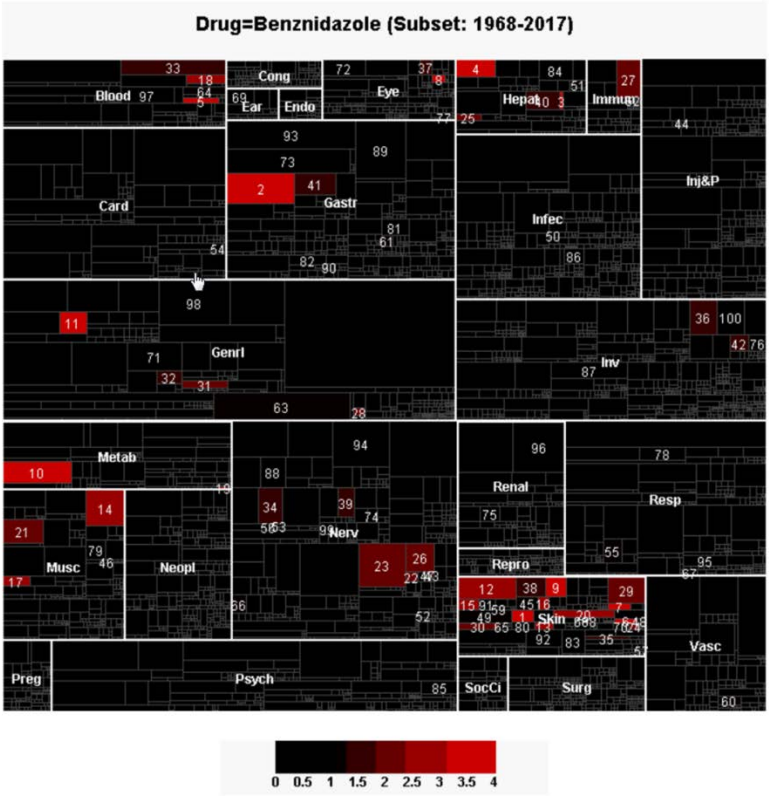

Rank	SOC	Term (PT)	EBGM
1	Skin	Drug reaction with eosinophilia and systemic symptoms	52.128
2	Gastr	Abdominal pain	6.538
3	Hepat	Hypertransaminasaemia	5.635
4	Hepat	Hepatitis	5.565
5	Blood	Eosinophilia	5.251
6	Skin	Dermatitis exfoliative	3.993
7	Skin	Rash pruritic	3.597
8	Eye	Eyelid oedema	3.490
9	Skin	Toxic epidermal necrolysis	3.421
10	Metab	Decreased appetite	3.229
11	Genrl	Unevaluable event	3.220
12	Skin	Rash	3.156
13	Skin	Toxic skin eruption	3.068
14	Musc	Arthralgia	3.026
15	Skin	Rash generalised	2.856
16	Skin	Erythema multiforme	2.749
17	Musc	Musculoskeletal pain	2.735
18	Blood	Agranulocytosis	2.492

Reference ID: 4134724

19	Metab Food intolerance	2.475
20	Skin Dermatitis	2.472

Notes

Run

Name:	2017Q2 Vigibase Generic by Year (S)
ID:	81
Created By:	Empirica Signal Administrator
Created Date:	23-Jun-2017 01:18:11 AM
Configuration:	2017Q2: Vigibase (S)
Drug Hierarchy:	WHO-DD 2017Q2
Event Hierarchy:	MedDRA 19.1 Final Release

Selection Criteria

Dimension:	2
Pattern:	Generic_Name (Abridged)(ATC5=Benznidazole) + PT
Subset:	1968-2017

Display Options

Color controlled by EBGM.
Size controlled by relative importance.
Maximum intensity at signal score of 4.0.
Omit rare terms used fewer than 100.0 times
List 100 top scores
Show score indexes.
Group by HLT
Group by HLGT
Group by SOC
Lowest level displayed is PT
Rare terms omitted: American trypanosomiasis(1), Lacrimation disorder(1)

Note: These data do not, by themselves, demonstrate causal associations; they may signal the need for further investigation.

Medical Reviewer's comment: *A review of cases reported to the FAERS and WHO databases reveal a consistent pattern of toxicities associated with benznidazole, similar to what is described in clinical studies and reported in the published literature, and described also in the drug class to which benznidazole belongs, the nitroimidazoles. An assessment of causality of the deaths reported cannot be made due to several confounding factors, mainly severe co-morbidities and concomitant medications, and additional details missing in the narratives. The vast majority of drug reactions associated with benznidazole are the various types of dermatitis from hypersensitivity, with a spectrum of manifestations ranging from a mild rash to generalized erythroderma, toxic epidermal necrolysis, Stevens Johnson's syndrome, edema, fever, lymph node enlargement. Associated manifestations of skin toxicities can include elevation of transaminases, neutropenia and eosinophilia. Gastrointestinal toxicities are*

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frequently reported, most commonly abdominal pain. The two cases of hepatitis reported in the FAERS database are confounded by the use of Posaconazole and the severe comorbidities in the second case, a liver transplant patient with hepatic artery thrombosis. Peripheral neuropathy has also been reported as well to the databases. This adverse reaction has been associated with temporary impairment, taking up to several months to recover.

8.4.2. **Serious Adverse Events**

A description of the data from submitted studies with patient-level data is presented, as well as those reported in the literature. The searches done in post-marketing databases (FAERS and WHO) are described in the previous Section 8.4.1, which include fatal and other severe adverse reactions that, even though some details are missing, would have met the requirements of serious adverse events (SAEs) as well.

Serious Adverse Events (SAEs) were not reported on any subjects from the two randomized and controlled studies in children. In a supplementary follow-up study of the serological progress of 252 Argentine children treated with benznidazole, including 46 of the children participating in the clinical trial mentioned above, Sosa Estani *et al*. (2002)[136] did not identify any long-term adverse events possibly meeting the definition of serious that could be attributed to benznidazole in this group. In the uncontrolled study of 38 pediatric patients ages 2 to 12 years, from which we have patient-level datasets, hospitalizations, deaths or disabilities were not observed. In a cohort study conducted with 95 children between 1 and 14 years old, with 64 of them treated with benznidazole at 5 mg/kg/day, split into two daily doss for 30 days, Streiger *et al*. (2004) noted good tolerability, not specifying the level of severity of adverse events observed, which were described as vomiting, widespread erythema with edema and itching, causing treatment interruption in two children. Another prospective cohort study, conducted between 2003 and 2007 at the Ricardo Gutierrez Pediatric Hospital in Buenos Aires (Argentina) by Altcheh *et al*. 2011[137] described adverse events in 107 children between 10 days and 19 years old (average of age 6.9 years), diagnosed with asymptomatic infections of *T. cruzi*, treated with benznidazole at 5 to 8 mg/kg/day, administered two or three times a day for 60 days, and monitored for a period of three years, did not observe serious adverse events. In all

[136] Sosa-Estani, Sergio, et al. "Evolución serológica a largo plazo en ninos infectados por Trypanosoma cruzi que cursan fase clınica indeterminada, tratados con Benznidazol." *Proceedings of the 2do Simposio Internacional de Enfermedad de Chagas en Internet*. Federación Argentina de Cardiología, 2002.

[137] Altcheh, Jaime, et al. "Adverse events after the use of benznidazole in infants and children with Chagas disease." *Pediatrics* 127.1 (2011): e212-e218.

Reference ID: 4134724

these studies, severe adverse events were reported, in all cases in low rates of 3% or below. However, in the MSF study (Yun et al., 2009), a higher rate of adverse reactions, including up to 10% severe, was noted in the programs conducted in Central America, compared with the Bolivian programs. Severe reactions reported at Yoro were neurological (neuromuscular disorders of the lower limbs appearing after six weeks of treatment in three patients); neuromuscular (2/63) and dermatological (1/63) in Olopa. In Entre Rios and Sucre severe adverse reactions occurred in 6 and 41 patients respectively, particularly a case of Lyell's syndrome in a 13-year old girl, which occurred in the 34th day of treatment, and 1 case of Stevens Johnson syndrome. In the weekly follow-up, this patient showed a generalized itchy rash with good general clinical status and was treated with oral antihistamine drugs. Two days later, a MSF physician was contacted and visited the child, who presented with high fever and general cutaneous rash with infected pustules. The patient was given intravenous fluids and ceftriaxone until admission to Tarija hospital. She was managed and discharged after 7 days with good clinical improvement.

All adverse events reported were resolved either spontaneously or with treatment suspension. No deaths were observed in these studies. The descriptions do not suggest that they would meet a regulatory definition of serious adverse events, however, since the source data is not available for review, we cannot completely rule out the occurrence of serious adverse events in children. The description of severe adverse reactions in this section is presented because some of these may have actually met the definition of a serious adverse event, but this could not be confirmed since we do not have access to source data.

In adult patients, the Viotti 2006 study does not mention adverse events described as meeting the characteristics of an SAE (no hospitalizations, deaths or diagnoses of cancer were described in the publication or the submitted database) in any of the 283 treated patients. In another study by Viotti *et al.* (1994)[138] of 131 patients, with an average age of 46 years, treated with benznidazole (5 mg/kg/day) during 30 days, adverse reactions were noted in 20% of those patients, and no serious adverse events were observed. Other studies in adults, such as the Gallerano 2000 and Fabbro 2007 studies indicate that all adverse events were mild, and do not describe hospitalizations or fatal events associated with benznidazole (or with any other drug). Other controlled study[139] conducted in Brazil, which included 26 patients receiving

[138] Viotti, Rodolfo, et al. "Treatment of chronic Chagas' disease with benznidazole: clinical and serologic evolution of patients with long-term follow-up." *American heart journal* 127.1 (1994): 151-162.

[139] Coura JR, de Abreu L, Willcox HP, Petana W 1997. Comparative controlled study on the use of benznidazole, nifurtimox and placebo, in the chronic form of Chagas' disease in a field area with interrupted transmission. I. Preliminary evaluation. Rev Soc Bras Med Trop 30: 139-144.

Reference ID: 4134724

benznidazole at 5 mg/kg/day for 30 days, only mild adverse events were identified in 29.1% of patients. In a cohort of 30 adult patients from the US[140], who received benznidazole at 5 mg/kg/day for 60 days, the mean age was 42 years (range, 17–67 years), 18 (60%) were female, 10 (33%) were male, no debilitating, irreversible or incapacitating adverse events were observed, although severe cases of dermatological rashes and angioedema were observed. Similar findings and conclusions regarding lack of serious adverse events in adult patients were reported by authors in Spain, n=105 (Pinazo et al, 2010)[141], and in Brazil, n=32 (Pontes, 2010)[142]. All three of these studies (conducted in the US, Spain and Brazil) used benznidazole at 5 mg/kg/day for 60 days. Even though serious adverse events were not reported, the rate of adverse reactions, in particular, peripheral neuropathy was higher (up to 22%).

In the DNDi E1224 study, the following 2 SAEs (2/184, 1.08%), were reported in the treatment arm, and none in the placebo arm. They were all resolved:

- Subject 01-188 (Acute Bronchitis)
- Subject 01-263 (Anembryonic Pregnancy)

None of these SAEs were attributed to benznidazole in the assessment of causality. All listings of adverse events were reviewed, as well as laboratory results. Regarding laboratory abnormalities, increased transaminases have been observed, sometimes up to 10 times the upper limit of normal. No Hy's law cases have been found in the datasets analyzed. All the elevations of transaminases included an elevation of alkaline phosphatase. All these changes resolved with treatment interruption or discontinuation. Neutropenia and thrombocytopenia have also been observed. There were 8 patients in the Altcheh study who presented neutropenia, none of them needed hospitalization, and all reversed after treatment interruption or discontinuation. Creatinine increases have been described as mild, and reversible as well.

8.4.3. Dropouts and/or Discontinuations Due to Adverse Effects

[140] Miller, David A., et al. "Tolerance of benznidazole in a United States Chagas disease clinic." *Clinical Infectious Diseases* 60.8 (2015): 1237-1240.

[141] Pinazo, María-Jesús, et al. "Tolerance of benznidazole in treatment of Chagas' disease in adults." *Antimicrobial agents and chemotherapy* 54.11 (2010): 4896-4899.

[142] Pontes, Vânia Maria Oliveira de, et al. "Adverse reactions in Chagas disease patients treated with benznidazole, in the State of Ceara." *Revista da Sociedade Brasileira de Medicina Tropical* 43.2 (2010): 182-187.

Reference ID: 4134724

The available data from datasets provided will be discussed first, followed by a review of the relevant published literature submitted by the Applicant and identified by this reviewer in an independent literature search.

Sosa Estani study
A total of 5 patients discontinued treatment due to Treatment Emergent Adverse Events. In four of these patients, rash and abdominal pain were the cause of treatment discontinuation. Details on these patients are shown in the table below.

Table 49: Discontinuations due to Adverse Events

USUBJID	Age	Sex	Comments
Sosa-Estani-999-0777	12	Male	Rash, Decreased appetite, Headache
Sosa-Estani-999-1322	12	Female	Transaminases increased
Sosa-Estani-999-1424	12	Male	Abdominal pain
Sosa-Estani-999-3435	10	Female	Rash, Abdominal pain, Nausea, Abdominal pain upper, Vomiting
Sosa-Estani-999-5515	9	Male	Rash, Abdominal pain, Nausea, Abdominal pain upper, Vomiting

De Andrade study Cutaneous maculopapular rash and pruritus during the treatment were more common in the benznidazole group than in the placebo group (eight and two cases, respectively; p=0·05), this side-effect was the reason for withdrawal of one patient from the benznidazole group.

Altcheh study Two patients (5.3%) did not complete treatment due to rash, eosinophilia and elevated alkaline phosphatase. A single benznidazole treated patient (0.4%) had a creatinine level > 3 x ULN and 4 benznidazole treated patients (1.7%) had a neutrophil level <1.0 x 109, requiring temporary treatment interruptions.

Viotti study: The side effects of benznidazole that required discontinuation of treatment (37 of 283 patients [13%]) were severe allergic dermatitis in 33 patients, 30 who required antihistamine treatment and 3 who required corticosteroids, and gastrointestinal disorders in 4 patients. These patients continued to be followed within the treated group, and only 6 patients (16%) were lost to follow-up.

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DNDi E1224 study: A total of 4/45 (8.9%) patients in the benznidazole arm discontinued treatment because of adverse events. The 4 patients (8.9%) from the benznidazole arm had 6 events. In all of these patients, drug hypersensitivity events were the cause of discontinuation, occurring at day 9 and day 10 of study treatment. Along with hypersensitivity, increased transaminases (ALT and GPT) were observed in one of these 4 patients, reported as the cause of discontinuation, and who also presented abdominal pain and neutropenia. All these were resolved after treatment discontinuation.
No patients from the placebo, or posaconazole high and low dose arms discontinued the study treatment due to adverse events.

Molina study: A total of 5/26 (19.2%) patients discontinued benznidazole treatment due to hypersensitivity drug reactions (allergic dermatitis). No patients discontinued treatment in the other arms of the study.

Review of relevant published literature

In this subsection, a review of the most common adverse reactions causing treatment discontinuations is presented. Some patients present more than one adverse reaction, usually in these two system organ classes discussed below.

Dermatological adverse reactions as cause of treatment interruption
The most common type of adverse events causing discontinuation in children and adults are skin lesions, described as allergic dermatitis or cutaneous hypersensitivity reactions, which range from mild rashes to generalized erythroderma, and may present popular or pustular characteristics, and are associated in some cases with marked eosinophilia and elevation of liver transaminases. The occurrence of these skin adverse reactions has not been associated with dose levels or a particular dose regimen. The median time of occurrence is around day 10 of treatment. In a pharmacovigilance study in Spain, benznidazole has been associated to DRESS and AGEP in 4 patients who required hospitalization. However, the four cases and not described in detail and since it is not known whether these patients had acute Chagas disease (an important differential diagnosis of most of DRESS symptoms), it is not possible to conclude on this association. However, several other case reports have described clearly the presence of toxic epidermolysis necrosis (TEN) and acute generalized exanthematous pustulosis (AGEP) with benznidazole treatment, sometimes needing hospitalization. Recovery of skin lesions has been reported with temporary or permanent discontinuation of treatment. Some patients needed corticosteroids to improve.

Reference ID: 4134724

In a prospective study of 107 children[143] (mean age 6.9 years), the most frequent adverse drug reactions (ADRs) were dermatologic (22 of 107 subjects [21%]), followed by gastrointestinal (9 of 107 subjects [8.5%]), central nervous system (CNS) (10 of 107 subjects [9%]), and neuromuscular (3 of 107 subjects [2.8%]) events. Mean duration of ADRs was 8.2 days (95% CI: 4.1–12). One patient, who experienced a generalized rash, was withdrawn from the study by the caregiver's decision. No target lesions, bullae, blisters, or skin necrosis developed, and symptoms resolved quickly with symptomatic treatment and drug discontinuation. Also, no fever was present.

Transient discontinuation of benznidazole because of ADRs was required in 12% (13 of 107) of children (mean duration: 8.9 days [95% CI: 2.6 –15.0]). Seven (7 of 13 [54%]) patients temporally discontinued treatment because of ADRs (4 because of rashes, 2 gastrointestinal discomforts, and 1 headache); 2 patients temporarily discontinued treatment because of a momentary lack of medication, 2 other patients because of patient noncompliance, and the last 2 patients because of mild viral illnesses.

Safety data summarized in this submission indicates a better tolerability for benznidazole among children, especially infants and toddlers, than adults, with treatment discontinuation due to adverse events being rare among newborns, and no greater than 10% in children in the indeterminate phase, while these rates may rise to 40% among adults, usually averaging at around 20%. The review of the studies submitted is consistent with findings from other published studies. A summary and discussion of other common adverse reactions causing withdrawals, reported in the literature is presented below.

Gastrointestinal intolerance
This is the second most frequently reported adverse reaction causing treatment discontinuations. The reported rates of discontinuations vary in the different series, and are usually around 5 to 10%. Abdominal pain, nausea and anorexia with weight loss have been reported. Most of the symptoms are mild and in a small percentage can be severe (1%) and cause treatment interruption or discontinuation. Gastrointestinal symptoms include appetite loss, anorexia, and "digestive intolerance" (including vomiting and abdominal pain). Appetite loss was observed in 17% of the benznidazole treated Chagas patients in the study reported by Fragata Filho et al[144]., and was sometimes accompanied by weight loss; drug treatment was

[143] Altcheh, Jaime, et al. "Adverse events after the use of benznidazole in infants and children with Chagas disease." *Pediatrics* 127.1 (2011): e212-e218.

[144] Fragata Filho, Abílio Augusto, Marco Aurélio Dias da Silva, and Elias Boainain. "Etiological treatment of acute and chronic Chagas' heart disease." *Sao Paulo Medical Journal* 113.2 (1995): 867-872.

Reference ID: 4134724

discontinued only in the more severe cases. Although anorexia in the study reported by Pinazo et al[89]. occurred in 40% of the BNZ treated patients, not needing discontinuation of benznidazole treatment. All symptoms are reported to resolve spontaneously or with treatment discontinuation.

Peripheral neuropathy
This is a serious toxic effect induced by benznidazole, with close connection with its therapeutic index. In most studies, it occurs towards the end of treatment, after 30 days in most cases, most frequently in adults. However, peripheral neuropathy is very difficult to evaluate in small children, particularly if it is mostly sensorial. It is interesting to note that patients perceive the first symptoms of polyneuritis, saying that cutting the nails and washing the hands in cold water become somewhat painful.
Other nervous system toxicities, such as neuromuscular (myalgia, gait problems) have been described early in the treatment course. All patients are susceptible to it; some authors state that it occurs when the treatment achieves the total dose around 18 grams[146]. However, there are no comparative trials in which different total doses have been compared; therefore, this is just a description on observations of the outcomes of different regimen durations and total doses used.

Depression of bone marrow
Another significant side effect is depression of bone marrow (neutropenia, agranulocytosis and thrombocytopenic purpura). These adverse reactions are occasionally causes of treatment interruption or discontinuation, in about 1% of cases[145]. They are all reported to be reversible and treated sometimes with corticosteroids and in some cases antimicrobial therapy, if infection is present. Since agranulocytosis can develop rapidly, periodic white-cell counts are recommended by most authors. Some authors advise[146] that *"patients should be advised that in case of sore throat, and fever, which are the first symptoms of agranulocytosis, or petechias, specially hemorrhagic blisters in the oral mucosa, which usually herald the onset of thrombocytopenic purpura, the drug should be immediately stopped and the physician informed, who would institute the adequate therapy"*.

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[145] Bern, Caryn. "Chagas' disease." *New England Journal of Medicine* 373.5 (2015): 456-466.

[146] CanÇado, J. Romeu. "Long term evaluation of etiological treatment of Chagas disease with benznidazole." *Revista do Instituto de Medicina Tropical de São Paulo* 44.1 (2002): 29-37.

Reference ID: 4134724

8.4.4. **Significant Adverse Events**

The most significant adverse reactions, frequently causing treatment interruption in the studies analyzed and in published literature were skin rashes and gastrointestinal disturbances, anorexia and weight loss. The rashes presented as morbilliform, generally pruriginous, exanthema, generalized erythroderma, Stevens Johnson and Toxic Epidermal Necrolysis (TEN). Patients also experienced neutropenia, thrombocytopenia, arthralgia, peripheral neuropathy, headache and toxic hepatitis/liver profile abnormalities.
All adverse events reported have resolved with temporary or permanent discontinuation of treatment.

Other types of side effects include reversible clastogenesis and mutagenesis[147] [148]with benznidazole without any associated manifestations, or increased risk of lymphomas in experimental animals have been described, but never demonstrated among a general population of infected patients undergoing treatment[149]. In a small series of 16 chagasic patients undergoing heart transplant, and 75 others transplant patients without Chagas disease, benznidazole was administered to 14 patients (4 of them received them as prophylaxis and 10 of them for treatment of Chagas reactivation). Six of 16 patients (37.5%) and 2 of the 75 (2.7%) non chagasic patients had malignant tumors in a period of 24 to 34 months of follow-up, respectively. In the chagasic group, lymphoproliferative disorders were diagnoses in 3 patients, Kaposi sarcoma in 2, and squamous cell carcinoma in 1. Four of these patients died and two survived in the follow-up up to 96 months. This study has several limitations: the confounding factor of immunosuppressive therapy, reactivation of Chagas disease and multiple treatments with benznidazole as prophylaxis and/or treatment in several of the patients, using doses of 10 mg/kg/day for 60 days.

8.4.5. **Treatment Emergent Adverse Events and Adverse Reactions**

Sosa Estani study

[147] Gorla, Nora B., et al. "Thirteenfold increase of chromosomal aberrations non-randomly distributed in chagasic children treated with nifurtimox." *Mutation Research/Genetic Toxicology* 224.2 (1989): 263-267.

[148] Moya, P. R., and G. T. Trombotto. "Chagas' disease: clastogenic effect of nifurtimox and benznidazole in children." *Medicina* 48.5 (1988): 487.

[149] Sosa-Estani, Sergio, Lisandro Colantonio, and Elsa Leonor Segura. "Therapy of Chagas disease: implications for levels of prevention." *Journal of tropical medicine* 2012 (2012).

Reference ID: 4134724

Overall the treatment was well tolerated. A total of 47/106 (44.3%) of all subjects reported treatment emergent adverse events during the study. A total of 33 of 55 subjects (60%) in the benznidazole arm and 14 of 51 subjects (27.5%) in the placebo arm reported TEAEs during the study follow-up. Even though there were fewer subjects in the ages 12 years and above (12 to 14 year), a trend towards more frequent adverse events with increasing age was noted in the children ages ≥12 to 14 years (n=15), where 12/15 (80%) presented TEAEs as compared to those 6 to <12 years old (n=40), where 21/40 (52.5%) presented TEAEs. In addition, a trend towards higher proportions of adverse events leading to discontinuations was observed in this group. In the 6 to 12 years group, 2/40 (5.4%) discontinued treatment due to adverse events. In the group of age 12 years and older, 3/15 (20%) discontinued treatment due to adverse events. This is shown in the table below (Table 46).

Table 50: Overview of Frequency of Treatment Emergent Adverse Events

Overview of Adverse Events – Sosa Estani study		
	Benznidazole (N=55)	Placebo (N=51)
Patients with TEAEs	33 (60%)	14 (27.5%)
Total number of TEAEs	55	18
Patients with TEAEs causing discontinuation	5 (9%)	0
Children 6 to <12 years old		
	Benznidazole (N=40)	Placebo (N=39)
Patients with TEAEs	21 (52.5%)	11 (28.2%)
Total number of TEAEs	37	15
Patients with TEAEs causing discontinuation	2 (5.4%)	0
Children ≥12 to <18 years old (maximum age: 14, two subjects were 14 years old, all others 12 years old)		
	Benznidazole (N=15)	Placebo (N=12)
Patients with TEAEs	12 (80%)	3 (25%)
Total number of TEAEs	18	3

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Patients with TEAEs causing discontinuation	3 (20%)	0

Source: ADSL, ADAE, Safety Population (TRTEMFL = 'Y') TRT01A

The distribution of adverse events by System Organ Class (SOC) is presented in the table below. The most common adverse events were in the Gastrointestinal SOC, abdominal pain was the most common preferred term, occurring in 11(20%) of subjects. The second most common preferred term was skin rash, observed in 10 (16.4%) subjects. Abdominal pain and rash were the most common adverse events preferred terms and also most common causes of treatment discontinuations. The second most frequent SOC was Investigations, in which weight decreased was observed in 7 (12.7%) subjects and transaminases elevations in 3 (5.5%). The adverse events distribution is shown in the table below (Table 47).

Table 51: Treatment Emergent Adverse Events – Sosa Estani

System Organ Class	**Adverse Event**	**Benznidazole N=55**	**Placebo N=51**	**Total (N)**	**Total (%)**
	Preferred Term	**n (%)**	**n (%)**		
Any Body System	Any Event	33 (60)	14 (27.5)	47	44.3
Gastrointestinal	Any Event in Gastrointestinal disorders	13 (23.6)	5 (9.8)	18	17
	Abdominal pain	11 (20)	3 (5.9)	14	13.2
	Abdominal pain upper	3 (5.5)	1 (2)	4	3.8
	Diarrhoea	2 (3.6)	0	2	1.9
	Nausea	3 (5.5)	1 (2)	4	3.8
	Vomiting	3 (5.5)	0	3	2.8
Investigations	Any Event in Investigations	10 (18.2)	1 (2)	11	10.4
	Weight decreased	7 (12.7)	1 (2)	8	7.5
	Transaminases increased	3 (5.5)	0	3	2.8
Skin and subcutaneous tissue	Any Event in Skin and subcutaneous tissue disorders	9 (16.4)	0	9	8.5
	Rash	9 (16.4)	0	9	8.5
Nervous system Disorders	Any Event in Nervous system disorders	7 (12.7)	6 (11.8)	13	12.3

	Headache	4 (7.3)	5 (9.8)	9	8.5
	Dizziness	2 (3.6)	2 (3.9)	4	3.8
	Neuropathy peripheral	1 (1.8)	0	1	0.9
	Tremor	1 (1.8)	0	1	0.9
Blood and lymphatic disorders	Any Event in Blood and lymphatic system disorders	3 (5.5)	5 (9.8)	8	7.5
	Uncoded: hematological changes	3 (5.5)	5 (9.8)	8	7.5
Metabolism and nutrition	Any Event in Metabolism and nutrition disorders	3 (5.5)	0	3	2.8
	Decreased appetite	3 (5.5)	0	3	2.8

To assess the frequency and distribution of adverse events in younger as compared to older children, I grouped the study participants into two age cohorts: ages 6 to <12, (treated, n=40; placebo, n=39) and those ages 12 years and above (two were 14 years old), treated, n=15; placebo, n=12. As mentioned before, the sample size of the older children group was smaller, and therefore comparisons have limitations. However, a trend towards higher rates of AEs could be observed in this older age group.

Events of the skin and soft tissue, and rash being the most common preferred term, were the most frequently observed adverse event in both age cohorts, observed in 7/40 (18.9%) of the children ages 6 to 12 years and 4 (26.7%) subjects ages 12 and older. Events in the Gastrointestinal SOC were common in both groups, and abdominal pain was the most common preferred term. Weight decreased 3/15 (20%) and decreased appetite 3/15 (20%), and increased transaminases in 2/15 (13%) were reported in children older than 12 years only.

Table 52: Treatment Emergent Adverse Events in Children ages 6 to <12 years - Sosa Estani

System Organ Class	**Adverse Event**	**Benznidazole N=40**	**Placebo N=39**
	Preferred Term	**n (%)**	**n (%)**
Any Body System	Any Event	21 (52.5%)	11 (28.2%)
Skin and soft tissue	Any Event in skin and soft tissue	7 (18.9%)	5 (12.5%)
	Rash	2 (5.4%)	5 (12.5%)

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	Skin Lesion	1 (2.7%)	0
	Macule	2 (5.4%)	0
	Rash maculopapular	2 (5.4%)	0
Gastrointestinal	Any event in gastrointestinal	11 (27.5%)	5 (12.8%)
	Abdominal pain	10 (25%)	5 (12.8%)
	Abdominal pain upper	3 (7.5%)	3 (7.7%)
	Vomiting	3 (7.5%)	1 (2.6%)
	Nausea	3 (7.5%)	1 (2.6%)
	Diarrhea	1 (2.5%)	0
Nervous system disorders	Any event in nervous system disorders	4 (10%)	4 (10.2%)
	Headache	3 (7.5%)	3 (7.7%)
	Dizziness	1 (2.5%)	2 (5.1%)
	Tremor	1 (2.5%)	0
Investigations	Any event in investigations	5 (12.5%)	1 (2.6%)
	Weight decreased	4 (10%)	1 (2.6%
	Transaminases increased	1 (2.5%)	
Blood and hematological disorders	Any event in blood and hematological disorders	2 (5%)	4 (10%)
	Uncoded: hematological changes	2 (5%)	4 (10%

In both age groups, skin rashes were the most common adverse event.

Table 53: Treatment Emergent Adverse Events in Children 12 to <18 years old – Sosa Estani

System Organ Class	**Adverse Event**	**Benznidazole N= 15**	**Placebo N=12 (%)**
	Preferred Term	**n (%)**	**n (%)**
Any Body System	Any Event	12 (80%)	3 (25%)
Skin and subcutaneous tissue disorders	Any event in skin and subcutaneous tissue disorders		
	Rash	4(26.7%)	4 (26.7%)
Investigations	Any event in investigations	5 (33.3%)	0
	Weight decrease	3 (20%)	0

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	Transaminases increased	2 (13.3%)	0
Metabolism and nutrition disorders	Any event in metabolism and nutrition disorders	3 (20%)	0
	Decreased appetite	3 (20%)	0
Nervous system disorders	Any event in nervous system disorders	3 (6.7%)	2 (16.7%)
	Dizziness	1 (6.7%)	
	Headache	1 (6.7%)	2 (16.7%)
	Neuropathy peripheral	1 (6.7%)	
Gastrointestinal disorders	Any event in gastrointestinal disorders	2 (13.3%)	0
	Abdominal pain	1 (6.7%)	0
	Diarrhea	1 (6.7%)	0
Blood and hematological disorders	Any event in blood and hematological disorders	1 (6.7%)	1 (8.3%)
	Uncoded: hematological changes	1 (6.7%)	1 (8.3%)

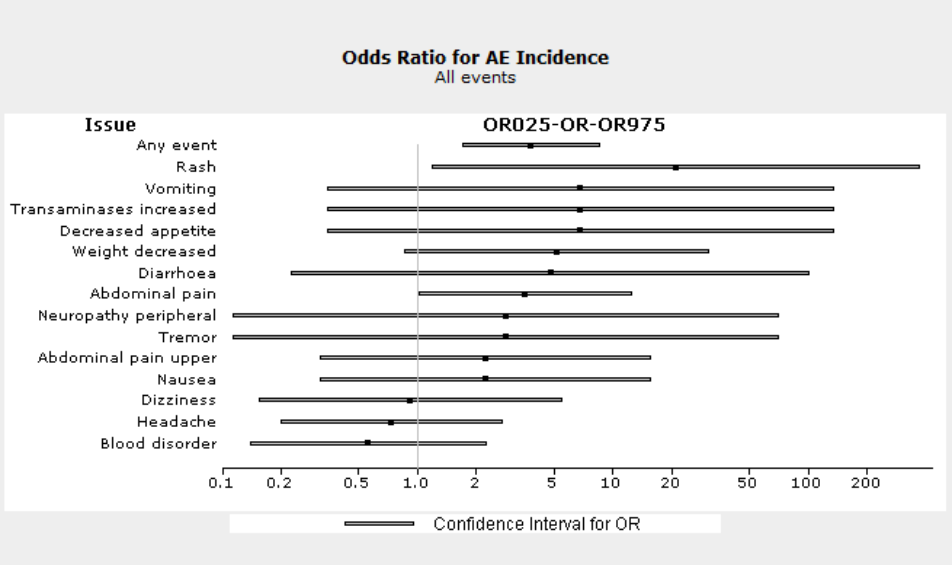

Figure 12: Odds Ratio for AE Incidence

These patient-level data suggest a significant incidence of skin rashes and abdominal pain associated with benznidazole treatment. More frequent adverse events occur in children older than 12 years as compared to those in the 6 to 12 years of age group. This observation has also been made in other prospective pediatric studies in the published literature (Altcheh, 2011). The patient-level data also suggests a tendency towards more frequent adverse events in

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females as compared to males. Several publications refer to this apparent predominance; however, some published studies could not confirm this same finding. The table below reflects the adverse events reported with the use of benznidazole in the published literature and other post-marketing safety databases (FAERS and WHO).

In the De Andrade study, only 7 treated children presented AEs, all skin lesions. In the placebo group, 2 subjects presented also skin lesions. All subjects but 1 of the 7 who presented AEs were older than 10 years old. The two placebo recipients with AEs were also older than 10 years of age. The AEs are not described in the patient-level database. The timing of the events was not provided. In the publication they are described as mild and reversible.

In the Altcheh study, 17 of 38 (44.7%) patients presented AEs, the most common events were in the skin and subcutaneous tissue SOC, and rash, followed by urticaria, macule and prurigo were the most common ones, observed in a total of 9 (23.7%) patients. Equally common events, observed in 9 patients (23.7%) in the Blood and Lymphatic disorders, eosinophilia was the most common in 6 (15.8%) patients and leukopenia and neutropenia observed in 4 patients (10.5%). Gastrointestinal disorders, elevation of alkaline phosphatase and myalgia were observed in 2.6% of patients. All adverse events resolved with treatment interruption or discontinuation.

Viotti *et al.* (2006): (information provided in the publication)
Adverse events of benznidazole in the group of patients who completed the treatment program (55 of 246 patients [22%]) were mild allergic dermatitis (36 patients [14.6%]), moderate allergic dermatitis (2 patients [0.8%]), headache (3 patients [1.2%]), gastrointestinal intolerance (11 patients [4.5%]), fever (1 patient [0.4%]), and pruritus (2 patients [0.8%]). The side effects of benznidazole that required discontinuation of treatment (37 of 283 patients [13%]) were severe allergic dermatitis in 33 patients, 30 who required antihistamine treatment and 3 who required corticosteroids, and gastrointestinal disorders in 4 patients.

Gallerano *et al*. (2000) of 130 patients between 10 and 79 years old (average age 33, 4 $\pm$ 14.2) treated with benznidazole at 4 to 8 mg/kg/day, for 45 to 60 days, adverse events were observed in 32% of patients treated with benznidazole, mainly gastric intolerance, cutaneous eruptions and peripheral neuropathy.

Fabbro *et al*. (2007) reported adverse events in 27% of 33 patients treated with benznidazole at a dose of 5m/kg/day for 30 days, consisting of maculo-papular erythema (18% or 6/33), edema (9% or 3/33), nausea (3% or 1/33), headache (3% or 1/33), itching (3% or 1/33) and a slight increase in the hepatic transaminases (3% or 1/33).

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Viotti *et al.* (1994) A total of 131 patients, with an average age of 46 years, treated with benznidazole (5 mg/kg/day) during 30 days, adverse reactions were noted in 20% of those patients, with the most frequent being moderate allergic dermatitis (77%), gastrointestinal intolerance (16%), widespread allergic dermatitis (7%). Other less significant effects were headache, itching and edema of the lower extremities.

Published studies reporting a higher rate of adverse events in adult patients

A higher rate of adverse events was reported in the following studies, summarized below, all of them included adult patients, and benznidazole treatment of 60 days. The manufacturer of benznidazole was from more than one manufacturer in these studies (Roche, LAFEPE, ELEA). One study (Molina et al., 2015) compared adverse events from two manufacturers (Roche and ELEA), and concluded that more neurological adverse events (paresthesia and dysgeusia) and arthromyalgia, were observed with the ELEA product. However, there was not a significant difference in the rate of discontinuations between patients who received the LAFEPE or the ELEA product.

Pinazo *et al*. (2010)[150] A total of 105 patients between 16 and 58 years old (average age 38.7 years) treated with benznidazole at 5 mg/kg/day for 60 days, 57.1% (60/105) presented adverse reactions, with 47% of them (27/60) presenting more than one adverse reaction. The most frequent adverse reactions were headache (56.2% or 59/105 patients), dermatopathies (50.5% or 53/105 patients), notably urticaria, rash and itching, anorexia (40% or 42/105 patients), joint disorders (36.2% or 38/105 patients), asthenia (30% or 32/105 patients), paresthesias (27.6% or 29/105 patients) and gastrointestinal disorders (15% or 16/105 patients), mainly epigastralgia. Ten patients discontinued treatment due to adverse reactions, eight of whom were due to severe urticaria and fever, and two were due to digestive intolerance.

[150] Pinazo, María-Jesús, et al. "Tolerance of benznidazole in treatment of Chagas' disease in adults." *Antimicrobial agents and chemotherapy* 54.11 (2010): 4896-4899.

Reference ID: 4134724

Pontes *et al.* (2010)[151] reported adverse reactions in 32 adults patients treated with benznidazole at 5 mg/kg/day, for 60 days, adverse events were observed in 87.5% of these patients. This study identified 20 different types of adverse reactions, with the most frequent being itching (50%), paresthesia (43.8%), asthenia (37.5%), headache (34.4%) cutaneous rash (31.3%) and peeling skin (25%). The dermatological system was the most severely affected, with 35% of the symptoms, followed by the central and peripheral nervous systems with 22% of the reported symptoms.

Molina *et al*. (2015)[152] 472 adult patients were treated with benznidazole, before 2012 with LAFEPE benznidazole (n=264) and after 2012 with ELEA benznidazole (n=208). A high proportion of patients (n = 360 [76%]) suffered AEs, the most frequent being those related to hypersensitivity (52.9% of patients), headache (12.5%), and epigastric pain (10.4%). In 72 (12.7%) cases, treatment was discontinued. Overall, women had a higher incidence of AEs compared to men (81.3% versus 66%, P = 0.001) and presented higher rates of hypersensitivity-related events. Dermatological events, digestive system manifestations, and general symptoms had a greater likelihood to appear around day 10 and neurological AEs (dysgeusia and paresthesis, in 7% of patients) around day 40 after starting treatment. With respect to liver function and hematological tests, the majority of patients did not present significant changes of liver enzymes or altered blood cell counts. However, 14 patients suffered from neutropenia, and 14 patients had aminotransferase levels that were more than four times the upper limit of the normal range. Patients treated with the ELEA benznidazole product experienced more arthromyalgia, neutropenia, and neurological disorders (mainly paresthesias) than those treated with the Roche product. Both drug products resulted in approximately the same percentage of permanent withdrawals. No gender differences were observed in the proportion of patients discontinuing benznidazole therapy as a result of AEs (females at 49 of 322 [15%] versus males at 24 of 150 [16%]).

Morillo et al. (2015)[153]: This was a randomized, placebo-controlled study of benznidazole given at 5mg/kg/day for 60 days to adult patients ages 18 to 73, with established Chagas

[151] Pontes, Vânia Maria Oliveira de, et al. "Adverse reactions in Chagas disease patients treated with benznidazole, in the State of Ceara." *Revista da Sociedade Brasileira de Medicina Tropical* 43.2 (2010): 182-187.

[152] Molina, I., et al. "Toxic profile of benznidazole in patients with chronic Chagas disease: risk factors and comparison of the product from two different manufacturers." *Antimicrobial agents and chemotherapy* 59.10 (2015): 6125-6131.

[153] Morillo, Carlos A., et al. "Randomized trial of benznidazole for chronic Chagas' cardiomyopathy." *New England Journal of Medicine* 373.14 (2015): 1295-1306.

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cardiomyopathy. A total of 2854 patients underwent randomization, with 1431 assigned to the benznidazole group and 1423 to the placebo group. The standard regimen (5 mg per kilogram of body weight per day for 60 days) was modified in February 2009 to the administration of a fixed dose of 300 mg per day and a variable duration of therapy (between 40 and 80 days) on the basis of the patient's weight, thereby preserving the total dose. The drug was initially from Roche (approximately half of the patients) and later from LAFEPE (the second half of patients who received benznidazole). A total of 342/1429 (23.9%) benznidazole recipients presented adverse events. Rash was the most common manifestation, occurring in 137/1429 (9.6%), gastrointestinal symptoms were observed in 112/1429 (7.8%), peripheral neuropathy was present in 52/1429 (3.6%) and a total of 192/1429 (13.4%) discontinued treatment due to adverse events, most commonly rash. Elevations of transaminases were observed in less than 5% of patients, and leukopenia in 0.1%.

Reviewer's comment: *Treatment with benznidazole for 60 days may be associated with higher rates of adverse events, and peripheral neuropathy and arthromyalgias appear to be reported more frequently in association with prolonged treatment. All adverse events are reported to be reversible with treatment interruption or discontinuation.*

Table 50 below shows the main adverse drug reactions reported in studies submitted with datasets, plus those reported in studies published in the literature and also in post-marketing reports.

Table 54: Main adverse drug reactions associated during treatment with benznidazole

Body Systems	Adverse Drug Reactions
Dermatological	• Maculo-papular cutaneous eruptions • Erythematous plaques • Rash • Rash, generalized • Rash, erythematous • Pruritic rash • Blistering eruptions • Peeling skin • Exfoliative dermatitis • Toxic epidermal necrolysis (TEN) • Acute generalized exanthematous pustulosis (AGEP) • Erythema multiforme • Drug reaction with eosinophilia and systemic symptoms
Neurological (central and peripheral nervous system)	• Paresthesia • Hypoesthesia • Tremors • Dizziness • Headaches • Insomnia • Convulsions • Inability to concentrate • Amnesia, temporary • Disorientation, temporary
Gastrointestinal	• Nausea • Vomiting • Diarrhea • Abdominal pain • Epigastric pain • Anorexia • Decreased appetite • Dry mouth • Ageusia

Hepatobiliary disorders	• Hepatitis • Toxic hepatitis
Skeletal Muscle	• Arthralgia • Myalgia • Musculoskeletal pain • Migratory arthritis
General / Constitutional Symptoms	• Fever • Asthenia • Fatigue
Lymphatic	• Generalized edema • Eyelid edema • Edema in the extremities • Lymph nodes enlargement • Lymphadenopathy
Bone Marrow	• Leukopenia • Thrombocytopenia • Granulocytopenia • Neutropenia • Agranulocytosis
Metabolism / laboratory	• Elevation of hepatic transaminases • Elevation of alkaline phosphatase • Elevation of bilirubin • Elevation of creatinine

8.4.6. **Laboratory Findings**

Transaminase elevations and hematological changes were noted in all studies submitted for review. All were transient and mostly mild, occurring in 10% or less among treated children and adult patients. There were no cases of Hy's law, all the elevations of transaminases and bilirubin were observed concomitantly with elevations of alkaline phosphatase. In a published series of 472 treated adult patients (68% were women, median age 37 years), transaminases elevations were found

Hematology and chemistries were similar in both benznidazole and placebo groups in the Sosa Estani study; transient increase in transaminases was observed in 3/55 (5.5%) patients

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(laboratory data was summarized in the publication, not available in the datasets submitted).

In the De Andrade study, the frequency of anemia (hemoglobin ⩽ 110 g/L) was similar in the two groups (15·4%) and no patient developed leucopenia (white-cell count <0·3 × 10^9/L) or neutropenia (neutrophil count <0·75 × 10^9/L). Results of liver and kidney function tests were within normal limits at baseline and did not change significantly during the study. ECG recorded at the end of the follow-up showed one (1·7%) incident case of complete right bundle branch block in the benznidazole group and four (6·9%) in the placebo group (p=0·36).

In the Altcheh study (n=38), one patient (1.2%) had a transient increase of creatinine more than 3 times the upper limit of normal and 4 patients (4.9%) had neutropenia (neutrophil count less than 1x10^9/L) . All these changes resolved after treatment.

8.4.7. **Vital Signs**

This section does not apply to this review, since benznidazole does not alter a specific vital sign as a result of its pharmacological action.

8.4.8. **Electrocardiograms (ECGs)**

Electrocardiograms were recorded and findings reported in the children and adults studies. The incidence of new changes was described, specifically new electrocardiographic changes typically associated to Chagas disease. Data are available from publications of clinical studies and review articles. No clinically significant changes specifically associated with the use of benznidazole were identified in pediatric or adult studies. Please see in the next Section on QT prolongation for more details.

8.4.9. **QT**

The Applicant presented available patient data and other relevant preclinical studies regarding the effects of benznidazole. A consult was requested to the CDER DCRP QT Interdisciplinary Review Team (IRT team) at FDA. The response, dated August 5, 2016, filed in DARRTS is summarized below.

The material for the safety review on QT prolongation, submitted by the Applicant included Chemo Research Study 50553926. This study evaluated the effect of BNZ in the hERG tail current recorded from HEK-293 cells stably transfected with hERG-1 cDNA. BNZ at a concentration of 30 and 100 µM showed a significant inhibition (7.9 ± 1.7% and 17.0 ± 2.7%, respectively) of the peak amplitude compared to the peak amplitude at the end of the vehicle

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perfusion period (one-tailed paired t-test, p<0.01). The positive control (0.1 µM E-4031) showed a significant inhibition of 91.8 ± 1.2%, supporting the reliability of the study. The study showed that the concentration of 100 µM was higher than the maximal anticipated therapeutic plasma concentration. The Cmax values for B Z are around 3.3 mg/L (12.8 µM) after single dose and 10 mg/L (38.4 µM) at the steady state.

In addition, six publications described clinical experience with benznidazole (5 clinical studies and 1 review article), plus detailed information submitted on 19 patients whose ECG reviewed by a cardiologist in one adult study (CHAGAZOL study) and other review from ECGs performed in participants from the DNDi E1224 study, were also available for review.

The team concluded that *"none of the studies, which span a period of almost 20 years (1996 to 2015), report general cardiac concerns or specific events of prolonged QT interval, torsades de pointes, or cardiac failure. Additionally, BNZ has been approved in several countries for treatment of Chagas disease, with no reports of QT prolongation, torsades de pointes, or cardiac failure".*

The final conclusions and recommendations were as follows:
"The hERG testing, observations of ECG tracings and the lack of cardiac toxicity reports in published placebo-controlled clinical trials of benznidazole are not sufficient to exclude small clinically relevant QTc prolongation (10 ms). However, we consider the world-wide clinical use of benznidazole is persuasive. Considering the totality of evidence, we agree with the sponsor that the likelihood of benznidazole to significantly prolong QTc interval is low and a TQT study is not needed for the planned NDA".

This consult response was discussed among the clinical and Clinical Pharmacology team, and the conclusions and recommendations from the IRT team were accepted. A TQT study was not required from the Applicant in this NDA.

8.4.10. Immunogenicity

There are no known descriptions of production of neutralizing antibodies against benznidazole. This section does not apply to this drug review.

8.5. Analysis of Submission-Specific Safety Issues

Significant safety risks described in animal studies include carcinogenicity, fetal toxicity, fertility impairment in male rats.

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These specific toxicities are discussed in in Section 4.4. For more details, please refer to the review by Dr. James Wild, Ph.D., Pharmacology and Toxicology Reviewer. Other significant and potentially serious adverse events have been described in sections 8.41, 8.4.2 and 8.4.4.

In human studies, even large cohorts with prolonged follow-up of more than 1000 patients[154], increased frequency of malignancies, fetal malformations or decreased fertility effects have not been reported. However, randomized controlled clinical trials have not been conducted to specifically look for evidence of these risks in benznidazole-treated patients compared to non-treated ones. Therefore, the occurrence of these potential risks is unknown.

8.6. Safety Analyses by Demographic Subgroups

Age and Adverse Events

In the Sosa Estani and De Andrade studies, a subgroup analysis exploring the frequency of adverse events by age was performed. A numerically higher number of AEs were observed in children older than 10 years of age in both studies. The risk difference was calculated in the groups of children younger and older than 10 years. The comparison was made to the matching placebo group. The risk difference appears higher in the younger group, probably as an effect of lower rates of the placebo recipients. The sample sizes are too small to make any conclusions. However, in children younger than 10, 14/55 (58.3%) presented adverse events and 19/55 (61.3%) in the group older than 10 presented adverse events in the Sosa Estani study. A similar trend was observed in the De Andrade study, where 6/40 (15%) of children older than 10 and only 1/24 in the 7 to 9 group (4.2%). Females had a higher rate of AEs in the Sosa Estani study, 21/30 females (70%) and 12/25 males (48%) presented AEs. However, this trend was not observed in the De Andrade study, where 5/38 males (13.2%) and 2/26 females (7.7%) presented AEs. The small number of subjects limits the conclusions regarding these trends. However, a review of the literature presented below showed descriptions of higher rates in older children and also among females in several studies.

[154] Viotti, Rodolfo, et al. "Side effects of benznidazole as treatment in chronic Chagas disease: fears and realities." *Expert review of anti-infective therapy* 7.2 (2009): 157-163.

Reference ID: 4134724

Table 55: Subgroup analysis of Sosa Estani study – AEs according to demographic characteristics

Subgroup	**Benznidazole 5mg kg for 60 Days N=55**		**Placebo N=51**		**Risk Difference (95% CI)**
	n(%)	**Total,N**	**n(%)**	**Total,N**	
Safety Subgroup (TRTEMFL = 'Y')	33 (60.0)	55	14 (27.5)	51	32.55 (14.73, 50.37)
SEX					
Male	12 (48.0)	25	6 (24.0)	25	24.00 (-1.76, 49.76)
Female	21 (70.0)	30	8 (30.8)	26	39.23 (15.07, 63.39)
AGE					
< 10	14 (58.3)	24	4 (19.0)	21	39.29 (13.38, 65.19)
>= 10	19 (61.3)	31	10 (33.3)	30	27.96 (3.90, 52.01)

Table 56: Subgroup analysis of De Andrade study - AEs according to demographic characteristics

Subgroup	**Benznidazole 7.5mg kg for 60 Days N=64**		**Placebo N=65**		**Risk Difference (95% CI)**
	n(%)	**Total,N**	**n(%)**	**Total,N**	
Safety Population (TRTEMFL = 'Y')	7 (10.9)	64	2 (3.1)	65	7.86 (-0.86, 16.58)
SEX					
Male	5 (13.2)	38	0 (0.0)	38	13.16 (2.41, 23.91)

Female	2 (7.7)	26	2 (7.4)	27	0.28 (-13.95, 14.51)
AGE Group					
10-12 years	6 (15.0)	40	2 (4.7)	43	10.35 (-2.38, 23.08)
7-9 years	1 (4.2)	24	0 (0.0)	22	4.17 (-3.83, 12.16)

Altcheh *et al*. 2011 described adverse events in 107 children between 10 days and 19 years old (average of age 6.9 years), diagnosed with asymptomatic infections of *T. cruzi*, treated with benznidazole at 5 to 8 mg/kg/day, administered two or three times a day for 60 days, and monitored for a period of three years. The statistical analysis showed that the occurrence of adverse events varied by age, being more frequent among older children. The average age of the children presenting adverse reactions was 9.9 years (95% CI: 8.2–12), significantly higher than the average age of the children with no adverse events (4.8 years [95% CI: 3.7– 6.0]; p<.001, *t* test). Moreover, 77.3% of the adverse events occurred in children more than seven years old, while only 18% of children less than two years old (7/38) presented adverse events, at rates significantly lower than those noted for older children (18% *vs* 53%; P <.001).

Gender and Adverse Events

An exploratory subgroup analysis of demographic characteristics and distribution of AEs was conducted in the two randomized controlled studies in children (Sosa Estani and De Andrade). Combining both study populations, there was a higher rate of adverse events in females, (23/56, 41.1%) as compared to males (17/63, 27%). This is illustrated in the table below.

Table 57: Subgroup Analysis - Integrated Safety, Sosa Estani and De Andrade studies

Study Population	**Benznidazole N=119**		**Placebo N=116**		**Risk Difference (95% CI)**
	n(%)	**Total, N**	**n(%)**	**Total, N**	
Safety Population (TRTEMFL = 'Y')	40 (33.6)	119	16 (13.8)	116	19.82 (9.27, 30.38)
SEX					

Male	17 (27.0)	63	6 (9.5)	63	17.46 (4.32, 30.60)
Female	23 (41.1)	56	10 (18.9)	53	22.20 (5.56, 38.85)
Source: adsl, adae - Safety Population (TRTEMFL = 'Y')					

Some studies have reported a more frequent occurrence of AEs in women, in both pediatric and adult patients. In one study of 190 patients[155], ages 13 to 65 years, in which 58.9% were male, the frequency of adverse events was significantly higher in women, occurring in 49 of 78 females (62.8%) and 44 of 68 (39.2%) males, p=0.001. In this study, there were more frequent discontinuations of treatment due to adverse events in women. In another study of 472 treated patients[156] (68% were women with a mean age of 37 years) women had an overall higher incidence of AEs compared to male patients (261 out of 322, 81.3% vs 99 out 150, 66% respectively, p=0.001), specifically digestive tract disturbances (77 out of 322, 23.9% vs 19 out of 150, 12.7%, p: 0.004) and also general symptoms (102 out of 31.7% vs 30 out of 150, 20%, p: 0.008). Women also had a higher proportion suffering hypersensitivity-related events, but this did not reach statistical significance (175 out of 322, 65.1% vs 69 out of 150, 46.3%, p=0.058). No differences were observed between the sexes in the numbers discontinuing benznidazole therapy as a result of AEs (females: 49 out of 322, 15% vs males: 24 out of 150, 16%).

8.7. Specific Safety Studies/Clinical Trials

No specific safety studies have been conducted with benznidazole.

[155] Hasslocher-Moreno, Alejandro M., et al. "Safety of benznidazole use in the treatment of chronic Chagas' disease." *Journal of antimicrobial chemotherapy* 67.5 (2012): 1261-1266.

[156] Molina, I., et al. "Toxic profile of benznidazole in patients with chronic Chagas disease: risk factors and comparison of the product from two different manufacturers." *Antimicrobial agents and chemotherapy* 59.10 (2015): 6125-6131.

Reference ID: 4134724

8.8. Additional Safety Explorations

8.8.1. Human Carcinogenicity or Tumor Development

The carcinogenicity of benznidazole was determined in mice and in rabbits (Teixeira, 1990a, 1990b, 1994) treated for up to 60 consecutive days by i.p. injection at doses of 8 mg/kg/day. Treatment-related lymphomas were induced in both species. The genotoxicity of benznidazole has been determined in vitro in several bacterial species and mammalian cell systems and in vivo in mammals including humans (Gorla, 1987, 1988 and 1991).

However, Gorla & Castro (1985)[157] did not report any significant increase in the formation of micronuclei in the bone marrow or spleen lymphocytes of rats treated with at dosages up to 2000 mg/kg, either p.o. or i.p., was observed, and Souza et al. (1991)[158] did not report any significant increase in the frequency of chromosome aberrations in bone marrow cells or the appearance of micronuclei in the peripheral blood cells of rats and Balb/c mice. In this study, rats were exposed to acute treatment with benznidazole (Rochagan) by gavage at total doses of 150, 300, 1500, 2000 and 3000 mg benznidazole/kg body weight and killed at different times. In the chronic treatments, healthy and chagasic Balb/c mice were treated with benznidazole by gavage at a dose of 100 mg bz/kg/day for 10 and 25 days. No significant increase in frequency of chromosomal aberrations in bone marrow cells or of micronuclei in peripheral blood cells was detected in the animals acutely or chronically exposed to benznidazole in vivo.

Prospective and controlled cohorts of a total of more than 1000 benznidazole-treated patients with long term follow-up averaging more than 10 years (0.1 to 27 years), have not reported the incidence of cancer. These trials have been discussed in Section 7.

A comprehensive safety review of benznidazole details the frequency of cancer in 1047 adult treated and 2228 non-treated patients[159] followed up for a mean of 7.5 years. A total of 7 of

[157] Gorla, N. B., and J. A. Castro. "Micronucleus formation in bone marrow of mice treated with nifurtimox or benznidazole." *Toxicology letters* 25.3 (1985): 259-263.

[158] Souza, Sandra C., Catarina S. Takahashi, and J. S. Da Silva. "Evaluation of the mutagenic potential of the antichagasic drug Rochagan in healthy and chagasic rodents." *Mutation Research/Genetic Toxicology* 259.2 (1991): 139-145.

[159] Viotti, Rodolfo, et al. "Side effects of benznidazole as treatment in chronic Chagas disease: fears and realities." *Expert review of anti-infective therapy* 7.2 (2009): 157-163.

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1047 (7%) and 17 of 2280 (7.5%) patients, with a mean age (at admission)of 47.7 and 53.1 years of age, treated and untreated, respectively, were diagnosed with cancer. The types of cancer were solid tumors of various organs (brain, breast, esophagus, gallbladder, kidney, leukemia, lung, ovary, pancreas, prostate, stomach, thyroid, bladder and uterus), without any predominance of one type of tumor in treated or untreated patients. The patients with a diagnosis of cancer had a similar distribution among males and females (3/7 in the treated group and 8/17 in the untreated group were males).

8.8.2. Human Reproduction and Pregnancy

Chagas Disease (CD) and Pregnancy

Chagas disease, or American trypanosomiasis, is a zoonosis caused by the parasite *Trypanosoma cruzi* (a flagellated protozoan parasite). World Health Organization (WHO) disease burden estimates place CD first among parasitic diseases in the Americas.[160] Twelve million people are estimated to have CD, and 25,000 CD-related deaths occur each year in the world.[161] On the basis of the size of the Latin American immigrant population and the estimates of *T. cruzi* prevalence in their home countries, it is estimated that 300,000 infected immigrants reside in the United States[162]. Infection is life-long in the absence of effective treatment. The most important consequence of *T. cruzi* infection is cardiomyopathy, which occurs in 20 to 30% of infected persons[163].

Maternal infection can cause adverse effects during pregnancy relating to fetal growth and maturity, abortion, prematurity, increased neonatal mortality and transmission of Chagas Disease.[164,165] Congenital transmission of *T. cruzi* is diagnosed when a neonate is born from an infected mother with positive serology or *T. cruzi* parasites circulating in the blood; when *T. cruzi* parasites are detected in the neonate at birth or shortly thereafter, or when *T. cruzi* antibodies not of maternal origin are detected after birth; and when transmission to the neonate by vectors or blood transfusion has been ruled out.[7]

[160] WHO technical report, 2008
[161] Corrêa VR *et al*. Uneventful benznidazole treatment of acute Chagas disease during pregnancy: a case report. Rev Soc Bras Med Trop. 2014. May-Jun; 47(3):397-400.
[162] Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis 2009; 49(5): e52-e54.
[163] Rassi A Jr, Rassi A, Marcondes de Rezende J. American trypanosomiasis (Chagas disease). Infect Dis Clin North Am 2012; 26: 275-91.
[164]Dao L. Otros casos de enfermedad de Chagas en el Estado Guárico (Venezuela): formas agudas y crônicas; observación sobre enfermedad de Chagas congénita. Rev Policlín Caracas 1949; 17:17-32.
[165] Carlier Y *et al*. Congenital Chagas disease: recommendations for diagnosis, treatment and control of newborns, siblings and pregnant women. PLoS Negl Trop Dis. 2011; 5:e1250.

Reference ID: 4134724

Only two drugs, nifurtimox and benznidazole are currently available for the treatment of Chagas Disease, both with similar effectiveness and limitations[166, 167]. Both drugs are associated with a high risk of toxicity in adults, especially dermatologic reactions[12]. Benznidazole is the most commonly used drug in South America, due to availability issues[12].

Because safety of benznidazole in pregnancy has not been established, it is not recommended for use in treating pregnant women[9]. Prevalence of vertical transmission of *T. cruzi* infection from immunocompetent women to their fetus varies from 0.1% to 18% among regions[168], and such transmission is strongly associated with the maternal blood-parasite load [9,169]. However, patients co-infected with HIV exhibit higher levels of parasitemia and a higher congenital transmission rate than those who are not co-infected[170]. Overall, 60-90% of congenitally infected children are asymptomatic. A small percentage of infected children present with clinical conditions common to other congenital infections, including hepatosplenomegaly, sepsis, myocarditis, hepatitis, meningoencephalitis, edema, fever, anemia, and jaundice.[171,172] Infected infants are presumed to carry the same 20–30% lifetime risk of cardiac or gastrointestinal disease as other infected individuals[173].

Pregnancy and Lactation Labeling

REVIEW

The following review of pregnancy and lactation labeling was performed by Dr. Jane Liedtka, M.D., Medical Officer from the Department of Maternal Health, CDER, FDA, in collaboration with DAIP Division staff (Pharmacology/Toxicology and Clinical reviewers and team leaders). The results of the literature searches were discussed at team meetings and agreements were reached to make labeling recommendations.

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[166] Jannin J, Villa L. An Overview of Chagas Disease Treatment. Mem Inst Oswaldo Cruz. 2007; 102 (Suppl 1): 95-7.
[167] Viotti R, Vigliano C. Etiological Treatment of Chronic Chagas Disease: Neglected "Evidence" by Evidence-Based Medicine. Expert Rev Anti Infect Ther. 2007; 5: 717-26.
[168] Carlier Y, Truyens C. Maternal-fetal transmission of Trypanosma cruzi, one hundred years of research. In: American trypanosomiasis: Chagas disease. London: Elsevier; 2010. p. 539–81.
[169] Bern C *et al*. Congenital Trypanosoma cruzi transmission in Santa Cruz, Bolivia. Clin Infect Dis. 2009; 49:1667–74.
[170] Scapellato PG *et al*. Mother-child transmission of Chagas disease: could coinfection with human immunodeficiency virus increase the risk? Rev Soc Bras Med Trop. 2009; 42:107–9.
[171]Carlier Y, Torrico F. Congenital infection with Trypanosoma cruzi: from mechanisms of transmission to strategies for diagnosis and control. Rev Soc Bras Med Trop 2003; 36: 767-771.
[172] Rassi A *et al*. Busca retrospectiva da transmissão maternal da infecção chagásica em pacientes na fase crônica. Rev Soc Bras Med Trop 2004; 37:485-489.
[173] Bern C *et al*. Acute and Congenital Chagas Disease. In: Advances in Parasitology, Volume 75. 2011. Elsevier Ltd.

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Pregnancy

Nonclinical Experience

In an embryo-fetal study in female rats, an oral dose of 150 mg/kg/day during organogenesis (days 6-17 of gestation) was associated with reduced fetal weights and smaller litter sizes. It also was associated with fetal malformations, principally eye abnormalities including microphthalmia and anophthalmia. Another fetal finding (anasarca) in rats treated at 50 and 150 mg/kg/day benznidazole was also considered to be associated with treatment. These effects are indicative of benznidazole-related developmental toxicity and teratogenicity in rats. The dose in this study, 50 mg/kg/day, is equivalent to approximately 0.6 times the maximum recommended dose in humans based on body surface area comparisons.

In an embryo-fetal study in pregnant rabbits, oral (gavage) administration of benznidazole during organogenesis (days 6 to 19 of gestation) at doses of 2.5, 7.5, and 25 mg/kg/day was associated with maternal effects at the high dose including reduced weight gain and food consumption and abortions in 2/20 females in this group. Despite the maternal effects, there was no adverse effect of treatment on fetal weights or embryo-fetal development at any dose. Benznidazole did not increase malformations and was not teratogenic in this study. The maternal NOAEL doses for maternal and fetal toxicity in this study were 7.5 and 25 mg/kg/day respectively which are equivalent to approximately 0.2 and 0.6 times the plasma AUC exposure associated with the maximum recommended dose in humans.

For further details, the reader is directed to the Nonclinical Review by James Wild, Ph.D.

Applicant's Review of Literature

The Applicant performed a literature search using the terms pregnancy and benznidazole in PubMed, Embase platform and LILACS database and identified nineteen publications. Of these, six were considered relevant and were submitted in full (the others were summarized or the abstract provided). Of the six, three case reports regarding benznidazole use in pregnancy were included (two in English, one in Spanish) and are summarized in Table 1 in the section of this review entitled "DPMH's Review of the Literature". One report of a screening program in Spain, which identified three pregnancies, is also included in Table #1.

DPMH's Review of the Literature

DPMH conducted a search of published literature in Embase using the search terms "benznidazole and pregnancy," "benznidazole and pregnant women," "benznidazole and

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pregnancy and birth defects," "benznidazole and pregnancy and congenital malformations," "benznidazole and pregnancy and stillbirth," "benznidazole and spontaneous abortion" and "benznidazole and pregnancy and miscarriage." No reports of adequate and well-controlled studies of benznidazole use in pregnant women were found. A few general articles discussing Chagas Disease with subsections on pregnancy were identified.

There is no reference to benznidazole in *Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk*[174]. Micromedex[175] has no information available on benznidazole. TERIS[10] notes that the "Magnitude of teratogenic risk to child born after exposure during gestation is "undetermined" and that the "quality and quantity of data on which risk estimate is based is "none'. TERIS does include a short summary of the case report by Correa[176] *et al*., (2014) included in Table 1 below.

Table 1 summarizes what is known about the cases of pregnancy associated with Chagas Disease that are reported in the literature.

[174] Briggs, GG. Freeman, RK. & Yaffe, SJ. (2015). Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk. Philadelphia, Pa, Lippincott Williams & Wilkins.
[175] Truven Health Analytics information, http://www.micromedexsolutions.com/. Accessed 5/31/17.
[176] Correa VR *et al*. Uneventful benznidazole treatment of acute Chagas disease during pregnancy: a case report. Rev Soc Bras Med Trop 47(3):397-400, 2014.

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Table 58: Pregnancies Exposed to Benznidazole Reported in the Published Literature

Pregnancy # Author/Year/Country	Maternal Age	Diagnosis	Timing of Exposure/ Dose	Confounders	Outcome
#1 Bisio[177] *et al* 2013 Argentina	33 years	HIV, Chagas reactivation with meningoencephalitis	Weeks 32-36, 5 mg/kg/day for 84 days	lamivudine, zidovudine, and nevirapine started at week 26 of pregnancy for HIV	Elective cesarean section at 36 weeks of 1700 gm female infant (low birth weight) with Apgars of 7/8 at 1+5 minutes, hospitalized for 32 days, negative for Chagas disease (at birth and one year) and HIV, no long term follow-up for infant reported
#2 Corrêa[21] *et al* 2014 Brazil	22 years	Chagas pericarditis and right bundle branch block (RBBB)	≈ first 2 weeks, 300mg/day X 4 weeks(wks), ↓200mg/day (for ↑ LFTs) X 3 months	None reported	Cesarean section at unreported gestational age (?term), negative for CD at 24 days and at 2-3 months, reported to have normal growth at that time
#3 Almuna[178] *et al (in Spanish)* 1981 Chile	25 years	Chagas Disease	Week 32, 5mg/kg/day X 60 days	Given oral corticosteroids at week 34 for rash, h/o tuberculous pleurisy	Cesarean section at 36 weeks, delivery of a 2250 gm infant with Tetralogy of Fallot, Apgars of 8/8 at 1+5 minutes, no evidence of Chagas disease at birth
#4-6 Salas[179] *et al*	Not reported,	Chagas Disease	2 women treated with	Not reported	No information reported except for the negative Chagas serology on the two infants of women

[177] Bisio M *et al*. Benznidazole Treatment of Chagasic Encephalitis in Pregnant Woman with AIDS. Emerging Infectious Diseases. 2013; 19, (9): 1490-1492.
[178] Almuna, R *et al*. Chagas disease and pregnancy. Review apropos of a clinical case. Treatment. Revista chilena de obstetricia y ginecologia 46.3 (1981): 107.
[179] Salas J *et al*. Chagas screening in pregnant women from Latin America: Experience in western Almeria. Tropical Medicine and International Health. 2011; 16 Suppl.1: 288.

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2011 Spain	288 women screened, 3 positive		unknown dose and duration, one lost to follow-up		from Bolivia

Source: Reviewer's Table

Table 59: Publications to Support Clinical Considerations in Pregnancy

Table 2: Publications to Support Clinical Considerations For Benznidazole

Author/ Year/ Country	Total # of patients	Diagnostic test for Chagas	Age Range (years)	Conclusions/ Outcomes	Comments
Torrico 2004 Bolivia	Cohort A[2]=1606 Cohort B[3]=3879 Within B: 100/762 M+B- pairs in study, 99/3070 M-B- pairs in study	A, M-B-[4]: 1162 A, M+B-[5]: 422 A, M+B+[6] : 22 B, M-B-[4]: 99 B, M+B-[5]: 100 B, M+B+[6]: 47 All with IHAT		Mothers of positive babies had 3-4 fold↑ in PROM[7], Apgars, birth weights and lengths, gestational ages and head circumference↓ in positive babies, Mortality was significantly ↑ in M+B+ babies in both cohorts, 5-6% transmission of Chagas in both cohorts	Maternal *T. cruzi* infection affects intrauterine growth and maturity of congenitally infected infants, but not of noninfected fetuses.
Schenone 1985 Chile	506 urban + rural women	266 IHAT[28] + 240 IHAT -	10-40	No difference in mean weight of infants at birth	

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Bittencourt 1984 ?Brazil				Compared the frequency of prematurity, abortion, stillbirths and neonatal deaths in chagasic and non chagasic mothers and in a group of chagasic women who transmitted the infection-only difference is ↑neonatal deaths in infected infants	
Hernandez-Matheson	604 patients	302 GMCFT[29]+ 302 GMCFT-		1.8 Fold ↑ in pregnancy loss (SAB[30], stillbirths, neonatal death in the first week of life) after adjustment for	No difference in rate of prematurity

[2]between 1992-1994, 1606 patients screened [3]between 1999-2001, 3879 patients screened [4]M-B- : Mother and baby negative for Chagas [5]M+B-: Mother positive and baby negative for Chagas [6] M+B+: Mother and baby positive for Chagas
[7] PROM= Premature rupture of membranes
[28] Indirect hemagglutination test
[29] Guerreiro and Machado complement fixation test
[30] SAB=spontaneous abortion

Author/ Year/ Country	Total # of patients	Diagnostic test for Chagas	Age Range (years)	Conclusions/ Outcomes	Comments
1983 Argentina				maternal age and birth order, ↑ in polyhydramnios and leg varicosities	
De Castilho 1976 ?Brazil	249 low birth weight (<2500 gm) live births	Indirect IF[31] test	Mean age≈ 26	No association between Chagas disease and prematurity after matching to controls of same sex, birth order and maternal age	Excluded known causes of premature birth such as placenta previa, eclampsia, HTN

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Oliveira 1966 ?Brazil	200	67 GMCFT[32]+ 133 GMCFT-		No difference in rate of spontaneous abortion	

Source: Reviewer's Table

Summary

Very limited human pregnancy outcome data (three cases with information on infant outcomes) were found in the published literature for benznidazole. Two of the three exposures were in the third trimester, and therefore, are uninformative with regards to potential teratogenicity. The third case was similarly uninformative due to very early exposure (within the first 2 weeks after conception, therefore likely before implantation). The findings in animal studies demonstrated increased fetal loss and eye anomalies at doses under the maximum human doses. Since there is evidence of fetal risk based on animal studies, a signal for mutagenicity and limited available data in humans, DPMH agrees with the Applicant and recommends that benznidazole not be used routinely during pregnancy (i.e., individual cases where maternal or fetal mortality or significant morbidity is threatened may be the exceptions). In addition, DPMH recommends adding a Warning and Precaution for embryo-fetal toxicity to the labeling for benznidazole.

Though information in the published literature is limited, there are reported increases in adverse pregnancy outcomes associated with Chagas Disease[10, 11]. These publications are summarized in Table 2 below and were used as a basis to craft the clinical considerations subsection for section 8.1 which will read as follows:

Disease-associated maternal and/or embryo/fetal risk

There are reports of increased pregnancy loss, prematurity and neonatal mortality in pregnant women who have chronic Chagas Disease.

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If a pregnant women presents with acute and symptomatic Chagas Disease, which is a rare occurrence, the risks versus benefits of (b) (4) treatment to the mother and the fetus should be evaluated on a case-by-case basis.

Lactation

Nonclinical Experience

There is no data available from animal studies regarding benznidazole's presence in milk or effects on lactation.

Applicant's Review of Literature

The Applicant performed a literature search using the terms lactation and benznidazole in PubMed, Embase platform and LILACS database and identified nine publications.
Those relevant to this review are included below in the discussion in the section of this review entitled "DPMH's Review of the Literature".

DPMH Review of the Literature

DPMH conducted a search of *Medications and Mother's Milk*[180], the Drugs and Lactation Database (LactMed),[181] Micromedex[182], and of published literature in Embase using the search terms "benznidazole and lactation" and "benznidazole and breastfeeding." No reports of adequate and well-controlled studies of benznidazole use in lactating women were found. The following publications were identified as relevant and are summarized below.

- Marson M[183] *et al* (2013) reported on development of an "accurate, sufficiently sensitive" method for the quantification of benznidazole in human milk. They obtained 17 breast milk samples at unspecified times from 10 women being treated for Chagas Disease with benznidazole 5-10 mg/kg/day. Breast milk concentration

[180] Hale, Thomas (2012) Medications and Mothers' Milk. Amarillo, Texas Hale Publishing, pg. 422-423.
[181] http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?LACT. The LactMed database is a National Library of Medicine (NLM) database with information on drugs and lactation geared toward healthcare practitioners and nursing women. The LactMed database provides information when available on maternal levels in breast milk, infant blood levels, any potential effects in the breastfed infants if known, alternative drugs that can be considered and the American Academy of Pediatrics category indicating the level of compatibility of the drug with breastfeeding.
[182] Truven Health Analytics information, http://www.micromedexsolutions.com/. Accessed 5/31/17.
[183] Marson M *et al*. A Simple and Efficient HPLC Method for Benznidazole Dosage in Human Breast Milk. Ther Drug Monit. 2013; 35:522–526.

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varied from undetectable (0.3 mg/L) and 9.8 mg/L, with a mean of 4.1 mg/L. The authors calculated that a fully breast fed infant would receive between 0.6-1.5 mg/kg daily dose

- Garcia-Bournissen,[184] *et al* reported on twelve lactating women treated for 30 days with benznidazole (BNZ) at an average dose of 5.65 mg/kg/day administered twice daily. The mean maternal age was 28.5 years. Median breast milk concentration was 3.8 mg/L (range 0.3-5.9) and 6.26 mg/L (range 0.3-12.6) in plasma. The median milk: plasma ratio was 0.52 (range 0.3-2.79). The median relative infant dose (assuming a daily milk intake of 150 ml/kg/day) was 12.3% of the maternal dose per kg (range 5.5-17%). No adverse effects were noted in the breastfed infants. The authors called this amount of milk transference "limited" and stated that in their opinion maternal BNZ treatment of Chagas disease was unlikely to present a risk to the breastfed infant.
- Padro JM *et al*. [185] reported on four women with Chagas disease who were treated with benznidazole 5 to 10 mg/kg daily in 2 divided doses and provided breastmilk samples for analysis after 4 to 10 days of therapy. The timing of the sample with respect to doses was not reported. Breastmilk concentrations of benznidazole ranged from nonquantifiable (<0.88 mg/L) to 7.1 mg/L.

Benznidazole is not referenced in *Medications and Mother's Milk* [23] or MicroMedex[10]. LactMed[9] notes the following in the "Summary of Use during Lactation"

Benznidazole is excreted into milk in dosages much lower than the treatment dosage for infants. Because of the low levels of benznidazole in breastmilk and safety when given directly to infants, its use is acceptable in nursing mothers.

Summary

There are data on the presence of benznidazole in human milk. A calculated relative infant dose of 12.3% was reported from a study of 12 lactating women[31]. Though LactMed finds this level acceptable and notes that it is much lower than doses given directly to infected infants, DPMH has concerns that the risk/benefit calculus for a non-infected infant in the United States (where alternatives to breast feeding our readily available) is less certain given the mutagenicity and

[184] Garcia-Bournissen F *et al.* Limited Infant Exposure to Benznidazole through Breast Milk during Maternal Treatment for Chagas Disease. Arch Dis Child. 2015; 100: 90-94.
[185] Padro JM *et al*. Development of an ionic-liquid-based dispersive liquid-liquid microextraction method for the determination of antichagasic drugs in human breast milk. Optimization by central composite design. J Sep Sci. 2015; 38:1591-600.

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carcinogenicity found in nonclinical models treated with doses of the product comparable to doses used for infected patients. This issue was discussed with the clinical team, the pharmacology/toxicology team and the clinical pharmacologist from DAIP and consensus was reached that "Breastfeeding is not recommended" during use of benznidazole.

Use in Females and Males of Reproductive Potential

Nonclinical Experience

In a pre- postnatal study in rats, first generation (F1) pups born to dams administered 15, 50, or 75 mg/kg/day benznidazole demonstrated normal pre-weaning behavior, physical and functional development, neurological findings, and reproductive parameters. However, cesarean section data for first generation (F1) females in the high-dose group included significantly higher pre-implantation loss and significantly lower mean values for corpora lutea counts, number of implantations, and number of live embryos. Also small testes and/or epididymides were observed in 1/20 and 2/20 F1 males in the mid- and high-dose groups respectively, and two of the affected animals failed to mate or induce pregnancy. However, the mean values for mating performance, fertility index, testes weight, testes and epididymides sperm counts, and epididymal sperm motility and progression were not altered in any of the F1 males in benznidazole treatment groups. The NOAEL value was considered to be 50 mg/kg/day which is equivalent to approximately 0.6 times the maximum recommended dose in humans based on whole body surface area comparisons.

The carcinogenicity of benznidazole was evaluated in mice and in rabbits (Teixeira, 1990a, 1990b, 1994) treated for up to 60 consecutive days by intraperitoneal injection at doses of 8 mg/kg/day. Treatment-related lymphomas were induced in both species. Genotoxicity of benznidazole has been shown to occur *in vitro* in several bacterial species and mammalian cell systems and *in vivo* in mammals including humans.

For further details, the reader is directed to the Nonclinical Review by James Wild, Ph.D.

Applicant's Review of Literature

The Applicant performed a literature search using the terms fertility and benznidazole in PubMed, Embase platform and LILACS database and identified four publications. The Applicant did not find these relevant to labeling and the articles were not provided.

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DPMH's Review of Literature

DPMH conducted a search of published literature in PubMed and Embase regarding benznidazole and its effects on fertility and found several nonclinical articles which will not be discussed further in this review as they are not to be included in labeling. Articles regarding the toxicity, mutagenicity and potential carcinogenicity of benznidazole were reviewed, and in addition to the animal findings, contributed to concerns about the use of this product in pregnancy and lactation.

Summary

There are no human data available on the effect of benznidazole on fertility. Based on animal fertility studies, benznidazole may impair fertility in male patients. Therefore, section 8.3 will also contain a section describing the potential for benznidazole to impair male fertility.
Based on the animal findings and the Warning and Precaution regarding embryo-fetal toxicity, recommendations for pregnancy testing and contraception will be added to section 8.3.

8.8.3. Pediatrics and Assessment of Effects on Growth

Benznidazole is administered in a course of 30 to 60 days, one time only. The described mechanism of action does not involve processes that would likely interfere with pediatric growth and development. Therefore, this specific effect has not been addressed.
Importantly, Chagas disease morbidity may affect fetal growth and early child development, together with other concomitant factors such as nutrition and access to health care. The use of benznidazole in children has been discussed in previous sections. Children tend to have fewer adverse events and discontinuations as compared to adults, especially children younger than 4 years of age.

8.8.4. Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Benznidazole does not belong to a class of drugs with drug abuse potential. This section does not apply. The effects of an overdose have not been reported; however, based on what is known about the drug profile, it is expected that if observed, these would be similar to those of the drug at usual doses, potentially with increased severity.

8.9. Safety in the Postmarket Setting

8.9.1. Safety Concerns Identified Through Postmarket Experience

Benznidazole has been in the market outside of the United States for more than 40 years outside of the United States. Using Empirica Signal, a search was conducted on May 24, 2017

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with key words “benznidazole”, “trypanocidal”, “Radanil” and “Rochagan”. Post-marketing safety has been discussed in section 8.4 Safety Results.

8.9.2. Expectations on Safety in the Postmarket Setting

It is expected that the Applicant will conduct a post-marketing study, which will collect additional safety data to further characterize the safety profile of their product. This study will evaluate (b) (4) and will serve as a confirmatory study for the Subpart H approval (b) (4). The safety database that supports the approval of the Applicant’s benznidazole product is based on a pharmacological bridge established with data from the original product that was used in the studies supporting this application. Therefore, a post-marketing study will allow for monitoring of adverse events and possibly detect new safety signals or different presentations of commonly described adverse events of benznidazole.

8.10. Additional Safety Issues From Other Disciplines

This has been discussed under Section 4.

8.11. Integrated Assessment of Safety

Benznidazole safety profile at oral doses ranging from 5 to 8 mg/kg/day for 30 to 60 days is well-established in children and adults, and adverse events and safety risks are consistent with those of its drug class, the nitroimidazoles. This is the only one of two treatment options available to treat Chagas disease worldwide. Overall, most patients complete treatment without serious adverse reactions, and tolerability is greatest among newborns, infants and young children. In the two randomized controlled studies in children, as well as in the adult controlled study, it was well tolerated, with few discontinuations and no serious adverse events reported. The frequency of adverse events is directly proportional with age. Discontinuation rates are rare among newborns and usually not more than 10% in children in the indeterminate phase, while these rates may be as high as 40% among adults, usually about 20%, and are primarily cutaneous adverse reactions.

Potential serious risks are indicated from preclinical data. Data from animal studies show potential risks of carcinogenicity with increased number of lymphomas in rabbits and mice, genotoxicity of in vitro in several bacterial species and mammalian cell systems and in vivo in mammals including humans. Reproductive toxicity was also observed in rats, including fetal toxicity, and decreased male fertility observed in rats. However, these risks in humans have not been confirmed or reported in over 40 years of use outside in endemic countries.

Adverse events that have been observed in Chagas patients undergoing treatment with benznidazole can be categorized into several groups: skin and soft tissue, of which hypersensitivity dermatitis is the most common, gastrointestinal toxicities, including abdominal pain, nausea, vomiting and anorexia, with associated weight loss in some cases, polyneuritis and peripheral neuropathy, neutropenia, anemia and thrombocytopenia. No fatal adverse events causally related to benznidazole have been reported. In all serious adverse events and severe events cases, confounding treatments and co-morbidities prevent an assessment of causality. The vast majority of adverse events are known to be reversible, even though in some rare cases, complete resolution may take several months.

Dermatologic adverse reactions, mediated by hypersensitivity mechanisms, are the most frequently observed manifestation and are the most common cause of treatment discontinuations in children and adults. Most of them are mild, however the spectrum include maculopapular rashes, blister formation and generalized erythroderma, accompanied by edema, pruritus, lymph node enlargement, fever, elevation of transaminases and eosinophilia. Cases of toxic epidermal necrolysis (TEN), Stevens Johnson syndrome and acute generalized exanthematous pustulosis (AGEP) have been associated with benznidazole. It is not dose dependent and it appears around the 10^{th} day of treatment. Most cases resolve with temporary or permanent discontinuation of treatment, and in some cases it requires corticosteroids or antihistamine medications.

Gastrointestinal intolerance
This is the second most frequently reported adverse reaction causing treatment discontinuations. The reported rates of discontinuations vary in the different series, and are usually around 5 to 10%. Abdominal pain, nausea and anorexia with weight loss have been reported. Most of the symptoms are mild and in a small percentage can be severe (1%) and cause treatment interruption or discontinuation. Gastrointestinal symptoms include appetite loss, anorexia, and “digestive intolerance” (including vomiting and abdominal pain). Appetite loss was observed in 17% of the benznidazole treated Chagas patients in one study of adult patients, and was sometimes accompanied by weight loss; drug treatment was discontinued only in the more severe cases. Although anorexia in another study in adults occurred in 40% of the benznidazole treated patients, there was no need for discontinuation of benznidazole treatment. All symptoms are reported to resolve spontaneously or with treatment discontinuation.

Peripheral neuropathy
This is a serious toxic effect induced by benznidazole, with close connection with its therapeutic index. In most studies, it occurs towards the end of treatment, after 30 days in most cases, most frequently in adults. However, peripheral neuropathy is very difficult to evaluate in small

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children, particularly if it is mostly sensorial. It is interesting to note that patients perceive the first symptoms of polyneuritis, saying that cutting the nails and washing the hands in cold water become somewhat painful.

Other nervous system toxicities, such as neuromuscular (myalgia, gait problems) have been described early in the treatment course. All patients are susceptible to it; some authors state that it occurs when the treatment achieves the total dose around 18 grams[146]. However, there are no comparative trials in which different total doses have been compared; therefore, this is just a description on observations of the outcomes of different regimen durations and total doses used.

Hepatobiliary disorders and Renal toxicity
Elevation of transaminases and alkaline phosphatase has been reported, in 1-20% range. No Hy's law cases have been observed in any of the submitted trials for review or in the literature. No liver failure cases caused by benznidazole have been reported. In severe hepatotoxicity, several confounding factors precluded a causality assessment. Transaminases elevations usually resolve with treatment interruption or discontinuation. Renal function changes and transient increases of creatinine have been observed in small percentages. No permanent renal impairment has been reported with the use of benznidazole.

Depression of bone marrow
Another significant side effect is depression of bone marrow (neutropenia, agranulocytosis and thrombocytopenic purpura). Anemia has also been reported. These adverse reactions are occasionally causes of treatment interruption or discontinuation, in about 1% of cases[186]. They are all reported to be reversible and treated sometimes with corticosteroids and in some cases antimicrobial therapy, if infection is present. Since agranulocytosis can develop rapidly, periodic white-cell counts are recommended by most authors.

Rare adverse events include ageusia, migratory arthritis, pain syndrome, sometimes requiring several months to resolve, have been described in the published literature.

In summary, benznidazole adverse reactions are well known, in most cases mild, and even if severe, they are manageable with monitoring and are reversible with temporary or permanent treatment discontinuation. Uncertainties remaining are the potential for carcinogenicity, fetal toxicity, which would preclude its use in pregnancy, and potential decrease on male fertility, which have not been reported in humans, however, the actual risk has not been formally

[186] Bern, Caryn. "Chagas' disease." *New England Journal of Medicine* 373.5 (2015): 456-466.

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evaluated. Considering the serious and life-threatening nature of Chagas disease, and the demonstrated effectiveness of benznidazole treatment with a single course of therapy at doses of 5 to 8 mg/kg/day for 60 days duration, the benefits of treatment outweigh the risks of both therapy and untreated disease.

9 Advisory Committee Meeting and Other External Consultations

No Advisory Committee meeting was held for this NDA submission.

10 Labeling Recommendations

10.1. Prescribing Information

The Applicant's proposed label is currently under review. The label will include warnings and precautions of the drug class (nitroimidazoles). Labeling will contain the following Warnings and Precautions:

- Potential for Carcinogenicity and Genotoxicity
- Embryo-Fetal Toxicity
- Cutaneous Hypersensitivity Reactions
- Central and Peripheral Nervous System
- Bone marrow depression

Safety and efficacy information will include data from the trials conducted with the benznidazole product for which the Applicant has demonstrated adequate comparability, and has submitted raw datasets. Additional safety information described in the literature, as well as data from safety reports submitted to post-marketing databases (FAERS and Vigibase, from WHO) will be included in Section 6.2 of the label, Post-marketing experience.

10.2. Patient Labeling

A Medication Guide is not considered necessary. Risk management will be sufficient through labeling and post-marketing surveillance.

10.3. Nonprescription Labeling

This section does not apply to this NDA.

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11 Risk Evaluation and Mitigation Strategies (REMS)

The Division of Risk Management was consulted and, based on the clinical review, and the fact that the benefit-risk profile is favorable, DRISK and DAIP agree that a REMS is not necessary to ensure that the benefits of benznidazole outweigh the risks. Risk management will be sufficient with label recommendations and post-marketing surveillance. (b) (4) (b) (4).

11.1. Safety Issue(s) that Warrant Consideration of a REMS

Benznidazole's safety profile is well known and adverse reactions are identified through monitoring. Adverse reactions resolve with temporary or permanent discontinuation of treatment, and/or treatment (corticosteroids, antihistamines in some cases). No REMS was recommended. A post-marketing safety study will be conducted, with the Applicant's product.

12 Postmarketing Requirements and Commitments

The following requirements and commitments were sent to the Applicant as part of the Late Cycle Meeting Background Package. These are still under discussion and may be subject to change.

<u>Postmarketing Requirements</u>

- Confirmatory study required as a condition of Subpart H approval: We are proposing an (b) (4), single-arm study (b) (4) A historical control (b) (4) PK (b) (4) (b) (4)
- ADME/mass balance study in humans
- Male fertility study in rats (agreed to in the End of Phase 2 meeting on April 27, 2016)

(b) (4)

13 Appendices

13.1. References

References are included as footnotes throughout the document.

13.2. Financial Disclosure

The Applicant has submitted adequate financial disclosure of the studies submitted in support of this application. Chemo Research S.L. has not any disclosable financial arrangements and interests in relation with the mentioned clinical studies.

Covered Clinical Study (Name and/or Number):

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 22		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: _____ Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in S Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to	Yes ☐	No ☐ (Request information

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minimize potential bias provided:		from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) _____		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MARIA C ALLENDE
08/03/2017

THOMAS D SMITH
08/04/2017