Clinical trial results:
Prospective, historically controlled study to evaluate the efficacy and safety of a new pediatric formulation of nifurtimox in children aged 0 to 17 years with Chagas' disease

Summary

EudraCT number	2022-001504-17
Trial protocol	Outside EU/EEA
Global end of trial date	10 August 2021

Results information

Result version number	v1 (current)
This version publication date	31 August 2022
First version publication date	31 August 2022

Trial information

Trial identification

Sponsor protocol code	16027

Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT02625974
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	Bayer AG
Sponsor organisation address	Kaiser-Wilhelm-Allee, Leverkusen, Germany, 51368
Public contact	Therapeutic Area Head, Bayer AG, clinical-trials-contact@bayer.com
Scientific contact	Therapeutic Area Head, Bayer AG, clinical-trials-contact@bayer.com

Notes:

Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)	Yes
EMA paediatric investigation plan number(s)	EMEA-003134-PIP01-21
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No
Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	10 August 2021
Is this the analysis of the primary completion data?	No

Global end of trial reached?	Yes
Global end of trial date	10 August 2021
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

The study consists of two parts: Part 1 (CHICO) comprised the treatment with nifurtimox including the 1-year follow up, and Part 2 (CHICO SECURE) comprises additional 3 years of follow-up after end of Part 1.

The primary objective of Part 1 was to assess the superiority of a 60-day regimen of nifurtimox to historical untreated control at the 12-month follow-up (360 days from end of treatment [EOT]) as:
- sero-reduction (defined as a ≥20% reduction in OD compared to baseline in subjects ≥8 months to <18 years of age at randomization; or
- sero-conversion (defined as negative IgG concentration) in all subjects

The primary objective of Part 2 was to assess the incidence of seronegative conversion in subjects who were randomized and received at least one dose of the 60-day nifurtimox treatment regimen in Part 1, compared to an external control group of historical placebo patients with Chagas' disease at the 4-year follow-up.

Protection of trial subjects:

The conduct of this clinical study met all local legal and regulatory requirements. The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and the International Conference on Harmonization guideline E6: Good Clinical Practice. Before entering the study, the informed consent form was read by and explained to all subjects. Participating subjects signed informed consent form and could withdraw from the study at any time without any disadvantage and without having to provide a reason for this decision. Only investigators qualified by training and experience were selected as appropriate experts to investigate the study drug.

Background therapy: -

Evidence for comparator: -

Actual start date of recruitment	27 January 2016
Long term follow-up planned	Yes
Long term follow-up rationale	Safety, Efficacy
Long term follow-up duration	4 Years
Independent data monitoring committee (IDMC) involvement?	Yes

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	Argentina: 178
Country: Number of subjects enrolled	Bolivia, Plurinational State of: 62
Country: Number of subjects enrolled	Colombia: 90
Worldwide total number of subjects	330
EEA total number of subjects	0

Notes:

Subjects enrolled per age group	
In utero	0
Preterm newborn - gestational age < 37 wk	0
Newborns (0-27 days)	7
Infants and toddlers (28 days-23 months)	37
Children (2-11 years)	146
Adolescents (12-17 years)	140
Adults (18-64 years)	0
From 65 to 84 years	0
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

Part 1 of the study was conducted from 27 JAN 2016 (First subject first visit) to 25 JUL 2018 (Last subject last visit) in Argentina, Bolivia and Colombia.

Part 2 of the study was conducted from 26 SEP 2018 (First subject first visit) to 10 AUG 2021 (Last subject last visit) in Argentina, Bolivia and Colombia.

Pre-assignment

Screening details:

Part 1: 330 subjects who were eligible to participate in the study were randomized in a 2:1 ratio to either a 60-Day or 30-Day regimen with nifurtimox tablets. 308 subjects completed treatment in Part 1, and 318 subjects completed Part 1.

Part 2: Of the 318 subjects completed Part 1, 295 subjects were included in Part 2.

Period 1

Period 1 title	Part 1
Is this the baseline period?	Yes
Allocation method	Randomised - controlled
Blinding used	Double blind
Roles blinded	Investigator, Subject

Arms

Are arms mutually exclusive?	Yes
Arm title	Nifurtimox 60 days / Arm 1

Arm description:

Nifurtimox tablets administered three times daily for 60 days (Days 1 – 60, active nifurtimox treatment)

Arm type	Experimental
Investigational medicinal product name	Nifurtimox
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Coated tablet
Routes of administration	Oral use

Dosage and administration details:

Nifurtimox tablets administered 3 times daily for 60 days (Days 1 – 60, active treatment, Treatment Group 1)

Arm title	Nifurtimox 30 days, then Placebo 30 days / Arm 2

Arm description:

Nifurtimox tablets administered three times daily for 30 days, followed by placebo administered three times daily for 30 days (Days 1 – 30, active nifurtimox treatment; Days 31 – 60, placebo)

Arm type	Experimental
Investigational medicinal product name	Nifurtimox
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Coated tablet
Routes of administration	Oral use

Dosage and administration details:

Nifurtimox tablets administered 3 times daily for 30 days (Days 1 – 30, active treatment; Treatment Group 2)

Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	

Pharmaceutical forms	Coated tablet
Routes of administration	Oral use

Dosage and administration details:

Matching placebo administered 3 times daily for 30 days (Days 31 – 60, placebo; Treatment Group 2)

Number of subjects in period 1	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2
Started	219	111
Received treatment	219	111
Completed	210	108
Not completed	9	3
Lost to follow-up	9	3

Period 2

Period 2 title	Part 2
Is this the baseline period?	No
Allocation method	Randomised - controlled
Blinding used	Not blinded

Arms

Are arms mutually exclusive?	Yes
Arm title	Nifurtimox 60 days / Arm 1

Arm description:

Nifurtimox tablets administered three times daily for 60 days (Days 1 – 60, active nifurtimox treatment)

Treatment administered in Part 1; no study drug was administered in Part 2

Arm type	Experimental
Investigational medicinal product name	Nifurtimox
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Coated tablet
Routes of administration	Oral use

Dosage and administration details:

Nifurtimox tablets administered 3 times daily for 60 days (Days 1 – 60, active treatment, Treatment Group 1)

Arm title	Nifurtimox 30 days, then Placebo 30 days / Arm 2

Arm description:

Nifurtimox tablets administered three times daily for 30 days, followed by placebo administered three times daily for 30 days (Days 1 – 30, active nifurtimox treatment; Days 31 – 60, placebo)

Treatment administered in Part 1; no study drug was administered in Part 2

Arm type	Experimental

Investigational medicinal product name	Nifurtimox
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Coated tablet
Routes of administration	Oral use

Dosage and administration details:

Nifurtimox tablets administered 3 times daily for 30 days (Days 1 – 30, active treatment; Treatment Group 2)

Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Coated tablet
Routes of administration	Oral use

Dosage and administration details:

Matching placebo administered 3 times daily for 30 days (Days 31 – 60, placebo; Treatment Group 2)

Number of subjects in period 2[1]	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2
Started	197	98
Completed	191	91
Not completed	6	7
Other, including due to the COVID-19 Pandemic	2	1
Lost to follow-up	4	6

Notes:

[1] - The number of subjects starting the period is not consistent with the number completing the preceding period. It is expected the number of subjects starting the subsequent period will be the same as the number completing the preceding period.
Justification: Of the 318 subjects completed Part 1, 295 subjects were included in Part 2

Baseline characteristics

Reporting groups	
Reporting group title	Nifurtimox 60 days / Arm 1
Reporting group description:	
Nifurtimox tablets administered three times daily for 60 days (Days 1 – 60, active nifurtimox treatment)	
Reporting group title	Nifurtimox 30 days, then Placebo 30 days / Arm 2
Reporting group description:	
Nifurtimox tablets administered three times daily for 30 days, followed by placebo administered three times daily for 30 days (Days 1 – 30, active nifurtimox treatment; Days 31 – 60, placebo)	

Reporting group values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2	Total
Number of subjects	219	111	330
Age Categorical Units: Subjects			
0 to 27 days	4	3	7
28 days to younger than 8 months	8	4	12
8 months to younger than 2 years	17	8	25
2 years to younger than 18 years	190	96	286
Sex: Female, Male Units: Subjects			
Female	119	59	178
Male	100	52	152
Race/Ethnicity, Customized Units: Subjects			
White	155	81	236
American Indian or Alaska native	64	30	94
Ethnicity (NIH/OMB) Units: Subjects			
Hispanic or Latino	217	108	325
Not Hispanic or Latino	2	3	5
Unknown or Not Reported	0	0	0
Total Purified Antigen enzyme-linked immunosorbent assay (ELISA) test results			
Chagas disease diagnosed by direct observation of T. cruzi by concentration test (subjects <8 months of age at randomization) or positive conventional ELISA results for both recombinant ELISA and total purified antigen ELISA (subjects ≥8 months to younger than 18 years of age at randomization) as determined by local laboratory test results were eligible for enrollment.			
Units: Subjects			
Reactive	219	110	329
Non Reactive	0	1	1
Recombinant ELISA test results			
Chagas disease diagnosed by direct observation of T. cruzi by concentration test (subjects <8 months of age at randomization) or positive conventional ELISA results for both recombinant ELISA and total purified antigen ELISA (subjects ≥8 months to younger than 18 years of age at randomization) as determined by local laboratory test results were eligible for enrollment.			
Units: Subjects			
Reactive	219	110	329
Non Reactive	0	1	1

Non conventional ELISA-F29 test results			
The non-conventional ELISA-F29 test is considered an early marker of treatment efficacy in chronic Chagas disease.			
Units: Subjects			
Reactive	142	72	214
Non Reactive	77	39	116
Concentration test for T. cruzi			
Chagas disease diagnosed by direct observation of T. cruzi by concentration test (subjects <8 months of age at randomization).			
Units: Subjects			
Positive	12	7	19
Negative	0	0	0
Missing	207	104	311
Total Purified Antigen ELISA optical density (OD) values			
Units: No dimension			
arithmetic mean	1.474	1.532	
standard deviation	± 0.553	± 0.533	-
Recombinant ELISA OD values			
Units: No dimension			
arithmetic mean	2.735	2.765	
standard deviation	± 0.640	± 0.605	-

End points

End points reporting groups

Reporting group title	Nifurtimox 60 days / Arm 1
Reporting group description:	
Nifurtimox tablets administered three times daily for 60 days (Days 1 – 60, active nifurtimox treatment)	
Reporting group title	Nifurtimox 30 days, then Placebo 30 days / Arm 2
Reporting group description:	
Nifurtimox tablets administered three times daily for 30 days, followed by placebo administered three times daily for 30 days (Days 1 – 30, active nifurtimox treatment; Days 31 – 60, placebo)	
Reporting group title	Nifurtimox 60 days / Arm 1
Reporting group description:	
Nifurtimox tablets administered three times daily for 60 days (Days 1 – 60, active nifurtimox treatment) Treatment administered in Part 1; no study drug was administered in Part 2	
Reporting group title	Nifurtimox 30 days, then Placebo 30 days / Arm 2
Reporting group description:	
Nifurtimox tablets administered three times daily for 30 days, followed by placebo administered three times daily for 30 days (Days 1 – 30, active nifurtimox treatment; Days 31 – 60, placebo) Treatment administered in Part 1; no study drug was administered in Part 2	
Subject analysis set title	Nifurtimox 60 days Reactive Detectable
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
Nifurtimox 60 days with Reactive Conventional Serologic Testing and Detectable qPCR	
Subject analysis set title	Nifurtimox 60 days Reactive Non-detectable
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
Nifurtimox 60 days with Reactive Conventional Serologic Testing and Non-detectable qPCR	
Subject analysis set title	Nifurtimox 60 days Non-reactive Detectable
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
Nifurtimox 60 days with Non-reactive Conventional Serologic Testing and Detectable qPCR	
Subject analysis set title	Nifurtimox 60 days Non-reactive Non-detectable
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
Nifurtimox 60 days with Non-reactive Conventional Serologic Testing and Non-detectable qPCR	
Subject analysis set title	Nifurtimox 60 days Reactive Non evaluable
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
Nifurtimox 60 days with Reactive Conventional Serologic Testing and Non evaluable qPCR	
Subject analysis set title	Nifurtimox 60 days Reactive qPCR Missing
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
Nifurtimox 60 days with Reactive Conventional Serologic Testing and missing qPCR	
Subject analysis set title	Nifurtimox 30 days Reactive Detectable
Subject analysis set type	Sub-group analysis
Subject analysis set description:	
Nifurtimox 30 days with Reactive Conventional Serologic Testing and Detectable qPCR	
Subject analysis set title	Nifurtimox 30 days Reactive Non-detectable
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 30 days with Reactive Conventional Serologic Testing and Non-detectable qPCR

Subject analysis set title	Nifurtimox 30 days Non-reactive Detectable
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 30 days with Non-reactive Conventional Serologic Testing and Detectable qPCR

Subject analysis set title	Nifurtimox 30 days Non-reactive Non-detectable
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 30 days with Non-reactive Conventional Serologic Testing and Non-detectable qPCR

Subject analysis set title	Nifurtimox 30 days Reactive Non evaluable
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 30 days with Reactive Conventional Serologic Testing and Non evaluable qPCR

Subject analysis set title	Nifurtimox 30 days Missing Conventional testing Non-detectable
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 30 days with missing Conventional Serologic Testing and Non-detectable qPCR

Subject analysis set title	Nifurtimox 60 days Reactive and Reactive
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 60 days with Reactive Conventional Serologic Testing and Reactive Non-conventional Serologic Testing

Subject analysis set title	Nifurtimox 60 days Reactive and Non-reactive
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 60 days with Reactive Conventional Serologic Testing and Non-reactive Non-conventional Serologic Testing

Subject analysis set title	Nifurtimox 60 days Non-reactive and Reactive
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 60 days with Non-reactive Conventional Serologic Testing and Reactive Non-conventional Serologic Testing

Subject analysis set title	Nifurtimox 60 days Non-reactive and Non-reactive
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 60 days with Non-reactive Conventional Serologic Testing and Non-reactive Non-conventional Serologic Testing

Subject analysis set title	Nifurtimox 30 days Reactive and Reactive
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 30 days with Reactive Conventional Serologic Testing and Reactive Non-conventional Serologic Testing

Subject analysis set title	Nifurtimox 30 days Reactive and Non-reactive
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 30 days with Reactive Conventional Serologic Testing and Non-reactive Non-conventional Serologic Testing

Subject analysis set title	Nifurtimox 30 days Non-reactive and Reactive
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 30 days with Non-reactive Conventional Serologic Testing and Reactive Non-conventional Serologic Testing

Subject analysis set title	Nifurtimox 30 days Non-reactive and Non-reactive
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Nifurtimox 30 days with Non-reactive Conventional Serologic Testing and Non-reactive Non-conventional Serologic Testing

Subject analysis set title	Non-reactive ELISA and Non-reactive IHA
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: Non-reactive
IHA results: Non-reactive

Subject analysis set title	Non-reactive ELISA and reactive IHA decrease
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: Non-reactive
IHA results: reactive with decreasing in titers

Subject analysis set title	Non-react ELISA and react IHA Nochange
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: Non-reactive
IHA results: reactive without change in titers

Subject analysis set title	Reactive ELISA: Sero-reduction and Non-react IHA
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: reactive, Sero-reduction
IHA results: Non-reactive

Subject analysis set title	Reactive ELISA: Sero reduction and reactive IHA decrease
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: reactive, Sero-reduction
IHA results: reactive with decreasing in titers

Subject analysis set title	Reactive ELISA: Sero-reduction and reactive IHA Nochange
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: reactive, Sero-reduction
IHA results: reactive without Change in titers

Subject analysis set title	Reactive ELISA: Others and Non-reactive IHA
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: reactive, Others
IHA results: Non-reactive

Subject analysis set title	Reactive ELISA: Others and react IHA decrease
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: reactive, Other
IHA: reactive with decreasing in titers

Subject analysis set title	Reactive ELISA: Others and react IHA Nochange
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: reactive, Other
IHA results: reactive without Change in titers

Subject analysis set title	Reactive ELISA: Others and Missing IHA
Subject analysis set type	Sub-group analysis

Subject analysis set description:

ELISA results: reactive, Others
IHA results: Missing

Subject analysis set title	Cure and Non reactive/reactive decreasing
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Elisa results: Cure
IHA results: Non reactive or reactive but decreasing in titer

Subject analysis set title	Cure and reactive non-decreasing
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Elisa results: Cure
IHA results: reactive but non-decreasing in titer

Subject analysis set title	No Cure and Non reactive/reactive decreasing
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Elisa results: No Cure
IHA results: Non reactive or reactive but decreasing in titer

Subject analysis set title	No Cure and reactive non-decreasing
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Elisa results: No Cure
IHA results: reactive but non-decreasing in titer

Subject analysis set title	No Cure and Missing IHA Testing
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Elisa results: No Cure
IHA results: Missing

Subject analysis set title	Nifurtimox 60 days: Chagas-related cardiomyopathy = Yes
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Serological response measured by two types of assays and ECG evaluation for signs of established Chagas-related cardiomyopathy by visit

Subject analysis set title	Nifurtimox 60 days: Chagas-related cardiomyopathy = No
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Serological response measured by two types of assays and ECG evaluation for signs of established Chagas-related cardiomyopathy by visit

Subject analysis set title	Nifurtimox 60 days: Chagas-related cardiomyopathy = Missing
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Serological response measured by two types of assays and ECG evaluation for signs of established Chagas-related cardiomyopathy by visit

Subject analysis set title	Nifurtimox 30 days: Chagas-related cardiomyopathy = Yes
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Serological response measured by two types of assays and ECG evaluation for signs of established Chagas-related cardiomyopathy by visit

Subject analysis set title	Nifurtimox 30 days: Chagas-related cardiomyopathy = No
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Serological response measured by two types of assays and ECG evaluation for signs of established Chagas-related cardiomyopathy by visit

Subject analysis set title	Nifurtimox 30 days: Chagas-related cardiomyopathy = Missing
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Serological response measured by two types of assays and ECG evaluation for signs of established Chagas-related cardiomyopathy by visit

Subject analysis set title	Historical Benznidazole 1998 - Sosa Estani et al. (1998)
Subject analysis set type	Sub-group analysis

Subject analysis set description:

istorical Benznidazole 1998, publication from Sosa Estani et al. (1998), 4-year follow-up data.

Subject analysis set title	Historical Benznidazole 1996, Andrade et al. (1996)
Subject analysis set type	Sub-group analysis

Subject analysis set description:

Historical Benznidazole 1996, publication from de Andrade et al. (1996), 3-year follow-up data.

Primary: Part 1 - Percentage of sero-reduction or sero-conversion (cured subjects)

End point title	Part 1 - Percentage of sero-reduction or sero-conversion (cured subjects)

End point description:

Cure is defined as sero-reduction (in subjects ≥8 months to <18 years of age at randomization) or sero-conversion (in all subjects). Sero-reduction is defined as a ≥20% reduction in optical density [OD]) measured by two conventional ELISA serology tests and sero-conversion is defined as negative Immunoglobulin G (IgG) concentration measured by two conventional ELISA serology tests.
Subjects who have missing conventional serology results at the 12 month time point were treated as failures (ie, no cure).

For the primary objective in the study, superiority over placebo was confirmed if the lower limit of the 95% Confidence Interval (CI) for the nifurtimox (60-day regimen) cure rate is greater than 16%, the larger of the upper limits of the 95% CIs for historical placebo control.

End point type	Primary

End point timeframe:

At 12 months post-treatment

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Percentage of subjects				
number (confidence interval 95%)	32.9 (26.4 to 39.3)	18.9 (11.2 to 26.7)		

Statistical analyses

Statistical analysis title	Historical Cure Rates

Statistical analysis description:

Historical Cure Rates for Placebo Publication 1: De Andrade et al 1996. Age range (years): 7-12. Sero-conversion rate (95% CI) in placebo patients: 3/65 = 5% (1%, 13%) Publication 2: Sosa et al 1998. Age range (years): 6-12. Sero-conversion rate (95% CI) in placebo patients: 2/44 = 5% (1%, 16%). CI = confidence interval

Comparison groups	Nifurtimox 60 days / Arm 1 v Nifurtimox 30 days, then Placebo 30 days / Arm 2

Number of subjects included in analysis	330
Analysis specification	Pre-specified
Analysis type	
Parameter estimate	Difference in cure rate
Point estimate	14
Confidence interval	
level	95 %
sides	2-sided
lower limit	3.7
upper limit	24.2

Primary: Part 2 - Incidence rate of seronegative conversion in subjects received at least one dose of the 60-day nifurtimox treatment regimen.

End point title	Part 2 - Incidence rate of seronegative conversion in subjects received at least one dose of the 60-day nifurtimox treatment regimen.[1]

End point description:

Seronegative conversion measured by two types of assay (recombinant ELISA and indirect hemagglutination assay [IHA]) in subjects who were randomized and received at least one dose of the 60-day nifurtimox treatment regimen compared to an external control group of historical placebo patients with Chagas' disease.
Incidence rate is the number of new cases of seronegative conversion over the study period (i.e., 4 years after end of nifurtimox treatment) divided by the person-time at risk. It was modelled using a Poisson distribution with a 2-sided 95% exact CI.

End point type	Primary

End point timeframe:

4 years after end of treatment

Notes:

[1] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: Descriptive statistics only

End point values	Nifurtimox 60 days / Arm 1			
Subject group type	Reporting group			
Number of subjects analysed	197			
Units: Incidence rate				
number (confidence interval 95%)	2.12 (1.21 to 3.45)			

Statistical analyses

No statistical analyses for this end point

Secondary: Part 2 - Incidence rate of seronegative conversion in subjects who received at least one dose of the 30-day nifurtimox treatment regimen

End point title	Part 2 - Incidence rate of seronegative conversion in subjects who received at least one dose of the 30-day nifurtimox treatment regimen

End point description:

Seronegative conversion measured by two types of assay (recombinant ELISA and indirect

hemagglutination assay [IHA]) in subjects who were randomized and received at least one dose of the 30-day nifurtimox treatment regimen.
Incidence rate is the number of new cases of seronegative conversion over the study period (i.e., 4 years after end of nifurtimox treatment) divided by the person-time at risk. It was modelled using a Poisson distribution with a 2-sided 95% exact CI.

End point type	Secondary

End point timeframe:

4 years after end of treatment

End point values	Nifurtimox 30 days, then Placebo 30 days / Arm 2			
Subject group type	Reporting group			
Number of subjects analysed	98			
Units: Incidence rate				
number (confidence interval 95%)	2.11 (0.91 to 4.16)			

Statistical analyses

No statistical analyses for this end point

Secondary: Part 2 - Serological response and ECG signs of established Chagas-related cardiomyopathy

End point title	Part 2 - Serological response and ECG signs of established Chagas-related cardiomyopathy

End point description:

Summary of subjects by serological response and evidence of established Chagas-related cardiomyopathy as measured by electrocardiogram (ECG).

Evidence of established Chagas-related cardiomyopathy: Total

End point type	Secondary

End point timeframe:

4 years after end of treatment

End point values	Nifurtimox 60 days: Chagas-related cardiomyopathy = Yes	Nifurtimox 60 days: Chagas-related cardiomyopathy = No	Nifurtimox 60 days: Chagas-related cardiomyopathy = Missing	Nifurtimox 30 days: Chagas-related cardiomyopathy = Yes
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	197	197	197	98
Units: Subjects				
number (not applicable)				
Year 2: Non Reactive	0	10	0	0
Year 2: Reactive	0	177	1	0
Year 2: Missing	0	0	9	0

Year 3: Non Reactive	0	12	0	0
Year 3: Reactive	0	163	0	0
Year 3: Missing	0	0	22	0
Year 4: Non-Reactive	0	14	0	0
Year 4: Reactive	0	174	2	0
Year 4: Missing	0	1	6	0

End point values	Nifurtimox 30 days: Chagas-related cardiomyopathy = No	Nifurtimox 30 days: Chagas-related cardiomyopathy = Missing		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	98	98		
Units: Subjects				
number (not applicable)				
Year 2: Non Reactive	3	1		
Year 2: Reactive	87	0		
Year 2: Missing	1	6		
Year 3: Non Reactive	5	1		
Year 3: Reactive	79	0		
Year 3: Missing	0	13		
Year 4: Non-Reactive	6	0		
Year 4: Reactive	83	1		
Year 4: Missing	1	7		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 + Part 2 - Serial reduction of optical density values measured by Total Purified Antigen ELISA

End point title	Part 1 + Part 2 - Serial reduction of optical density values measured by Total Purified Antigen ELISA

End point description:

Summary and change from baseline of optical density values measured by total purified antigen ELISA.

End point type	Secondary

End point timeframe:

Baseline and 4 years after end of treatment

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	197	98		
Units: Optical density				
arithmetic mean (standard deviation)				
Part 1 - Baseline (Visit 1)	1.47 (± 0.55)	1.52 (± 0.55)		
Part 1 - Up to 420 days post-treatment (Visit 11)	1.24 (± 0.62)	1.30 (± 0.61)		
Part 1 - Change from Baseline (Visit 11)	-0.24 (± 0.29)	-0.23 (± 0.41)		
Part 2 - Year 2: (FU Visit 1)	1.23 (± 0.59)	1.29 (± 0.61)		
Part 2 - Year 2: Change from Baseline (FU Visit 1)	-0.25 (± 0.41)	-0.23 (± 0.49)		
Part 2 - Year 3 (FU Visit 3)	1.16 (± 0.58)	1.23 (± 0.58)		
Part 2 - Year 3: Change from Baseline (FU Visit 3)	-0.30 (± 0.42)	-0.30 (± 0.50)		
Part 2 - Year 4 (FU Visit 5)	1.12 (± 0.57)	1.23 (± 0.59)		
Part 2 - Year 4: Change from Baseline (FU Visit 5)	-0.35 (± 0.40)	-0.30 (± 0.46)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 + Part 2 - Serial reduction of optical density values measured by Recombinant ELISA

End point title	Part 1 + Part 2 - Serial reduction of optical density values measured by Recombinant ELISA

End point description:

Summary and change from baseline of optical density values measured by recombinant ELISA.

End point type	Secondary

End point timeframe:

Baseline and 4 years after end of treatment

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	197	98		
Units: Optical density				
arithmetic mean (standard deviation)				
Part 1 - Baseline (Visit 1)	2.74 (± 0.63)	2.73 (± 0.63)		
Part 1 - Up to 420 days post-treatment (Visit 11)	2.27 (± 0.98)	2.31 (± 1.00)		
Part 1 - Change from Baseline (Visit 11)	-0.47 (± 0.74)	-0.41 (± 0.79)		
Part 2 - Year 2: (FU Visit 1)	2.5 (± 0.95)	2.57 (± 0.88)		

Part 2 - Year 2: Change from Baseline (FU Visit 1)	-0.23 (± 0.87)	-0.17 (± 0.90)		
Part 2 - Year 3 (FU Visit 3)	2.17 (± 0.98)	2.25 (± 0.91)		
Part 2 - Year 3: Change from Baseline (FU Visit 3)	-0.56 (± 0.86)	-0.45 (± 0.87)		
Part 2 - Year 4 (FU Visit 5)	2.09 (± 1.01)	2.22 (± 1.00)		
Part 2 - Year 4: Change from Baseline (FU Visit 5)	-0.64 (± 0.89)	-0.48 (± 0.87)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 1

End point title	Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 1

End point description:

The evaluation was based on clinical examinations.

End point type	Secondary

End point timeframe:

At Visit 1 (before treatment started)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
Known ECG abnormality	2	0		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 3

End point title	Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 3

End point description:

The evaluation was based on clinical examinations.

End point type	Secondary

End point timeframe:

Up to 7 days (Visit 3)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	110		
Units: Subjects				
Anemia	1	0		
Chagas disease	2	0		
Hepatomegaly	1	0		
Known ECG abnormality	4	0		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 6

End point title	Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 6

End point description:

The evaluation was based on clinical examinations.

End point type	Secondary

End point timeframe:

Up to 30 days (Visit 6)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	215	110		
Units: Subjects				
Known ECG abnormality	1	0		
Romana sign	0	1		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 8

End point title	Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 8

End point description:

The evaluation was based on clinical examinations.

End point type	Secondary

End point timeframe:

Up to 60 days (Visit 8; end of treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	217	109		
Units: Subjects				
Anemia	1	0		
Chagas disease	1	0		
Known ECG abnormality	1	0		
Lymphadenopathy	1	0		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 9

End point title	Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 9

End point description:

The evaluation was based on clinical examinations.

End point type	Secondary

End point timeframe:

Up to 90 days (Visit 9 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	213	107		
Units: Subjects				
Known ECG abnormality	1	0		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 10

End point title	Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 10

End point description:

The evaluation was based on clinical examinations.

End point type	Secondary

End point timeframe:

Up to 240 days (Visit 10 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	212	109		
Units: Subjects				
Known ECG abnormality	1	0		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 11

End point title	Part 1 - Number of subjects with Clinical signs/ symptoms of Chagas disease at Visit 11

End point description:

The evaluation was based on clinical examinations.

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	210	108		
Units: Subjects				
Known ECG abnormality	3	3		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with positive results in concentration test for T. cruzi (for subjects <8 months of age)

End point title	Part 1 - Number of subjects with positive results in concentration test for T. cruzi (for subjects <8 months of age)

End point description:

End point type	Secondary

End point timeframe:

Up to 90 days (Visit 9 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	12	7		
Units: Subjects				
Visit 1|Positive	12	7		
Visit 3|Positive	1	1		
Visit 6|Positive	0	0		
Visit 8|Positive	0	0		
Visit 9|Positive	1	0		
Visit 1|Negative	0	0		
Visit 3|Negative	11	5		
Visit 6|Negative	12	7		
Visit 8|Negative	12	7		
Visit 9|Negative	11	7		
Visit 1|Missing	0	0		
Visit 3|Missing	0	1		
Visit 6|Missing	0	0		
Visit 8|Missing	0	0		
Visit 9|Missing	0	0		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with a positive serological response using non-conventional enzyme-linked immunosorbent assay-F29 (ELISAF29) test

End point title	Part 1 - Number of subjects with a positive serological response using non-conventional enzyme-linked immunosorbent assay-F29 (ELISAF29) test

End point description:

The non-conventional ELISA-F29 test is considered an early marker of treatment efficacy in chronic Chagas disease.

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
Visit 1|Reactive	142	72		
Visit 3|Reactive	133	64		
Visit 6|Reactive	131	67		
Visit 8|Reactive	124	63		
Visit 10|Reactive	110	61		
Visit 11|Reactive	96	54		
Visit 1|Non-reactive	77	39		
Visit 3|Non-reactive	85	44		
Visit 6|Non-reactive	83	43		
Visit 8|Non-reactive	91	44		
Visit 10|Non-reactive	101	48		
Visit 11|Non-reactive	114	54		
Visit 1|Missing	0	0		
Visit 3|Missing	1	3		
Visit 6|Missing	5	1		
Visit 8|Missing	4	4		
Visit 10|Missing	8	2		
Visit 11|Missing	9	3		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with positive quantitative polymerase chain reaction (qPCR) results

End point title	Part 1 - Number of subjects with positive quantitative polymerase chain reaction (qPCR) results

End point description:

The qPCR is molecular technique, considered a tool to diagnose acute and congenital Chagas disease, as well as a marker to measure treatment failure when demonstrating positive (detectable) results

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
Visit 1|Non-detectable	99	53		
Visit 3|Non-detectable	171	86		
Visit 6|Non-detectable	207	105		
Visit 8|Non-detectable	210	105		
Visit 10|Non-detectable	206	105		
Visit 11|Non-detectable	205	102		
Visit 1|Detectable	117	57		
Visit 3|Detectable	46	21		
Visit 6|Detectable	4	3		
Visit 8|Detectable	3	1		
Visit 10|Detectable	3	2		
Visit 11|Detectable	3	5		
Visit 1|Non-evaluable	1	1		
Visit 3|Non-evaluable	0	1		
Visit 6|Non-evaluable	3	2		
Visit 8|Non-evaluable	2	2		
Visit 10|Non-evaluable	2	2		
Visit 11|Non-evaluable	1	1		
Visit 1|Missing	2	0		
Visit 3|Missing	2	3		
Visit 6|Missing	5	1		
Visit 8|Missing	4	3		
Visit 10|Missing	8	2		
Visit 11|Missing	10	3		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of Subjects With Treatment-emergent Adverse Events (TEAEs) and Treatment-emergent Serious Adverse Events (TESAEs)

End point title	Part 1 - Number of Subjects With Treatment-emergent Adverse Events (TEAEs) and Treatment-emergent Serious Adverse Events (TESAEs)

End point description:

TEAEs comprised events which first occurred or worsened at or after first application of study drug during the course of the study up to and including 7 days after last application of study drug

End point type	Secondary

End point timeframe:

up to 7 days after last application of study drug

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
Any treatment-emergent adverse event (TEAE)	147	66		
Any Serious TEAE	6	3		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 2 - Number of Subjects With Treatment-emergent Adverse Events (TEAEs) and Treatment-emergent Serious Adverse Events (TESAEs)

End point title	Part 2 - Number of Subjects With Treatment-emergent Adverse Events (TEAEs) and Treatment-emergent Serious Adverse Events (TESAEs)

End point description:

TEAEs comprised events which first occurred or worsened at study start up to end of study in part 2.

In Part 2, only AEs considered at least possibly related to nifurtimox (administered in part 1) and those caused by protocol-related procedures were reported.

End point type	Secondary

End point timeframe:

up to 3 years after start of study part 2

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	197	98		
Units: Subjects				
Any AE	0	0		
Any study drug-related AE	0	0		
Any AE related to protocol	0	0		
Any SAE	0	0		
Any study drug-related SAE	0	0		
Any SAE related to protocol	0	0		
AE with outcome death	0	0		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with treatment-emergent high blood chemistry abnormalities by treatment

End point title	Part 1 - Number of subjects with treatment-emergent high blood chemistry abnormalities by treatment

End point description:

The Number Analyzed represents the number of subjects at baseline with a normal or lower than normal laboratory assessment who also had at least one valid laboratory value after start of treatment.
The number of subjects represents subjects with at least one high laboratory assessment after start of treatment who had a normal or lower than normal laboratory assessment at baseline.

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
Creatinine (mg/dL)	3	2		
Albumin (g/dL)	49	21		
Alkaline Phosphatase (U/L)	11	3		
Alanine Aminotransferase (U/L)	19	7		
Aspartate Aminotransferase (U/L)	27	7		
Bilirubin (mg/dL)	19	9		
Direct Bilirubin (mg/dL)	21	9		
Blood Urea Nitrogen (mg/dL)	11	3		
Urate (mg/dL)	7	1		
Glucose (mg/dL)	15	13		
Protein (g/dL)	42	14		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with treatment-emergent low blood chemistry abnormalities by treatment

End point title	Part 1 - Number of subjects with treatment-emergent low blood chemistry abnormalities by treatment

End point description:

The number analyzed represents the number of subjects at baseline with a normal or higher than normal laboratory assessment who also had at least one valid laboratory value after start of treatment. The number of subjects represents subjects with at least one low laboratory assessment after start of treatment who had a normal or higher than normal laboratory assessment at baseline.

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
Creatinine (mg/dL)	22	10		
Albumin (g/dL)	14	4		
Alkaline Phosphatase (U/L)	4	4		
Alanine Aminotransferase (U/L)	6	3		
Aspartate Aminotransferase (U/L)	1	5		
Bilirubin (mg/dL)	7	3		
Direct Bilirubin (mg/dL)	0	0		
Blood Urea Nitrogen (mg/dL)	31	15		
Urate (mg/dL)	32	18		
Glucose (mg/dL)	29	18		
Protein (g/dL)	17	15		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with treatment-emergent high hematology abnormalities by treatment

End point title	Part 1 - Number of subjects with treatment-emergent high hematology abnormalities by treatment

End point description:

The number analyzed represents the number of subjects at baseline with a normal or lower than normal laboratory assessment who also had at least one valid laboratory value after start of treatment. Subjects with missing or high abnormal values at baseline are not included in the number analyzed. The number of subjects represents subjects with at least one high laboratory assessment after start of treatment who had a normal or lower than normal laboratory assessment at baseline.

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
Hematocrit (%)	19	8		
Hemoglobin (g/dL)	15	3		
Erythrocytes (T/L) in Blood	21	9		
Ery. Mean Corpuscular Volume (fL) in Blood	20	7		
Ery. Mean Corpuscular Hemoglobin (pg) in Blood	14	5		
Ery. Mean Corpuscular HGB Conc. (g/dL) in Blood	23	6		
Leukocytes (GIGA/L) in Blood	41	16		
Neutrophils/Leukocytes (%)	38	12		
Neutrophils (GIGA/L)	36	8		
Lymphocytes/Leukocytes (%)	35	17		
Lymphocytes (GIGA/L)	21	7		
Monocytes/Leukocytes (%)	33	17		
Monocytes (GIGA/L)	17	15		
Eosinophils/Leukocytes (%)	58	26		
Eosinophils (GIGA/L)	53	22		
Basophils/Leukocytes (%)	20	7		
Basophils (GIGA/L)	10	2		
Platelets (GIGA/L) in Blood	27	13		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with treatment-emergent low hematology abnormalities by treatment

End point title	Part 1 - Number of subjects with treatment-emergent low hematology abnormalities by treatment

End point description:

The number analyzed represents the number of subjects at baseline with a normal or higher than normal laboratory assessment who also had at least one valid laboratory value after start of treatment. The number of subjects represents subjects with at least one low laboratory assessment after start of treatment who had a normal or higher than normal laboratory assessment at baseline.

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Participants				
Hematocrit (%)	30	17		
Hemoglobin (g/dL)	23	17		
Erythrocytes (T/L) in Blood	15	9		
Ery. Mean Corpuscular Volume (fL) in Blood	16	6		
Ery. Mean Corpuscular Hemoglobin (pg) in Blood	19	10		
Ery. Mean Corpuscular HGB Conc. (g/dL) in Blood	29	21		
Leukocytes (GIGA/L) in Blood	39	18		
Neutrophils/Leukocytes (%)	53	27		
Neutrophils (GIGA/L)	46	25		
Lymphocytes/Leukocytes (%)	40	9		
Lymphocytes (GIGA/L)	5	0		
Monocytes/Leukocytes (%)	54	28		
Monocytes (GIGA/L)	33	22		
Eosinophils/Leukocytes (%)	13	5		
Eosinophils (GIGA/L)	14	5		
Basophils/Leukocytes (%)	1	2		
Basophils (GIGA/L)	1	2		
Platelets (GIGA/L) in Blood	6	3		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with treatment-emergent high coagulation abnormalities by treatment

End point title	Part 1 - Number of subjects with treatment-emergent high coagulation abnormalities by treatment

End point description:

The Number Analyzed represents the number of subjects at baseline with a normal or lower than normal laboratory assessment who also had at least one valid laboratory value after start of treatment.
The number of subjects represents subjects with at least one high laboratory assessment after start of treatment who had a normal or lower than normal laboratory assessment at baseline.

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
Prothrombin Time (sec) in Plasma	38	23		
Activated Partial Thromboplastin	9	10		
Time in Plasma Prothrombin Intl. Normalized Ratio	21	13		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with treatment-emergent low coagulation abnormalities by treatment

End point title	Part 1 - Number of subjects with treatment-emergent low coagulation abnormalities by treatment

End point description:

The number analyzed represents the number of subjects at baseline with a normal or higher than normal laboratory assessment who also had at least one valid laboratory value after start of treatment. The number of subjects represents subjects with at least one low laboratory assessment after start of treatment who had a normal or higher than normal laboratory assessment at baseline.

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Participants				
Prothrombin Time (sec) in Plasma	10	5		
Activated Partial Thromboplastin	15	2		
Time in Plasma Prothrombin Intl. Normalized Ratio	14	5		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with abnormal Urinalysis findings considered as clinically significant or reported as Adverse Events (AEs)

End point title	Part 1 - Number of subjects with abnormal Urinalysis findings

	considered as clinically significant or reported as Adverse Events (AEs)

End point description:

Urinalysis was performed and the following parameters evaluated: bilirubin, blood (red blood cells, white blood cells), chorionic gonadotropin β, glucose, ketones, leukocytes, nitrite, pH, protein, specific gravity, and urobilinogen.

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
abnormal Urinalysis findings	0	0		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Number of subjects with abnormal ECG findings considered as clinically significant by investigators

End point title	Part 1 - Number of subjects with abnormal ECG findings considered as clinically significant by investigators

End point description:

Clinical significance of abnormal ECG was based on the judgement of the investigator

End point type	Secondary

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: Subjects				
abnormal ECG findings	0	0		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Mean changes in vital signs (Systolic Blood Pressure) between the treatment groups from baseline

End point title	Part 1 - Mean changes in vital signs (Systolic Blood Pressure) between the treatment groups from baseline
End point description: Systolic Blood Pressure	
End point type	Secondary
End point timeframe: Baseline and up to 420 days (Visit 11 post-treatment)	

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: mmHg				
arithmetic mean (standard deviation)				
VISIT 1	1.09 (± 7.79)	0.69 (± 7.85)		
VISIT 2	-3.75 (± 7.50)	-5.00 (± 7.07)		
VISIT 3	1.08 (± 9.98)	-0.50 (± 8.45)		
VISIT 6	-0.74 (± 10.70)	-1.12 (± 9.89)		
VISIT 8	-0.41 (± 11.01)	0.48 (± 11.24)		
VISIT 9	-0.13 (± 11.11)	-0.45 (± 10.49)		
VISIT 10	-0.02 (± 10.46)	-1.48 (± 10.95)		
VISIT 11	1.20 (± 11.52)	1.57 (± 10.70)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Mean changes in vital signs (Diastolic Blood Pressure) between the treatment groups from baseline

End point title	Part 1 - Mean changes in vital signs (Diastolic Blood Pressure) between the treatment groups from baseline
End point description: Diastolic Blood Pressure	
End point type	Secondary
End point timeframe: Baseline and up to 420 days (Visit 11 post-treatment)	

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: mmHg				
arithmetic mean (standard deviation)				
VISIT 1	1.00 (± 7.72)	0.90 (± 7.90)		
VISIT 2	-1.25 (± 2.50)	-1.00 (± 2.00)		
VISIT 3	0.76 (± 8.36)	0.26 (± 8.05)		
VISIT 6	0.26 (± 10.87)	-0.14 (± 10.21)		
VISIT 8	0.08 (± 9.91)	0.93 (± 10.17)		
VISIT 9	-0.68 (± 8.81)	-0.21 (± 10.03)		
VISIT 10	0.99 (± 9.90)	0.85 (± 9.53)		
VISIT 11	2.30 (± 11.06)	3.25 (± 10.95)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Mean changes in vital signs (Respiratory Rate) between the treatment groups from baseline

End point title	Part 1 - Mean changes in vital signs (Respiratory Rate) between the treatment groups from baseline
End point description:	
Respiratory Rate	
End point type	Secondary
End point timeframe:	
Baseline and up to 420 days (Visit 11 post-treatment)	

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: BREATHS/MIN				
arithmetic mean (standard deviation)				
VISIT 1	0.12 (± 4.10)	0.47 (± 2.91)		
VISIT 2	-1.50 (± 3.42)	-1.00 (± 1.15)		
VISIT 3	-0.05 (± 3.15)	-0.14 (± 3.91)		
VISIT 6	-0.57 (± 4.16)	-0.06 (± 3.87)		

VISIT 8	-0.77 (± 4.04)	0.04 (± 4.58)		
VISIT 9	-1.01 (± 4.06)	-0.50 (± 3.76)		
VISIT 10	-1.36 (± 4.50)	-0.24 (± 3.60)		
VISIT 11	-1.93 (± 5.10)	-0.74 (± 4.61)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Mean changes in vital signs (Heart Rate) between the treatment groups from baseline

End point title	Part 1 - Mean changes in vital signs (Heart Rate) between the treatment groups from baseline

End point description:

Heart Rate

End point type	Secondary

End point timeframe:

Baseline and up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: BEATS/MIN				
arithmetic mean (standard deviation)				
VISIT 1	-1.08 (± 10.19)	0.26 (± 11.37)		
VISIT 2	-6.75 (± 2.22)	-0.75 (± 2.99)		
VISIT 3	0.04 (± 10.11)	-0.51 (± 11.46)		
VISIT 6	-0.78 (± 11.49)	-0.75 (± 11.92)		
VISIT 8	-1.27 (± 11.65)	-1.34 (± 11.03)		
VISIT 9	-1.26 (± 11.97)	-1.63 (± 10.56)		
VISIT 10	-3.28 (± 11.87)	-3.28 (± 11.61)		
VISIT 11	-3.57 (± 12.57)	-4.66 (± 14.73)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Mean changes in vital signs (Body Temperature) between the treatment groups from baseline

End point title	Part 1 - Mean changes in vital signs (Body Temperature) between the treatment groups from baseline

End point description:

Temperature

End point type	Secondary

End point timeframe:

Baseline and up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	219	111		
Units: °C				
arithmetic mean (standard deviation)				
VISIT 1	0.01 (± 0.39)	0.04 (± 0.36)		
VISIT 2	-0.35 (± 0.45)	-0.03 (± 0.39)		
VISIT 3	-0.10 (± 0.36)	0.04 (± 0.43)		
VISIT 6	-0.06 (± 0.44)	0 (± 0.44)		
VISIT 8	-0.06 (± 0.45)	0.05 (± 0.42)		
VISIT 9	-0.03 (± 0.44)	0.02 (± 0.47)		
VISIT 10	-0.07 (± 0.49)	-0.01 (± 0.52)		
VISIT 11	-0.14 (± 0.51)	-0.10 (± 0.50)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Nifurtimox concentration over time in plasma at Visit 2

End point title	Part 1 - Nifurtimox concentration over time in plasma at Visit 2

End point description:

Measured in sub-population. 00000 = data not available.

End point type	Secondary

End point timeframe:

At Visit 2 (Day 1): Pre-dose and Post-dose at 5-10 minutes, 10-120 minutes, 2-4 hours, and 4-8 hours

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	40	23		
Units: ug/L				
median (full range (min-max))				
Predose	00000 (00000 to 26.8)	00000 (00000 to 00000)		
5 - 10 MIN POST	00000 (00000 to 154.8)	21.1 (00000 to 695.0)		
10 - 120 MIN POST	78.2 (5.8 to 847.5)	49.0 (00000 to 524.3)		
2 - 4 HOURS POST	215.4 (77.9 to 516.3)	300.7 (106.9 to 533.5)		
4 - 8 HOURS POST	267.6 (40.2 to 508.1)	289.6 (103.2 to 301.0)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Nifurtimox concentration over time in plasma at Visit 3

End point title	Part 1 - Nifurtimox concentration over time in plasma at Visit 3

End point description:

Measured in sub-population. 00000 = data not available.

End point type	Secondary

End point timeframe:

At Visit 3 (Day 7): Pre-dose and Post-dose at 5-10 minutes, 10-120 minutes, 2-4 hours, and 4-8 hours

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	40	22		
Units: ug/L				
median (full range (min-max))				
Predose	47.7 (00000 to 407.6)	38.3 (00000 to 472.3)		
5 - 10 MIN POST	82.6 (00000 to 561.6)	56.0 (00000 to 442.8)		
10 - 120 MIN POST	250.6 (13.8 to 1035.6)	232.3 (13.9 to 706.3)		
2 - 4 HOURS POST	497.2 (57.1 to 1027.4)	257.7 (46.3 to 660.2)		
4 - 8 HOURS POST	427.5 (141.2 to 778.2)	267.0 (00000 to 339.6)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Nifurtimox concentration over time in plasma at Visit 6

End point title	Part 1 - Nifurtimox concentration over time in plasma at Visit 6

End point description:

Measured in sub-population. 00000 = data not available.

End point type	Secondary

End point timeframe:

At Visit 6 (Day 30): Pre-dose and Post-dose at 5-10 minutes, 10-120 minutes, 2-4 hours, and 4-8 hours

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	35	24		
Units: ug/L				
median (full range (min-max))				
Predose	23.8 (00000 to 459.3)	40.8 (00000 to 284.3)		
5 - 10 MIN POST	71.7 (00000 to 153.7)	73.7 (00000 to 266.7)		
10 - 120 MIN POST	135.0 (00000 to 932.4)	172.7 (14.1 to 387.3)		
2 - 4 HOURS POST	369.5 (92.1 to 1277.0)	103.8 (12.3 to 1107.2)		
4 - 8 HOURS POST	249.7 (70.9 to 504.3)	165.2 (84.9 to 1089.3)		

Statistical analyses

No statistical analyses for this end point

Secondary: Part 1 - Nifurtimox concentration over time in plasma at Visit 8

End point title	Part 1 - Nifurtimox concentration over time in plasma at Visit 8

End point description:

Measured in sub-population. 00000 = data not available.

End point type	Secondary

End point timeframe:

At Visit 8 (Day 60): Pre-dose and Post-dose at 5-10 minutes, 10-120 minutes, 2-4 hours, and 4-8 hours

End point values	Nifurtimox 60 days / Arm 1	Nifurtimox 30 days, then Placebo 30 days / Arm 2		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	36	19		
Units: ug/L				
median (full range (min-max))				
Predose	00000 (00000 to 258.7)	00000 (00000 to 00000)		
5 - 10 MIN POST	92.4 (15.9 to 255.1)	00000 (00000 to 00000)		
10 - 120 MIN POST	139.1 (23.6 to 711.4)	00000 (00000 to 21.5)		
2 - 4 HOURS POST	395.6 (166.9 to 883.0)	00000 (00000 to 00000)		
4 - 8 HOURS POST	300.6 (28.5 to 1217.2)	00000 (00000 to 00000)		

Statistical analyses

No statistical analyses for this end point

Other pre-specified: Part 1: Number of subjects cured with 60-day regimen compared with historical active control (benznidazole)

End point title	Part 1: Number of subjects cured with 60-day regimen compared with historical active control (benznidazole)[2]

End point description:

This exploratory efficacy analysis evaluated the cure rate assessed as seroconversion of nifurtimox after 1-year post-treatment follow-up with that of published data for benznidazole (Sosa Estani et al. 1998 and de Andrade et al. 1996) at 4- and 3-year post-treatment follow-up, respectively, used as historical control.

End point type	Other pre-specified

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

Notes:

[2] - The end point is not reporting statistics for all the arms in the baseline period. It is expected all the baseline period arms will be reported on when providing values for an end point on the baseline period.
Justification: Descriptive statistics only

End point values	Nifurtimox 60 days / Arm 1	Historical Benznidazole 1998 - Sosa Estani et al. (1998)	Historical Benznidazole 1996, Andrade et al. (1996)	
Subject group type	Reporting group	Subject analysis set	Subject analysis set	
Number of subjects analysed	219	53	64	
Units: Subjects	10	4	4	

Statistical analyses

No statistical analyses for this end point

Other pre-specified: Part 1 - Relationship of conventional serology (total purified antigen ELISA) and qPCR testing by Visit

End point title	Part 1 - Relationship of conventional serology (total purified antigen ELISA) and qPCR testing by Visit

End point description:

Using frequencies of matches and mismatches to assess agreement

Reactive = Reac ELISA
Detectable = Detec qPCR
Non-reactive = Nonreac ELISA
Non-detectable = Nondetec qPCR
Non evaluable = Noneval qPCR
qPCR Missing = Miss qPCR
Missing conventional testing = Miss ELISA

End point type	Other pre-specified

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days Reactive Detectable	Nifurtimox 60 days Reactive Non-detectable	Nifurtimox 60 days Non-reactive Detectable	Nifurtimox 60 days Non-reactive Non-detectable
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	219[3]	219[4]	219[5]	219[6]
Units: Subjects				
Visit 1	117	99	0	0
Visit 8	3	210	0	0
Visit 10	3	195	0	11
Visit 11	3	194	0	11

Notes:

[3] - Nifurtimox 60 days with Reac ELISA and Detec qPCR

[4] - Nifurtimox 60 days with Reac ELISA and Nondetec qPCR

[5] - Nifurtimox 60 days with Nonreac ELISA and Detec qPCR

[6] - Nifurtimox 60 days with Nonreac ELISA and Nondetec qPCR

End point values	Nifurtimox 60 days Reactive Non evaluable	Nifurtimox 60 days Reactive qPCR Missing	Nifurtimox 30 days Reactive Detectable	Nifurtimox 30 days Reactive Non-detectable
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	219[7]	219[8]	111[9]	111[10]
Units: Subjects				
Visit 1	1	2	57	52
Visit 8	2	0	1	103

Visit 10	2	0	2	99
Visit 11	1	1	5	96

Notes:

[7] - Nifurtimox 60 days with Reac ELISA and Non evaluable qPCR

[8] - Nifurtimox 60 days with Reac ELISA and missing qPCR

[9] - Nifurtimox 30 days with Reac ELISA and Detec qPCR

[10] - Nifurtimox 30 days with Reac ELISA and Nondetec qPCR

End point values	Nifurtimox 30 days Non-reactive Detectable	Nifurtimox 30 days Non-reactive Non-detectable	Nifurtimox 30 days Reactive Non evaluable	Nifurtimox 30 days Missing Conventional testing Non-detectable
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	111[11]	111[12]	111[13]	111[14]
Units: Subjects				
Visit 1	0	1	1	0
Visit 8	0	1	2	1
Visit 10	0	6	2	0
Visit 11	0	6	1	0

Notes:

[11] - Nifurtimox 30 days with Nonreac ELISA and Detec qPCR

[12] - Nifurtimox 30 days with Nonreac ELISA and Nondetec qPCR

[13] - Nifurtimox 30 days with Reac ELISA and Non evaluable qPCR

[14] - Nifurtimox 30 days with Miss ELISA and Nondetec qPCR

Statistical analyses

No statistical analyses for this end point

Other pre-specified: Part 1 - Relationship of conventional serology (total purified antigen ELISA) and non-conventional (ELISA-F29) serologic testing by visit

End point title	Part 1 - Relationship of conventional serology (total purified antigen ELISA) and non-conventional (ELISA-F29) serologic testing by visit

End point description:

Using frequencies of matches and mismatches to assess agreement

Reactive = Reac ELISA
Reactive = Reac F29
Non-reactive = Nonreac ELISA
Non-reactive = Nonreac F29

End point type	Other pre-specified

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days Reactive and Reactive	Nifurtimox 60 days Reactive and Non-reactive	Nifurtimox 60 days Non-reactive and Reactive	Nifurtimox 60 days Non-reactive and Non-reactive
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	219[15]	219[16]	219[17]	219[18]
Units: Subjects				

Visit 1	142	77	0	0
Visit 3	133	85	0	0
Visit 6	131	83	0	0
Visit 8	124	91	0	0
Visit 10	108	92	2	9
Visit 11	93	106	3	8

Notes:

[15] - Nifurtimox 60 days with Reac ELISA and Reac F29

[16] - Nifurtimox 60 days with Reac ELISA and Nonreac F29

[17] - Nifurtimox 60 days with Nonreac ELISA and Reac F29

[18] - Nifurtimox 60 days with Nonreac ELISA and Nonreac F29

End point values	Nifurtimox 30 days Reactive and Reactive	Nifurtimox 30 days Reactive and Non-reactive	Nifurtimox 30 days Non-reactive and Reactive	Nifurtimox 30 days Non-reactive and Non-reactive
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	111[19]	111[20]	111[21]	111[22]
Units: Subjects				
Visit 1	72	38	0	1
Visit 3	64	43	0	1
Visit 6	67	42	0	1
Visit 8	63	43	0	1
Visit 10	61	42	0	6
Visit 11	54	48	0	6

Notes:

[19] - Nifurtimox 30 days with Reac ELISA and Reac F29

[20] - Nifurtimox 30 days with Reac ELISA and Nonreac F29

[21] - Nifurtimox 30 days with Nonreac ELISA and Reac F29g

[22] - Nifurtimox 30 days Nonreac ELISA and Nonreac F29

Statistical analyses

No statistical analyses for this end point

Other pre-specified: Part 1 - Relationship of conventional serology (recombinant ELISA) and non-conventional (ELISA-F29) serologic testing by visit

End point title	Part 1 - Relationship of conventional serology (recombinant ELISA) and non-conventional (ELISA-F29) serologic testing by visit

End point description:

Using frequencies of matches and mismatches to assess agreement

Reactive = Reac ELISA
Reactive = Reac F29
Non-reactive = Nonreac ELISA
Non-reactive= Nonreac F29

End point type	Other pre-specified

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Nifurtimox 60 days Reactive and Reactive	Nifurtimox 60 days Reactive and Non-reactive	Nifurtimox 60 days Non-reactive and Reactive	Nifurtimox 60 days Non-reactive and Non-reactive
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	219[23]	219[24]	219[25]	219[26]
Units: Subjects				
Visit 1	142	77	0	0
Visit 3	133	85	0	0
Visit 6	131	81	0	2
Visit 8	124	90	0	1
Visit 10	108	91	2	10
Visit 11	93	106	3	8

Notes:

[23] - Nifurtimox 60 days Reac ELISA and Reac F29

[24] - Nifurtimox 60 days with Reac ELISA and Nonreac F29

[25] - Nifurtimox 60 days with Nonreac ELISA and Reac F29

[26] - Nifurtimox 60 days Nonreac ELISA and Nonreac F29

End point values	Nifurtimox 30 days Reactive and Reactive	Nifurtimox 30 days Reactive and Non-reactive	Nifurtimox 30 days Non-reactive and Reactive	Nifurtimox 30 days Non-reactive and Non-reactive
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	111[27]	111[28]	111[29]	111[30]
Units: Subjects				
Visit 1	72	38	0	1
Visit 3	64	43	0	1
Visit 6	67	42	0	1
Visit 8	63	43	0	1
Visit 10	59	43	2	5
Visit 11	53	48	1	6

Notes:

[27] - Nifurtimox 30 days with Reactive ConvReac ELISA and Reac F29

[28] - Nifurtimox 30 days with Reac ELISA and Nonreac F29

[29] - Nifurtimox 30 days with Nonreac ELISA and Reac F29

[30] - Nifurtimox 30 days with Nonreac ELISA and Nonreac F29

Statistical analyses

No statistical analyses for this end point

Other pre-specified: Part 1 - Relationship of conventional serology (ELISA) to Indirect hemagglutination assay (IHA) results

End point title	Part 1 - Relationship of conventional serology (ELISA) to Indirect hemagglutination assay (IHA) results

End point description:

Sero-reduction is defined as a => 20% reduction in optical density [OD]) using two conventional ELISA serology tests in subjects => 8 months to < 18 years of age at randomization; Others: reactive results that are not sero-reduction in subjects => 8 months to < 18 years of age at randomization; or reactive results in subjects < 8 months of age at randomization.

Non-reactive ELISA = Nonreac ELISA
Non-reactive IHA = Nonreac IHA
Reactive IHA decrease = Reac IHA dec
React IHA nochange = Reac IHA nochange
Reactive ELISA: seroreduction = Reac ELISA reduc

Reactive ELISA: others = Reac ELISA other

End point type	Other pre-specified

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Non-reactive ELISA and Non-reactive IHA	Non-reactive ELISA and reactive IHA decrease	Non-react ELISA and react IHA Nochange	Reactive ELISA: Sero-reduction and Non-react IHA
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	318[31]	318[32]	318[33]	318[34]
Units: Subjects				
Nifurtimox 60 days	8	2	0	2
Nifurtimox 30 days	4	1	0	0

Notes:

[31] - Nonreac ELISA and Nonreac IHA

[32] - Nonreac ELISA and Reac IHA dec

[33] - Nonreac ELISA and Reac IHA nochange

[34] - Reac ELISA and Nonreac IHA

End point values	Reactive ELISA: Sero reduction and reactive IHA decrease	Reactive ELISA: Sero-reduction and reactive IHA Nochange	Reactive ELISA: Others and Non-reactive IHA	Reactive ELISA: Others and react IHA decrease
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	318[35]	318[36]	318[37]	318[38]
Units: Subjects				
Nifurtimox 60 days	40	20	1	47
Nifurtimox 30 days	12	4	1	30

Notes:

[35] - Reac ELISA reduc and Reac IHA dec

[36] - Reac ELISA reduc and Reac IHA nochange

[37] - Reac ELISA other and Nonreac IHA

[38] - Reac ELISA other and Reac IHA dec

End point values	Reactive ELISA: Others and react IHA Nochange	Reactive ELISA: Others and Missing IHA		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	318[39]	318[40]		
Units: Subjects				
Nifurtimox 60 days	89	1		
Nifurtimox 30 days	58	0		

Notes:

[39] - Reac ELISA other and Reac IHA nochange

[40] - Reac ELISA other and IHA missing

Statistical analyses

No statistical analyses for this end point

Other pre-specified: Part 1 - Relationship between conventional ELISA results in terms of Cure or No Cure and IHA results in all patients

End point title	Part 1 - Relationship between conventional ELISA results in terms of Cure or No Cure and IHA results in all patients

End point description:

Cure is defined as sero-reduction (in subjects => 8 months to < 18 years of age at randomization) or sero-conversion (in all subjects). Sero-reduction is defined as a => 20% reduction in optical density [OD]) measured by two conventional ELISA serology tests and sero-conversion is defined as negative Immunoglobulin G [IgG] concentration measured by two conventional ELISA serology tests.

Cure = Cure
Non reactive/reactive decreasing = Nonreac/reac dec
Reactive non-decreasing = Reac nondec
No cure = No Cure
Missing IHA testing = IHA missing

End point type	Other pre-specified

End point timeframe:

Up to 420 days (Visit 11 post-treatment)

End point values	Cure and Non reactive/reactive decreasing	Cure and reactive non-decreasing	No Cure and Non reactive/reactive decreasing	No Cure and reactive non-decreasing
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	318[41]	318[42]	318[43]	318[44]
Units: Subjects				
Nifurtimox 60 days	52	20	48	89
Nifurtimox 30 days	17	4	29	58

Notes:

[41] - ELISA Cure and IHA Nonreac/reac dec

[42] - ELISA Cure and IHA Reac nondec

[43] - ELISA No Cure and IHA results: Nonreac/reac dec

[44] - ELISA No Cure and IHA Reac nondec

End point values	No Cure and Missing IHA Testing			
Subject group type	Subject analysis set			
Number of subjects analysed	318[45]			
Units: Subjects				
Nifurtimox 60 days	1			
Nifurtimox 30 days	0			

Notes:

[45] - ELISA No Cure and IHA missing

Statistical analyses

No statistical analyses for this end point

Adverse events

Adverse events information

Timeframe for reporting adverse events:

Part 1: From first application of study drug up to and including 7 days after last application of study drug
Part 2: first occurred or worsened at start 0f study part 2 up to 3 years.

Adverse event reporting additional description:

Part 1: TEAS first occurred or worsened after first application of study drug up to 7 days after last application.
Part 2: only AEs considered at least possibly related to nifurtimox (administered in part 1) and those caused by protocol-related procedures were reported.

Assessment type	Non-systematic

Dictionary used

Dictionary name	MedDRA
Dictionary version	20.0

Reporting groups

Reporting group title	Nifurtimox 30 days

Reporting group description:

Nifurtimox tablets administered three times daily for 30 days, followed by placebo administered three timesdaily for 30 days (Days 1 - 30, active nifurtimox treatment; Days 31 - 60, placebo)

Reporting group title	Nifurtimox 60 days

Reporting group description:

Nifurtimox tablets administered three times daily for 60 days (Days 1 - 60, active nifurtimox treatment)

Serious adverse events	Nifurtimox 30 days	Nifurtimox 60 days	
Total subjects affected by serious adverse events			
subjects affected / exposed	3 / 111 (2.70%)	6 / 219 (2.74%)	
number of deaths (all causes)	0	1	
number of deaths resulting from adverse events	0	0	
Nervous system disorders			
Febrile convulsion			
subjects affected / exposed	1 / 111 (0.90%)	0 / 219 (0.00%)	
occurrences causally related to treatment / all	0 / 1	2 / 2	
deaths causally related to treatment / all	0 / 0	0 / 0	
Seizure			
subjects affected / exposed	0 / 111 (0.00%)	1 / 219 (0.46%)	
occurrences causally related to treatment / all	0 / 0	0 / 2	
deaths causally related to treatment / all	0 / 0	0 / 0	
Syncope			

subjects affected / exposed	0 / 111 (0.00%)	1 / 219 (0.46%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Gastrointestinal disorders			
Abdominal pain			
subjects affected / exposed	1 / 111 (0.90%)	0 / 219 (0.00%)	
occurrences causally related to treatment / all	1 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Respiratory, thoracic and mediastinal disorders			
Apnoea			
subjects affected / exposed	0 / 111 (0.00%)	1 / 219 (0.46%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Skin and subcutaneous tissue disorders			
Urticaria			
subjects affected / exposed	0 / 111 (0.00%)	1 / 219 (0.46%)	
occurrences causally related to treatment / all	0 / 0	1 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Infections and infestations			
Cellulitis			
subjects affected / exposed	0 / 111 (0.00%)	1 / 219 (0.46%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Pelvic inflammatory disease			
subjects affected / exposed	0 / 111 (0.00%)	1 / 219 (0.46%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Ear infection			
subjects affected / exposed	1 / 111 (0.90%)	0 / 219 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Pneumonia			

subjects affected / exposed	1 / 111 (0.90%)	0 / 219 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	

Frequency threshold for reporting non-serious adverse events: 2 %

Non-serious adverse events	Nifurtimox 30 days	Nifurtimox 60 days	
Total subjects affected by non-serious adverse events			
subjects affected / exposed	44 / 111 (39.64%)	98 / 219 (44.75%)	
Nervous system disorders			
Headache			
subjects affected / exposed	16 / 111 (14.41%)	28 / 219 (12.79%)	
occurrences (all)	22	41	
General disorders and administration site conditions			
Pyrexia			
subjects affected / exposed	3 / 111 (2.70%)	16 / 219 (7.31%)	
occurrences (all)	3	17	
Gastrointestinal disorders			
Abdominal pain			
subjects affected / exposed	7 / 111 (6.31%)	15 / 219 (6.85%)	
occurrences (all)	9	23	
Abdominal pain upper			
subjects affected / exposed	4 / 111 (3.60%)	14 / 219 (6.39%)	
occurrences (all)	5	19	
Diarrhoea			
subjects affected / exposed	6 / 111 (5.41%)	10 / 219 (4.57%)	
occurrences (all)	7	10	
Nausea			
subjects affected / exposed	14 / 111 (12.61%)	18 / 219 (8.22%)	
occurrences (all)	15	23	
Vomiting			
subjects affected / exposed	9 / 111 (8.11%)	32 / 219 (14.61%)	
occurrences (all)	10	56	
Infections and infestations			
Nasopharyngitis			

subjects affected / exposed occurrences (all)	6 / 111 (5.41%) 6	14 / 219 (6.39%) 17	
Metabolism and nutrition disorders Decreased appetite subjects affected / exposed occurrences (all)	 8 / 111 (7.21%) 8	 23 / 219 (10.50%) 24	

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
02 April 2015	- Text added to indicate that ECGs will be obtained at the time PK samples are collected when nifurtimox has reached Cmax or peak steady-state concentrations (i.e., at the 2 – 4 hour time point). - Clarification of study objectives and efficacy variables regarding sero-reduction and sero-conversion; change in tests required for confirmation of Chagas' disease (recombinant ELISA and total purified antigen ELISA). - Baseline specimens for serological tests to be collected for diagnosis, and frozen as a subject control. - Change in urine color to be reinforced to subjects at the visits where study drug is dispensed. - Information about laboratory procedures, assay variability and cutoffs, and facilities to be used for all assays was added. - A safety and compliance questionnaire/script (Phone Contact Form) for use by site staff when conducting the telephone assessments was added. - For standardization of adverse event reporting, the terms "mild", "moderate", and "severe" were defined within the protocol. - A telephone assessment at Day 14 was added. - Measurement of ECGs will be optional assessments for subjects < 5 years of age, and required for subjects 5 years of age and older. - A neurological examination to be performed at the time of each physical examination in the study was added. - Detailed procedures regarding follow-up on all subjects who prematurely discontinue study drug was added. - Monitoring of treatment compliance was included in the Visit 6 (Day 30) study assessments to be conducted via telephone; this is reflected in the Phone Contact Form. - A Phone Contact Form was developed and added as an appendix to the protocol. - A Subject Diary was developed and added as an appendix to the protocol.
19 October 2015	- To address FDA comments regarding handling of the analyses of data for the primary efficacy endpoint. - To provide textual clarifications resulting from comments received from Ethics Committees and ex-US Health Authorities. - Overall editorial corrections and clarifications.
07 April 2016	- To delete a phrase "and ECG abnormalities" which was added in error to amendment 2.
15 November 2016	- To implement a PK subject diary which will allow PK subjects to record the meals consumed with study drug administration. - Clarifying editorial changes were made
12 October 2017	- To introduce an additional test (Immunofluorescent antibody, IFA), which would validate the current ELISA test results, as requested by FDA. This test will be carried out at Visit 1 and Visit 11 using random back-up samples from the study subjects.
20 December 2017	- To replace the immunofluorescent antibody (IFA) test introduced via Amendment 5 by the indirect hemagglutination assay (IHA). - Use of another serological test such as IFA or IHA was requested by FDA to validate the current ELISA test results. This test will be carried out at Visit 1 and Visit 11 using random back-up samples from the study subjects.

11 May 2018	- To satisfy FDA's request to "further describe and verify the clinical benefit of nifurtimox" according to the FDA meeting minutes of the Type C meeting in August 2017 by following the subjects in Study 16027 for a reasonable amount of time to demonstrate reversion of serology to negative. - Changes include extending the current Study 16027 with the long-term follow-up (LTFU), and detailed study design and procedures for this LTFU considering FDA's recommendations to the detailed study concept for the LTFU received in March 2018.
23 July 2019	To incorporate feedback received from study site personnel at the investigator meeting for part 2 of the study in APR 2019. None of the changes implemented in this amendment impact the benefit-risk evaluation of the study.
18 October 2019	- To incorporate feedback received from the FDA on 19 SEP 2019. It will allow subjects who were randomized and received at least one dose of their assigned nifurtimox treatment regimen and who are otherwise eligible to participate in the long-term follow-up portion of the study (Part 2, CHICO SECURE), regardless of subsequent treatment for Chagas' disease. None of the changes implemented in this amendment impact the benefit-risk evaluation of the study.
03 November 2020	- To incorporate feedback received from the FDA on 10 JUL 2020. The Agency requested inclusion of non-conventional ELISA in Part 2 (CHICO SECURE) of the study and that antibody titers by serial dilution be obtained for all available serum samples. None of the changes implemented in this amendment impacted the benefit-risk evaluation of the study.

Notes:

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

Limitations of the trial such as small numbers of subjects analysed or technical problems leading to unreliable data.

Abnormal findings on neurological examination by physical examination after Screening will be documented as AEs.

Notes:

Online references

http://www.ncbi.nlm.nih.gov/pubmed/33412557