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STATISTICAL REVIEW(S)



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STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

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Applicant: Chemo Research S.L

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1 EXECUTIVE SUMMARY

In this submission the Applicant, Chemo Research, S.L. is seeking the approval of (b) (4) (benznidazole) for the indication of the treatment of Chagas disease. Benznidazole has been used for years to treat Chagas disease but is not approved in the United States. Chagas disease is acquired from the bite of a triatomine bug infected with the protozoan *Trypanosoma cruzi* (*T. cruzi*) or congenitally from an infected mother. Chagas disease can be difficult to diagnose and assess for cure making choice of an efficacy endpoint complicated. It is typically diagnosed by conventional serologic tests which measure *T. cruzi* antibodies. These conventional serologic tests include an enzymatic immunoassay (EIA/Conventional ELISA), indirect hemagglutination assay (IHA), and indirect immunofluorescence assay (IFA). Additional, “unconventional” serologic tests are also used. One problem with these serologic tests is that it may take years for subjects to seroconvert (i.e., have their antibody level be reduced to a degree that it can be concluded they are no longer infected with Chagas disease). Xenodiagnosis methods are also used. This method allows an uninfected triatomine bug to feed on the blood from a subject. If the bug becomes infected with Chagas, it can be concluded that the subject was infected. However, false negatives are common making this test less useful as a conclusive endpoint. The ultimate endpoint of interest would be cardiac events which can occur decades after initial infection.

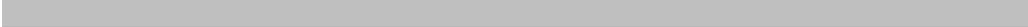
The Applicant’s submission includes five controlled efficacy studies of which two are pediatric studies (De Andrade study and Sosa-Estani study) and three are adult studies (Viotti, Molina and DNDi-CH-E1244). With the exception of DNDi-CH-E1244, all of the studies were literature-based. None of the studies were conducted under the Applicant’s IND, but patient-level data from all of the studies was submitted. The De Andrade and Sosa-Estani studies were conducted to test the superiority of benznidazole compared to placebo and measured serology as the primary endpoint. The Viotti study included long term follow-up and measured both serology and clinical endpoints. The other two adult studies (Molina and DNDi-CH-E1244) were conducted to assess the efficacy of a test drug versus benznidazole, as the active control. Due to a limited follow-up period in this adult population and the use of a polymerase chain reaction (PCR) endpoint, these studies are considered as supportive only.

The Sosa-Estani study was a randomized, parallel-group, double-blind, placebo-controlled study to evaluate the efficacy and safety of benznidazole in pediatrics 6 to 12 years of age. A total of 106 patients were enrolled and randomized into the study (55 in the benznidazole group and 51 in the placebo group). Subjects were followed for 4 years. Four serologic tests and xenodiagnosis were measured at various time points throughout the study. These serologic tests included EIA, IHA, IFA, and an unconventional assay, F29. Efficacy analyses were conducted using a modified-intent-to- treat (mITT) population which included patients who were positive using the specific serologic test at baseline. Only the unconventional F29 assay found a significant effect in the proportion of subjects who seroconverted during the trial. However, all of the assays showed significant reduction in the amount of antibodies in the benznidazole arm compared to control. The results of xenodiagnoses were only borderline significant.

The De Andrade study was a randomized, double-blind, parallel-group, placebo-controlled study to evaluate the efficacy and safety of benznidazole in pediatric patients aged 7 to 12 years. A total of 64 patients were randomized to benznidazole and 65 patients were randomized to placebo. Subjects were followed for 3 years. Similar results were seen in this trial with significant results in the proportion of subjects who seroconverted using the unconventional assay, and all of the assays showed significant reductions in the amount of antibodies.

The Viotti study was a non-randomized, no treatment controlled, parallel-group study to evaluate the long-term outcomes of patients aged 30 - 50 with non-acute Chagas disease. The study compared benznidazole to no treatment and followed subjects for up to 15 years. A total of 566 patients were included in this study. Benznidazole showed an effect in the proportions of patients converting to negative serology, the proportions of patients having new ECG abnormalities, and mortality compared to the untreated group. However, there remain some questions regarding the conduct of the trial that reduces our confidence in the results.

It is recommended that the results of the De Andrade study and Sosa-Estani study be considered adequate evidence of efficacy to support the indication of treatment of Chagas disease in pediatric patients for benznidazole. The Viotti study provides some support of the effectiveness of benznidazole in the adult population, along with the Molina and DNDi-CH-E1244. ^{(b) (4)}



APPEARS THIS WAY ON ORIGINAL



2 INTRODUCTION

2.1 Overview

This NDA is for (b) (4) (Benznidazole), a nitroimidazole derivate, for the proposed indication of the treatment of Chagas disease. An estimated 8 million adults and children are infected with Chagas disease worldwide with more than 10,000 deaths per year. The Centers for Disease Control (CDC) had estimated that over 300,000 persons infected with *T. cruzi* live in the United States. Benznidazole was originally marketed under Roche in Brazil, Argentina, Bolivia, Uruguay, Peru, and Nicaragua. Benznidazole was also marketed under the trade names Radanil®, Ragonil® or Rochagan® in Japan in the 1970s. In 2003, Roche gave up commercial rights and the technology to manufacture benznidazole to the Brazilian government as a generic version and withdrew its registration.

Benznidazole has not been previously approved in the United States. The Investigational New Drug application (IND) 118976 for benznidazole was submitted by Chemo Research in June 2013. This product was granted Orphan Drug Designation.

2.1.1 Specific Studies Reviewed

The Applicant's submission includes five studies, two randomized, controlled pediatric studies (De Andrade study and Sosa-Estani study) and three controlled adult studies (Viotti, Molina and DNDi-CH-E1244). None of the studies were conducted under the IND. The applicant obtained study information including patient-level data from the individual sponsors of the studies which were conducted years earlier. The amount of information available for each study is variable. The De Andrade and Sosa-Estani studies were conducted to test the superiority of benznidazole to placebo and measured serology as the primary endpoint. The Viotti study included long term follow-up and measured both serology and clinical endpoints. The other adult studies (Molina and DNDi-CH-E1244) were conducted to assess the efficacy of a test drug versus benznidazole as the active control. In both studies, the test drug failed to show adequate efficacy compared to benznidazole. These two adult studies measured polymerase chain reaction (PCR) and had only a limited follow-up period. The duration of follow-up was not sufficient to show a change in serology. Due to a lack of information on the clinical relevance of the PCR endpoint and the short duration of follow up, these studies are considered as supportive only. Please see the statistical review by Janelle Charles, Ph.D located in DARRTS for a full discussion of these studies. The focus of this review will be the De Andrade study, the Sosa-Estani study and the Viotti study. A brief summary of all studies are provided in [Table 1](#).

Table 1: List of All Efficacy Studies Included in the NDA

Protocol	Phase and Design	Dosing Regimen	Dosing Duration	# of Subject per Arm	Study Population
Sosa-Estani study ¹	Phase 2 randomized, double-blind, placebo control, parallel-group	BZN: 5 mg Placebo	60 days of treatment, 48 months of follow-up	BZN: N= 51 Placebo: N= 55	Pediatric patients 6 – 12 years of age in the indeterminate phase of infection by <i>T. cruzi</i>
De Andrade study ²	Phase 2 randomized, placebo control, parallel-group	BZN: 7.5 mg/kg Placebo	60 days of treatment, 36 months of follow-up	BZN: N= 64 Placebo: N= 65	Pediatric patients 7 – 12 years of age in the early chronic phase of <i>T. cruzi</i> infection
Viotti study ³	Non-randomized, no treatment control, parallel-group	BZN: 5 mg/kg No treatment	30 days of treatment, 15years of follow-up	BZN: N= 283 Placebo: N= 283	Adult patients: 30 – 50 years of age with 3 positive results on serologic tests for <i>T. cruzi</i> infection and no clinical signs of heart failure (Kuschnir groups 0, I, or II)
Molina study ⁴	Phase 2 randomized, open-label, active-control, and parallel-group	BZN: 150 mg twice daily Posaconazole LD: 100 mg twice daily Posaconazole HD: 400 mg twice daily	60 days of treatment, 12 months of follow-up	BZN: N=26 Posaconazole LD-100: N= 26 Posaconazole HD- 400: N= 26	Adults patients 18 years or older with chronic Chagas disease (CD) in both its indeterminate and symptomatic form
DNDi-CH-E1244 study	Phase 2 randomized, placebo, active-control, and placebo-blinded parallel-group	BZN:100 mg twice daily E1224 HD: 400mg E1224: LD 200mg E1224 SD: 400mg for 3 weeks Placebo	8 weeks of treatment, 12 months of follow-up	BZN: N=45 E1224 HD: N=45 E1224 LD: N=48 E1224 SD: N=46 Placebo: N=47	Adults patients 18 years or older with chronic indeterminate Chagas disease

2.2 Data Sources

The data analyzed in this review come from three randomized controlled trials, two pediatric studies and one adult study submitted to support the efficacy and safety of benznidazole. The submission included publications based on the studies as well as the patient-level data. In addition, statistical analysis plans were submitted for the publications based on studies. These can be found in the electronic submission located at: <\\CDSESUB1\evsprod\NDA209570\0001>, <\\CDSESUB1\evsprod\NDA209570\0006> and <\\CDSESUB1\evsprod\NDA209570\0007>. Protocols were not included for these three studies.

The Molina and DNDi-CH-E1244 studies were reviewed by Dr. Janelle Charles in a separate statistical review and are only briefly discussed in this review.

3 STATISTICAL EVALUATION

3.1 Data and Analysis Quality

The datasets submitted were of acceptable quality. However, the analysis as reported in the Sosa-Estani paper could not be reproduced using the data submitted. Discrepancies found are discussed in the review. All tables and figures presented in this review are based on analyses conducted by the reviewer, unless otherwise indicated.

3.2 Evaluation of Efficacy

3.2.1 Sosa-Estani study

3.2.1.1 Study Design and Endpoints

Sosa-Estani was a randomized, parallel-group, double-blind, placebo controlled study to evaluate the efficacy and safety of benznidazole in patients in the indeterminate phase of infection of *T. cruzi*¹. The study was conducted from 1991- 1995. The article based on the study was published in 1998. The indeterminate phase lasts approximately 6 months after infection but prior to the chronic phase in which patients present with clinical evidence of heart disease. The trial was conducted in the Province of Salta in northwestern Argentina. Patients with at least two positive results for antibodies to *T. cruzi* by serologic tests were included in the study. Pediatric patients 6 to 12 years of age with antibodies to *T. cruzi* from 14 localities were matched within each locality by age to receive benznidazole (5 mg/kg/day) or placebo (no details given). A total of 55 patients were randomized to benznidazole and 51 patients were randomized to placebo. Key exclusion criteria included lack of consent, presence of any chronic health condition, presence of acute infectious disease and unstable residence.

Reviewer's Comment: *Based on the information provided in the article, it is not clear if matched means stratified. There was no information provided about the randomization method used in the study.*

Treatment was administered by trained parents, teachers, or nurses from the health services. The duration of treatment was 60 days. Study visits were conducted on Day 21, Day 60, and Months 3, 6, 12, 18, 24, and 48 after start of treatment.

Four serologic tests and xenodiagnosis were performed at various time points throughout the study. These serologic tests included an enzymatic immunoassay (EIA/Conventional ELISA), indirect hemagglutination assay (IHA), indirect immunofluorescence assay (IFA), and chemoluminescent enzymatic immunoassay (F29). Enrollment required two positive tests on EIA, IHA and/or IFA. F29 is considered an unconventional assay with less well characterized operating characteristics. Xenodiagnosis is a diagnostic method used to determine the presence of infectious disease microorganisms or pathogens. This method allows an uninfected triatomine bug to feed on the blood from a subject. If the bug becomes infected with Chagas, it can be concluded that the subject was infected. However, false negatives are common making this test less useful as a conclusive endpoint.⁶ The data contained both continuous and binary (positive/negative) values for the different serologic tests and binary values only for the xenodiagnosis test. The primary endpoint as stated in the statistical analysis plan was seroconversion (a binary endpoint) at the end of the 4 year follow-up period using EIA/Conventional ELISA. The publication based on the study focused on the chemoluminescent enzymatic immunoassay (F29) in the evaluation of seroconversion at end of follow-up. This review will consider all four serologic tests and xenodiagnosis in the evaluation of seroconversion and change from baseline in serological titers at the end of the 4 year follow-up period as well as at various timepoints. In addition, we will evaluate the correlation among the different endpoints.

Reviewer's comment: *See clinical and microbiology review for the discussion of various methods used to measure seroconversion.*

3.2.1.2 Statistical Methodologies

We examined an intent-to-treat (ITT) population of all randomized patients and a modified-intent-to-treat (mITT) population which included patients who were positive using the specific serologic test at baseline. The efficacy analyses presented in this review will focus on the mITT population. No patients had xenodiagnoses conducted at baseline so analyses of xenodiagnoses will be conducted in the ITT population.

Efficacy analyses evaluated the proportion of patients with a seronegative response for F29, EIA, IHA, IFA and xenodiagnosis at time points when most of the patients were measured. These time points varied by test. Two-sided 95% confidence intervals (CI) for the observed differences between benznidazole and placebo in the proportions with seronegative response were calculated using an exact method. Seronegative response was defined as a subject having a measurement below a specific cutoff for each serologic test. Changes from baseline in the mean titers were evaluated for each treatment as well as the mean difference between treatment groups. Analysis of covariance was used to compare the mean difference between treatment groups with baseline

as a covariate. IHA and IFA values were given as dilutions (undiluted, 16:1, 32:1, 64:1, etc.). These values were log-transformed in the analyses with a 1 representing undiluted samples. A correlation matrix was used to evaluate the correlation between the various serology tests. Additional analyses evaluated if patients were seronegative using both conventional serology and F29 assays. For the efficacy analysis, missing data for the binary values were imputed as positive. For continuous values, no imputation was conducted and patients with missing values were excluded from the analysis.

Reviewer’s comment: *We defer to the microbiology team regarding the acceptability of the various cutoffs chosen for the serologic tests and xenodiagnoses.*

Reviewer’s comment: *Neither the protocol nor publication reported information regarding the determination of sample size and power calculation.*

In this review, we refer to significant results as those with two-sided p-values less than 0.05. Note however that there is no control for the type I error. Our conclusions will be based on an assessment of the overall results of the trial and not based on any particular “significant” p-value.

3.2.1.3 Patient Disposition, Demographic and Baseline Characteristics

Reviewer’s comment: *Limited data were provided in the submission.*

A total of 106 patients were randomized into the study (55 in the benznidazole group and 51 in the placebo group). Of the 106 patients randomized, a total of 101 patients completed treatment. The numbers of patients in each analysis population are summarized in [Table 2](#). Only a few patients were excluded from the EIA-mITT, IHA-mITT, and IFA-mITT population. Exclusions were due to patients being negative or having missing values at baseline. None of the patients randomized were excluded from the safety analysis population.

Table 2: Sosa-Estani Analysis Populations by Treatment

	Benznidazole (N = 55)	Placebo (N = 51)	Total (N= 106)
ITT, n (%)	55 (100.0)	51 (100.0)	106 (100.0)
F29-mITT, n (%)	40 (72.7)	37 (72.5)	77 (72.6)
EIA-mITT, n (%)	53 (96.4)	50 (98.0)	103 (97.2)
IHA-mITT, n (%)	52 (94.5)	50 (98.0)	102 (96.2)
IFA-mITT, n (%)	53 (96.4)	48 (94.1)	101 (95.3)
Safety, n (%)	55 (100.0)	51 (100.0)	106 (100.0)

[Table 3](#) summarizes the demographic and baseline characteristics of patients in the ITT population. There were no significant differences in demographic characteristics across treatment groups.

Table 3:
Sosa-Estani Demographic Characteristics (ITT)

	Benznidazole (N = 55)	Placebo (N = 51)
Age		
mean (SD)	9.7 (2.0)	9.9 (1.8)
Min, max	6, 14	6, 14
Gender, n (%)		
Male	25 (45.5)	25 (49.0)
Female	30 (54.6)	26 (51.0)

3.2.1.4 Results

The article states that 62% of patients in the benznidazole group and no patients in the placebo group were seronegative using F29 at the end of the 4 year follow-up period. We were not able to reproduce the results from the article with the data submitted. When including all patients regardless of their status at baseline and considering missing values at the end of the follow-up period as positive, our results show that the percentage of patients who were seronegative at the end of follow-up was 63.6% in the benznidazole group and 25.5% in the placebo group. Additional analyses presented in the article could also not be reproduced using the data submitted with the NDA. The results provided below are based on this reviewer’s analyses of each serologic test endpoint and xenodiagnoses endpoint using the data submitted.

[Table 4](#) displays the F29 serologic response at pre-treatment in the ITT population. The percentage of patients classified as seronegative at baseline in the benznidazole group was 23.6% and 23.5% in the placebo group. In addition, 2 patients in each treatment group were missing baseline values. 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.

[Table 5](#) displays the percentage of patients classified as seronegative at various time points using F29 in the mITT population. 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.

[Table 6](#) contains the mean and range of the titers of the F29 assay at baseline in the ITT population. Note that these continuous values were used to determine seropositive (value > 0.17) and seronegative (value < 0.17) values in the binary analysis discussed above. The average change from baseline in serologic titer at various time points using F29 for those considered seropositive at baseline (mITT population) is displayed in [Table 7](#). There is a significant difference between treatment groups at months 6, 24 and 48 for the average change from baseline in serologic titers.

**Table 4: Sosa-Estani Study
Serologic Response at Pre-treatment using
Chemoluminescent Enzymatic Immunoassay (F29) (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 rates (Benznidazole treatment group minus placebo group) was calculated using an exact method.

**Table 5: Sosa-Estani Study Serologic
Response at various time points using
Chemoluminescent Enzymatic Immunoassay (F29) (mITT)**

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	3	

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

**Table 6: Sosa-Estani Study Serologic
Titer at Pre-treatment using Chemoluminescent Enzymatic Immunoassay (F29) (ITT)**

Visit	Titer	Benznidazole (N=55)	Placebo (N=51)	Difference* (95%CI)	P-value**
Pre-treatment	Mean (SD) Range Missing	0.251 (0.12) [0.012, 0.522] 2	0.281 (0.14) [0.050, 0.595] 2	-0.030 (-0.081, 0.020)	0.2312

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

Table 7: Sosa-Estani Study Serologic Titer at Various Time Points using Chemoluminescent Enzymatic Immunoassay (F29) (mITT)

Visit	Titer	Benznidazole (N=40)	Placebo (N=37)	Difference* (95%CI)	P-value**
Pre-treatment	Mean (SD)	0.299 (0.09)	0.336 (0.12)	-0.037	0.2312
	Range	[0.188, 0.522]	[0.174, 0.595]	(-0.084, 0.010)	
	Missing	0	0		
Change from Baseline					
Month 6	Mean (SD)	-0.055 (0.11)	0.010 (0.11)	-0.065	0.0020
	Range	[-0.406, 0.135]	[-0.392, 0.237]	(-0.121, -0.010)	
	Missing	9	5		
Month 12	Mean (SD)	-0.046 (0.11)	-0.018 (.11)	-0.028	0.0884
	Range	[-0.392, 0.204]	[-0.339, 0.342]	(-0.082, 0.026)	
	Missing	8	5		
Month 24	Mean (SD)	-0.107 (0.11)	-0.013 (0.14)	-0.095	<.0001
	Range	[-0.397, 0.163]	[-0.374, 0.433]	(-0.159, -0.031)	
	Missing	8	4		
Month 48	Mean (SD)	-0.135 (0.13)	-0.021 (0.12)	-0.114	<.0001
	Range	[-0.454, 0.184]	[-0.359, 0.372]	(-0.174, -0.053)	
	Missing	1	4		

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

Table 8 displays the EIA serologic response at pre-treatment in the ITT population. There were 2 patients in the benznidazole group and 1 patient in the placebo group that were missing an EIA response at baseline. The remaining subjects were positive. Table 9 displays the results from the efficacy analysis of serologic response at various time points using EIA in the mITT population which included only those who were positive at baseline. There were no statistically significant differences between treatment groups in the proportion of patients with negative seroconversion for *T. cruzi* antibodies at the various time points.

Table 10 contains the mean and range of the titers of the EIA assay at baseline in the ITT population. Note that these continuous values were used to determine seropositive (value > 0.200) and seronegative (value < 0.200) values in the binary analysis discussed above. The average change from baseline in serologic titer at various time points using EIA for those considered seropositive at baseline (mITT population) is displayed in Table 11. Despite there being a lack of significant differences over the various time points in the binary endpoint, there were significant differences between treatment groups in the average change from baseline in serologic titer at all the post baseline time points.

**Table 8: Sosa-Estani Study
Serologic Response at Pre-treatment using
Enzymatic Immunoassay (EIA) (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 9: Sosa-Estani Study
Serologic Response at Various Time Points using
Enzymatic Immunoassay (EIA) (mITT)**

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-values for the differences in seronegative rates (Benznidazole treatment group minus placebo group) were calculated using an exact method.

**Table 10: Sosa-Estani Study Serologic
Titer at Pre-treatment using Enzymatic Immunoassay (EIA) (ITT)**

Visit	Titer	Benznidazole (N=55)	Placebo (N=51)	Difference* (95%CI)	P-value**
Pre-treatment	Mean (SD)	0.465 (0.10)	0.474 (0.09)	-0.009	0.6314
	Range	[0.226, 0.690]	[0.298, 0.723]	(-0.047, 0.029)	
	Missing	2	1		

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

**Table 11: Sosa-Estani Study Serologic Titer
at various time points using Enzymatic Immunoassay (EIA) (mITT)**

Visit	Titer	Benznidazole (N=53)	Placebo (N=50)	Difference* (95%CI)	P-value**
Pre-treatment	Mean (SD)	0.465 (0.10)	0.474 (0.09)	-0.009	0.6314
	Range	[0.226, 0.690]	[0.298, 0.723]	(-0.047, 0.029)	
	Missing	0	0		
Change from Baseline					
Month 3	Mean (SD)	-0.074 (0.10)	0.013 (0.06)	-0.0874	<.0001
	Range	[-0.432, 0.045]	[-0.108, 0.173]	(-0.122, -0.053)	
	Missing	4	6		
Month 6	Mean (SD)	-0.101 (0.09)	0.004	-0.105	<.0001
	Range	[-0.441, 0.046]	[-0.084, 0.159]	(-0.138, -0.072)	
	Missing	5	11		
Month 12	Mean (SD)	-0.103 (0.09)	0.006 (.05)	-0.109	<.0001
	Range	[-0.453, 0.114]	[-0.130, 0.150]	(-0.139, -0.080)	
	Missing	1	3		
Month 18	Mean (SD)	-0.113 (.10)	-0.012	-0.101	<.0001
	Range	[-0.491, 0.092]	[-0.184, 0.079]	(-0.135, -0.068)	
	Missing	3	2		
Month 24	Mean (SD)	-0.143 (.09)	0.004 (.05)	-0.147	<.0001
	Range	[-0.459, 0.090]	[-0.134, 0.145]	(-0.176, -0.118)	
	Missing	3	1		
Month 48	Mean (SD)	-0.129 (.10)	-0.031 (0.09)	-0.160	<.0001
	Range	[-0.512, 0.123]	[-0.226, 0.233]	(-0.201, -0.119)	
	Missing	5	6		

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

Table 12 displays the IFA serologic response at pre-treatment in the ITT population. There were two patients in the placebo group that were negative at baseline. In addition, there were 2 patients in the benznidazole group and 1 patient in the placebo group that were missing an IFA response at baseline. The remaining subjects were positive. Table 13 displays the results from the efficacy analysis of serologic response at various time points using IFA in the mITT population. There were no statistically significant differences between treatment groups in the proportion of patients with negative seroconversion for *T. cruzi* antibodies at the various time points.

Table 14 contains the mean and range of the titers of the IFA assay at baseline in the ITT population. These continuous values were used to determine seropositive (value ≥ 32 or $\ln(\text{value}) > 3.4$) and seronegative (value < 32 or $\ln(\text{value}) < 3.4$) values in the binary analysis discussed above. The average change from baseline (mITT population) is displayed in Table 14. Although there were no significant differences over the various time points in the binary endpoint, there were significant differences between treatment groups in the average change from baseline in serologic titer at months 6, 12, 24, and 48 (Table 14).

**Table 12: Sosa-Estani Study
Serologic Response at Pre-treatment using
Indirect Immunofluorescence (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 13: Serologic Response at Various Time
Points using Indirect Immunofluorescence (IFA) (mITT)**

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 (5.7)	1 (2.0)	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	9 (17.0)	3 (6.3)	0.128
Positive	40	44	
Missing	3	1	
Month 48, n (%)			
Negative	3 (5.7)	0 (0.0)	0.244
Positive	45	42	
Missing	5	6	

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

Table 14: Sosa-Estani Study Serologic Titer at Pre-treatment using Indirect Immunofluorescence (IFA) (ITT)

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

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

Table 15: Serologic Titer at Various Time Points using Indirect Immunofluorescence (IFA) (mITT)

Visit	Titer	Benznidazole (N=53)	Placebo (N=48)	Difference* (95%CI)**	P-value**
Pre-treatment	Mean (SD)	4.85 (0.79)	4.78 (0.76)	0.07	0.5000
	Range	[3.47, 6.24]	[3.5, 6.24]	(-0.24, 0.38)	
	Missing	0	0		
Change from Baseline					
Month 3	Mean (SD)	-.59 (0.75)	-0.26 (0.76)	-0.34	0.1362
	Range	[-3.47, 0.69]	[-1.39, 1.39]	(-0.65, -0.02)	
	Missing	4	5		
Month 6	Mean (SD)	-0.78 (1.08)	-0.24 (1.28)	-0.54	0.0361
	Range	[-4.85, 1.39]	[-2.08, 2.07]	(-1.05, -0.03)	
	Missing	5	11		

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 16 displays the IHA serologic response in the ITT population. There was 1 patient in the benznidazole group that was negative at baseline. In addition, there were 2 patients in the benznidazole group and 1 patient in the placebo group that were missing an IHA response at baseline. The remaining subjects were positive. Table 17 displays the results from the efficacy analysis of serologic response at various time points using IHA. There were no statistically significant differences between treatment groups in the proportion of patients with negative seroconversion for *T. cruzi* antibodies at the various time points.

Table 18 contains the mean and range of the titers of the IHA assay at baseline in the ITT population. The continuous values were used to determine seropositive (value ≥ 32) and seronegative (value < 32) values in the binary analysis discussed above. The average change from baseline (mITT population) is displayed in Table 19. As seen with the previous assays, there were significant differences between treatment groups in the average change from baseline in serologic titer at all time points.

**Table 16: Sosa-Estani Study
Serologic Response at Pre-treatment using
Indirect Hemagglutination (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 17: Sosa-Estani Study
Serologic Response at Various Time Points using
Indirect Hemagglutination (IHA) (mITT)**

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-values for the differences in seronegative rates (Benznidazole treatment group minus placebo group) were calculated using an exact method.

**Table 18: Sosa-Estani Study Serologic
Titer at Pre-treatment 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 19: Sosa-Estani Study Serologic Titer at Various Time Points Using Indirect Hemagglutination (IHA) (mITT)

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

*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.

Response using xenodiagnoses is displayed in [Table 20](#). 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).

Table 20: Sosa-Estani Study Response at Various Time Points using Xenodiagnosis (ITT)

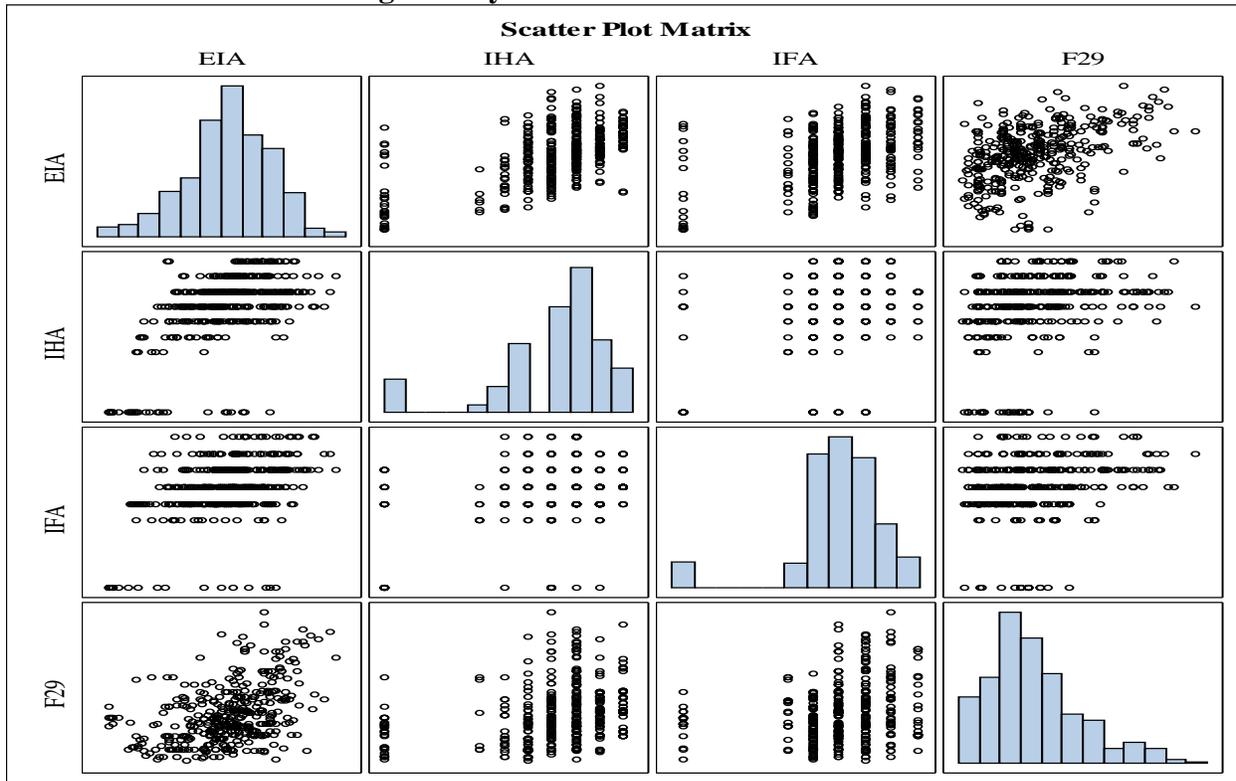
Visit*	Benznidazole (N=55)	Placebo (N=51)	P-value**
Month 24			
Negative	23 (41.8)	13 (25.5)	0.101
Positive	27	36	

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.

Since F29, which showed the largest treatment effect, is considered an unconventional assay, we explored the correlation among the 4 serologic tests. We focused on the results from the benznidazole group because placebo values were likely only changing randomly over time, whereas benznidazole might be able to better assess the correlation related to changes due to the effect of treatment. Figure 1 displays a correlation matrix for the four serologic assays used to measure serology in the benznidazole treatment group. 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 EIA and F29 ($r=.515$; Pearson). Both IHA ($r=0.385$; Spearman) and IFA ($r=0.282$; Spearman) display a weak correlation with F29.

Figure 1: Sosa-Estani Study Scatter Plot Matrix of Serologic Assays Results in the Benznidazole Arm



*IFA and IHA are log transformed data

We also conducted an analysis to evaluate if patients were negative using both conventional serology (EIA, IHA, and IFA) and F29. Of the 4 patients in the benznidazole group and 2 patients in the placebo group that were classified as seronegative at the 4 year follow-up using EIA, 3 patients in the benznidazole group and 2 in the placebo group were also classified as negative using F29. In addition, of the 5 patients in the benznidazole group that were classified as seronegative at the 4 year follow-up using IHA, 3 patients were also classified as negative using F29. Further, of the 3 patients in the benznidazole group that were classified as seronegative at the 4 year follow-up using IFA, 2 patients were also classified as negative using F29.

An additional analysis evaluated if patients were negative using both xenodiagnosis and F29. Of the 20 patients in the benznidazole group and 9 patients in the placebo group that were classified as negative at the 4 year follow-up using xenodiagnosis, only 8 patients in the benznidazole group and 2 in the placebo group were also classified as negative using F29 as shown in Table 21. Of the 28 patients in the benznidazole group and 35 patients in the placebo group that were classified as positive at the 4 year follow-up using xenodiagnosis, only 14 patients in the benznidazole group and 1 in the placebo group were classified as negative using F29.

Table 21: Sosa-Estani Study Response using Xenodiagnosis and F29 (ITT)

Xenodiagnosis at 48 months	F29 at 48 months - benznidazole			F29 at 48 months - Placebo		
	Sero-	Sero+	missing	Sero-	Sero+	missing
Negative, N=29	8	4	11	2	4	0
Positive, N=63	14	10	13	1	25	1
Missing , N=14	2	1	7	2	0	2

3.2.1.5 Conclusions

The Sosa-Estani study examined the effect of benznidazole in children 6-12 years of age with indeterminate Chagas disease. The results of the Sosa-Estani study showed a significant effect of benznidazole in rates of seroconversion using the F29 unconventional assay from 12 months to 48 months. In addition, benznidazole also showed significant effects in change in serologic titers for all the serologic assays in this study, as well as, in rates of xenodiagnosis at 48 months. According to our clinical colleagues the 48 month duration of follow-up was likely not long enough to see an effect of benznidazole on clinical response.

3.2.2 De Andrade study

3.2.2.1 Study Design and Endpoint

The De Andrade study was a randomized, double-blind, parallel-group, placebo controlled study to evaluate the efficacy and safety of benznidazole in patients in the early chronic phase of *T. cruzi* infection. The trial was conducted in central Brazil from 1991 - 1995. Patients were recruited from the following communities: Posse, Simolândia, and Guarani de Goiás. To be eligible for the trial, patients had to be seropositive on all three of the following tests: indirect

immunofluorescence (IFA), indirect haemagglutination (IHA), and enzymatic immunoassay (EIA). A total of 130 pediatric patients 7 to 12 years of age with antibodies to *T. cruzi* were enrolled into the study. A total of 64 patients were randomized to benznidazole and 65 patients were randomized to placebo. Randomization was stratified by school, age group and sex using blocks of 6.

Patients received benznidazole or placebo given at a dose of 7.5 mg per kg bodyweight divided in two daily doses for 60 days. Serum samples were taken on day 60 and months 3, 6, 12, and 36; however, only baseline and month 36 data were provided in the submission.

Four serologic tests were conducted at the 3 year follow-up time point. These serologic tests included EIA, IHA, IFA, and chemoluminescent enzymatic immunoassay (CLEIA), considered an unconventional assay. The data reported both the continuous values as well as binary (positive/negative) for the different serologic tests. Seronegative results were based on having a test results < 1.2 for EIA, ≤ 8 for IHA, ≤ 20 for IFA, and < 1 for CLEIA. The primary endpoint stated in the statistical analysis plan was seroconversion at the end of the 3 year follow-up period using EIA; however, the publication focuses on the CLEIA in the evaluation of seroconversion at the end of the 3 year follow-up. This review will consider all four serologic tests in the evaluation of seroconversion at end of the 3 year follow-up period and the correlation among them. In addition, we will also evaluate change from baseline in serological titers at various time points using the four tests. A subsequent publication⁷ of this trial reported results of follow-up of CLEIA out to 6 years. This six year follow-up data of was not submitted to the NDA.

Reviewer's comment: *See clinical and microbiology review for the discussion of various methods used to measure seroconversion.*

Reviewer's comment: *We defer to the microbiology team regarding the acceptability of the various cutoffs chosen for the serologic tests.*

3.2.2.2 Statistical Methodologies

We examined an intent-to-treat (ITT) population defined in the article as all randomized patients who received at least one week of treatment.

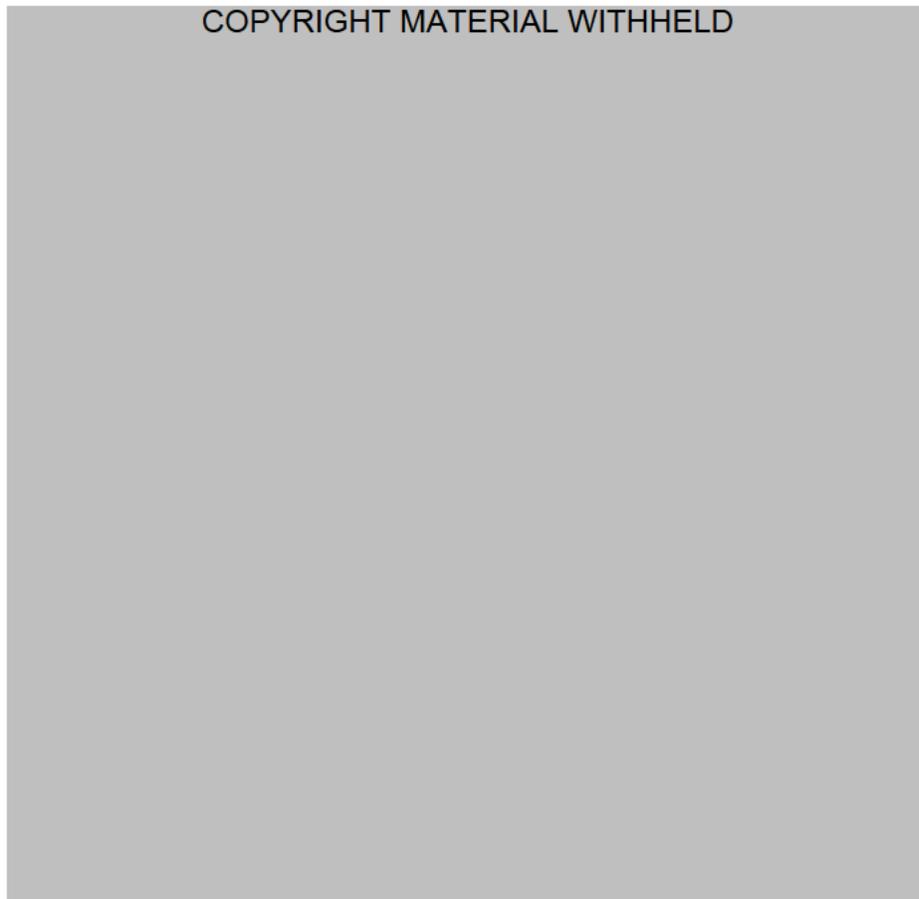
Efficacy analyses evaluated the proportion of patients with a seronegative response at 36 months using the four serology tests (CLEIA, EIA, IHA and IFA). Two-sided 95% confidence intervals (CI) for the observed differences in seronegative rates were calculated using an exact method. Other efficacy analyses evaluated trends in titers of the serological tests. Change from baseline in the mean titers was evaluated for each treatment as well as the mean difference between treatment groups. Analysis of covariance was used to compare the mean difference in results at 36 months between treatment groups with baseline in the model. IHA and IFA values were given as dilutions (4:1, 16:1, 32:1, 64:1, etc.). These values were log-transformed in the analyses. A correlation matrix was used to evaluate the correlation between the various serologic tests. Additional analyses evaluated if patients were seronegative using both conventional serology and CLEIA.

3.2.2.3 Patient Disposition, Demographic and Baseline Characteristics

Reviewer's comment: Limited data were provided in the submission.

A total of 130 patients were enrolled and randomized into the study. However, a patient randomized to benznidazole moved away right after randomization and is excluded from all analyses. Therefore, there were a total of 64 patients in the benznidazole group and 65 patients in the placebo group who remained in the study and were considered in the ITT population. Of the 129 patients randomized, a total of 112 patients completed treatment (Figure 2). Table 22 summarizes the demographic and baseline characteristics of patients in the ITT population. There were no significant differences in demographic characteristics across treatment groups.

Figure 2: De Andrade Study Trial Profile



Source: Figure 1¹

Table 22: De Andrade Study Demographic and Selected Baseline Characteristics (ITT)

	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)

3.2.2.4 Results

The article states that 37/64 (58%) patients in the benznidazole group and 3/65 (5%) patients in the placebo group were seronegative using CLEIA at the end of the 3 year follow-up period. Based on the data submitted in the NDA, 35 patients in the benznidazole group were seronegative. The results provided below are based on this reviewer's analyses of each serologic endpoint using the data submitted. [Table 23](#) displays the results from the efficacy analysis of serologic response at baseline and Month 36 using CLEIA 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 CLEIA is displayed in [Table 24](#) There is a significant difference between treatment groups at month 36 for the average change from baseline in serologic titers ($p < .0001$). Six year follow-up of CLEIA results, reported in a subsequent publication, showed the trend continuing with significantly more seroconversion on the benznidazole arm (47/53) compared to placebo (12/46). Fourteen additional subjects were lost to follow-up (data not submitted to the NDA).

**Table 23: De Andrade Study
Serologic response at Pre-Treatment and Month 36
using Chemoluminescent Enzymatic Immunoassay (CLEIA) (ITT)**

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	23	51	
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 24: De Andrade Study Serologic Titer at Pre-Treatment and Month 36
using Chemoluminescent Enzymatic Immunoassay (CLEIA) (ITT)**

Visit	Titer	Benznidazole (N=64)	Placebo (N=65)	Difference* (95%CI)	P-value**
Pre-Treatment	Mean(SD) Range	4.028 (2.95) [1.001, 18.109]	5.075 (4.04) [1.005, 22.363]	-1.047 (-2.281, 0.188)	0.0909
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 25 displays the results from the efficacy analysis of serologic response at pre-treatment and Month 36 using EIA in the ITT population. The percentages of patients who were seronegative 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 is a significant difference between treatment groups in the average change from baseline in serologic titer at the 36 month follow-up (Table 26).

**Table 25: De Andrade Study
Serologic Response at Pre-Treatment and Month 36 using
Enzymatic Immunoassay (EIA) (ITT)**

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	4 (6.25)	0 (0.0)	0.0577
Positive	54	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 26: De Andrade Study Serologic Titer at Pre-Treatment and Month 36 using Enzymatic Immunoassay (EIA) (ITT)

Visit	Titer	Benznidazole (N=64)	Placebo (N=65)	Difference* (95%CI)	P-value**
Pre-Treatment	Mean (SD)	2.728 (0.56)	2.901 (0.61)	-0.173	0.0895
	Range	[1.200, 4.000]	[1.600, 4.300]	(-0.377, 0.030)	
Change from Baseline					
Month 36	Mean (SD)	-0.870 (0.539)	-0.198 (0.06)	-0.673	<.0001
	Range	[-2.500, 0.600]	[-1.300, 0.800]	(-0.861, -0.484)	
	Missing	6	11		

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

Table 27 displays the results from the efficacy analysis of serologic response at baseline and Month 36 using IFA in the ITT population. There was no statistically significant difference between treatment groups in the proportion of patients with negative seroconversion for *T. cruzi* antibodies at the 36 month follow-up. Similarly to EIA, there was a significant difference between treatment groups in the average change from baseline in serologic titer at the 36 month follow-up (Table 28).

Table 27: De Andrade Study Serologic Response at Pre-Treatment and Month 36 using Indirect Immunofluorescence (IFA) (ITT)

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	3 (4.29)	0 (0.0)	0.1192
Positive	55	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 28: De Andrade Study Serologic Titer at Pre-Treatment and Month 36 using Indirect Immunofluorescence (IFA) (ITT)

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

Table 29 displays the results from the efficacy analysis of serologic response at baseline and Month 36 using IHA in the ITT population. There is a significant difference between treatment groups in the proportion of patients with negative seroconversion for *T. cruzi* antibodies at the 36 month follow-up (p= 0.0013). In addition, there is a significant difference between treatment groups in the average change from baseline in serologic titer at the 36 month follow-up (Table 30).

Table 29: De Andrade Study Serologic Response at Baseline and Month 36 using Indirect Hemagglutination (IHA) (ITT)

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.

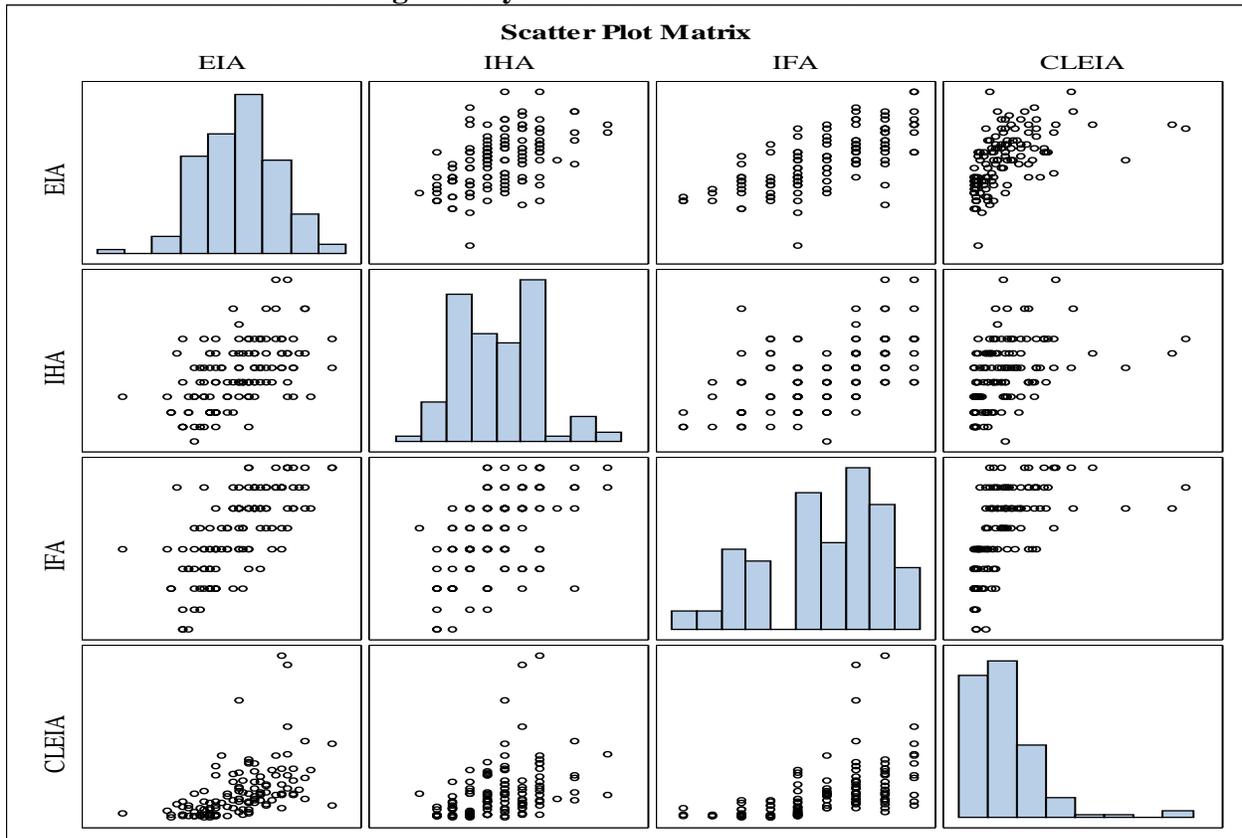
Table 30: De Andrade Study Serologic Titer at Baseline and Month 36 using Indirect Hemagglutination (IHA) (ITT)

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

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

Figure 3 displays a correlation matrix for the four serologic assays use to measure serology in benznidazole treatment group. 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 EIA and CLEIA ($r=0.51$; Pearson). Both IHA and IFA display a weak correlation with CLEIA.

Figure 3: De Andrade Study Scatter Plot Matrix of Serologic Assays Results in the Benznidazole Arm



*IFA and IHA are log transformed data

As in the previous study, we conducted additional analyses to evaluate if patients were negative using both conventional serology (EIA, IHA, and IFA) and CLEIA. All 4 patients in the benznidazole group that were classified as seronegative at the 3 year follow-up using EIA were also classified as seronegative using CLEIA. In addition, of the 3 patients in the benznidazole group that was classified as seronegative at the 3 year follow-up using IFA, 2 of those patients were also classified as negative using CLEIA. Further, of the 9 patients in the benznidazole group that were classified as seronegative at the 3 year follow-up using IHA, 7 of those patients were also classified as negative using CLEIA.

3.2.2.5 Conclusion

The De Andrade Study examined the effect of benznidazole in children 7- 12 years of age with early chronic phase of Chagas disease. The results of the study showed a significant effect of benznidazole on rates of seroconversion at 36 months using the CLEIA and IHA assays. In addition, benznidazole also showed an effect in the change in serologic titers for all the assays at 36 months. The study duration was likely not sufficient to see a clinical response.

3.2.3 Viotti Study

3.2.3.1 Study Design and Endpoint

The Viotti study was a non-randomized, no treatment controlled, parallel-group study to evaluate the long-term outcomes of patients with nonacute Chagas disease. The trial was conducted in Buenos Aires, Argentina. A total of 1968 patients with chronic Chagas disease were evaluated in the Chagas Section at Hospital Eva Peron between 1984 and 2001. During this period, patients received physical examinations and clinical tests. Patients were also classified in the Kuschnir groups defined in [Table 31](#).

Table 31: Viotti Study Kuschnir Groups

Kuschnir Group	Serology	Electrocardiography	Chest Radiography	Cardiac Enlargement
Group 0	Positive results	Normal	Normal	No enlargement
Group 1	Positive results	Abnormal	Normal	No enlargement
Group 2	Positive results	Abnormal	Abnormal	Enlargement but no signs of heart failure
Group 3	Positive results	Abnormal	Abnormal	Enlargement with signs of heart failure

To be eligible for the trial, patients had to be 30 – 50 years of age and seropositive on three of the following tests: complement fixation, indirect hemagglutination, immunofluorescence, or enzyme-linked immunosorbent assay for *T. cruzi* infection, with no clinical signs of heart failure (Kuschnir groups 0, 1, or 2). Patients with a history of previous treatment for *T. cruzi* infection, concomitant disorders, or overt heart failure were excluded from the study. A total of 598 patients were enrolled into the study.

Patients received either benznidazole twice per day, at a maximum dosage of 5 mg/kg per day for 30 consecutive days or no treatment. Patients were assigned by using a non randomized alternating sequence where every other individual enrolled was assigned to treatment and the alternate individuals were assigned to the control group. For patients who withdrew from the study or declined to participate, the physician not involved in the clinical evaluation would assign the next eligible patient to the respective group. The article states that patients were informed about possible side effects of benznidazole therapy and were advised to consult the study physician immediately if a symptom occurred. Follow-up visits and results of electrocardiography were recorded every 6, 4, and 3 months in patients in Kuschnir groups 0, 1, and 2, respectively.

The primary objective of the study was to compare long-term outcomes of patients with non-acute Chagas disease treated with benznidazole versus outcomes of those who did not receive treatment. The primary endpoint was change from a lower to a more advanced Kuschnir group or cardiac death. Secondary endpoints included new abnormalities on electrocardiography (ECG), persistence of 3 positive results on serologic evaluation and completed negative seroconversion on the last serologic test done for each patient.

3.2.3.2 Statistical Methodologies

We examined an Intent-to-treat (ITT) population defined in the article as all patients assigned to treated and untreated groups.

The primary efficacy analyses evaluated the proportion of patients who changed from a lower to a more advanced Kuschnir group or experienced cardiac death. Secondary efficacy analyses evaluated the proportion of patients who had new ECG abnormalities, positive results on three serologic tests, and completed negative seroconversion. Additional analyses evaluated the mortality rate. For the secondary and additional efficacy analyses, the numbers and percentages for the treated and untreated group was tabulated. Analyses of the secondary endpoints were analyzed using a two-sided 95% CI to observe the difference in proportions between the treated and untreated groups using an exact method.

The sample size was calculated based on a previous study with 8 years of follow-up. The planned sample size was calculated to be 319 patients per group. Interim analyses were conducted every 5 years. 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.

3.2.3.3 Patient Disposition, Demographic and Baseline Characteristics

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. Due to this being a non-randomized, open label trial, the withdrawal of those 32 patients could have affected the results of the study. Demographic and selected baseline characteristics for the ITT population are summarized in [Table 32](#). Demographic and baseline disease characteristics appeared balanced between treatment groups. In addition, [Table 33](#) summarizes the years of follow-up for each group. The follow-up time was fairly balanced between the two groups with a median 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. In addition [Figure 4](#) and [Figure 5](#) present the distribution of years of follow-up for the treated and untreated groups.

Table 32: Viotti Study Demographic and Selected Baseline Characteristics (ITT)

	Benznidazole (N = 283)	No treatment (N = 283)
Gender, n (%)		
Male	134 (47.3)	127 (44.9)
Female	149 (52.7)	156 (55.1)
Age		
mean (SD)	39.4 (5.3)	39.4 (5.8)
Min, max	30, 49	30, 49
Kuschnir Group, n(%)		
Group 0	180 (63.6)	180 (63.6)
Group 1	73 (25.8)	75 (26.5)
Group 2	30 (10.6)	28 (9.9)
Age by Kuschnir Group		
Group 0, mean [range]	39.1 [30, 49]	38.6 [30, 49]
Group 1, mean [range]	39.5 [30, 49]	40.8 [30, 49]
Group 2, mean [range]	40.9 [30, 49]	41.4 [31, 49]
Baseline symptoms, n (%)	162 (57.2)	157 (55.4)
Baseline palpitations, n (%)	72 (25.4)	81 (28.6)
Baseline atypical chest pain, n (%)	78 (27.6)	65 (23.0)
Baseline Dizziness, n (%)	19 (6.7)	23 (8.1)
Baseline Other Symptoms, n (%)	35 (12.4)	34 (12.0)
Baseline Conduction Abnormalities	66 (23.3)	63 (22.2)

Baseline Heart Rate		
mean (SD)	71.5 (12.4)	69.7 (14.9)
Min, max	47, 108	35, 200
Baseline Systolic Blood		
mean (SD)	119.3 (13.1)	119.4 (14.3)
Min, max	90, 160	90, 180
Baseline Diastolic Blood		
mean (SD)	80.2 (8.9)	79.9 (9.7)
Min, max	60, 105	60, 110

Table 33: Viotti Study Years of Follow-up (ITT)

	Benznidazole (N = 283)	No treatment (N = 283)
Years		
mean (SD)	11.2 (6.5)	10.2 (5.8)
IQR	5.8, 16.3	5.4, 13.9

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Figure 4: Viotti Study Distribution of Years of Follow-up in the Benznidazole Group

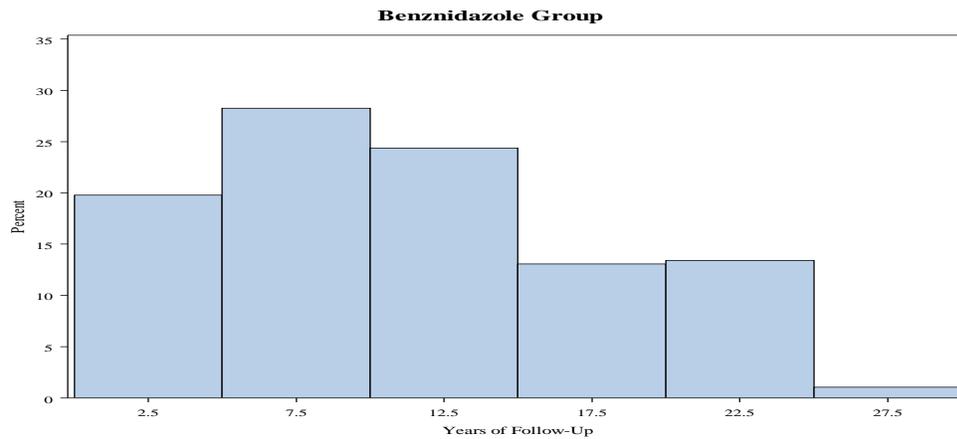
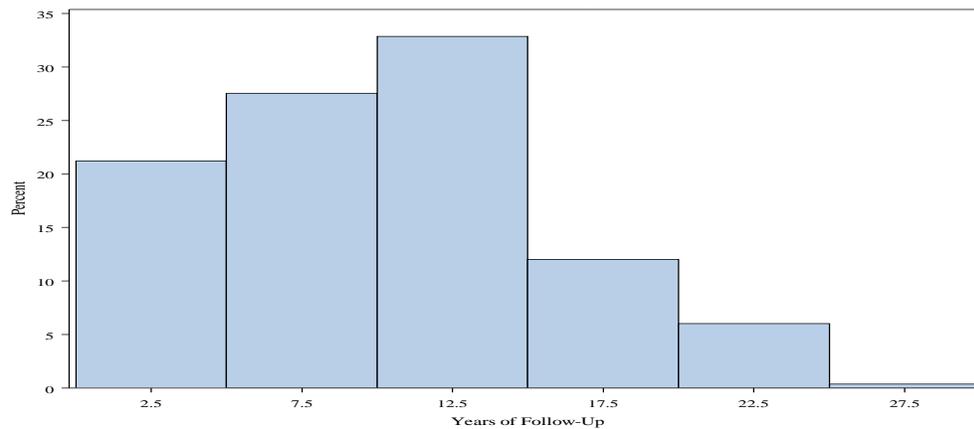


Figure 5: Viotti Study Distribution of Years of Follow-up in the Untreated Group



The article states that there were 54 (19%) and 57 (20%) patients lost to follow-up in the benznidazole and untreated arms respectively. However, it is not clear why some patients were considered lost to follow-up while others were not. 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.

3.2.3.4 Results

Reviewer's Comment: The applicant's submission does not include event times in the data.

Table 34 displays the results from the primary efficacy analysis of change in Kuschnir groups or cardiac death in the ITT population. 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], $p < 0.0001$). Three times as many patients in the untreated group ($n=41$) changed from a lower to a more advanced Kuschnir group or experienced a cardiac death compared to the benznidazole group ($n=12$).

Reviewer's comment: *The only information given was if a subject increased in Kushnir group. Since the Kushnir group definition requires positive serology, all subjects who increased Kushnir group would be required to have a positive serology. If this interpretation of the definition is correct, because of its strong dependence on serology, the Kushnir group endpoint cannot be considered a good clinical outcome on which to validate a serologic endpoint.*

Table 34: Viotti Study Change in Kuschnir Groups or Cardiac Death

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%)

Table 35 displays the results from the secondary efficacy analysis of patient serologic response based on the last serologic test done for each patient in the ITT population. Patients who were classified as having a negative seroconversion had no positive serologic tests. The percentage of patients that had a negative seroconversion in the benznidazole group was 11.3% and 4.2% in the untreated group. The observed difference in seroconversion rate was 7.0% (95% CI: 2.7, 11.4). Table 36 displays the seroconversion rates based on baseline Kuschnir Groups. There does not seem to be a trend with a higher or lower likelihood of seroconversion based on baseline Kuschnir group.

Table 35: Viotti Study Seroconversion to Negative (ITT)

Results	Benznidazole (N=283)	Untreated (N=283)	Difference (95%CI)
Conversion n(%)	32 (11.3)	12 (4.2)	7.0 (2.7, 11.4)
No Conversion	184 (65.0)	196 (69.3)	
Missing	64 (22.6)	63 (22.3)	
Death	3 (1.1)	12 (4.2)	

**Table 36: Viotti Study
Seroconversion based on Baseline Kuschnir Group (ITT)**

	Baseline Kuschnir Group		
Benznidazole, N= 283	0 (n= 180)	1 (n=73)	2 (n=30)
Conversion, n (%)	19 (10.6%)	11 (15.1%)	2 (6.7%)
Untreated, N= 283	0 (n=180)	1 (n=75)	2 (n=28)
Conversion, n (%)	9 (5%)	3 (4%)	0

Table 37 displays the results of additional efficacy analyses evaluating positive results on three serologic tests, new ECG abnormalities, and mortality in the ITT population. 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 serologic tests and mortality.

Table 37: Viotti Study Additional Outcomes (ITT)

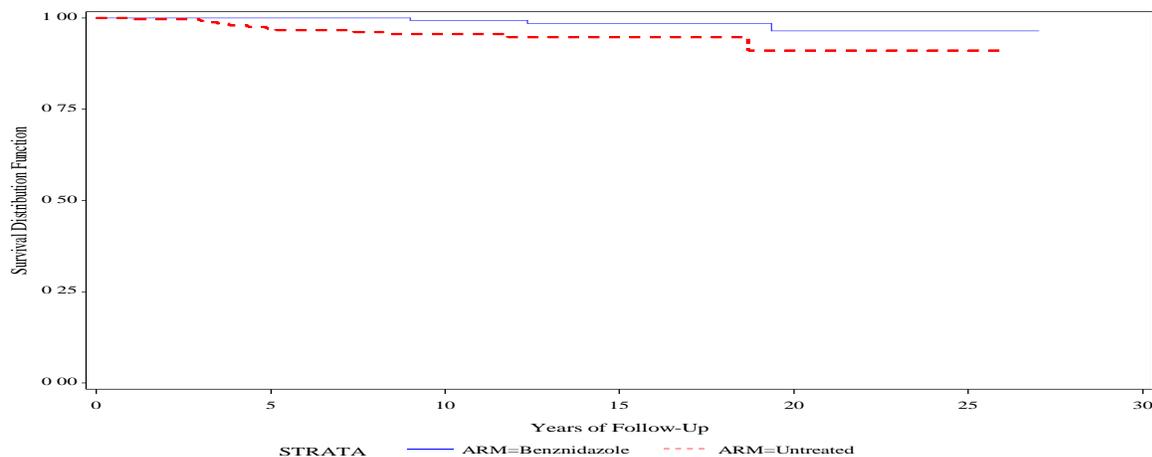
Event	Benznidazole, N= 283	Untreated, N= 283	P-value
Positive Results on Three Serologic Tests, n (%)	130 (59.6)	177 (83.5)	<0.0001
New ECG Abnormalities, n(%)	15 (5.3)	45 (15.9)	<0.0001
Mortality, (%)	3 (1.1)	12 (4.2)	0.033

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.

We 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 (Figure 6).

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). There were no deaths among those who seroconverted.

Figure 6: Viotti Study Overall Survival by Treated and Untreated Group.



3.2.3.5 Conclusion

The Viotti study enrolled adult subjects between the ages of 30 and 49 who were not currently experiencing heart failure. 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 in Kuschner groups. 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. Other drawbacks included the lack of blinding, the replacement of subjects who declined to participate after treatment assignments, the lack of granular data that would allow for the assessment of the timing of outcomes, and the 20% lost to follow-up as reported in the article.

3.3 Evaluation of Safety

Reviewer's comment: This review will only briefly describe the safety reported for the three studies covered in this review. Please see the medical officer's review for a detailed review of the safety data for benznidazole.

A summary of the safety data for the Sosa-Estani study is presented in [Table 38](#). There were more adverse events in the benznidazole treatment group compared to the placebo group. The majority of the adverse events in the benznidazole group were colic, weight loss, and rash. The majority of the adverse events in the placebo group were headaches.

Table 38: Summary of Adverse Events in the Safety Population

Body System	Adverse Event	Benznidazole (N=55)	Placebo (N=51)
Gastrointestinal Disorders, n	Epigas	3	1
	Nausea	3	1
	Diarrhea	2	0
	Vomiting	3	0
	Colic	11	3
Investigations, n	Mobilisation of transaminases	3	0
	Weightloss	7	1
Metabolism and nutrition disorders, n	Anorexia	3	0
Nervous system disorders, n	Dizziness	2	2
	Headache	4	5
	Paraesthesia or Hyperaesthesia	1	0
	Trembling	1	1
Skin and subcutaneous tissue disorders, n	Rash	9	0

The safety data for the De Andrade study was not provided in the applicant's submission. The article reported that less than 5% of patients in the benznidazole group experienced slight adverse effects such as nausea, anorexia, headache, stomach-ache and arthralgia. However, there was no significant difference between treatments. In addition, the article further states that the results of liver and kidney function tests did not change significantly during the study.

Limited data was provided in the applicant's submission in reference to safety for the Viotti study. In the study, 31.8% of patients in the benznidazole group experienced side effects. There were 3 deaths in the benznidazole group and 12 deaths in the untreated group. Causes of death in both arms were listed as heart failure and sudden death³.

4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

4.1 Gender, Race, Age, and Geographic Region

This section provides information on gender and age subgroups since the Applicant only submitted data for these two subgroup populations. The submission does not include data on race and geographic region as it is not relevant since each study contained subjects from a narrow geographic area. Further, this section will focus on Month 48 (Sosa-Estani study) and Month 36 (De Andrade study) for the unconventional assays (F29 and CLEIA) and conventional ELISA

(EIA). These studies had a limited age range; therefore, analysis by age is limited. For the Viotti study, the discussion below will focus on seroconversion, mortality and new ECG abnormalities by age and gender.

[Table 39](#) presents the results for the subgroup analysis by gender of the percentage of patients classified as seronegative at Month 48 using F29 in the mITT population for the Sosa-Estani study. [Table 40](#) presents the average change from baseline in serologic titer at Month 48 using F29 by gender. Similarly, [Table 41](#) presents the results for the subgroup analysis by gender of the percentage of patients classified as seronegative at Month 48 using EIA in the mITT population. [Table 42](#) presents the average change from baseline in serologic titer at Month 48 using EIA by gender. The results of these analyses were consistent with the overall study results.

We modeled the effect of age and treatment on seroconversion using F29 assay at 48 months using a model with age, treatment, and treatment*age interaction. Treatment was statistically significant ($p=0.0001$) and age, and treatment*age interaction were not statistically significant ($p=0.6565$, and $p=0.4895$, respectively). The insignificance of the interaction between treatment and age, which looks for a difference in the treatment effect over different ages, could be due to the narrow age range of 6- 12 years of age in the study. In addition, we modeled the effect of treatment, age, and treatment*age interaction on serologic titers using F29. There was no statistical significant treatment*age interaction on serologic titers. Further, when modeling the effect of treatment*age interaction on serologic titers using EIA assay at 48 months, there was no statistically significant interaction.

[Table 43](#) and [Table 45](#) presents the results for the subgroup analysis by gender for the percentage of patients classified as seronegative at Month 36 using CLEIA and EIA in the ITT population for the De Andrade study. [Table 44](#) presents the average change from baseline in serologic titer at various time points using CLEIA by gender. Further, [Table 46](#) presents the average change from baseline in serologic titer at various time points using EIA by gender.

In addition, [Table 47](#) and [Table 48](#) present the percentage of patients classified as seronegative at Month 36 by age group using CLEIA and EIA in the ITT population. These results were consistent with the overall De Andrade study results. We modeled the effect of age and treatment on seroconversion using CLEIA assay at 36 months using a model with age, treatment, and treatment*age interaction. Age and treatment*age interaction were not statistically significant ($p=0.5728$ and $p=0.5490$, respectively), however treatment was statistically significant ($p < .0001$). In addition we modeled the effect of treatment, age, and treatment*age interaction on serologic titers using CLEIA. Similar to the Sosa-Estani study, there was no statistical significant treatment*age interaction on serologic titers. When modeling the effect of treatment*age interaction on serologic titers using EIA assay at 36 months, there was no statistically significant interaction. Similarly, this insignificance is likely due to the narrow age range of 7- 12 years of age in the study.

Table 39: Sosa-Estani Study Serologic Response at Month 48 by Gender using Chemoluminescent Enzymatic Immunoassay (F29) (mITT)

Visit*	Benznidazole	Placebo	P-value**
Female N=42, n (%)			
Negative	15 (62.5)	1 (5.7)	0.0003
Positive	8	16	
Missing	1	1	
Male N =35, n (%)			
Negative	9 (56.3)	4 (21.1)	0.0425
Positive	7	13	
Missing	0	2	

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

Table 40: Sosa-Estani Study Serologic Titer Month 48 using Chemoluminescent Enzymatic Immunoassay (F29) by gender (mITT)

Visit	Titer	Benznidazole	Placebo	Difference* (95%CI)**	P-value**
Change from Baseline Males					
Month 48	Mean (SD)	-0.10 (.10)	-0.01 (0.15)	-0.09	0.0016
	Range	[-0.21, 0.18]	[-0.36, 0.37]	(-0.19, 0.003)	
	Missing	0	2		
	N	16	19		
Change from Baseline Females					
Month 48	Mean (SD)	-0.16 (0.14)	-0.04 (0.09)	-0.12	<.0001
	Range	[-0.45, 0.14]	[-0.21, 0.13]	(-0.20, -0.04)	
	Missing	1	1		
	N	24	18		

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

Table 41: Sosa-Estani Study Serologic response at Month 48 by Gender using Enzymatic Immunoassay (EIA) (mITT)

Gender*	Benznidazole	Placebo	P-value**
Female N=54, n (%)			
Negative	4 (14.3)	0 (0.0)	0.1120
Positive	22	22	
Missing	2	4	
Male N =49, n (%)			
Negative	0 (0.0)	2 (8.3)	0.2347
Positive	22	20	
Missing	3	2	

*Patients with missing values were imputed as positive. ** The p-values for the differences in seronegative rate (Benznidazole treatment group minus placebo group)

group) were calculated using an exact method.

Table 42: Sosa-Estani Study Serologic Titer Month 48 using Enzymatic Immunoassay (EIA) By Gender (mITT)

Visit	Titer	Benznidazole	Placebo	Difference* (95%CI)**	P-value**
Change from Baseline Males					
Month 48	Mean (SD)	-0.10 (0.08)	-0.03 (0.10)	-0.09	<.0001
	Range	[-0.24, 0.12]	[-0.22, 0.23]	(-0.19, -0.09)	
	Missing	3	2		
	N	25	24		
Change from Baseline Females					
Month 48	Mean (SD)	-0.14 (0.11)	0.02 (0.03)	-0.17	<.0001
	Range	[-0.51, 0.03]	[-0.14, 0.16]	(-0.23, -0.11)	
	Missing	2	4		
	N	28	26		

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

Table 43: De Andrade Study Serologic response at Month 48 by Gender using Chemoluminescent Enzymatic Immunoassay (CLEIA) (ITT)

Gender	Benznidazole	Placebo	P-value*
Female N=53, n (%)			
Negative	15 (57.7)	2 (7.4)	<.0001
Positive	11	25	
Male N =76, n (%)			
Negative	20 (53.6)	1 (2.6)	<.0001
Positive	18	37	

* The p-values for the differences in seronegative rate (Benznidazole treatment group minus placebo group) were calculated using an exact method.

Table 44: De Andrade Study Serologic Titer at Month 48 using Chemoluminescent Enzymatic Immunoassay (CLEIA) by Gender (ITT)

Visit	Titer	Benznidazole	Placebo	Difference* (95%CI)**	P-value**
Change from Baseline Males					
Month 48	Mean (SD)	-2.42 (1.94)	0.26 (3.08)	-2.70	<.0001
	Range	[-6.46, 1.76]	[-6.46, 11.44]	(-3.95, -1.44)	
	N	25	24		
Change from Baseline Females					
Month 48	Mean (SD)	-2.67 (2.45)	0.93 (1.30)	-3.59	0.0011
	Range	[-11.84, 0.93]	[-20.02, 13.89]	(-6.32, -0.87)	
	N	28	26		

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

Table 45: De Andrade Study Serologic Response at Month 48 by Gender using Enzymatic Immunoassay (EIA) (ITT)

Gender	Benznidazole	Placebo	P-value*
Female N=53, n (%)			
Negative	2 (7.69)	0 (0.0)	0.2358
Positive	24	27	
Male N =49, n (%)			
Negative	2 (5.26)	0 (0.0)	0.4933
Positive	36	38	

*The p-values for the differences in seronegative rate (Benznidazole treatment group minus placebo group) were calculated using an exact method.

Table 46: De Andrade Study Serologic Titer Month 48 using Enzymatic Immunoassay (EIA) by Gender (ITT)

Visit	Titer	Benznidazole	Placebo	Difference* (95%CI)**	P-value**
Change from Baseline Males					
Month 48	Mean (SD)	-0.87 (0.52)	-0.19 (0.47)	-0.68	<.0001
	Range	[-2.50, 0.20]	[-1.20, 0.50]	(-0.92, -0.43)	
	N	25	24		
Change from Baseline Females					
Month 48	Mean (SD)	-0.87 (0.58)	-0.20 (0.47)	-0.67	<.0001
	Range	[-2.30, 0.60]	[-1.30, 0.80]	(-0.98, -0.35)	
	N	28	26		

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

Table 47: De Andrade Study Serologic response at Month 48 by Age Group Chemoluminescent Enzymatic Immunoassay (CLEIA) (ITT)

Age Group	Benznidazole	Placebo	P-value*
7-9, N=46, n (%)			
Negative	16 (66.67)	1 (0.0)	<.0001
Positive	8	21	
10-12, N =83, n (%)			
Negative	19 (47.50)	2 (4.65)	<.0001
Positive	22	41	

*The p-values for the differences in seronegative rate (Benznidazole treatment group minus placebo group) were calculated using an exact method.

Table 48: De Andrade Study Serologic response at Month 48 by Age Group using Enzymatic Immunoassay (EIA) (ITT)

Age Group	Benznidazole	Placebo	P-value*
7-9, N=46, n (%)			
Negative	2 (8.33)	0 (0.0)	0.4899
Positive	22	22	
10-12, N =83, n (%)			
Negative	2 (5.00)	0 (0.0)	0.2292
Positive	38	43	

*The p-values for the differences in seronegative rate (Benznidazole treatment group minus placebo group) were calculated using an exact method.

Table 49 displays secondary outcomes by gender for the Viotti Study. There is a statistically significant difference in the proportion of patients with three positive serologic tests, new ECG abnormalities, and mortality for females. In addition, there is a statistically significant difference in the proportion of patients with the three positive serologic tests in males with similar trends in the other two endpoints. When modeling the effect of age, treatment and treatment*age interaction on three positive serologic tests, new ECG abnormalities, and mortality, treatment*age interaction was not statistically significant. This is likely due to the narrow age range in the study.

Table 49: Viotti Study Additional Outcomes by Gender

Event	Benznidazole, N= 283	Untreated, N= 283	P-value
Males			
Positive Results on Three Serologic Tests	59/134 (62.7)	76/134 (84.4)	0.0014
New ECG Abnormalities	8/134 (5.97)	16/127 (12.60)	0.0856
Mortality	1/134 (.75)	4/127 (3.15)	0.2032
Females			
Positive Results on Three Serologic Tests	71/149 (57.3)	101/156 (82.8)	<.0001
New ECG Abnormalities	7/149 (4.70)	29/156 (18.59)	0.0002
Mortality	2/149 (1.34)	8/156 (5.13)	0.0483

4.2 Other Special/Subgroup Populations

There were no additional subgroups assessed.

5 SUMMARY AND CONCLUSIONS

5.1 Statistical Issues

None of the studies in this review were conducted under an IND. The Applicant contacted the authors of the publications and obtained some amount of patient-level data. This was critical in order to independently assess the outcomes of the studies, but the quantity of information was much less than we would typically attain from studies conducted under an IND. Additionally, we did not have access to the protocols and were unable to obtain additional information regarding the data provided. Some statistical issues specific for each study are given below.

There was no information provided about the randomization method used for the Sosa Estani study. In addition, we were not able to reproduce the results from the article for serologic response using F29 at the end of the 4 year follow-up period with the data submitted.

The Viotti study was a non-randomized study. Patients were assigned by using a non-randomized alternating sequence where every other individual enrolled was assigned to treatment and the alternate individuals were assigned to the control group. A total of 32 subjects withdrew after treatment assignment and were replaced (11 in the benznidazole arm and 21 untreated controls). We do not know if this led to a biased sample with sicker subjects more or less likely to remain on the benznidazole arm. In addition, the planned sample size was 319 patients per group; however, a total of 283 patients per group were actually enrolled into the study. The study also included interim analyses every 5 years with a plan 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 in the study. This could lead to unsubstantiated claims for the effectiveness of benznidazole due to a possible inflated rate of false positive conclusion. There was a large amount of subjects who were lost to follow-up; however, it is not clear why certain subjects were labeled as lost to follow-up despite long follow-up times and why others were not labeled as lost to follow-up despite short follow-up times. Furthermore, no event times were provided in the data submitted by the sponsor. Our conclusion is that although these were limitations we are still able to assess whether there is activity of the benznidazole.

5.2 Collective Evidence

For this review, the pivotal evidence to support the efficacy and safety of benznidazole for the treatment of Chagas disease was based on two randomized controlled pediatric studies (De Andrade study and Sosa-Estani study) and one adult study (Viotti study). This NDA submission also included 2 additional adult studies (Molina and DNDi-CH-E1244) as additional evidence to support the efficacy of benznidazole. None of the studies were conducted under the Applicant's IND, but patient-level data from all of the studies was submitted.

The De Andrade and Sosa-Estani studies were conducted in subjects 6 to 12 years old to test the superiority of benznidazole versus placebo and measured serology as the primary endpoint with 2 to 3 years of follow-up. The two pediatric studies were consistent in showing an effect of

benznidazole in the rate of seroconversion using an unconventional assay as well as reduction in serologic titers using both conventional and unconventional serology.

The Viotti study conducted in subjects 30 to 50 years old without heart failure contained long term follow-up and measured both serology and clinical endpoints. This study showed an effect of benznidazole on clinical outcomes as well as serology; however, there were limitations of the study that impacted the ability to draw definitive conclusions. This study was also beneficial in that it did show some relationship between clinical response and serologic titers.

The additional two adult studies (Molina and DNDi-CH-E1244) were conducted to assess the efficacy of a test drug with benznidazole included as an active comparator. The primary endpoint for the Molina study was parasite suppression measured by real-time PCR at 12 months after starting therapy. The primary endpoint for the DNDi-CH-E1244 study was RT-PCR at day 65; however, PCR was also measured up to month 12. Secondary endpoints for the two additional adult studies evaluated serologic response at month 12 using ELISA. These studies included patients in the indeterminate phase of Chagas disease. Due to a limited follow-up period and a less relevant endpoint, these studies are considered as supportive only. Benznidazole was superior to the active arms in the Molina study, and benznidazole was superior to the active and placebo arms in the DNDi-CH-E1244 study at 12 months for the PCR endpoint. Further, sustained PCR response over time showed significantly better results in the benznidazole group compared to all other arms in both studies. No significant results were seen with serology. Both the Molina and DNDi-CH-E1244 studies provide some support of effectiveness of benznidazole. For a full discussion of these studies, see statistical review by Janelle Charles, Ph.D.

Interpretation of the collective results is complicated by the limited clinical evidence and the limited information on the correlation of serology and PCR with clinical response.

5.3 Conclusions and Recommendations

Based on the studies submitted, benznidazole has been shown to have an effect on serology in both adults and pediatrics aged from 6 to 12 years old. There is evidence from a non-randomized trial that benznidazole also has an effect on clinical outcome in adults patients from 30 to 50 years old who do not yet have signs of heart failure. Supportive results in adults were seen using a more exploratory endpoint of PCR at 12 months.

It is recommended that the results of the De Andrade study and Sosa-Estani study be considered adequate evidence of efficacy to support the indication of treatment of Chagas disease in pediatric patients for benznidazole. The Viotti, Molina, and DNDi-CH-E1244 studies provide some support of the effectiveness of benznidazole in the adult population. (b) (4)

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Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA#: 209570

Proposed Drug Name: (b) (4) (benznidazole) tablets

Indication(s): Treatment of Chagas disease

Applicant: Chemo Research

Date(s): December 29, 2016 (Receipt Date)
March 10, 2017 (Assignment Date to Statistical Reviewer)
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Biometrics Division: Division of Biometrics IV

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Keywords: serology, PCR

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1 EXECUTIVE SUMMARY

This is a statistical review of New Drug Application (NDA209570), submitted by Chemo Research (hereafter referred to as the Applicant) for (b) (4) (benznidazole) tablets with the proposed indication of treatment of Chagas disease. The Applicant states that this disease, which is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*), is the third most common parasitic disease globally and that it causes incapacity in infected individuals and more than 10,000 deaths per year. Benznidazole is one of two products that have been used as standard-of-care for this disease; however, there are no currently approved products for this indication in the United States. The Applicant proposes a dose of (b) (4)

(b) (4) The main objective of this review is to evaluate data from two studies in adults with chronic Chagas disease, Molina and DNDi-CH-E1224-001, included in the submission, as part of supportive information to the efficacy evaluation for this NDA. Neither of these studies was conducted by the Applicant; they were conducted by the respective sponsors to investigate alternative treatments to benznidazole, given its known toxicities, with benznidazole included as a control. These studies are being reviewed differently from the originally planned study objectives and analyses in an attempt to assess the efficacy of benznidazole based on these data. Conventional serology assays have traditionally been used in the diagnosis and assessment of cure of Chagas disease; therefore, an important issue in this review is whether or not findings for polymerase chain reaction (PCR) observed in this studies can be interpreted as clinical benefit with benznidazole. Both of these studies use the LAFEPE company benznidazole 100 mg tablet, which is different from the proposed product; defer to clinical pharmacology for evaluation of this. Also, refer to the statistical review by Dr. Felicia Griffin for evaluation of the other studies included in the submission that are pertinent to the evaluation of the efficacy of benznidazole.

Molina, which was published by Molina *et al*¹, is a prospective, 1:1:1 randomized, open-label, active-controlled clinical trial in adults aged 23 to 62 years with chronic Chagas disease, in both its indeterminate and symptomatic form, diagnosed by detection of *T. cruzi* on two different serology tests and a positive result of a real-time polymerase chain reaction (PCR) assay for *T. cruzi* DNA. A total of 78 eligible patients were randomized in the study to receive the LAFEPE company benznidazole at a dose of 150 mg (26 patients), low-dose (LD) posaconazole (26 patients), or high-dose (HD) posaconazole (26 patients). Each treatment was to be orally administered twice daily for 60 days with a planned post-treatment follow-up of 10 months, i.e. total study duration of 12 months. The majority of patients in the study (65%) were classified by the study authors as having an indeterminate form of the disease, i.e. chronic *T. cruzi* infections with no clinical, radiological, or electrocardiographic evidence of visceral involvement. PCR response was measured in this study at various time points including end of treatment (Day 60) and at Month 12. Sustained PCR response, i.e. PCR negative at end of treatment and at each subsequent time point through end of study, and seroconversion to negative at various time points using conventional ELISA and recombinant ELISA (Biokit) are among the outcomes assessed for this study.

¹ Molina, I. *et al*. Randomized Trial of Posaconazole and Benznidazole for Chronic Chagas' Disease. *N Engl J Med* 370; 20. May 15, 2014.

DNDi-CH-E1224-001, shortened DNDi, is a prospective, 1:1:1:1:1 randomized, placebo- and active-controlled, assessor-blind, clinical trial in adults aged 18 to 49 years with chronic indeterminate Chagas disease determined by a minimum of two out of three serology tests and serial qualitative PCR (three samples taken over 7 days at least one of which must be positive). A total of 231 patients were randomized to receive the LAFEPE company benznidazole at a dose of 150 mg twice daily (45 patients), HD E1224² (45 patients), short-dose (SD) E1224 (46 patients), LD E1224 (48 patients), or placebo (47 patients). The placebo and E1224 arms were to receive double-blind oral treatment for 8 weeks and benznidazole patients were to receive open-label treatment for 60 days; refer to Section 3.2.2.1. A post-treatment follow-up was planned for a duration of 10 months, i.e. total study duration of 12 months. Patients with signs or symptoms of chronic cardiac and/or digestive form of Chagas disease were to be excluded from the study. Similar outcomes are evaluated in this study as defined for the Molina study. Additionally, seroconversion to negative at Month 12, among other time points, is also assessed for this study using the non-conventional AT CL-ELISA.

Both studies showed a statistically significant effect of benznidazole for PCR response at Month 12 as well as the sustained PCR response. In the Molina study, the differences in sustained PCR negative rates were 38.5%, 95% CI (9.8%, 62.6%) and 50.0%, 95% CI (22.1%, 71.9%) for comparison of benznidazole to HD and LD posaconazole arms, respectively; see Table 1. Most of the benznidazole patients who did not sustain PCR negative response are patients who had missing measurement at some time point after end of treatment whereas most of the posaconazole patients who were negative at end of treatment actually had a positive result recorded at a post-treatment follow-up visit Table 1.

Table 1 Sustained PCR Response in Molina and DNDi Studies – ITT Population

Molina Study					
	BNZ N=26	HD Posaconazole N=26	LD Posaconazole N=26		
Negative, n (%)	14 (53.9)	4 (15.4)	1 (3.9)		
Positive, n (%)	1 (3.9)	20 (76.9)	16 (61.5)		
Missing, n (%)	11 (42.3)	2 (7.8)	9 (34.6)		
Difference ¹ (95% CI)		38.5 (9.8, 62.6)	50.0 (22.1,71.9)		
DNDi Study					
	BNZ N=45	HD E1224 N=45	SD E1224 N=46	LD E1224 N=48	PBO N=47
Negative, n (%)	37 (82.2)	12 (26.7)	5 (10.9)	3 (6.3)	4 (8.5)
Positive, n (%)	4 (8.9)	26 (57.8)	40 (90.0)	39 (81.3)	42 (89.4)
Missing, n (%)	4 (8.9)	7 (15.6)	1 (2.2)	5 (10.4)	1 (2.1)
Difference ¹ (95% CI)		55.6 (38.5, 72.6)	71.4 (57.0, 85.7)	76.0 (56.8, 87.3)	73.7 (56.8, 85.7)

ITT=intent-to-treat comprising all randomized patients who were PCR positive at baseline, BNZ=benznidazole, PBO=placebo
 Missing includes patients who were missing all measurements after end of treatment or had at least one missing and all other measurements negative.
¹Difference in sustained PCR negative rates, expressed as percentages, and CIs based on normal approximation or exact method if less than 5.
 Source: Created by the statistical reviewer using dataset "adlb1row.xpt" for respective study.

² E1224 is drug substance that is equivalent to ravuconazole.

In the DNDi study, substantially higher percentage rates of sustained PCR negative response were observed for benznidazole patients compared to each of the other treatment arms. Notably, for comparison of benznidazole to placebo, a difference of 73.7%, 95% CI (56.8%, 85.7%) is observed.

In both studies, there were no patients with seroconversion to negative, based on conventional ELISA and recombinant ELISA (i.e. Biokit in Molina or Wiener in DNDi), at any time point for which serology measurements were taken. At Month 12 in the DNDi study, 5 (11.4%) benznidazole patients compared to 2 (4.3%) placebo patients were identified (based on cut-offs <0.9 negative, >1 positive) as having seroconversion to negative using the non-conventional AT CL-ELISA; a finding that was not statistically significant: difference of 7.1%, 95% CI (-13.8%, 27.0%).

Regarding safety, gastrointestinal disorders (e.g. upper abdominal pain and nausea) were among the most frequently reported treatment emergent adverse events, i.e. occurring in at least 10% of patients, across the treatment arms in the studies. There were no patients in the Molina study and in the DNDi study, one placebo and no benznidazole patients reported to have adverse events that were classified as cardiac disorders. The incidence of serious adverse events appears to be low across the studies and there were no deaths reported.

The use of PCR as an endpoint and the short duration of follow-up of only one year need to be carefully considered when interpreting the findings presented in this review. A recent study by Fabbro *et al*³ indicates that it takes years and up to decades to observe seroconversion to negative using non-conventional ELISA (average of 14.7 years based on ELISA-F29) and conventional ELISA (average of 22 years). Given the short follow-up in the Molina and DNDi studies, several assessments that might have been useful in understanding the efficacy of benznidazole for treatment of Chagas disease could not be performed in this review. For instance, these studies cannot be used to assess seroconversion to negative, since there were no such patients, or for assessing the relationship between non-conventional and conventional ELISAs. Further, because progression⁴ to clinically evident cardiac, gastrointestinal disease or both in patients who initially have the indeterminate form of Chagas disease may happen over a period of years, the short duration of follow-up in the Molina and DNDi studies limits the ability to adequately assess benznidazole effect on clinical outcomes.

In conclusion, the results presented in this review show that benznidazole is superior to the different test drugs in the respective studies and to placebo with respect to negative PCR at Month 12 and sustained PCR response. At the Month 12 time point, both studies showed that the test drugs had a large number of subjects reverting to PCR positive after having converted to negative while the benznidazole arm did not. Missing PCR measurements at various time points after end of treatment, notably in the Molina study, introduces a little uncertainty about the benznidazole rates. However, the fact that there were very few PCR reversions to positive for benznidazole patients (1 in the Molina study and 4 in the DNDi) at any time point during the

³ Fabbro D., et al. Evaluation of the ELISA-F29 Test as an Early Marker of Therapeutic Efficacy in Adults with Chronic Chagas Disease. *Rev. Inst. Med. Trop. Sao Paulo* 55(3): 167-172, May-June 2013.

⁴ C. Bern. Antitrypanosomal Therapy for Chronic Chagas Disease. *N Engl J Med* 2011; 364:2527-34.

post-treatment follow-up is encouraging. We defer to clinical expertise for whether PCR can be utilized as a surrogate of clinical response in this setting and whether or not these studies can be supportive of the efficacy of benznidazole.

2 INTRODUCTION

2.1 Overview and Regulatory Background

This is a statistical review of the original 505(b)(2) New Drug Application, NDA209570, that was submitted by Chemo Research, also referred to as the Applicant, on December 29, 2016 for (b) (4) (benznidazole) tablets. Benznidazole is an antiprotozoal agent, which the Applicant proposes to be indicated for the treatment of Chagas disease. The proposed dosing (b) (4)

(b) (4) This product was granted Orphan Drug Designation⁵.

Chagas disease, also known as American trypanosomiasis, is a chronic parasitic infection caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). According to the World Health Organization (WHO)⁶, approximately 6-7 million people are infected with *T. cruzi*. Chagas disease is found mainly in endemic areas of Latin American countries; however, the WHO indicates that in the past decade it has been increasingly detected in the US, Canada, Europe and some Western Pacific countries. The disease is mostly transmitted to humans through contact with feces or urine of triatomine bugs, also known as ‘kissing bugs’. The incubation period after exposure is one to two weeks. The disease presents in 2 phases: an acute phase and a chronic phase. The initial acute phase lasts for about 2 months after infection during which time patients may have symptoms such as, fever, headache, enlarged lymph glands, and abdominal or chest pains. In the chronic phase, the parasites are hidden mainly in the heart and digestive muscles. People in the chronic phase, but without signs or symptoms of disease, are considered to have the indeterminate form of the disease. Approximately 20% - 30% of individuals infected in the chronic indeterminate stage will progress to the chronic stage of disease exhibiting symptoms of cardiac disorders (e.g. conduction-system abnormalities, cardiomyopathy) and/or digestive disorders (e.g. megaesophagus, megacolon); progression may take years and up to decades from initial infection⁷. In later years, the infection can lead to sudden death due to cardiac arrhythmias or progressive heart failure caused by the destruction of the heart muscle.

Conventional serologic tests including enzyme linked immunosorbent assay (ELISA) kits using parasite lysate or recombinant antigens, direct agglutination (DA), and indirect immunofluorescence (IIF) have been used for diagnosis of chronic Chagas disease. The results of conventional serologic assays tend to remain positive for years or even decades after treatment.

⁵ Refer to Orphan Drug Designation Letter dated April 14, 2014.

⁶ Fact Sheet Chagas disease (American trypanosomiasis) <http://www.who.int/mediacentre/factsheets/fs340/en/>. Accessed April 7, 2017.

⁷ Bern C. Antitrypanosomal Therapy for Chronic Chagas’ Disease. N Engl J Med 2011; 364: 2527 – 34.

Non-conventional serology, such as, the chemoluminescent enzymatic (F29) ELISA and trypanolytic anti- α -Gal antibodies (AT CL-ELISA), have been explored as early markers of seronegativity. Parasitological tests, such as polymerase-chain reaction (PCR) assays from blood samples, have also been investigated as alternatives to serology testing for early diagnosis of chronic Chagas disease. However, PCR sensitivity might be limited because circulating parasite load in the blood is low in the chronic phase. Once diagnosed, Chagas disease has been treated with benznidazole and also nifurtimox (NFX); but, there are no currently approved treatments for this disease in the United States. The WHO states that both medicines are almost 100% effective in curing the disease if given soon after infection at the onset of the acute phase, including the cases of congenital transmission. The WHO further states that the efficacy of both diminishes, however, the longer a person has been infected and there are toxicity concerns with use of these products. Some adverse events reported⁷ to be associated with benznidazole use include allergic dermatitis, paresthesia, peripheral neuropathy, nausea, and leukopenia.

The efficacy and safety data included in the Applicant's benznidazole application for approval in the US comes from five controlled clinical studies: two studies conducted in children and three studies conducted in adults. The two studies in children, which measured serologic endpoints, are as follows:

- De Andrade⁸ – A 1:1 randomized, placebo-controlled, parallel-group Phase 2 study with a primary objective to compare the proportion of patients without specific antibodies, i.e., seronegative, at the end of 3 years of follow up between benznidazole 7.5 mg/kg and placebo. This study was conducted in 129 symptom-free children aged 7 to 12 years with antibodies for *T. cruzi*. The proportion of patients who were negative for *T. cruzi* antibodies at the end of the 3-year follow-up was statistically significantly higher in benznidazole (55%) compared to placebo (5%) using a non-conventional F29 ELISA assay; no significant difference was observed using conventional ELISA assay.
- Sosa-Estani⁹ – A 1:1 randomized double-blind, placebo-controlled, parallel-group Phase 2 study with a primary objective to assess the proportion of children seronegative against *T. cruzi* at 4 years between benznidazole 5 mg and placebo. This study was conducted in 106 children aged 6 years to 12 years with antibodies for *T. cruzi*. The proportion of patients who were negative for *T. cruzi* antibodies at the end of the 4-year follow-up was statistically significantly higher in benznidazole (60%) compared to placebo (14%) using a non-conventional F29 ELISA assay; no significant difference was observed using conventional ELISA assay.

Data from a long-term cardiac outcomes study¹⁰ in adult patients was later submitted to the NDA in February 2017. This study reported that statistically significantly fewer benznidazole patients

⁸ de Andrade, A. *et al.* Randomized trial of efficacy of benznidazole in the treatment of early *Trypanosoma cruzi* infection. *Lancet* 1996; 348: 1407 – 13.

⁹ Sosa-Estani, S. *et al.* Efficacy of chemotherapy with benznidazole in children in indeterminate phase of Chagas disease. *Am J Trop Med Hyg.* 1998; 59 (6): 526 – 529.

¹⁰ Viotti, R. *et al.* Long-Term Cardiac Outcomes of Treating Chronic Chagas Disease with Benznidazole versus No Treatment. *Ann Intern Med.* 2006; 144:724-734.

(4%) had progression of disease compared to untreated patients (14%) or developed abnormalities on electrocardiography (5% vs. 16%).

Given the outcomes measured in the two pediatric studies and the long-term cardiac study in adults, these studies comprise the main data for evaluation of efficacy in this NDA. Refer to statistical review by Dr. Felicia Griffin for detailed review these studies. This statistical review focuses on the two adult studies, Molina and DNDi-CH-E1224-001 (or shortened DNDi) included in the original NDA, which are randomized, parallel-group Phase 2 studies. A summary of the designs and outcomes analyzed in this review from these studies is shown in Table 2. It should be noted that both studies were planned to investigate test drugs (posaconazole in Molina and ravuconazole in DNDi) as alternatives to benznidazole based on the primary endpoint of proportion of patients with negativization of the parasite burden measured by PCR; benznidazole was included in these studies as a control. The timing for the definition of the PCR primary endpoint varied between the study protocols (Month 12 for Molina and Day 65 for DNDi); however, it is of interest in this review to focus on the effect at late time point (i.e. Month 12) for both studies. It is also notable that the PCR endpoint in these studies differs from the primary endpoint of seronegative conversion that was assessed in the two studies in children; seronegative conversion is also measured in these adult studies and assessed in this review. As mentioned earlier, serologic assays have traditionally been used in the diagnosis of Chagas disease, so results based on seronegative conversion are more readily interpretable. PCR, however, is being evaluated as an exploratory endpoint in this review. The PCR results from these studies are considered as supportive information in the efficacy evaluation in this NDA.

(b) (4)

It is acknowledged that the benznidazole product (i.e. the LAFEPE benznidazole 100 mg tablet) used in the DNDi and Molina studies is different from the proposed to-be-marketed product. Defer to clinical pharmacology review for assessment of this issue.

¹¹ Refer to Type B Meeting Minutes dated November 8, 2013.

Table 2 Summary of Trial Designs – Molina and DNDi

Protocol Number: Title	Planned Treatment Regimen ITT Patients	Study Endpoints Analyzed in Review
Molina: A Phase 2, Randomized, Open-Label, Clinical Trial for the Etiological Treatment of Chronic Chagas Disease with Posaconazole and Benznidazole	Benznidazole (LAFEPE Benznidazole) 150 mg (i.e. 1 ½ tablet) taken orally every 12 hours after breakfast or dinner for 60 days. Posaconazole (Noxafil®) 400 mg taken orally every 12 hours for 60 days at meal time. Posaconazole (Noxafil®) 100mg taken orally every 12 hours for 60 days at meal time. <u>ITT Patients:</u> Benznidazole – 26 Posaconazole 400 mg – 26 Posaconazole 100 mg – 26	Negativization of parasite burden measured by real-time PCR at Day 60 (end of treatment), at Month 4, Month 6, Month 8, and Month 12 after initiation of treatment. Seronegative conversion as measured by conventional ELISA and Biokit ELISA at Months 8 and 12.
DNDi-CH-E1224-001: A Phase 2 Randomized, Multicenter, Placebo-Controlled, Safety and Efficacy Study to Evaluate Three Oral Dosing Regimens and Benznidazole for the Treatment of Adult Patients with Chronic Intermediate Chagas Disease	Benznidazole (LAFEPE Benznidazole) 5mg/kg/day taken orally divided in two daily doses for 60 days. High dose ravuconazole (E1224) at loading dose 400 mg QD on Days 1-3 followed by 400 mg once weekly starting on Day 8 for seven weeks. Low dose E1224 at loading dose 200 mg QD and placebo on Days 1-3 followed by 200 mg and placebo once weekly starting on Day 8 to complete 7 weeks of treatment. Short dose E1224 loading dose of 400 mg QD on Days 1-3 followed by 400 mg once weekly starting on Day 8 for three weeks followed by placebo to complete 7 weeks of treatment. E1224 matching placebo for 8 weeks. <u>ITT Patients:</u> Benznidazole – 45 High dose E1224 – 45 Low dose E1224 – 46 Short dose E1224 – 48 Placebo – 47	Parasitological cure rate as determined by serial negative qualitative PCR results (3 negative PCR results from 3 samples collected over 7 days) at end of treatment (Day 65), at Month 4, Month 6, Month 8, and Month 12. Seronegative conversion as measured by conventional ELISA, Wiener ELISA, and non-conventional AT CL-ELISA at Day 65, Month 4, Month 6, and Month 12..

ITT=intent-to-treat comprising all randomized patients who are PCR positive at baseline, QD=once daily
 Source: Created by the statistical reviewer using protocols and clinical study reports for each study

2.2 Data Sources

The NDA was submitted electronically and includes a full study report for the DNDi study. A study report was not provided for the Molina study; instead a publication of this study from *The New England Journal of Medicine* was submitted in the application. The application also includes standardized patient-level data for the Molina and DNDi studies that are relevant for the analyses presented in this review. Analysis datasets and corresponding definition files can be found at the following locations:

- <\\CDSESUB1\evsprod\NDA209570\0001\m5\datasets\Molina\analysis\adam\datasets>
- <\\CDSESUB1\evsprod\NDA209570\0001\m5\datasets\DNDi-CH-E1224-001\analysis\adam\datasets>
- <\\CDSESUB1\evsprod\NDA209570\0004\m5\datasets\dndi-ch-e1224-001\analysis\adam\datasets>
- <\\CDSESUB1\evsprod\NDA209570\0004\m5\datasets\molina\analysis\adam\datasets>

For each study, the following datasets submitted by the Applicant are used in this statistical review:

- adsl.xpt contains the demographic data
- adlbf.xpt contains the efficacy data based on PCR and serology responses
- adae.xpt contains the adverse event data
- adds.xpt contains the disposition data
- adlb1row.xpt contains a restructured efficacy dataset comprising one row per patients that was submitted in February 2017 in response to request from microbiology reviewer, Dr. Shukal Bala
- adtte.xpt contains the times to reversion to PCR negative

The original protocol for the Molina study was dated April 12, 2010 and was amended once; final version dated September 18, 2012. The original protocol for the DNDi study was dated November 24, 2010, which was amended three times with final version dated February 22, 2013. The final versions of the protocols for these studies are utilized in this review.

The quality and integrity of the data included in the submission is discussed in Section 3.1.

3 STATISTICAL EVALUATION

3.1 Data and Analysis Quality

In general, the data submitted for the Molina and DNDi studies are adequate to allow the reviewer to perform the statistical analyses presented in this review. However, there are statistical issues related to datasets, serology data in the DNDi study, lack of information in the data definition files to confirm the qualitative measurements, missing data, and concerns with the Applicant's proposal for the USPI. Further, the extent of study follow-up in both studies needs to be considered when interpreting the findings of this statistical review. An overarching issue with these studies is whether PCR is a meaningful clinical endpoint in chronic Chagas disease. Defer to clinical review by Dr. Maria Allende for discussion of this issue.

A major statistical issue was identified during the review regarding the serology measurements reported in the datasets for the DNDi study. Although the study protocol states that two serology tests, i.e. conventional ELISA and Wiener ELISA, were used throughout the study after the baseline visit, the datasets reported two additional variables indicative of more serology tests

being performed than had been identified in the protocol. Upon our request concerning this finding, the Applicant stated¹² that in addition to the planned measurements in the protocol, which were recorded in the case report forms (CRFs), at the end of a patient's follow-up all of the patient's samples were re-tested using the same two ELISAs that had been specified in the protocol. The samples were re-read at the same time in the same laboratory and results of these additional tests were recorded on an Excel spreadsheet, not included in the submission, which was transferred to the contract research organization (CRO) for statistical analyses. The analyses presented in the study report submitted by the Applicant incorporate results from the re-read samples whenever available instead of from CRFs. It is unclear why these re-readings were performed and whether these re-readings were performed in a blinded fashion. Also, given that these additional tests were performed in most cases months after the samples had been obtained (e.g. samples taken at baseline or at Day 65), it calls into question the viability of these samples and consequently the accuracy of test results from re-reading. Therefore, all analyses conducted by the statistical reviewer use the measurements from the CRF only. It is noted however that the reviewer's conclusions based on the CRF datasets are consistent with those in the clinical study report submitted for this study. In addition to this issue of re-read data, there are unexpectedly similar trends observed for serology responses (i.e. conventional, Wiener, and AT-CL-ELISA) between placebo and benznidazole arms throughout this study's follow-up. This raises questions about the serology data for this study in general.

Another statistical issue is that the datasets for both studies did not report complete information about last date of treatment. For example, in the DNDi study all patients treated with benznidazole were missing last treatment date. This limits the ability to compare exposure distributions, i.e. number of days patients exposed, across treatment arms as part of the safety evaluation in this review.

Additionally, the definition files submitted for the efficacy analysis datasets for both studies did not provide the derivation rules used to convert quantitative PCR or quantitative serology measurements to the corresponding qualitative responses. Therefore, the reviewer is unable to verify the qualitative responses for PCR and serology assays presented in this review. Defer to clinical microbiology review by Dr. Shukul Bala for discussion regarding the reliability of the PCR and serology assays used in these studies and the consistency of quantitative and qualitative serology and PCR measurements in these studies.

Missing PCR measurements at various time points after end of treatment, notably in the Molina study, introduces a little uncertainty about the benznidazole response rates. However, the fact that there were very few PCR reversions to positive for benznidazole patients (1 in the Molina study and 4 in the DNDi) at any time point during the post-treatment follow-up is encouraging.

(b) (4)

¹² Refer to Response to Information Request dated April 28, 2017

The short duration of patient follow-up of only one year in the Molina and DNDi studies limits the ability to perform a number of important assessments needed to understand the efficacy of benznidazole; namely, a comparison of proportion of patients with seroconversion, i.e. from positive at baseline to negative, between benznidazole and other treatments in the studies, and an investigation of the relationship between PCR and serology. A related issue with this short follow-up is the inability to assess the relationship between the favorable PCR findings observed in the studies and clinical outcome; progression to clinically evident cardiac disease, or gastrointestinal disease or both, in patients with chronic indeterminate Chagas disease, may happen over a period of years or decades¹³. This limitation and other less significant ones are discussed in more detail in Section 5 of this review.

3.2 Evaluation of Efficacy

This section presents the statistical analyses of data from the Molina and DNDi studies that are considered supportive information to the evaluation of efficacy for benznidazole presented in the statistical review by Dr. Felicia Griffin. It is important to note that although these studies were originally intended to assess the efficacy of investigational products, posaconazole and E1224 (ravuconazole) with benznidazole as control in both studies, all analyses presented in this review are being performed to support the efficacy evaluation of benznidazole. All statistical analyses are performed at the 0.05 significance level (two-sided) and there are no adjustments to control for multiple comparisons, since these assessments are being performed after the results of the studies are known. Conclusions from these analyses are based on whether large, consistent, and durable effects are seen with particular focus on the results at the late time points in the studies.

3.2.1 Evaluation of Efficacy in the Molina Study

3.2.1.1 Design and Endpoints

The Molina study is a randomized, open-label, active-controlled, Phase 2 study in adult patients with chronic Chagas disease in both its indeterminate and symptomatic phases. The study was conducted at three sites in South America and comprised three periods: a Recruitment Period, a Treatment Period, and a Follow-up Period. Patients eligible for the study had to meet the following criteria:

- Adults aged 18 years or older

¹³ Bern, C. Antitrypanosomal Therapy for Chronic Chagas' Disease. *N Engl J Med* 2011; 264:2527-34

- Diagnosed with Chagas disease by positive reactions in two serology tests that use different antigens
- Completed the site's diagnostic protocol for patients with Chagas disease
- Positive real-time (RT) PCR at the time of diagnosis
- Women of childbearing potential must use adequate birth control or abstain from sexual relations while they were taking the study drugs
- Had lab work at the recruitment visit within normal limits
- Provided written informed consent

Patients who had chronic *T. cruzi* infection but had no clinical, radiologic, or electrocardiographic evidence of visceral involvement were categorized as having an indeterminate Chagas disease. Women who were pregnant or breastfeeding were to be excluded from the study. The protocol lists 7 additional exclusion criteria.

Patients who met all study eligibility criteria were to be randomized in a 1:1:1 ratio to receive one of three treatments:

- Benznidazole (LAFEPE Benznidazole, 100 mg tablet): 300 mg daily dose, i.e. 1½ of a 100 mg tablet taken twice daily, administered orally after breakfast or dinner.
- High-dose Posaconazole (Noxafil^{®14}): 400 mg every 12 hours administered orally with fat-rich foods
- Low-dose Posaconazole (Noxafil[®]): 100 mg every 12 hours administered orally with fat-rich foods

Randomization codes were to be computer generated using variable block sizes. The investigator was to remain unaware of the block size and of the patient's assignment until the time of inclusion in the study. The planned treatment duration was 60 days.

Reviewer's Comment: The Molina study was originally conducted to evaluate the efficacy, safety, and side-effect profile of posaconazole as an alternative to benznidazole. The study was published¹⁵ in May 2014 and the authors concluded that in patients with chronic Chagas disease, treatment with low-dose or high-dose posaconazole resulted in significantly worse efficacy compared to benznidazole.

Following the recruitment visit, patients were to return for periodic visits during treatment and up to Month 12. The evaluations that were to be performed at each visit are shown in Figure 1.

¹⁴ Noxafil[®] (posaconazole) is an antifungal medication available as injection, delayed-release tablets, and oral suspension for prophylaxis of invasive *Aspergillus* and *Candida* infections in patients who are at high risk of developing these infections due to being severely immunocompromised, such as hematopoietic stem cell transplant recipients with graft-versus-host disease or those with hematologic malignancies with prolonged neutropenia from chemotherapy. As oral suspension, it is also indicated for the treatment of oropharyngeal candidiasis, including oropharyngeal candidiasis refractory to itraconazole and/or fluconazole.

¹⁵ Molina, I. et al. Randomized Trial of Posaconazole and Benznidazole for Chronic Chagas Disease. *N. Engl. J. Med* 370; 20. May 15, 2014.

There were 5 PCR measurements taken during the treatment period and 4 PCR measurements taken during the post-treatment follow-up period. It should be noted that PCR was only reported as qualitative responses in this study. For serology, two enzyme-linked immunosorbent assays (ELISAs) were used: one that used recombinant antigen (Bioelisa Chagas, Biokit) and another that used a crude antigen, *T. cruzi* ELISA, Ortho-Clinical Diagnostics. These ELISAs are referred to as Biokit ELISA and conventional ELISA, respectively. Serology testing was performed at baseline, at Month 8, and at Month 12 and reported as qualitative and quantitative responses.

Figure 1 Schedule of Procedures for Molina Study

	0d	7d	14d	28d	45d	60d	4m	6m	8m	12m
Medical visit	x	x	x	x	x	x	x	x	x	x
Labwork (CBC + chemistry)	x	x	x	x		x				
Serology	x								x	x
RT-PCR	x	x	x	x	x	x	x	x	x	x
ECG	x	x	x	x	x	x				x
Chest X-ray	x									x
Echocardiography	x									
Digestive tract study	x									

Source: Protocol for Molina study (page 32)

According to the protocol, patients may have been withdrawn from the study for any of the following reasons:

- When they present with adverse events (AEs) considered serious and that require discontinuation of study medication, regardless of the study arm assigned
- Whenever the patient chooses
- If another treatment could be beneficial in the physician’s opinion

The efficacy endpoints presented in this statistical review are as follows:

- PCR response at Day 60 (end of treatment), at Month 8, and at Month 12
- Sustained PCR response defined as negative PCR at the end of treatment and each subsequent visit through Month 12
- Serology response, based on qualitative (i.e. negative/positive) and quantitative response, at Month 8 and Month 12 using both serology tests

Discussions of the analyses of these endpoints are provided in Section 3.2.1.2.

3.2.1.2 Statistical Methodologies

All statistical analyses of PCR are based on an intent-to-treat (ITT) population comprising all randomized patients who were PCR positive at baseline. Analyses of the qualitative serology are based on a modified-ITT (MITT) population comprising ITT patients who were positive at baseline for the respective serology test.

The following are the analyses performed by the reviewer for this study:

- A comparison of the proportions of patients with negative PCR at Day 60, at Month 6, at Month 8, and at Month 12 between benznidazole vs. high-dose posaconazole and benznidazole vs. low-dose posaconazole.
- A comparison of the proportions of patients with sustained PCR response between benznidazole vs. high-dose posaconazole and benznidazole vs. low-dose posaconazole. Patients who had missing measurements at all visits after end of treatment or at least one missing measurement and all other measurements negative are imputed as positive in the analysis.
- A Kaplan-Meier survival plot of time to reappearance of parasite, i.e. reversion to PCR positive after Day 60, in patients who have cleared parasite at Day 60, is presented for each treatment arm. Patients who were missing at Day 60 are excluded from this analysis. Patients who had missing measurements for all time points after Day 60 or at least one missing measurement with PCR negative for the remaining recorded time points are censored at the date of last non-missing measurement reported.
- For qualitative serology, a comparison of the proportions of patients with seronegative conversion at Month 8 and at Month 12 between benznidazole vs. high-dose posaconazole and benznidazole vs. low-dose posaconazole. This analysis is performed for conventional ELISA and Biokit ELISA.
- For quantitative serology, change from baseline in mean response at Month 8 and Month 12 is estimated using analysis of covariance model with post-treatment response (i.e. at Month 8 or Month 12) as the dependent variable, treatment an independent variable, site as a fixed effect and baseline response at a covariate. This analysis is performed for conventional ELISA and Biokit ELISA.

Subgroup analyses of PCR at Month 12 are presented by age, sex, and site. Given that no patients were over age 65, an analysis by age groups (<65, ≥65) cannot be performed in this review. Instead, a logistic model is used to assess the effect of age as a continuous variable. In this model, PCR response at Month 12 is used as the outcome variable and treatment and age as the independent variables; treatment by age interaction is also assessed with this model. Additionally, an analysis looking at results above and below the median age is performed.

For analyses of all qualitative responses, the 95% confidence intervals (CIs) are presented for differences in proportions using normal approximations or exact methods when responses are less than 5.

3.2.1.3 Patient Disposition, Demographic and Baseline Characteristics

The ITT population for the Molina study comprises a total of 78 patients: 26 benznidazole, 26 high-dose posaconazole, and 26 low-dose posaconazole. The ITT population excludes one HD posaconazole patient who was randomized, but was actually PCR negative at baseline. The 78 ITT patients were enrolled in 2 sites, 58 enrolled in Site 01 and 20 enrolled in Site 02. There was equal allocation of patients across the treatment arms at each of these sites.

The majority of patients (78.2%) completed the study; see Table 3. The most common reason for study withdrawal across the treatment arms was lost to follow-up, which was reported in 15.4% of the benznidazole patients and 15.4% of the low-dose posaconazole patients; no high-dose posaconazole patients were lost to follow-up. The HD posaconazole arm had the lowest overall study withdrawal rate (7.7%) with “unknown” as the only reason reported for withdrawal. Benznidazole had the highest withdrawal rate (38.5%) across the treatment arms and was the only treatment arm for which patients were withdrawn due to adverse events.

Table 3 Patient Disposition in Molina Study – ITT Population

Disposition Event	Benznidazole	HD Posaconazole	LD Posaconazole	Total
	N=26 n (%)	N=26 n (%)	N=26 n (%)	N=78 n (%)
Study Completion	16 (61.5)	24 (92.3)	21 (80.7)	61 (78.2)
Study Withdrawal	10 (38.5)	2 (7.7)	5 (19.2)	17 (21.8)
<u>Primary Reason for Withdrawal</u>				
Adverse Event	4 (15.4)	0 (0)	0 (0)	4 (5.1)
Lost to Follow-Up	4 (15.4)	0 (0)	4 (15.4)	8 (10.3)
Unknown	1 (3.9)	2 (7.7)	1 (3.9)	4 (5.1)
Withdrawal by Subject	1(3.9)	0 (0)	0 (0)	1 (1.3)

HD=high-dose, LD=low-dose
 Source: Created by the Statistical Reviewer using adds.xpt

Table 4 shows that the demographic characteristics are generally similar across the treatment arms in this study. The mean age in the study was 39.2 years, range: 23 – 62 years. There were no elderly patients, i.e. over 65 in this study. The majority of patients were female (60.3%) and from Bolivia (96.2%). Most patients resided in the city of Barcelona, Bolivia (77%); not shown in the table. There are some imbalances in the clinical involvement of Chagas disease across the treatment arms. Most patients in the study were reported as having indeterminate Chagas disease (65.4%), but with a higher percentage in the high-dose posaconazole (80.8%) compared to benznidazole (57.7%) and low-dose posaconazole (57.7%).

Reviewer’s Comment: The distributions of clinical involvement of Chagas disease were obtained from the Molina et al. publication because the reviewer was unable to find this information in the submitted datasets. No statistical assessments based on this patient characteristic are presented in this review.

Table 4 Demographic and Baseline Characteristics in Molina Study – ITT Population

Demographic Characteristics	HD		LD	Total N=78
	Benznidazole N=26	Posaconazole N=26	Posaconazole N=26	
Age, in years				
Mean (SD)	40.3 (8.6)	37.8 (8.8)	39.4 (10.2)	39.2 (9.1)
Median	39.0	36.5	36.5	37.0
Range	27 – 60	23 – 60	24 – 62	23 – 62
Sex, n (%)				
Female	15 (57.7)	16 (61.5)	16 (61.5)	47 (60.3)
Male	11 (42.3)	10 (38.5)	10 (38.5)	31 (39.7)
Country, n (%)				
Bolivia	24 (92.2)	25 (96.1)	26 (100)	75 (96.2)
Brazil	1 (3.9)	0 (0)	0 (0)	1 (1.3)
Paraguay	1 (3.9)	1 (3.9)	(0)	2 (2.6)
Clinical Involvement, n (%)				
Indeterminate	15 (57.7)	21 (80.8)	15 (57.7)	51 (65.4)
Cardiac	8 (30.8)	3 (11.5)	6 (23.1)	17 (21.8)
Gastrointestinal	1 (3.9)	1 (3.9)	3 (11.5)	5 (6.4)
Mixed	2 (7.7)	1 (3.9)	2 (7.7)	5 (6.4)

Source: Created by the statistical reviewer using adsl.xpt and Molina et al. publication

Table 5 shows that the majority of patients (at least 95%) had a positive serology test result at baseline for either conventional ELISA or Biokit ELISA.

Table 5 Baseline Quantitative and Qualitative Serology in Molina Study – ITT Population

	HD		LD	Total N=78
	Benznidazole N=26	Posaconazole N=26	Posaconazole N=26	
Conventional ELISA				
Qualitative Result				
Positive	24 (92.3)	25 (96.2)	25 (96.2)	74 (94.9)
Negative	0 (0)	0 (0)	0 (0)	0 (0)
Missing	2 (7.7)	1 (3.9)	1 (3.9)	4 (5.1)
Quantitative Result*				
Mean (SD)	6.4 (1.3)	6.2 (1.2)	6.3 (1.6)	6.3 (1.3)
Median	6.2	6.0	6.1	6.1
Range	4.3 – 9.1	4.3 – 9.1	4.3 – 12.0	4.3 – 12.0
Biokit ELISA				
Qualitative Result				
Positive	24 (92.3)	26 (100)	25 (96.2)	75 (96.2)
Negative	0 (0)	0 (0)	0 (0)	0 (0)
Missing	2 (7.7)	0 (0)	1 (3.9)	3 (3.9)
Quantitative Result*				
Mean (SD)	6.0 (2.3)	5.8 (1.6)	5.8 (2.4)	5.9 (2.3)
Median	6.8	6.4	7.2	6.7
Range	0.6 – 9.5	1.1 – 8.6	1.3 – 9.2	0.6 – 9.5

*Quantitative result summarized only for patients with non-missing serology result at baseline.
Source: Created by the statistical reviewer using adsl.xpt and adlb1row.xpt

The overall mean optical density (OD) for the conventional ELISA was 6.3 and the distributions were similar across the treatment arms. The overall mean OD for Biokit ELISA was 5.9 and with similar distributions for response across the treatment arms. There were few patients with missing serology measurement at baseline.

3.2.1.4 Results and Conclusions

This section summarizes the findings from analyses of PCR and serology in the Molina study for the entire ITT population (or MITT for qualitative serology). Results for the subgroup analyses of this study are presented in Section 4 of this review.

Results for Qualitative PCR

The results from the analysis of qualitative PCR response at Month 12, the latest time point in the study, are shown in the table that follows; recall that no quantitative PCR responses were reported in this study.

Table 6 Analyses of Qualitative PCR over Time in Molina Study – ITT Population

Time Point PCR Result	Benznidazole N=26	High-dose Posaconazole N=26	Low-dose Posaconazole N=26
<u>Day 60</u>			
Negative	22 (84.6)	25 (96.2)	18 (69.2)
Positive	0 (0)	0 (0)	0 (0)
Missing*	4 (15.4)	1 (3.9)	8 (30.8)
Difference [#] , % (95% CI)		-11.5 (-39.1, 17.4)**	15.4 (-13.6, 42.5)**
<u>Month 6</u>			
Negative	19 (73.1)	15 (57.9)	6 (23.1)
Positive	1 (3.9)	10 (38.5)	15 (57.7)
Missing*	6 (23.1)	1 (3.9)	5 (19.2)
Difference [#] , % (95% CI)		15.4 (-10.1, 40.9)	50.0 (26.5, 73.5)
<u>Month 8</u>			
Negative	18 (69.2)	11 (42.3)	7 (26.9)
Positive	0 (0)	12 (46.2)	13 (50.0)
Missing*	8 (30.8)	3 (11.5)	6 (23.1)
Difference [#] , % (95% CI)		26.9 (0.9, 52.9)	42.3 (17.7, 66.9)
<u>Month 12</u>			
Negative	16 (61.5)	7 (26.9)	6 (23.1)
Positive	0 (0)	17 (65.4)	13 (50.0)
Missing*	10 (38.5)	2 (7.7)	7 (36.8)
Difference [#] , % (95% CI)		34.6 (9.3, 59.9)	38.5 (13.7, 63.2)

N=randomized patients who were PCR qualitative positive at baseline
 *Missing represents patients who did not have a PCR measurement reported at respective time point and imputed as PCR positive, i.e. failures, in analysis.
[#]Difference in PCR negative rates (benznidazole – high-dose posaconazole) or (benznidazole – low dose posaconazole), expressed as a percentage, and 95% CI based on normal approximations or using exact method if presented with double asterisks **
 Source: Created by the statistical reviewer using datasets “adsl.xpt” and “adlb1row.xpt”

The proportion of patients with negative PCR at Month 12 was higher in benznidazole (61.5%) compared to high-dose posaconazole (26.9%) or low-dose posaconazole (23.1%). Based on these proportions, the difference in PCR negative rates was 34.6%, 95% CI (9.3%, 59.9%) when

benznidazole was compared to high-dose posaconazole and 38.5%, 95% CI (13.7%, 63.2%) when compared to low-dose posaconazole. These results show that benznidazole is statistically significantly better than either dose of posaconazole with respect to negative PCR at Month 12. Further, it is notable that there were no benznidazole patients who were reported as PCR positive at Month 12 compared to the majority of patients in the posaconazole arms (65.4% in HD posaconazole and 50% in LD posaconazole).

The results of the analyses of PCR response at Day 60 (end of treatment), at Month 6, and at Month 8 are also shown in this table. At Day 60, the proportion of patients with negative PCR response was higher in the HD posaconazole arm (96.2%) compared to benznidazole (84.6%) and LD posaconazole (69.2%). At Month 6, a higher proportion of PCR negative was observed for benznidazole patients (73.1%) compared to HD posaconazole (57.9%) and LD posaconazole (23.1%). At Month 8, a statistically significantly better proportion of PCR negative results are observed for benznidazole (69.2%) compared to the posaconazole arms (42.3% HD posaconazole and 26.9% LD posaconazole). This finding is driven by the substantial number of patients in the posaconazole arms who were reported to have PCR positive at Month 8, suggesting a high rate of reversion to PCR positive after the end of treatment in the posaconazole arms.

To further explore this finding, an analysis was performed to investigate the sustained PCR response, i.e. negative PCR result from Day 60 through Month 12; shown in Table 7. Patients who had a missing measurement at any time point after Day 60 or had at least one missing and all other measurements negative were imputed as PCR positive in this analysis. A higher proportion of sustained PCR negative response was observed for benznidazole 53.9% (14 patients) compared to high-dose posaconazole 15.4% (4 patients) and compared to low-dose posaconazole 3.9% (1 patient). The difference for sustained PCR response was statistically significant: 38.5%, 95% CI (14.8%, 62.1%) and 50.0%, 95% CI (29.5%, 70.5%) for comparisons of benznidazole to HD posaconazole and to LD posaconazole, respectively. Note that these CIs have not been adjusted for multiple comparisons.

Table 7 Sustained PCR Response at Month 12 – ITT Population

Sustained PCR Response	Benznidazole N=26	High-dose Posaconazole N=26	Low-dose Posaconazole N=26
Negative	14 (53.9)	4 (15.4)	1 (3.9)
Positive	1 (3.9)	20 (76.9)	16 (61.5)
Missing*	11 (42.3)	2 (7.8)	9 (34.6)
Difference [#] , % (95% CI)		38.5 (14.8, 62.1)	50.0 (29.5, 70.5)

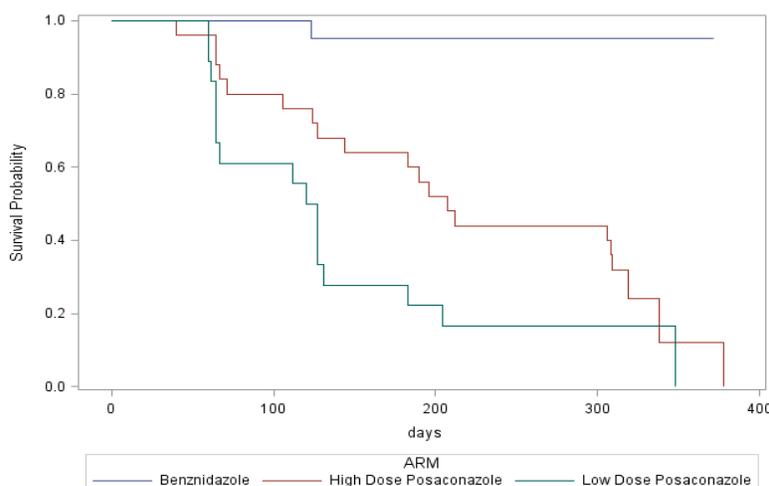
N=randomized patients who were PCR qualitative positive at baseline
 *Missing represents patients who did not have a PCR measurement reported all time points after Day 60 or had at least one time point missing and all other measurements negative at remaining time points. Patients with missing measurements are imputed as positive in the analysis, i.e. did not have sustained PCR response.
[#]Difference in PCR-negative rates (benznidazole – high-dose posaconazole) or (benznidazole – low dose posaconazole), expressed as a percentage, and 95% CI based on normal approximations or using exact method if presented with double asterisks**
 Source: Created by the statistical reviewer using datasets “adsl.xpt” and “adlb1row.xpt”

Reviewer’s Comment: The Molina et al publication also concluded statistically significant differences in sustained PCR negative favoring benznidazole compared to the posaconazole

arms. However, the publication used an alternative approach, whereby patients who had missing measurements at follow-up visits were considered to have sustained PCR negative if all available measurements at the observed visits were negative. This approach resulted in slightly different sustained suppression rates: 61.5% (16 patients) benznidazole, 19.2% (5 patients) HD posaconazole, and 7.7% (2 patients) LD posaconazole.

The Kaplan-Meier plot shown in Figure 2 illustrates the time to PCR reversion to positive, i.e. reappearance of parasite, across all three treatment arms in patients who were PCR negative at end of treatment (Day 60); see Table 6. The 13 patients (4 benznidazole, 1 HD posaconazole, and 8 LD posaconazole) with missing measurements at Day 60 are excluded from this analysis. Patients with missing measurements for all time points or missing for at least one time point and otherwise negative are censored at the last non-missing visit date for this analysis. The plot shows separation between the curves favoring benznidazole at about 50-60 days, i.e. after the Day 60 evaluation. This separation in the curves, favoring benznidazole, continues through Month 12. As stated previously, there were many patients who reverted to PCR positive (shown by each step in this plot) after end of treatment in the posaconazole arms compared to only one benznidazole patient who reverted to PCR positive. It is unclear whether this favorable trend on PCR reversion observed for benznidazole would be expected to continue with longer follow-up.

Figure 2 Kaplan-Meier Plot of Time to Reversion to PCR Positive in Molina Study



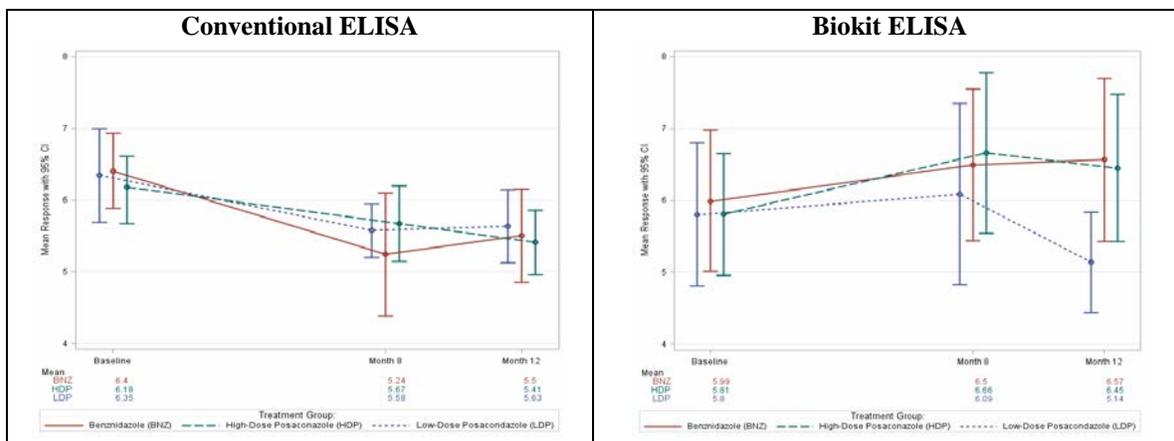
The horizontal axis shows number of days from the Day 60 evaluation. The vertical axis shows the proportion of patients who remain PCR negative. Patients with all missing measurements or at least one missing measurement and all other measurements recorded as negative at any time point after Day 60 are censored in this plot. Each step indicates a reappearance of parasite in blood samples, i.e. a PCR positive response. The plot includes only those ITT patients who were PCR negative at Day 60.
Source: Created by the statistical reviewer using “adlbeff.xpt” and “adtte.xpt”

Results for Qualitative and Quantitative Serology

Analyses of qualitative serology using conventional ELISA and Biokit ELISA testing, not presented in this review, found that no patients had seroconversion to negative at Month 8 and Month 12 in any of the treatment arms in this study. The remainder of this section presents the findings of analyses of change in mean quantitative serology response at these time points.

Graphical presentations of the mean serology responses for the conventional and Biokit ELISA tests throughout the study are shown in Figure 3.

Figure 3 Plot of Mean Serologic Response over Time in Molina Study – ITT Population



Missing responses at Month 8 and Month 12 have not been imputed. At each time point, 95% CI for the mean is based on normal approximation and the mean response at latter time points have not been adjusted for baseline.
 Source: Created by the statistical reviewer using datasets “adsl.xpt” and “adlb1row.xpt”

These graphs show very little change from baseline to Month 8 and from baseline to Month 12 for either ELISA. Note that because no additional time points were measured beyond those indicated in the plot, caution is advised in concluding a linear trend (decrease or increase) as suggested by the lines between time points. The findings from the analyses of mean change from baseline at Month 8 and at Month 12 in quantitative serology response for the conventional ELISA and Biokit ELISA are presented in Table 8. There is a large number of missing patients in the benznidazole arm (~38% - 50%); missing measurements are excluded from analysis. These results show no difference in mean change from baseline in serology response between benznidazole and either low- or high-dose posaconazole in using either ELISA test.

3.2.1.5 Conclusions for Molina Study

There is a statistically significant difference in negative PCR rates favoring benznidazole over both posaconazole doses at the end of the study (Month 12). Analyses of sustained PCR response from Day 65 (end of treatment) through study completion provide evidence to support this conclusion. Only one benznidazole patient had a positive PCR response after the end of treatment compared to several patients who reverted to positive after completing treatment with posaconazole.

There was no patient who was serology positive at baseline who converted to seronegative at any later time point measured in the study. Further, there appears to be no statistical difference in mean change from baseline in quantitative serology response at Month 8 or Month 12 using either conventional or Biokit ELISA.

Table 8 Analyses of Mean Serologic Response over Time in Molina Study – ITT Population

	<u>Conventional ELISA</u>			<u>Biokit ELISA</u>		
	Benznidazole	High-Dose Posaconazole	Low-Dose Posaconazole	Benznidazole	High-Dose Posaconazole	Low-Dose Posaconazole
<u>Baseline</u>	N=24	N=25	N=25	N=24	N=26	N=25
Mean (SD)	6.40 (1.27)	6.18 (1.18)	6.35 (1.59)	5.99 (2.33)	5.81 (2.10)	5.81 (2.41)
<u>Month 8</u>	N=12	N=20	N=18	N=12	N=20	N=18
Mean (95% CI)	5.24 (4.38, 6.10)	5.67 (5.14, 6.20)	5.58 (5.20, 5.95)	6.50 (5.45, 7.55)	6.66 (5.54, 7.78)	6.09 (4.83, 7.35)
Difference* (95% CI)		-0.38 (-1.18, 0.41)	-0.36 (-1.16, 0.45)		-0.06 (-1.67, 1.55)	0.39 (-1.25, 2.02)
p-value		0.33	0.38		0.94	
<u>Month 12</u>	N=15	N=24	N=20	N=16	N=24	N=20
Mean (95% CI)	5.50 (4.85, 6.15)	5.41 (4.96, 5.86)	5.63 (5.13, 6.13)	6.57 (5.43, 7.70)	6.45 (5.43, 7.48)	5.14 (4.44, 5.84)
Difference* (95% CI)		0.07 (-0.64, 0.79)	-0.31 (-1.06, 0.43)		0.08 (-1.21, 1.37)	1.01 (-0.23, 2.25)
p-value		0.84	0.40		0.90	0.12

N=randomized patients who were PCR positive at baseline and had quantitative serology response recorded at specified time point.

*Estimate of difference in mean change from baseline between the treatment groups, (benznidazole – high-dose posaconazole) or (benznidazole – low-dose posaconazole), 95% CI, and p-value obtained from ANCOVA model with post-treatment responses as the dependent variables, treatment as independent variable, site as fixed effect and baseline response as a covariate. Responses have not been imputed for patients with missing data.

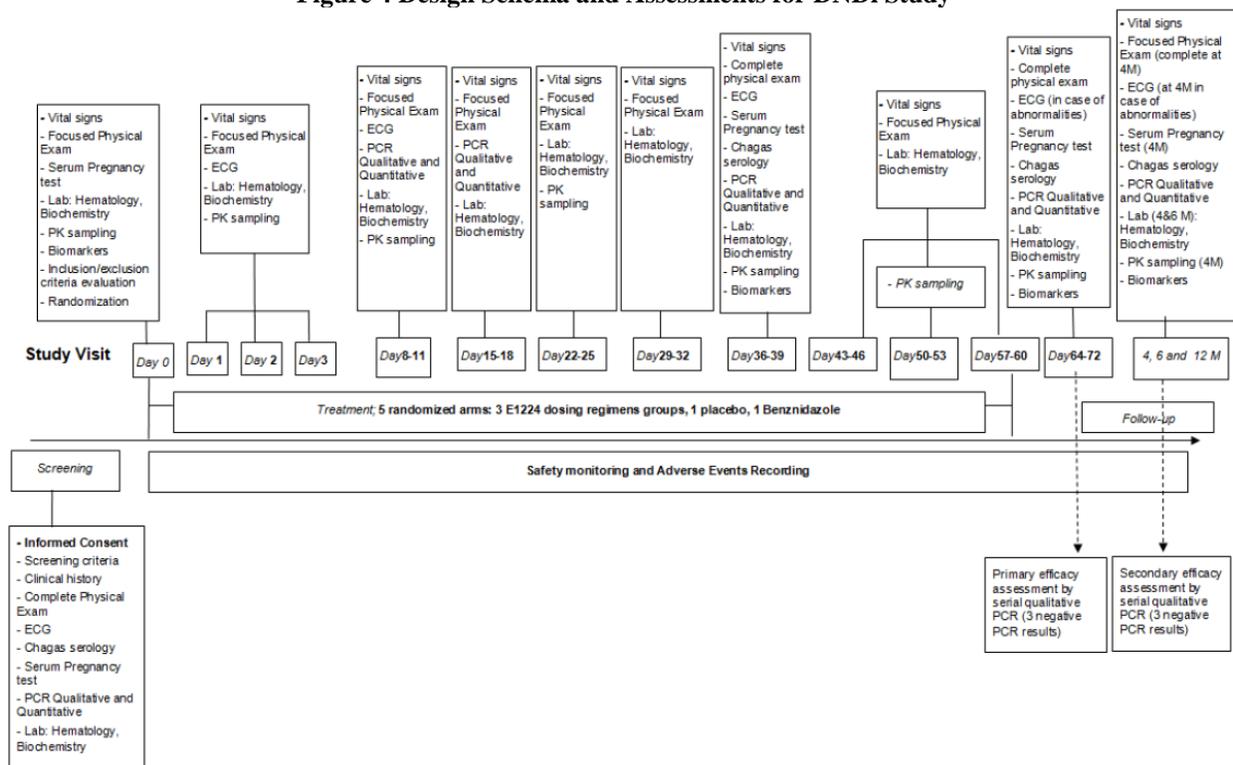
Source: Created by the statistical reviewer using “adsl.xpt” and “adlbfef.xpt”

3.2.2 Evaluation of Efficacy in the DNDi Study

3.2.2.1 Design and Endpoints

The DNDi study is a randomized, placebo and active-controlled, prospective, assessor-blind, parallel-group, Phase 2 study in adult patients with chronic indeterminate Chagas disease. The study was to be conducted in at most three clinical sites in Bolivia. The study comprised of three periods: a Screening Period of up to 20 days, an 8-week Treatment Period, and a Follow-up Period of up to 12 months after treatment initiation; see Figure 4. Adult patients aged 18 years to 50 years (inclusive) and weight at least 40 kg with serologic tests (a minimum of 2 out of 3 positive tests, i.e. conventional ELISA, Wiener ELISA, Biokit ELISA, IIF, or Indirect Hemagglutination (HAI)) confirming a diagnosis of *T. cruzi* infection were eligible to participate in the study provided they also met 12 screening criteria listed in the protocol.

Figure 4 Design Schema and Assessments for DNDi Study



Source: Study Report for DNDi Study (Figure 1, page 36)

Following the screening period, patients were to meet all of the following three inclusion criteria to be eligible for randomization:

- Confirmed diagnosis of *T. cruzi* infection by:
 - Serial qualitative PCR (three samples collected over 7 days, at least one of which must be positive) AND

- Conventional serology (a minimum of two out of three must be positive tests [conventional ELISA, Wiener ELISA, Biokit ELISA, IIF, or HAI])
- Women in reproductive age must have a negative serum pregnancy test at screening, must not be breastfeeding, and must consistently use and/or have partner consistently use a non-hormonal, highly effective contraceptive method during the entire treatment phase of the trial up to 4 months follow-up visit
- Normal ECG ($PR \leq 200$ msec, $QRS \leq 120$ msec, and $400 \text{ msec} \leq QTc \leq 450$ msec interval durations) at screening

Reviewer's Comment: The Applicant states that there is increased PCR sensitivity using multiple blood samples¹⁶.

Patients with signs or symptoms of the chronic cardiac and/or digestive form of Chagas disease were to be excluded from the study. The protocol lists 6 additional exclusion criteria for the study.

Eligible patients were to be equally randomized to one of five treatment regimens:

- High-dose E1224 (E1224 is a drug substance equivalent to ravuconazole): Loading dose of E1224 (400 mg once daily (QD) on Days 1-3) followed by 400 mg administered once weekly (starting on Day 8) for seven weeks (total dose: 4000 mg)
- Low-dose (LD) E1224: Loading dose of E1224 and placebo (200 mg QD on Days 1-3) followed by 200 mg and placebo administered once weekly (starting on Day 8) for seven weeks (total dose: 2000 mg)
- Short-dose (SD) E1224: Loading dose of E1224 (400 mg QD on Days 1-3) followed by 400 mg administered once weekly (starting on Day 8) for three weeks, to be followed by placebo to complete 7 weeks (total dose: 2400 mg)
- Placebo: E1224 matched placebo tablets comprising 4 tablets QD on Day 1-3, followed by 4 tablets administered once weekly (starting on Day 8) for seven weeks
- Benznidazole (LAFEPE Benznidazole tablet 100 mg): 5 mg/Kg/day divided in two daily doses, for 60 days. Patients were to be instructed on the exact dose to be taken and advised how the treatment must be taken, i.e., in two divided doses and with a meal.

E1224 was supplied as 100 mg tablets. Double-blinding was to be adopted for the E1224 and matching placebo treatments only. Patients randomized to benznidazole were to receive open-label treatment.

¹⁶ Refer to Type B Meeting Minutes dated November 11, 2013.

Reviewer's Comment: As noted earlier, this study was primarily conducted to determine whether at least one of the three dosing regimens of orally administered E1224 is more efficacious than placebo; benznidazole was included as a control to assess the sensitivity of study findings, given that it is current standard-of-care. The study data is being reviewed as supportive information to the evaluation of efficacy for this benznidazole application.

Rescue treatment might have been administered during the study according to the following regimens listed in the protocol:

- Patients who received doses of E1224 and did not present parasitological eradication (i.e. negative PCR) were to be offered benznidazole for 60 days at the end of his/her participation in the study
- Patients who received benznidazole and did not present parasitological cure at the end of his/her participation in the study were to be offered NFX for 60 days
- Patients who did not tolerate the study treatment were to be withdrawn from the study and were to receive treatment with NFX for 60 days
- Patients who received placebo and who presented with “parasitological clearance” were to be offered benznidazole for 60 days at the end of the study after the study had been unblinded.

Reviewer's Comment: The reviewer assumes that rescue medication would be administered only if a patient had not been cleared of the parasite, so the statement in the last bullet above about administering rescue to placebo patients who presented with “parasitological clearance” is likely a typo in the protocol.

A physical examination, assessment of vital signs, evaluation of biomarkers and other clinical assessments were to be performed at the baseline visit prior to initiation of assigned treatment. Following these baseline evaluations, patients were to return for periodic visits during the treatment period and up to 12 months from treatment initiation. The timing of these visits and summary of the evaluations performed are shown in Figure 4. Three blood samples were to be taken at Day 36, Day 65 (end of treatment), and at the 4, 6, and 12 month follow-up visit, and a single blood sample taken at Day 8 and Day 15 for PCR analyses. Measurements were to be recorded qualitatively and quantitatively using real-time PCR. Two conventional ELISAs (referred to as conventional ELISA and Wiener ELISA) and one non-conventional ELISA (trypanolytic anti- α -Gal antibodies - AT CL-ELISA) were to be recorded at Day 36, Day 65, and at the 4, 6, and 12 month follow-up visits. Serology measurements were quantitatively and qualitatively recorded. All PCR, other laboratory assessments, ECGs and clinical safety assessments were to be performed with the assessor blinded to treatment allocation for all groups. At each visit, patients received enough study treatment to last until the next scheduled visit.

According to the protocol, patients were considered to have completed the study if they satisfied all entry criteria, completed the course of treatment, and attended the three follow-up visits after end of treatment. Patients may have withdrawn from the study before the full course of treatment was completed. Additionally, patients may have discontinued treatment but remained in the

study. The protocol notes that reasons for treatment discontinuation and study withdrawal were to be recorded on the case report forms.

A Data Safety Monitoring Committee composed of 5 members, independent of the study sponsor, was established to review safety data on an ongoing basis throughout the study. The Principal Investigator and other study personnel were to remain blinded through the end of the study at Month 12. The study statisticians were not to have access to the unblinded data prior to the end of the study. An independent statistician was to perform the planned primary efficacy analysis as soon as the end of treatment follow-up had been completed, as well as an “interim” analysis of sustained PCR response at Month 6. To allow for “administrative decisions”, the unblinded results of the interim analysis were disclosed to Joint Development Committee (JDC) composed of the DNDi Project Leader, DNDi Medical Director, and members from the EISAI team and Wellcome Trust.

Reviewer’s Comment: The administrative reasons that warranted disclosure of unblinded “interim analysis” results are unclear. As noted in FDA DMC Guidance¹⁷, “Knowledge of unblinded interim comparisons from a clinical trial is generally not necessary for those conducting or sponsoring the trial; further, such knowledge can bias the outcome of the study by inappropriately influencing its continuing conduct or the plan of analyses. Unblinded interim data and the results of comparative interim analyses, therefore, should generally not be accessible by anyone other than DMC members or the statistician(s) performing these analyses and presenting them to the DMC.” Given that this interim analysis took place after Month 6, it is not expected that this unblinding would have influenced findings prior to this time point.

The following efficacy endpoints are evaluated in this statistical review for this study:

- PCR response at Day 65 (end of treatment), and at 4, 6, and 12 months after treatment initiation
- Sustained PCR response defined as PCR negative at the end of treatment through Month 12
- Serology response, based on qualitative (i.e. negative/positive) responses, at Day 65, and at 4, 6, and 12 months after initiation of treatment using conventional and non-conventional ELISAs
- Change in quantitative PCR and change in quantitative serology response (conventional and non-conventional ELISAs) at Day 65, and at 4, 6, and 12 months after initiation of treatment.

Discussion of the analyses of these endpoints is provided in the next section. These analyses are comparable to those described previously for the Molina study.

¹⁷FDA Guidance for Clinical Trial Sponsors. *Establishment and Operation of Clinical Trial Data Monitoring Committees*, dated March 2006.

3.2.2.2 Statistical Methodologies

Similar to the Molina study, all statistical analyses of PCR in the DNDi study are based on an intent-to-treat (ITT) population comprising all randomized patients who were PCR positive at baseline. Analysis of the qualitative serology responses are based on a modified-ITT (MITT) population comprising of ITT patients who were seropositive at baseline for the respective serology test.

The following analyses are performed by the reviewer for DNDi:

- Comparisons of the proportions of patients with PCR negative response at end of treatment (Day 65) and 4, 6, and 12 months between benznidazole and placebo and benznidazole and each of the E1224 arms. For these comparisons, patients are considered PCR negative if all 3 samples at each respective time point are negative. Patients who have missing measurements for all samples for each respective time point or have a missing measurement for at least one sample and all other samples negative are presented as missing. Missing measurements are imputed as positive in the analyses.
- A Kaplan-Meier survival plot of time to relapse, i.e. reversion to positive PCR after Day 65, in patients who had PCR negative response at Day 65, is presented for each treatment arm in the study. Patients who were missing measurements at Day 65 are excluded from this analysis. Patients who had missing measurements for all samples collected after Day 65 or at least one missing measurement with PCR negative for the remaining recorded samples are censored at the date of last non-missing measurement.
- A comparison of the proportions of patients with sustained PCR response at end of treatment through Month 12 between benznidazole and each treatment arm. Patients who had missing measurements at all visits after end of treatment or at least one missing measurement and all other measurements negative are imputed as positive in the analysis.
- For qualitative serology response, comparisons of the proportions of patients with seronegative conversion at Day 65, and at the 4, 6, and 12 month follow-up visits between benznidazole and each treatment arm. These analyses are performed for conventional and non-conventional serology assays.
- For quantitative serology response, change from baseline in mean response between benznidazole and each treatment arm at Day 65, and at the 4, 6, and 12 month follow-up visits are estimated separately using analysis of covariance (ANCOVA) model with post-treatment response as the dependent variable, treatment an independent variable, site as a fixed effect and baseline response as a covariate. This analysis is performed for conventional and non-conventional ELISAs. For non-conventional AT CL-ELISA, the analysis is based on log transformations of the baseline and post-treatment responses at each time point to account for the skewed nature of the distribution.

- Analysis of quantitative PCR response to assess the mean change from baseline between benznidazole and each treatment arm uses the same method, i.e., via ANCOVA, as specified in the point above for quantitative serology. At each time point, a patient's PCR result is determined as the average of the PCR results from the available samples at that time point. The analysis utilizes a log transformation to account for the skewed distribution for quantitative PCR.
- Analyses to assess the correlation between the conventional and non-conventional ELISAs as well as between PCR and the different serologic assays are performed based on the Pearson's correlation coefficient.

Subgroup analyses of PCR negative response at Month 12 are presented by age, sex, and site. The effect of age is assessed using similar methods as described for the Molina study in Section 3.2.1.2.

For analyses of qualitative responses, the 95% confidence intervals (CIs) are presented for differences in proportions using normal approximations or exact methods when responses are less than 5.

3.2.2.3 Patient Disposition, Demographic and Baseline Characteristics

A total 231 patients were randomized in the DNDi study, thereby comprising the ITT population for this study. These patients were allocated to study treatments as follows: 45 to benznidazole, 45 to HD E1224, 46 to SD E1224, 48 to LD E1224, and 47 to placebo. One hundred and sixteen patients were enrolled at Site 01 and 115 at Site 02; treatment arms were balanced within each site. More than 90% of patients in each treatment arm completed the study, i.e. continued participation through Month 12; see Table 9. The reasons for study withdrawal were lost to follow-up (1 benznidazole and 1 in SD E1224), other (2 HD E1224), and consent withdrawn (2 HD E1224 and 1 placebo). Patients who completed the study may have prematurely discontinued treatment but continued to be followed in the study. As shown in this table, the number of patients who prematurely discontinued treatment was low across all treatment arms. The most common reason for premature treatment discontinuation was due to adverse events, which occurred in 3 benznidazole patients and 4 HD E1224 patients. All patients in the LD E1224 were reported to have completed treatment and continued to be followed through study completion.

Table 10 shows that demographic characteristics were generally similar across the treatment arms. The average age of patients was 30 years with a range of 18 to 49 years. Most patients were female (74.4%) and resided in the city of Tarija, Bolivia (47.2%).

Table 9 Patient Disposition in DNDi Study – ITT Population

Disposition Event	Benznidazole N=45 n (%)	HD E1224 N=45 n (%)	SD E1224 N=46 n (%)	LD E1224 N=48 n (%)	Placebo N=47 n (%)	Total N=231 n (%)
Study Completion	44 (97.8)	41 (91.1)	45 (97.8)	48 (100)	46 (97.9)	224 (97.0)
Study Withdrawal	1 (2.2)	4 (8.8)	1 (2.2)	0 (0)	1 (2.1)	7 (3.0)
Reason for Withdrawal						
Lost to Follow-Up	1 (2.2)	0 (0)	1 (2.2)	0 (0)	0 (0)	2 (0.9)
Other	0 (0)	2 (4.4)	0 (0)	0 (0)	0 (0)	2 (0.9)
Consent Withdrawn	0 (0)	2 (4.4)	0 (0)	0 (0)	1 (2.1)	3 (1.2)
Treatment Completed	41 (91.1)	39 (86.7)	43 (93.5)	48 (100)	46 (97.9)	217 (93.9)
Treatment Discontinued	4 (8.9)	6 (13.3)	3 (6.5)	0 (0)	1 (2.1)	14 (6.1)
Discontinuation Reason						
Adverse Event	3 (6.7)	4 (8.9)	0 (0)	0 (0)	0 (0)	7 (3.0)
Lost to Follow-Up	0 (0)	0 (0)	1 (2.2)	0 (0)	0 (0)	1 (0.4)
Other	1 (2.2)	1 (2.2)	2 (4.3)	0 (0)	0 (0)	4 (1.7)
Consent Withdrawn	0 (0)	1 (2.2)	0 (0)	0 (0)	1 (2.1)	2 (0.9)

HD=high-dose, LD=low-dose, SD=short-dose
 Source: Created by the Statistical Reviewer using adsl.xpt

Table 10 Demographic Characteristics in DNDi Study – ITT Population

Demographic Characteristics	Benznidazole N=45 n (%)	HD E1224 N=45 n (%)	SD E1224 N=46 n (%)	LD E1224 N=48 n (%)	Placebo N=47 n (%)	Total N=231 n (%)
Age						
Mean (SD)	30.7 (9.0)	30.2 (8.9)	27.8 (8.3)	31.3 (8.8)	31.0 (9.2)	30.2 (8.8)
Median	29	30	26	30	30	28
Range	18 – 47	18 – 49	18 – 47	19 – 49	18 – 46	18 – 49
Sex, n (%)						
Female	31 (68.9)	32 (71.1)	35 (76.1)	37 (77.1)	37 (78.7)	172 (74.4)
Male	14 (31.1)	13 (28.9)	11 (23.9)	11 (22.9)	10 (21.3)	59 (25.5)
City of Residence*, n (%)						
Cochabamba	12 (26.7)	8 (17.8)	14 (30.4)	15 (31.3)	12 (25.5)	61 (26.4)
Other	10 (22.2)	17 (37.8)	9 (19.6)	12 (25.0)	13 (27.7)	61 (26.4)
Tarija	23 (51.1)	20 (44.4)	23 (50.0)	21 (43.8)	22 (46.8)	109 (47.2)

*This study was conducted only in Bolivia.
 Source: Created by the statistical reviewer using adsl.xpt.

Table 11 shows the serology (quantitative and qualitative) results and quantitative PCR results at baseline. The conventional ELISA was performed for all patients and Wiener ELISA test was performed for all patients but for one benznidazole patient. Most patients had a positive qualitative result for these tests. There were four patients (3 benznidazole and 1 HD E1224) who did not have the test performed or had an indeterminate or negative result for either one of these tests as shown in this table. Two of these benznidazole patients had additional test with HAI which were positive and the remaining two patients had a second ELISA (ELISA recombinant Biokit) that was positive, thereby qualifying them for randomization in the study. Note that HAI and ELISA recombinant (Biokit) were not used for testing at subsequent time points in the study.

Table 11 Baseline Serology, Quantitative PCR, and AT CL-ELISA in DNDi Study – ITT Population

	Benznidazole N=45	HD E1224 N=45	SD E1224 N=46	LD E1224 N=48	Placebo N=47	Total N=231
Conventional ELISA, n (%)						
Test Performed	45 (100)	45 (100)	46 (100)	48 (100)	47 (100)	231 (100)
<u>Qualitative Result</u>						
Positive	44 (97.8)	44 (97.8)	46 (100)	48 (100)	47 (100)	229 (99.2)
Negative	0 (0)	1 (2.2)	0 (0)	0 (0)	0 (0)	1 (0.4)
Indeterminate	1 (2.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)
<u>Quantitative Result*</u>						
Mean (SD)	2.9 (0.3)	2.8 (0.5)	2.9 (0.3)	2.8 (0.3)	2.9 (0.2)	2.9 (0.3)
Median	3.0	3.0	3.0	3.0	3.0	3.0
Range	2.0 – 3.2	0.6 – 3.2	1.5 – 3.2	1.5 – 3.2	2.0 – 3.2	0.6 – 3.2
Wiener ELISA, n (%)						
Test Performed	44 (97.8)	45 (100)	46 (100)	48 (100)	47 (100)	230 (99.6)
<u>Qualitative Result</u>						
Positive	43 (95.6)	45 (100)	46 (100)	48 (100)	47 (100)	229 (99.2)
Negative	1 (2.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)
Indeterminate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Missing	1 (2.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)
<u>Quantitative Result*</u>						
Mean (SD)	2.8 (0.5)	2.9 (0.3)	2.9 (0.3)	2.9 (0.3)	3.0 (0.2)	2.9 (0.3)
Median	3.0	3.0	3.0	3.0	3.0	3.0
Range	0.8 – 3.2	1.4 – 3.5	1.4 – 3.2	2.0 – 3.2	2.1 – 3.8	0.8 – 3.8
Quantitative PCR**, pEq/mL						
Mean (SD)	0.6 (0.8)	0.7 (1.0)	0.5 (0.7)	1.3 (2.0)	1.0 (2.4)	0.8 (1.6)
Median	0.3	0.3	0.3	0.4	0.3	0.3
Range ¹	0.0 – 2.6	0.0 – 3.6	0.0 – 2.9	0.0 – 8.5	0.0 – 12.6	0.0 – 12.6
AT CL-ELISA***						
<u>Qualitative Result***</u>						
Positive	44 (97.8)	45 (100)	46 (100)	46 (95.8)	47 (100)	228 (98.7)
Negative	1 (2.2)	0 (0)	0 (0)	2 (4.2)	0 (0)	3 (1.3)
<u>Quantitative Result*</u>						
Mean (SD)	5.6 (4.1)	5.9 (3.9)	6.5 (3.5)	5.5 (3.7)	6.7 (4.9)	6.0 (4.1)
Median	4.2	5.3	5.4	4.3	5.5	5.0
Range	0.6 – 17.2	1.1 – 18.2	1.7 – 14.8	0.6 – 16.2	1.5 – 23.1	0.6 – 23.1

*Quantitative serology presented only for those patients with non-negative result for respective test
 **Quantitative PCR, parasites equivalents/mL(pEq/mL), is reported for all ITT patients based on average results from three baseline samples
 ***Per study report, less than 0.9 is equivalent to negative result and >1.0 equivalent to positive
¹Actual minimum value for PCR is 0.0072
 Source: Created by the statistical reviewer using adlbef xpt

The overall mean OD for the conventional ELISA was 2.9 (range: 0.6 – 3.2) and similar across the treatment arms. The overall mean OD for the Wiener ELISA was 2.9 (range: 0.9 – 3.2) and similar across the treatment arms. The distributions for parasite burden measured by quantitative PCR was similar for the benznidazole, HD E1224, and SD E1224 with mean parasite burden of 0.6, 0.7, and 0.5, respectively. The distributions for the LD E1224 and placebo arms were right skewed and the means were somewhat higher, i.e. 1.3 and 1.0, respectively, than that observed in the other treatment arms. The study report states that these apparent differences in the distributions of PCR are due to random fluctuations.

Reviewer's Comment: Baseline results for conventional and Wiener ELISAs presented in this table differ from the findings presented in the study report. For example, the study report presents a mean of 2.2 for benznidazole compared to the reviewer's mean of 2.9 for conventional ELISA. As described in Section 3.1, these differences are because the analyses in the study report incorporate results from re-read samples after study completion whereas the reviewer's analyses are based only on results obtained during the course of the study.

In addition to the serology and PCR tests performed, this table also shows AT CL-ELISA lytic antibody levels; a non-conventional serology test. The overall mean for AT CL-ELISA was 6.0 (range: 0.6 – 23.1). There are some differences in the distributions for AT CL-ELISA across the treatment arms; placebo and SD E1224 appear to have higher mean baseline values compared to the other treatment arms. The distributions for AT CL-ELISA are right skewed as indicated by the smaller median than mean. Three patients had AT CL-ELISA lytic antibodies titer less than 0.9 at baseline (i.e. negative) and all other patients had baseline value greater than 1.0, i.e. positive; 0.9 and 1.0 are the cut-off values specified in the study report.

3.2.2.4 Results and Conclusions

This section presents the results for the analyses of PCR and serology in the DNDi study; all subgroup analyses for the study are presented in Section 4. Given that E1224 is not currently approved for Chagas disease, estimates for benznidazole treatment effect are presented primarily using comparisons of benznidazole response rates to placebo. However, if it could be assumed that E1224 has a slight effect, then results presented in this section that compare benznidazole to E1224 may be considered as more conservative estimates of benznidazole effect.

Results for Qualitative and Quantitative PCR

The findings from the analysis of qualitative PCR at various time points in the DNDi study are shown in Table 12. The proportion of patients in the benznidazole arm (93.3%) with negative PCR at Month 12 is statistically significantly greater than in the placebo arm (21.3%). The estimated treatment effect for benznidazole is 72.1% with 95% CI (55.8%, 84.5%). This table also shows that the PCR negative rate for benznidazole is statistically significantly higher in comparison to the E1224 arms at this time point. The results at end of treatment (Day 65) are comparable between benznidazole and the E1224 arms, but substantial differences are observed at Month 4 and Month 6. The PCR negative rates for benznidazole are relatively unchanged at each time point following Day 65. However, an increasing number of patients in the E1224 arms become PCR positive after end of treatment. Note that the CIs presented in this table have not been adjusted for multiple comparisons.

Table 13 shows the results of sustained PCR response from end of treatment at Day 65 through Month 12. The highest proportion of sustained PCR negative was observed in benznidazole patients (82.2%), which is statistically significantly better compared to E1224 patients (26.7% HD, 10.9% SD, and 6.3% LD) and placebo patients (8.5%). The estimated treatment effect for sustained PCR response at Month 12, i.e., with respect to placebo, is 73.7% with 95% CI (56.8%, 85.7%). Note that these CIs have not been adjusted for multiple comparisons.

Table 12 Analyses of Qualitative PCR over Time in DNDi Study – ITT Population

Time Point PCR Response	Benznidazole N=45	HD E1224 N=45	SD E1224 N=46	LD E1224 N=48	Placebo N=47
Day 65					
Negative	41 (91.1)	33 (73.3)	41 (89.1)	42 (87.5)	12 (25.5)
Positive	3 (6.7)	8 (17.8)	4 (8.7)	5 (10.4)	34 (72.3)
Missing	1 (2.2)	4 (8.9)	1 (2.2)	1 (2.1)	1 (2.1)
% Difference ¹ (95% CI)		17.8 (-4.0, 38.3)	0.02 (-18.2, 22.4)	0.04 (-24.1, 16.7)	65.6 (48.4, 79.5)
Month 4					
Negative	43 (95.6)	32 (71.1)	13 (28.3)	30 (62.5)	9 (19.2)
Positive	0 (0)	9 (20.0)	31 (67.4)	18 (37.5)	37 (78.7)
Missing	2 (4.4)	4 (8.9)	2 (4.4)	0 (0)	1 (2.1)
% Difference ¹ (95% CI)		24.4 (2.7, 44.5)	67.3 (51.0, 81.0)	33.1 (12.5, 51.3)	76.4 (61.1, 87.8)
Month 6					
Negative	41 (91.1)	26 (57.8)	10 (21.7)	16 (33.3)	6 (12.8)
Positive	2 (4.4)	14 (31.1)	33 (71.7)	32 (66.7)	40 (85.1)
Missing	2 (4.4)	5 (11.1)	3 (6.5)	0 (0)	1 (2.1)
% Difference ¹ (95% CI)		33.3 (11.9, 52.5)	69.4 (52.4, 82.6)	57.8 (39.7, 72.8)	78.4 (62.6, 89.2)
Month 12					
Negative	42 (93.3)	18 (40.0)	7 (15.2)	11 (22.9)	10 (21.3)
Positive	2 (4.4)	23 (51.1)	38 (82.6)	36 (75.0)	36 (76.6)
Missing	1 (2.2)	4 (8.9)	1 (2.2)	1 (2.1)	1 (2.1)
% Difference ¹ (95% CI)		53.3 (33.1, 69.9)	78.1 (62.4, 89.2)	70.4 (54.2, 83.1)	72.1 (55.8, 84.5)

N=all randomized patients PCR positive at baseline

Negative includes patients with all PCR tests negative from 3 samples at respective time point, positive includes patients with at least one positive result in the 3 samples at the respective time point; otherwise results are presented as missing. Missing measurements are imputed as positive in the analysis.

¹Difference in negative response rates obtained using exact methods. CIs have not been adjusted for multiple comparisons.

Source: Created by the statistical reviewer using datasets adsl.xpt and adlb1row.xpt

Table 13 Analysis of Sustained PCR Response in DNDi Study – ITT Population

Sustained PCR Response	Benznidazole N=45	HD E1224 N=45	SD E1224 N=46	LD E1224 N=48	Placebo N=47
Negative	37 (82.2)	12 (26.7)	5 (10.9)	3 (6.3)	4 (8.5)
Positive	4 (8.9)	26 (57.8)	40 (90.0)	39 (81.3)	42 (89.4)
Missing	4 (8.9)	7 (15.6)	1 (2.2)	5 (10.4)	1 (2.1)
% Difference ¹ (95% CI)		55.6 (38.5, 72.6)	71.4 (57.0, 85.7)	76.0 (59.6, 87.3)	73.7 (56.8, 85.7)

LD=low-dose, SD=short-dose, HD=high-dose E1224, N=all randomized patients PCR positive at baseline

Negative response includes patients with sustained parasitological cure, i.e. patients who had all 3 samples negative at Day 65, and at each subsequent time point through Month 12. Positive includes patients with at least one positive measurement for any of the subsequent time points, but were negative at Day 65, or had a positive response at Day 65 which was sustained at subsequent time points. Otherwise, patients are presented as missing; missing measurements are imputed as positive responses in the analysis.

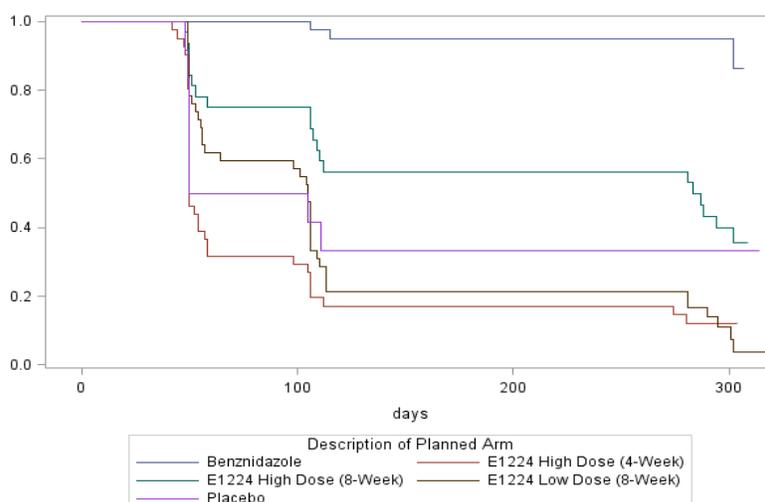
¹Difference in sustained PCR response rates between benznidazole and each treatment arm, expressed as percentage, and confidence interval based on normal approximation or exact method where cell count less than 5.

Source: Created by the statistical reviewer using datasets adsl.xpt, adtte.xpt, andadlb1row.xpt

Among the patients who were PCR negative at Day 65 (see Table 12), reappearance occurred at a subsequent time point in 8 of 12 placebo patients, 20 of 33 HD E1224 patients, 36 of 41 SD

E1224 patients, 39 of 42 LD E1224 patients, and 4 of 41 benznidazole patients. A Kaplan-Meier plot of time to first reappearance of parasite, illustrated in Figure 5, based only on those patients who were PCR negative at Day 65, shows that the earliest reappearance in benznidazole patients occurred at around 110 days after end of treatment compared to approximately 50-60 days (i.e. Month 4 visit) for the E1224 and placebo arms. Given the differences in PCR negative rates observed across the treatment arms (Table 12), it is not assumed that patients included in this analysis are similar at Day 65. However, these observed trends are very noteworthy and show large effect of benznidazole compared to each of other treatment arms in the study. It is uncertain whether these favorable results for benznidazole within the 10 months of post-treatment follow-up for this study would be observed with a lengthier follow-up duration.

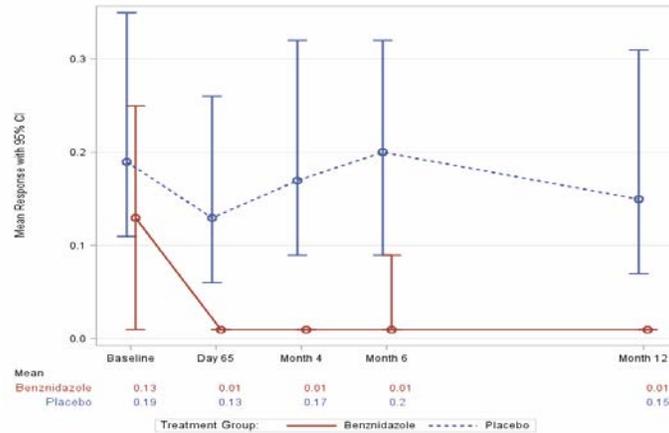
Figure 5 Time to First Parasite Reappearance after Day 65 in DNDi Study



The vertical axis shows the proportion of patients without reappearance in parasite. This figure includes only those ITT patients who were PCR negative at Day 65. E1224 High Dose (4-week) is equivalent to SD E1224. Patients with missing measurements for all time points after Day 65 or for whom at least one sample was missing and all others negative are censored in this plot at the date of the last reported non-missing measurement. Each step in this plot indicates a reappearance of parasite, i.e. positive PCR.
Source: Created by the statistical reviewer using adlb1row.xpt, adlbfef, and adtte.xpt.

A graphical presentation of the geometric mean responses for PCR over time in the benznidazole and placebo arms is shown in the figure that follows. There is a clear separation in mean responses between benznidazole and placebo at Day 65 through end of study follow-up at Month 12. Notably, at Day 65, there is a statistically significant reduction (approximately 95% reduction) in geometric mean from baseline favoring benznidazole over placebo; see Table 14. This effect is maintained at each visit through end of study follow-up. The results from analyses comparing benznidazole to each of the E1224 arms, not presented in this review, showed similar geometric mean responses across the treatment arms at Day 65. However, at each subsequent time point, the geometric mean response for benznidazole was considerably less compared to the E1224 arms; this is consistent with what was observed in the analyses of qualitative PCR.

Figure 6 Plot of Geometric Mean PCR Responses over Time in DNDi Study – ITT Population



Source: Created by the statistical reviewer using adsl.xpt and adlb1row.xpt

Table 14 Analyses of Geometric Mean PCR Responses over Time in DNDi Study – ITT Population

	<u>PCR</u>	
	Benznidazole	Placebo
Baseline*	N=45	N=47
Geometric Mean	0.13	0.19
(95% CI)	(0.01, 0.25)	(0.11, 0.35)
Day 65	N=44	N=46
Geometric Mean	0.01	0.13
(95% CI)	(0.01, 0.01)	(0.06, 0.26)
Ratio ¹ (95% CI)	0.06 (0.03, 0.12)	
p-value	<0.0001	
Month 4	N=43	N=46
Geometric Mean	0.01	0.17
(95% CI)	(0.01, 0.01)	(0.09, 0.32)
Ratio ¹ (95% CI)	0.05 (0.03, 0.09)	
p-value	<0.0001	
Month 6	N=43	N=46
Geometric Mean	0.01	0.20
(95% CI)	(0.01, 0.09)	(0.11, 0.39)
Ratio ¹ (95% CI)	0.04 (0.02, 0.08)	
p-value	<0.0001	
Month 12	N=44	N=46
Geometric Mean	0.01	0.15
(95% CI)	(0.01, 0.01)	(0.07, 0.31)
Ratio ¹ (95% CI)	0.06 (0.03, 0.11)	
p-value	<0.0001	

N=randomized patients with non-missing measurement at baseline for respective test

*There are no statistically significant differences at baseline.

¹For each time point, the ratio of geometric mean changes from baseline, confidence interval, and p-value obtained from ANCOVA model with post-treatment response as the dependent variable, treatment as independent variable, site as fixed effect and baseline response as covariate. Patients with missing data excluded from analysis. Analysis uses log transformation of the baseline and post-treatment responses to account for skewed distributions for PCR. Patients with missing measurements have not been imputed.

Source: Created by the statistical reviewer using adsl.xpt and adlb1row.xpt

Results for Quantitative and Qualitative Serology

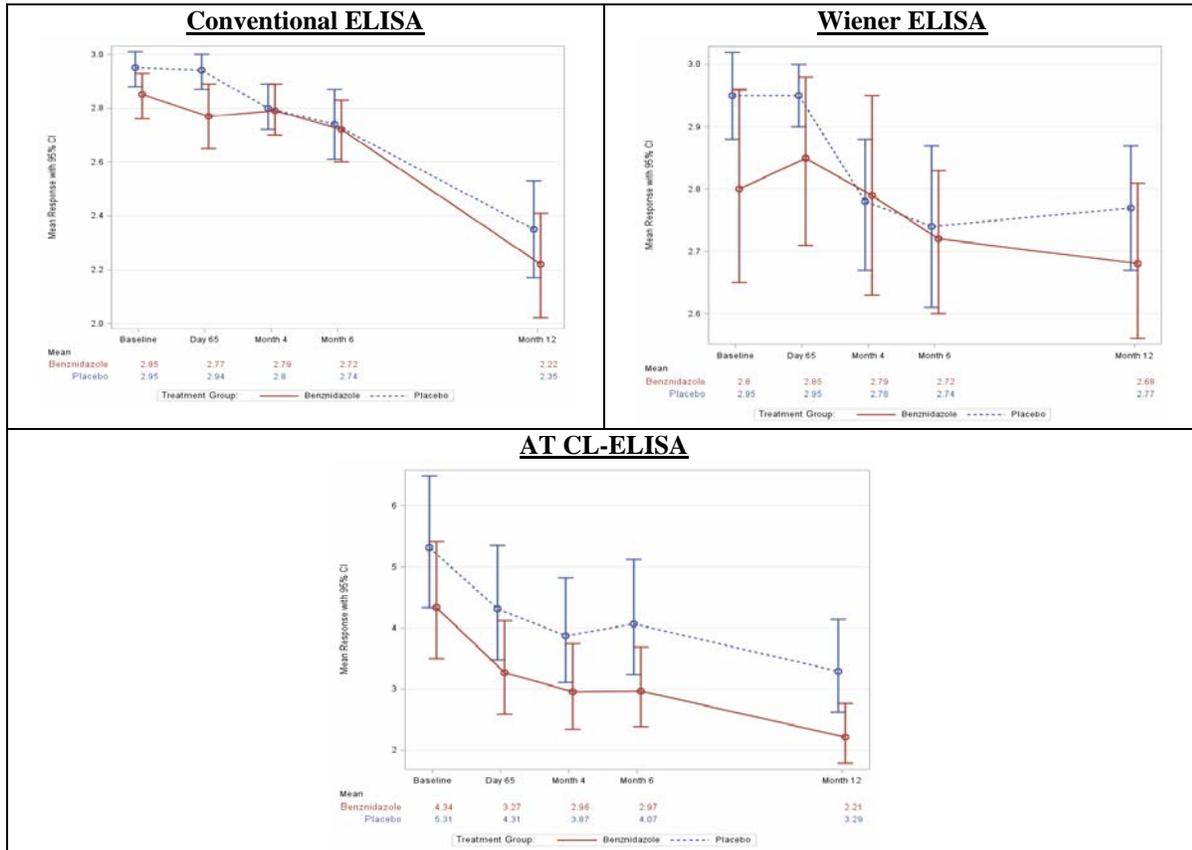
There were no patients, regardless of treatment assigned, who converted to seronegative at Month 12 or at any prior time point in the study based on conventional ELISA or Wiener ELISA. For the non-conventional AT CL-ELISA, the reviewer identified, using cut-off values of <0.9 negative and >1 positive as indicated in the study report for baseline qualitative responses for this assay, a higher percentage of benznidazole patients (11.4%, 5 patients) compared to placebo (4.3%, 2 patients) who converted to seronegative at Month 12. However, the difference 7.1%, 95% CI (-13.8%, 27.0%) was not statistically significant. For the E1224 arms, the reviewer identified 4 patients (8.9%) in the HD E1224 arm, no patient in SD E1224, and 3 patients in the LD E1224 arms who were seronegative at Month 12. No notable differences in conversion to seronegative between benznidazole and placebo or benznidazole compared to each of the E1224 arms was observed at any other time point for the non-conventional AT CL-ELISA.

Reviewer's Comment: *In response to an information request dated February 2017, the Applicant stated that seronegative conversion for the AT CL-ELISA was found in 7 benznidazole patients (15.9%) versus 3 placebo patients (6.5%) at the end of the 1-year study follow-up, which is different from the results described above from the reviewer's analysis. The datasets submitted by the Applicant did not specify the qualitative responses for the AT CL-ELISA. A subsequent information request was sent to the Applicant in May 2017 to clarify these minor numerical differences between the statistical reviewer's findings and those reported in the Applicant's response. A response to the May information request was not received at the time of this review. However, this discrepancy does not change the overall conclusions about these results.*

Figure 7 shows the mean response from serology tests (geometric mean for AT CL-ELISA) for benznidazole and placebo at baseline, Day 65, Month 4, Month 6, and Month 12. The responses for the conventional and Wiener ELISA tests were generally consistent with the exception of Month 12 where lower mean responses for both treatment arms are observed for the conventional ELISA. Following Day 65 (end of treatment), the placebo mean response appears similar to that of benznidazole through Month 12 for the conventional and Wiener ELISAs. For the AT-CL-ELISA, the curves show a difference of about one unit in geometric mean at baseline and the curves are parallel thereafter.

Reviewer's Comment: *The observed similarity in mean serology response for the different ELISAs between the benznidazole and placebo shown in this figure, most notably after Day 65 for the conventional ELISA, is unexpected and difficult to interpret. It is notable that means for PCR, presented earlier in this review, show substantial distinction between benznidazole and placebo. Brief inspection of the mean serology responses for the E1224 arms, not presented in this review, show somewhat similar patterns to what is presented in this figure and as such, do not aid in the interpretation of the serology data. An information request to the Applicant regarding these observations was pending at the time of this statistical review. In light of these strange patterns observed in this figure, the utility of the serology data is questionable.*

Figure 7 Mean Serology Responses over Time in DNDi Study – ITT Population



Geometric mean is presented for AT CL-ELISA and arithmetic mean for conventional and Wiener ELISAs. Patients with missing measurements have not been imputed.

Source: Created by the statistical reviewer using adsl.xpt and adlb1row.xpt

The results for analyses of the mean change from baseline over time for conventional ELISAs and ratio of geometric means for non-conventional AT CL-ELISA are presented in Table 15 for benznidazole and placebo. There are generally no significant differences between benznidazole and placebo for the serology tests; this is not surprising given the similarities observed in the previous figure. There are two instances, one at Day 65 for conventional ELISA and one at Month 12 for AT CL-ELISA, where an apparent statistically significant (or marginally statistically significant) result is observed. For conventional ELISA, the mean change for benznidazole was 0.16 lower than placebo at Day 65, i.e., estimate of -0.16 with 95% CI (-0.30, -0.03). For AT CL-ELISA, a reduction in geometric mean from baseline of 19% for benznidazole compared to placebo was observed at Month 12, i.e. ratio estimate 0.81, 95% CI (0.65, 1.0). It cannot be excluded that these findings were due to chance.

Assessments of correlation resulted in low (<0.3) Pearson correlation coefficient between conventional (or Wiener) ELISA and non-conventional ELISA. The correlation coefficients showed no relationship between PCR and conventional (or Wiener) ELISA (Pearson coefficient ~0.05 in either case) and a slight, but unimpressive relationship between PCR and non-conventional ELISA (Pearson coefficient <0.1). These findings are not unexpected in light of the graphical presentations for PCR and serology discussed previously. These results for the

correlation assessments are not very informative and therefore not presented in further detail in this review.

3.2.2.5 Conclusions for DNDi Study

The findings in the DNDi study show that benznidazole is superior to placebo and the E1224 arms with respect to PCR response at Month 12. Analysis of the sustained PCR response endpoint provides evidence to support this conclusion. Additionally, mean change from baseline in PCR result appears significantly better for benznidazole compared to placebo over the course of the study.

There were no patients treated with benznidazole or placebo who converted to sero-negative using conventional serology from baseline to any later time point measured in the study. Using the AT-CL-ELISA, there were few patients (at a higher frequency for benznidazole compared to placebo) who converted to negative. There appears to be no statistical difference between benznidazole and placebo in mean change from baseline for conventional serology response over the course of the study; this result is not surprising given the similar and unexpected trend in mean response over time between these treatment arms. For AT CL-ELISA, a marginally statistically significant reduction in geometric mean from baseline at Month 12 is observed; but it's possible that this is a chance finding, especially considering the observed difference at baseline that remains constant throughout the study follow-up.

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Table 15 Analyses of Quantitative Serology over Time in DNDi Study – ITT Population

	<u>Conventional ELISA</u>		<u>Wiener ELISA</u>		<u>AT CL-ELISA</u>	
	Benznidazole	Placebo	Benznidazole	Placebo	Benznidazole	Placebo
Baseline*	N=44	N=47	N=43	N=47	N=45	N=47
Mean	2.85	2.95	2.80	2.95	4.34	5.31
(95% CI)	(2.76, 2.94)	(2.88, 3.01)	(2.64, 2.96)	(2.88, 3.02)	(3.49, 5.41)	(4.34, 6.49)
Day 65	N=43	N=46	N=42	N=46	N=44	N=46
Mean	2.77	2.94	2.85	2.95	3.27	4.31
(95% CI)	(2.65, 2.89)	(2.87, 3.00)	(2.71, 2.98)	(2.90, 3.00)	(2.59, 4.12)	(3.47, 5.35)
Difference ¹ (95% CI)	-0.16 (-0.30, -0.03)		-0.04 (-0.16, 0.08)		0.91 (0.76, 1.12)	
p-value	0.02		0.51		0.39	
Month 4	N=42	N=46	N=42	N=46	N=43	N=46
Mean	2.79	2.80	2.79	2.78	2.96	3.87
(95% CI)	(2.70, 2.89)	(2.72, 2.89)	(2.63, 2.95)	(2.67, 2.88)	(2.34, 3.74)	(3.11, 4.82)
Difference ¹ (95% CI)	0.01 (-0.18, 0.14)		0.10 (-0.07, 0.27)		0.91 (0.72, 1.15)	
p-value	0.84		0.25		0.42	
Month 6	N=42	N=46	N=42	N=46	N=43	N=46
Mean	2.72	2.74	2.76	2.80	2.97	4.07
(95% CI)	(2.60, 2.83)	(2.61, 2.87)	(2.63, 2.90)	(2.70, 2.90)	(2.38, 3.69)	(3.24, 5.12)
Difference ¹ (95% CI)	0.003 (-0.17, 0.17)		0.05 (-0.09, 0.19)		0.87 (0.69, 1.09)	
p-value	0.97		0.49		0.23	
Month 12	N=43	N=46	N=41	N=46	N=44	N=46
Mean	2.22	2.35	2.68	2.77	2.21	3.29
(95% CI)	(2.02, 2.41)	(2.17, 2.53)	(2.56, 2.81)	(2.67, 2.87)	(1.78, 2.76)	(2.62, 4.14)
Difference ¹ (95% CI)	-0.09 (-0.35, 0.16)		-0.07 (-0.23, 0.09)		0.81 (0.65, 1.00)	
p-value	0.48		0.40		0.05	

N=randomized patients with non-missing measurement at baseline for respective test

Arithmetic mean presented for ELISA conventional and Wiener ELISA. Geometric mean presented for AT CL-ELISA.

*There are no statistically significant differences at baseline.

¹For each time point, difference in means (ratio of geometric means for PCR and AT CL-ELISA) changes from baseline, confidence interval, and p-value obtained from ANCOVA model with post-treatment response as the dependent variable, treatment as independent variable, site as fixed effect and baseline response as covariate. Patients with missing data excluded from analysis. Analysis uses log transformation of the baseline and post-treatment responses to account for skewed distributions for AT CL-ELISA and PCR. Patients with missing measurements have not been imputed.

Source: Created by the statistical reviewer using adsl.xpt and adlb1row.xpt

3.3 Evaluation of Safety

This section presents descriptive summaries of the percentages of treatment-emergent adverse events (TEAEs) in the Molina and DNDi studies; TEAEs are presented regardless of the investigator’s conclusion about relatedness to treatment. These summaries are provided for the safety analysis population, which is defined as all randomized patients who receive at least 1 dose of study medication in the respective study.

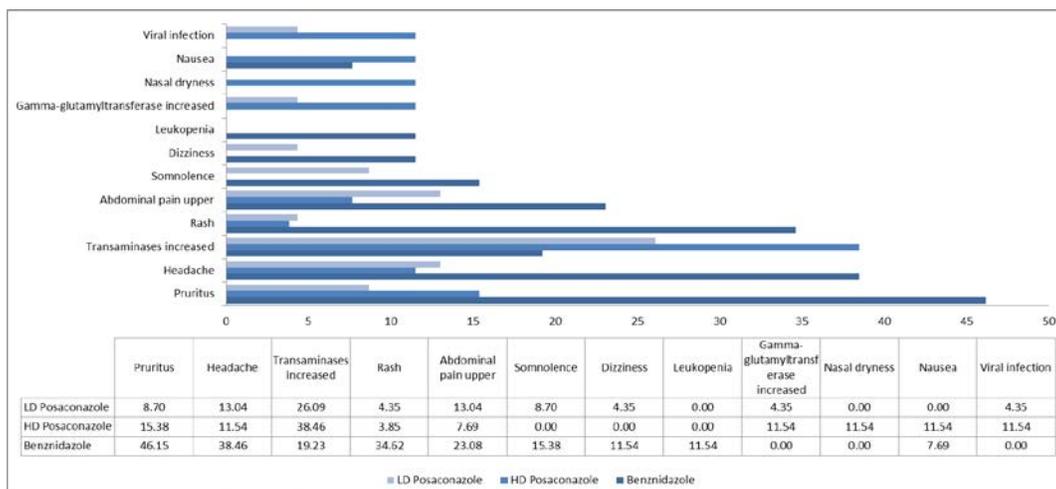
Reviewer’s Comment: *The reviewer was unable to find date of last treatment for patients in the Molina study from the submitted datasets. Also, it appears that date of last treatment was not reported for the benznidazole arm in the DNDi study. Therefore, a comparison of the distributions of treatment exposure cannot be presented in this statistical review.*

3.3.1 Evaluation of Safety in the Molina Study

The safety analysis population in the Molina study comprises 75 patients, 26 benznidazole, 26 HD posaconazole and 23 LD posaconazole. The proportion of patients with at least one TEAE was high across all treatment arms: 88.5% (23 patients) benznidazole, 76.9% (20 patients) HD posaconazole, and 73.9% (17 patients) LD posaconazole. There were no TEAEs reported in the cardiac disorders MedDRA system organ class (SOC) and the number of patients experiencing TEAEs in the gastrointestinal disorders SOC was higher in benznidazole compared to posaconazole treatments: 26.9% (7 patients) benznidazole, 19.2% (5 patients) HD posaconazole, and 17.3% (4 patients) LD posaconazole. Most of these gastrointestinal disorders were abdominal pain and nausea. Defer to clinical expertise for whether the gastrointestinal disorders reported in this study are related to progression of Chagas disease.

The most commonly reported TEAEs, i.e. occurring in at least 10% of patients in either treatment arm, are shown in Figure 8.

Figure 8 Treatment-Emergent Adverse Events in Molina Study – Safety Analysis Population



HD=High-dose, LD=low-dose

Horizontal axis represents percentage of patients experiencing the adverse event. Adverse events were coded using the MedDRA

Source: Created by the statistical reviewer using dataset adae.xpt using the Dictionary Derived Term

For these TEAEs, the proportion of benznidazole patients experiencing the following events was notably higher compared to the posaconazole arms: leukopenia (11.5%), dizziness (11.5%), somnolence (15.4%), abdominal pain upper (23.1%), rash (34.6%), transaminases increased (19.2%), headache (38.5%) and pruritus (46.2%). Leukopenia was reported only in benznidazole patients, which may be consistent with known toxicities with this product.

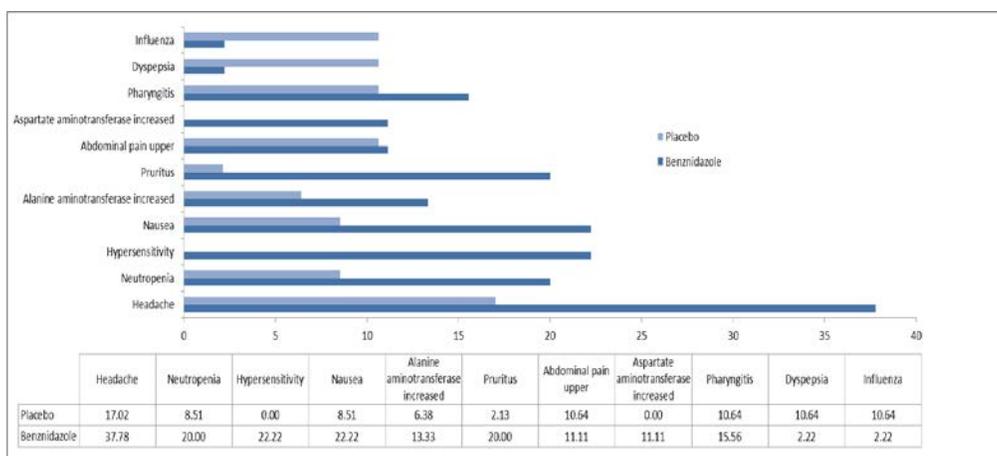
Five benznidazole patients were discontinued from treatment due to treatment-emergent rash, pruritus, or arthralgia. There were no adverse events that led to discontinuation of the posaconazole treatments. One low dose posaconazole patient had a treatment-emergent vertigo that was reported as serious because the patient was unable to work or perform daily activities. No other serious adverse events or deaths were reported in this study.

3.3.2 Evaluation of Safety in the DNDi Study

The safety analysis population for the DNDi study comprises 45 benznidazole patients and 47 placebo patients. There were 39 (86.7%) benznidazole patients and 38 (80.9%) placebo patients who experienced at least one TEAE during this study. One placebo patient (2.1%) and no benznidazole patient had a TEAE classified as a cardiac disorder. The number of patients with TEAEs classified as gastrointestinal disorders was higher in placebo patients compared to benznidazole: 14 patients (31.1%) benznidazole and 17 patients (36.2%) placebo. This finding appears to be driven by the higher percentage of placebo patients experiencing dyspepsia as shown in the figure below. Defer to clinical expertise regarding this finding.

The most commonly reported TEAEs, i.e. occurring in at least 10% of patients in either treatment arm, are shown in Figure 9.

Figure 9 Treatment-Emergent Adverse Events in DNDi Study – Safety Analysis Population



Horizontal axis represents percentage of patients. Adverse events were coded using the MedDRA.
 Source: Created by the statistical reviewer using dataset adae.xpt for DNDi study using the Dictionary Derived Term

For these TEAEs, the proportion of patients experiencing the following events was notably higher in benznidazole compared to placebo: headache (37.8% vs. 17.0%), neutropenia (20% vs. 8.5%), hypersensitivity (22.2% vs. 0%), nausea (22.2% vs. 8.5%), alanine amionotransferase

increase (33.3% vs. 6.4%), pruritus (20% vs. 2.1%), abdominal pain upper (11.1% vs. 10.6%), asparatate aminotransferase increased (11.1% vs. 0%). Three patients were discontinued from benznidazole patients due to events reported as hypersensitivity or drug hypersensitivity. No placebo patients were discontinued from treatment due to adverse events. Two benznidazole patients had serious adverse events and no placebo patients were reported to have serious adverse events.

Reviewer's Comment: No further assessments of safety are provided in this statistical review; defer to clinical review by Dr. Maria Allende for detailed assessment of benznidazole safety from all available data in the NDA.

4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

This section summarizes the results of analyses of PCR response at Month 12 within the specified subgroups in the Molina and DNDi studies; all subgroups are defined based on pre-treatment measurements. These subgroup analyses have not been adjusted for multiple tests and thus are being presented for exploratory purposes only. Further, given the small sample sizes, confidence intervals for subgroups that include the null values of zero should not be interpreted as a lack of effect for benznidazole for that particular subgroup.

4.1 Age, Sex, and Site

Table 16 shows results from subgroup analyses of PCR negative response by age (categorized by the median age in this study), sex, and site in the Molina Study.

Table 16 Negative PCR Response at Month 12 by Age, Sex, and Site in Molina Study – ITT Population

Demographic Characteristic	Benznidazole N=26	HD Posaconazole N=26	LD Posaconazole N=26	Difference ¹ (95% CI)	Difference ² (95% CI)
Overall	16/26 (61.5)	7/26 (26.9)	6/26 (23.1)	34.6 (9.3, 59.9)	38.5 (13.7, 63.2)
Age[*], in years					
23 – 37	5/10 (50.0)	3/13 (23.1)	4/13 (30.8)	26.9 (-15.2, 63.0) [*]	19.2 (-22.9, 56.9) [*]
37 – 62	11/16 (68.8)	4/13 (30.8)	2/13 (15.4)	38.0 (0.3, 68.2) [*]	53.4 (17.4, 79.2) [*]
Sex					
Female	9/15 (60.0)	6/16 (37.5)	5/16 (31.3)	22.5 (-11.8, 56.8)	28.8 (-4.9, 62.4)
Male	7/11 (63.6)	1/10 (10.0)	1/10 (10.0)	53.6 (11.7, 83.5) [*]	53.6 (11.7, 83.5) [*]
Site					
01	12/19 (62.2)	6/19 (31.6)	5/20 (25.0)	31.6 (1.5, 61.7)	38.2 (9.3, 67.0)
02	4/7 (57.1)	1/7 (14.3)	1/6 (16.7)	42.8 (-16.2, 83.2) [*]	40.5 (-16.5, 81.6) [*]

N=number of randomized patients who were PCR positive at baseline, LD=low dose, HD=high dose

¹Difference, expressed as percentage, in negative PCR response between benznidazole and HD posaconazole.

²Difference, expressed as percentage, in negative PCR response between benznidazole and LD posaconazole.

CIs based on normal approximations or by exact method if indicated by an asterisk (*). Subgroup analyses have not been adjusted for multiple comparisons.

*Age categorized by median age of 37 years in the study. In a logistic model whereby age is treated as a continuous predictor, treatment as an independent variable and PCR response at Month 12 as a dependent (outcome) variable, age was not found to be a significant predictor of PCR response. There was no statistically significant treatment by age interaction observed.

Source: Created by the statistical reviewer using adsl.xpt and adlb1row.xpt

As shown in the table, the effect of benznidazole appears to be more substantial in males compared to females and in older patients (i.e. at least 37 years) compared younger patients (i.e. 18 to less than 37). Caution is advised against interpreting these findings as a lack of effect in women or individuals younger than 37 years. The findings within sites appear consistent with the overall ITT population results.

Table 17 presents the findings for subgroup analyses of PCR negative response at Month 12 by age, sex and site in the DNDi study. The findings within these subgroups are generally consistent with the results from the analysis in the entire ITT population.

Table 17 Negative PCR Response by Age, Sex, and Site in DNDi Study– ITT Population

Demographic Characteristic	Benznidazole N=45	Placebo N=47	Difference (95%CI)
Overall	42/45 (93.3)	10/47 (21.3)	72.1 (55.8, 84.5)
Age[*], in years			
18 – 28	18/19 (94.7)	4/21 (19.1)	75.7 (49.8, 91.6)
28 – 49	24/26 (92.3)	6/26 (23.1)	69.2 (43.8, 86.2)
Sex			
Female	30/31 (96.8)	9/37 (24.3)	72.5 (52.4, 86.1)
Male	12/14 (85.7)	1/10 (10.0)	75.7 (38.0, 94.3)
Site			
01	19/22 (86.4)	6/24 (25.0)	61.4 (34.6, 81.1)
02	23/23 (100)	4/23 (17.4)	82.6 (83.4, 95.2)

N=number of randomized patients who were PCR positive at baseline
 Difference, expressed as percentage, in negative PCR rates between benznidazole and placebo; CIs based on exact method. Subgroup analyses have not been adjusted for multiple comparisons.
^{*}Age categorized by median age of 28 years in the study. In a logistic model whereby age is treated as a continuous predictor, treatment as an independent variable and PCR response at Month 12 as a dependent (outcome) variable, age was not found to be a significant predictor of PCR response. There was no statistically significant treatment by age interaction observed.
 Source: Created by the statistical reviewer using adsl.xpt and adlb1row.xpt

4.2 Other Special/Subgroup Populations

No other subgroup analyses are presented in this review.

5 SUMMARY AND CONCLUSIONS

5.1 Statistical Issues

There are some important statistical issues as well as limitations with the Molina and DNDi studies that should be considered when interpreting the findings of this statistical review.

The statistical issues are summarized as follows:

- The data utilized for the analyses presented in the DNDi study report for quantitative serology using conventional ELISA and Wiener ELISA is questionable. These analyses

incorporate results from re-readings at the end of the study of samples that were taken throughout the course of the study. It is unclear why these re-readings were performed and whether these re-readings were performed in a blinded fashion. Also, given that these re-readings were performed in most cases months after the samples had been obtained (e.g. samples taken at baseline or at Day 65) it raises uncertainty about the viability of these samples and consequently the accuracy of test results from re-reading. We note, however, that the conclusions presented in this review are consistent with those presented in the study report. Aside from this issue, the unexpectedly similar patterns in mean responses observed between benznidazole and placebo for the conventional ELISA, Wiener ELISA, and AT CL-ELISA ELISAs is concerning.

- The datasets for both studies did not report complete information about last date of treatment. This limits the ability to compare exposure distributions, i.e. number of days patients exposed, across treatment arms as part of the safety evaluation in this review.
- Missing PCR measurements at various time points after end of treatment, notably in the Molina study, introduces a little uncertainty about the benznidazole response rates.



Regarding the study limitations, the main issues are the use of PCR as an endpoint and the short duration of study follow-up of only one year in both studies. According to Fabbro *et al.*¹⁸, progressive decrease in serologic titers usually takes years or decades until serology becomes negative; this diminution occurs more slowly in adults than in children. In this publication, the median time to seroconversion was approximately 15 years based on non-conventional ELISA (F-29 ELISA, a non-conventional test which is not used in these studies) and approximately 22 years for conventional ELISA. Therefore, it is not surprising that there were no patients in either the Molina or DNDi studies who had seroconversion to negative based on conventional ELISAs and very few patients in the DNDi study with seroconversion to negative based on the non-conventional AT CL-ELISA. Nonetheless, the lack of seroconversion in these studies limits the ability to relate findings from PCR to serology or to assess the relationship between conventional and non-conventional ELISA assays. Further, progression to clinically evident cardiac disease, or gastrointestinal disease or both in patients with indeterminate stage of disease may happen over a

¹⁸ Fabbro, C. *et al.* Evaluation of the ELISA-F29 Test as an Early Marker of Therapeutic Efficacy in Adults with Chronic Chagas Disease. *Rev. Inst. Med. Trop. Sao Paulo.* 55(3): 167-17. 2013.

period of years or decades¹⁹ in patients who initially have chronic indeterminate Chagas disease. As such, the short follow-up in the study limits the ability to assess whether the favorable PCR findings relate to clinical cure of disease from these study data.

Additionally, it is acknowledged that the benznidazole product (i.e. the LAFEPE benznidazole 100 mg tablet) is different from the proposed to-be-marketed product. We defer to clinical pharmacology review for assessment of the different benznidazole products in the studies submitted in support of the NDA.

5.2 Collective Evidence

The results from the Molina and DNDi studies evaluated in this review are very similar in that the early PCR response, i.e., at end of treatment, showed somewhat similar effects (or at least not significantly different effect) between benznidazole and the respective test drug in the studies, and significant effects compared to placebo. This finding is seen with both the PCR response at Month 12 and a sustained PCR response requiring all negative PCR response at all post treatment visits. At the Month 12 time point, both studies showed that the test drugs had a large number of subjects reverting to PCR positive while the benznidazole arm did not. Missing PCR measurements at various time points after end of treatment, notably in the Molina study, introduces a little uncertainty about the benznidazole response rates. However, given there were very few positive PCR results for benznidazole patients (1 in the Molina study and 4 in the DNDi) after one year is encouraging.

There were no patients with seroconversion to negative using conventional ELISA in both studies and only a few patients, with higher proportion in benznidazole compared to placebo, had seroconversion using AT-CL-ELISA; these findings for serology are not unexpected given the short duration of follow-up in these studies.

With respect to safety, gastrointestinal disorders were among the most frequently reported treatment-emergent adverse events observed in these studies. It is uncertain if these events are related to progression of disease. The clinical review will provide a more complete assessment of the safety of the product.

Given the effect on PCR, a less well understood endpoint, these studies are considered as supportive information to the efficacy evaluation for benznidazole. Refer to statistical review by Dr. Felicia Griffin for discussion of the additional studies submitted for review in this NDA.

5.3 Conclusions and Recommendations

The findings from the analyses presented in this statistical review show that benznidazole is superior to the different test arms and placebo in negative PCR at Month 12 and sustained PCR response. We defer to clinical expertise for whether PCR can be utilized as a surrogate of clinical response in this setting and whether or not these studies can be supportive of the efficacy of benznidazole.

¹⁹ Bern C. Antitrypanosomal Therapy for Chronic Chagas' Disease. *N Engl J Med* 2011;364:2527-34.

5.4 Labeling Recommendations

(b) (4)



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/s/

JANELLE K CHARLES
06/07/2017

KAREN M HIGGINS
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I concur.

DIONNE L PRICE
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concur