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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

This protocol version is an integration of the following documents / sections:

- **Original protocol**, Version 1.0, dated 04 NOV 2014
- **Amendment no. 1** (described in Section [15.1](#))
forming integrated protocol Version 2.0, dated 02 APR 2015
- **Amendment no. 2** (described in Section [15.2](#))
forming integrated protocol Version 3.0, dated 19 OCT 2015
- **Amendment no. 3** (described in Section [15.3](#))
forming integrated protocol Version 4.0, dated 07 APR 2016
- **Amendment no. 4** (described in Section [15.4](#))
forming integrated protocol Version 5.0, dated 15 NOV 2016
- **Amendment no. 5** (described in Section [15.5](#))
forming integrated protocol Version 6.0, dated 12 OCT 2017
- **Amendment no. 6** (described in Section [15.6](#))
forming integrated protocol Version 7.0, dated 20 DEC 2017
- **Amendment no. 7** (described in Section [15.7](#))
forming integrated protocol Version 8.0, dated 11 MAY 2018
- **Amendment no. 8** (described in Section [15.8](#))
forming integrated protocol Version 9.0, dated 23 JUL 2019
- **Amendment no. 9** (described in Section [15.9](#))
forming integrated protocol Version 10.0, dated 18 OCT 2019
- **Amendment no. 10** (global)
forming integrated protocol Version 11.0, dated 03 NOV 2020

Amendments not included in the consecutive numbering of amendments are local amendments not forming part of this integrated global protocol.

1. Title page

Study title: 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

Short title: Prospective study of a pediatric nifurtimox formulation for Chagas' disease

Test drug: BAY No. A2502/Nifurtimox

Study purpose: To evaluate efficacy and safety, and characterize the pharmacokinetics, of nifurtimox in children with Chagas' disease using 30-day and 60-day treatment durations

Clinical study phase: 3 Date: 03 NOV 2020

Registration: EudraCT: NA Version no.: 11.0

Sponsor's study no.: 16027

Sponsor: **Bayer AG, D-51368 Leverkusen, Germany**

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The study will be conducted in compliance with the protocol, ICH-GCP and any applicable regulatory requirements.

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Signature of principal investigator

The signatory agrees to the content of the final clinical study protocol as presented.

Name:

Affiliation:

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Signed copies of this signature page are stored in the sponsor's study file and in the respective center's investigator site file.

2. Synopsis

Title	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
Short title	Prospective study of a pediatric nifurtimox formulation for Chagas' disease
Clinical study phase	3
Study objective(s)	<p>This study consists of two parts:</p> <ul style="list-style-type: none"> Part 1 comprises the treatment with nifurtimox including the 1-year follow up (CHICO) Part 2 comprises the 3-year long-term follow-up (LTFU) of all subjects who were randomized and received at least one dose of their assigned nifurtimox treatment regimen in CHICO (CHICO SECURE) <p>Part 1 (CHICO)</p> <p>The primary objective of this Phase 3 clinical study is:</p> <ul style="list-style-type: none"> 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 $\geq 20\%$ reduction in optical density [OD] measured by conventional enzyme-linked immune sorbent assay [ELISA]) compared to baseline in subjects ≥ 8 months to < 18 years of age at randomization; or, sero-conversion (defined as negative Immunoglobulin G concentration in all subjects)¹ <p>Secondary objectives of the study are:²</p> <ul style="list-style-type: none"> To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox as sero-reduction or sero-conversion at the 12-month follow-up (360 days from EOT)³ To evaluate the safety/tolerability profile of nifurtimox by laboratory parameters (hematology, blood chemistry, urinalysis), electrocardiogram (ECG) monitoring, vital sign measurements (blood pressure, heart rate, respiratory rate, temperature), adverse event (AE) monitoring, and physical examinations, including neurological examinations To evaluate the pharmacokinetics (PK)/pharmacodynamics (PD) of nifurtimox in children receiving the drug for treatment of Chagas' disease (to be described in a separate report) <p>Exploratory objectives of the study are:⁴</p> <ul style="list-style-type: none"> To assess the comparability of a 60-day regimen of nifurtimox to historical active control (benznidazole)⁵ as sero-reduction or sero-conversion at the 12-month follow-up (360 days from EOT)⁶

¹ Text modified as per amendment 2

² Text of first 3 secondary objectives modified, and fourth secondary objective was added, per Modification 2 (Amendment 1)

³ Text modified per Modification 2 (Amendment 1)

⁴ Exploratory objectives added as per amendment 2.

⁵ Sero-conversion for the historical controls will be estimated from cure rates as presented in two publications.[15][16] See Section 5, Study Design, Justification of the design.

- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox using quantitative polymerase chain reaction (qPCR) at the 12-month follow-up (360 days from EOT)⁷
- To evaluate the relationship of conventional serology (as sero-reduction or sero-conversion) to qPCR using frequencies of matches and mismatches to assess agreement⁸
- To evaluate the relationship of non-conventional serology to conventional serology⁹

Part 2 (CHICO SECURE)

The primary objective of the LTFU part of the study is:

- Assess the incidence of seronegative conversion as confirmed 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 untreated patients with Chagas' disease at the 4-year follow-up.

The secondary objectives are:

- Assess the incidence of seronegative conversion, as confirmed by two types of assay (recombinant ELISA and IHA), in subjects who were randomized and received at least one dose of the 30-day nifurtimox treatment regimen at the 4-year follow-up.
- Evaluate the proportion of responders who show both seronegative conversion as confirmed by two types of assay (recombinant ELISA and IHA) and no evidence of established cardiomyopathy as evaluated in electrocardiogram (ECG) recordings.
- Assess serial reduction in OD measured by recombinant ELISA and total purified antigen ELISA, in subjects who were randomized and received at least one dose of either the 60- or 30-day nifurtimox treatment regimens compared to Visit 1 in Part 1.

The exploratory objectives are:

- Assess the incidence of seronegative conversion as confirmed by two types of assay (recombinant ELISA and IHA) in subjects by age categories (≤ 2 years, > 2 years to ≤ 6 years, > 6 to ≤ 12 years, > 12 to < 18 years; age is defined as subject's age at randomization).
- Assess the proportion of responders who show seronegative conversion and the incidence of seronegative conversion as confirmed by non-conventional serology in subjects who were randomized and received at least one dose of either 60- or 30-day nifurtimox treatment regimens.
- Assess the occurrence of congenital infection with *T. cruzi* in children

⁶ Text modified per Modification 2 (Amendment 1)

⁷ Text modified per Modification 2 (Amendment 1)

⁸ Text modified per Modification 2 (Amendment 1)

⁹ Fourth objective added per Modification 2 (Amendment 1)

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	born of female subjects who were randomized and received at least one dose of the 60- or 30-day nifurtimox treatment regimen. The congenital infection will be confirmed by parasitological method in children ≤ 8 months of age and by serological method in children > 8 months of age.
Test drug(s)	Nifurtimox 30-mg and 120-mg tablets with score lines to be divided into two equal halves to allow for 15-mg and 60-mg dose increments, respectively.
Name of active ingredient	Nifurtimox
Dose(s)	For pediatric subjects with body weight ≤ 40 kg: 10-20 mg/kg/day in three divided doses For pediatric subjects with body weight > 40 kg: 8 – 10 mg/kg/day in three divided doses. The planned mg/kg per day dosages are as in the Company Core Data Sheet.
Route of administration	Oral tablets, to be administered with food
Duration of treatment	60 days of nifurtimox (Treatment Group 1), or 30 days of nifurtimox followed by 30 days of placebo (Treatment Group 2)
Reference drug(s)	None
Indication	Treatment of Chagas' disease in children younger than 18 years of age
Diagnosis and main criteria for inclusion/exclusion	<p>Part 1 (CHICO)</p> <ul style="list-style-type: none"> Male and female pediatric subjects aged 0 days to younger than 18 years Chagas' disease diagnosed/confirmed by: <ul style="list-style-type: none"> Subjects < 8 months of age at randomization must demonstrate direct observation of <i>Trypanosoma cruzi</i> by concentration test Subjects ≥ 8 months to < 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis: <ul style="list-style-type: none"> Recombinant ELISA Total purified antigen ELISA <p>Additionally, a non-conventional ELISA test and indirect hemagglutination assay (IHA) will be obtained; however, a positive result will not be required for diagnosis. ^{10,11}</p> <p>Subjects will also be eligible if there is acceptable documentation of positive Chagas' disease within three months prior to screening and they have not had prior anti-trypanocidal or anti-parasitic treatment. ¹²</p> <p>Written informed consent by the subject and/or parent(s) or legally authorized representative(s) according to the age established per local regulations must be obtained from all subjects prior to screening for the study. In addition, depending on the subject's age, subject's assent is required as applicable by local laws and regulations (varies in each country).</p> <p>Females of childbearing potential (i.e., female subjects who have experienced menarche) and male subjects must agree to use adequate contraception if sexually active from the time of signing the informed consent/assent form until 3 months after the last study drug administration. Depending on the country,</p>

¹⁰ IFA test added per Amendment 5

¹¹ Replaced IFA with IHA per Amendment 6.

¹² Text modified/added to clarify test required for Chagas' disease diagnosis per Modification 2, text "at randomization" added for clarity (Amendment 1)

study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.

Subjects aged 0 to 27 days who, at birth, were pre-term (i.e., gestational age less than 37 weeks), weighed less than 2500 g, or had a maximum Apgar score < 7 at 5 minutes will be excluded from the study. Subjects with contraindications/warnings to nifurtimox administration (or with conditions that may increase the risk of the undesirable effects of nifurtimox), are immuno-compromised, or have had previous treatment with trypanocidal agents or an accepted indication for anti-parasitic therapy (e.g., reactivation of Chagas' infection due to immunosuppression by several diseases or treatment with prolonged oral steroids) will also be excluded from the study.

Part 2 (CHICO SECURE)

Inclusion criteria

1. Male and female subjects who were randomized and received at least one dose of their assigned 60- or 30-day regimen of nifurtimox treatment
2. Written informed consent by the subject and/or parent(s) or legally authorized representative(s) according to the age established per local regulations must be obtained from all subjects who are screened for the study. In addition, depending on the subject's age, subject's assent is required as applicable by local laws and regulations (varies in each country)

Exclusion criteria

1. Subjects with acute or chronic health conditions or congenital disorders which, in the opinion of the investigator, would make them unsuitable for participation in the clinical study
2. Subjects living in housing conditions where there is no active or effective vector-control to *T. cruzi* reinfection as determined by Ministry of Health guideline of the respective country and the judgement of the investigator
3. Subjects with clinical manifestations of Chagas' disease, such as:
 - Known evidence of Chagas' disease-related gastrointestinal dysfunction (e.g., megaesophagus, megacolon, or both) or Chagas' digestive disease
 - Serious manifestations of acute Chagas' disease, including myocarditis, meningoencephalitis, or pneumonitis
4. Immuno-compromised subjects (e.g., those with human immunodeficiency virus infection, primary immunodeficiency, or prolonged treatment with corticosteroids or other immunosuppressive drugs)
5. Subjects who the investigator considers unlikely to adhere to the protocol, or complete the long-term follow-up
6. Subjects with close affiliation with the investigational site; e.g., a close relative of the investigator, dependent person (e.g., employee or student of the investigational site)

Study design	<p>Part 1 (CHICO)</p> <p>Prospective, randomized (to dosing regimen), age-stratified, double-blind, parallel-group study. Subjects will be randomized into four strata as follows:</p> <p>Stratum 1: 0 to 27 days</p> <p>Stratum 2: 28 days to younger than 8 months</p> <p>Stratum 3: 8 months to younger than 2 years</p> <p>Stratum 4: 2 years to younger than 18 years</p> <p>A minimum of 38 subjects in each age stratum is targeted, but not required, in order to be able to derive meaningful safety conclusions.¹³</p> <p>Part 2 (CHICO SECURE)</p> <p>Prospective, long-term follow-up to evaluate seronegative conversion and serial reduction in OD. Subjects will be followed up for a total of 4 years after end of nifurtimox treatment. The first year of follow-up period is included in part 1 and 3 years of follow-up are included in part 2 of the study.</p> <p>The following age categories will be considered for part 2 of the study: ≤ 2 years, > 2 years to ≤ 6 years, > 6 to ≤ 12 years, > 12 to < 18 years (age is defined as subject's age at randomization).</p>
Methodology	<p>Part 1 (CHICO)</p> <p>No study-specific procedures will be performed before the subject/legally authorized representative has signed the informed consent form, or a minor subject has provided assent, including asking a potential subject to fast (if applicable, depending on the age of the subject at the discretion of the investigator) prior to the screening blood samples. Informed consent/assent for optional PK blood sampling will also be obtained. After the consents/assents have been obtained, screening assessments will be performed from 1 to 14 days prior to randomization at Visit 2.¹⁴</p> <p>After study eligibility has been confirmed and safety assessments have been performed at Visit 2 (Day 1), subjects will be randomized via Interactive Voice Response System/Interactive Web Response System in a 2:1 ratio (60-day regimen vs. 30-day regimen) to one of two treatment groups:¹⁵</p> <p>Nifurtimox tablets administered three times daily for 60 days (Days 1 – 60, active nifurtimox treatment, Treatment Group 1) or</p> <p>Nifurtimox tablets administered three times daily for 30 days, followed by nifurtimox placebo administered three times daily for 30 days (Days 1 – 30, active nifurtimox treatment; Days 31 – 60, placebo; Treatment Group 2).</p> <p>The first dose of study drug will be administered at Visit 2. Pre- and post-dose PK blood samples will be obtained at specified time points from those subjects consenting to PK assessments. At the 2 – 4 hour post-dose time point of PK blood sampling (i.e., at the time of maximum concentration), an ECG (optional for subjects < 5 years of age at the discretion of the investigator) will be obtained to allow for PK/PD investigations. Study drug will be dispensed and instructions for study drug administration will be provided to all subjects.¹⁶</p>

¹³ Text modified as of amendment 2

¹⁴ Text added for clarity (Amendment 1)

¹⁵ Text modified for clarity (Amendment 1)

¹⁶ Text modified/added for clarity and per recommendations per Modification 1 (Amendment 1)

Subjects will return to the investigational site for efficacy and safety assessments at Visits 3 (Day 7±1) and 6 (Day 30). Subjects who have not consented to PK assessments will take that day's doses of study drug as instructed. Subjects consenting to PK assessments must hold the morning dose of study drug; for these subjects, a pre-dose PK blood sample will be obtained, then the morning dose of study drug will be administered. Post-dose PK blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point. A pre-paid phone card will be provided to all subjects to facilitate contact between study site personnel and the subjects/subjects' authorized representatives.¹⁷

At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.¹⁸

Visits 4 (Day 14), 5 (Day 21), and 7 (Day 42) will be telephone assessments where study site personnel will contact the subject/legally authorized representative via telephone to assess the occurrence of AEs, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone assessments will be provided to all sites.¹⁹

Subjects will return to the investigational site for efficacy and safety assessments on Day 60 (Visit 8), which will be the EOT for both treatment groups. Subjects who have not consented to PK assessments will take that day's doses of study drug as instructed. Subjects consenting to PK assessments must hold the morning dose of study drug. A pre-dose PK blood sample will be collected, then the morning dose of study drug will be administered. Post-dose PK blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point. Study drug will be collected, and no additional study drug will be dispensed.²⁰

After the EOT visit (Visit 8), subjects will return to the site on Days 90 (Visit 9), 240 (Visit 10), and 420 (Visit 11) for additional efficacy and safety assessments. The total duration of each subject's participation is expected to be approximately 14 months.²¹

Safety will be assessed via laboratory parameters (hematology, blood chemistry, urinalysis), vital sign measurements (blood pressure [optional in subjects < 5 years of age at the discretion of the investigator], heart rate, respiratory rate, temperature), monitoring of ECGs (optional in subjects < 5 years of age at the discretion of the investigator), monitoring of AEs, and physical examinations, including neurological examinations.²²

Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visit 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (±3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for

¹⁷ Text modified/added for clarity and per recommendations of Modification 1 (Amendment 1)

¹⁸ Text added for clarity (Amendment 1)

¹⁹ Text added to describe telephone assessments per Modification 8 (Amendment 1)

²⁰ Text modified/added for clarity and per ECG assessments at PK sampling per Modification 1 (Amendment 1)

²¹ Text modified for clarity (Amendment 1)

²² Text modified for clarity and to include neurological examinations per Modification 10 (Amendment 1)

	<p>the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments.²³</p> <p>Part 2 (CHICO SECURE)</p> <p>No study-specific procedures will be performed before the subject and/or parent(s) or legally authorized representative(s) has signed the informed consent form, or a minor subject has provided assent for part 2 of the study. After the consents/assents have been obtained, the assessments scheduled for follow up (FU) Visit 1 including eligibility assessments will be performed.</p> <p>The first visit should be performed as soon as possible after availability of regulatory and ethics committee approval for all subjects who have already completed part 1 of the study for 1 year \pm 6 weeks or longer. Subjects who are entering the study beyond the time window for FU Visit 1 (2 years \pm 6 weeks) will have their eligibility assessments as soon as possible provided that consents/assents have been obtained.</p> <p>Subjects will have annual visits at the investigational site for efficacy and safety assessments for 3 years. Timing of study visits is relative to end of treatment in part 1 of the study.</p> <p>At each investigational site visit, blood samples for serology tests (recombinant ELISA, total purified antigen ELISA, IHA and a non-conventional ELISA test) will be collected. Blood samples will be taken under fasting conditions, if applicable, depending on the age of the subject, at the discretion of the investigator. Subjects will undergo a physical examination, body weight, height/length and vital signs will be measured and protocol specified AEs will be recorded. A 12-lead electrocardiogram (ECG) will be obtained at each annual visit. The subject's concomitant medications will be recorded. Signs and symptoms of Chagas' disease will be assessed and blood will be taken for detection of <i>T. cruzi</i>'s deoxyribonucleic acid (DNA) using quantitative polymerase chain reaction (qPCR). If during the course of the study, conventional ELISA OD increases, the subject has positive qPCR results and shows signs or symptoms of Chagas' disease, it is up to the investigator's discretion to initiate antitrypanosomal treatment.</p> <p>Whenever an AE considered at least possibly related to nifurtimox or caused by protocol-required procedures occurs, the subject should report it to the investigator.</p> <p>Female subjects who gave birth before end of part 2 of the study will be invited to test their child for the presence or absence of infection with <i>T. cruzi</i>.</p> <p>Between the annual visits at the investigational site, subjects and/or his or her parent(s) or legally authorized representative(s) will be contacted by qualified personnel by a method agreed (e.g., phone call) about 6 months after a visit at the investigational site to identify the occurrence of potential clinical symptoms of Chagas' disease or any pathology mentioned in the exclusion criteria using a structured interview. In case of suspected findings, an unscheduled visit at the investigational site to initiate appropriate diagnostic evaluations will be requested.</p>
Type of control	<p>For primary objective: historical</p> <p>For secondary objectives: historical and 30-day vs. 60-day treatment</p>

²³ Text added to include assessments for subjects who prematurely discontinued study drug per Modification 11 (Amendment 1)

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Data Monitoring Committee	An independent Data Monitoring Committee will meet periodically to review the safety data of enrolled subjects, as well as the continuing scientific merit of the trial. (Not applicable to part 2)
Number of subjects	Approximately 300 pediatric subjects will be randomized (2:1 randomization, 60-day regimen vs. 30-day regimen) in part 1 (CHICO); all subjects who were randomized and received at least one dose of their assigned 60- or 30-day nifurtimox treatment regimen will be assessed for eligibility for part 2 (CHICO SECURE) providing the consent has been obtained.
Primary variable(s)	Part 1 (CHICO) The primary efficacy variable will be sero-reduction or sero-conversion at 12 months post-treatment using two conventional ELISA serology tests as the measure of efficacy. ²⁴ Part 2 (CHICO SECURE) The primary efficacy variable will be the incidence rate of seronegative conversion in subjects who were randomized and received at least one dose of the 60-day nifurtimox treatment, measured and confirmed by two types of assay (recombinant ELISA and IHA). Both test results should be negative for the subject to be considered as seroconverted.
Time point/frame of measurement for primary variable(s)	Part 1 (CHICO): 12 months post-treatment Part 2 (CHICO SECURE): 48 months post-treatment

²⁴ Text modified per Modification 2 (Amendment 1)

Plan for statistical analysis	<p data-bbox="502 250 699 291">Part 1 (CHICO)</p> <p data-bbox="502 291 783 331"><u>Primary efficacy analysis</u></p> <p data-bbox="502 331 1369 465">The difference in the proportion of nifurtimox subjects with sero-reduction or sero-conversion (60-day regimen) and the proportion estimated from historical data will be tested using a 2-sided 95% confidence interval (CI) for a single proportion.²⁵</p> <p data-bbox="502 465 1369 600">Superiority will be confirmed if the lower limit of the CI for the proportion of nifurtimox subjects (60-day regimen) with sero--reduction or sero-conversion is greater than the proportion of historical placebo subjects with sero-conversion.²⁶</p> <p data-bbox="502 600 810 640"><u>Secondary efficacy analysis</u></p> <p data-bbox="502 640 1369 775">A secondary analysis will be done to compare the proportion of subjects with sero-reduction or sero-conversion for the 60-day and 30-day nifurtimox regimens. This will be performed using a 2-sided 95% CI for the difference of two independent proportions.²⁷</p> <p data-bbox="502 775 1369 842">The analyses specified above will also be done using data from the EOT visit, and 3 and 6 months after EOT.</p> <p data-bbox="502 842 1369 999">The relationship of conventional serology results to qPCR results will be done using frequencies of matches and mismatches on the determination of disease status, and phi-correlation and kappa coefficient to assess the degree of agreement. The relationship of non-conventional serology to conventional serology will be analyzed in the same way.²⁸</p> <p data-bbox="502 999 1369 1133">The primary efficacy analysis will be done using the full analysis set, which is the set of subjects who received at least one dose of study drug. Analyses will also be done using the per-protocol set, which is comprised of subjects treated with study drug who have no major protocol deviations.</p> <p data-bbox="502 1133 1369 1267">According to Guhl et al. (2004), nifurtimox 12 months post- treatment cure rate is about 55%, with a sample size of 200 for the 60-day regimen, the power is 99% for the lower limit of the 95% CI to be greater than 16%, a performance goal based on historical study.²⁹</p> <p data-bbox="502 1301 810 1341">Part 2 (CHICO SECURE)</p> <p data-bbox="502 1346 783 1386"><u>Primary efficacy analysis</u></p> <p data-bbox="502 1386 1369 1487">The difference in the incidence rate of nifurtimox subjects with seronegative conversion (60-day regimen) and the rate estimated from historical data will be tested using a Poisson two-sided 95% confidence interval (CI).</p> <p data-bbox="502 1487 1369 1621">The incidence rate of seronegative conversion for the primary efficacy variable will be modelled using a Poisson distribution with a 2-sided 95% CI. The rate will be estimated as the number of seronegative conversion divided by the time at risk of event (person-year) during the study.</p> <p data-bbox="502 1621 1369 1794">This study uses an external control group of untreated patients with Chagas' disease in the 4-year follow-up period presented in Sosa et al. [15, 35]. In the reference, seroconversion to a negative result in the placebo group after 4-year follow-up was detected in 2 subjects with conventional serology and in 0 subjects with IHA test. Thus, none of the subjects in the placebo group was</p>
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²⁵ Text modified per Modification 2 (Amendment 1)

²⁶ Text modified per Modification 2 (Amendment 1)

²⁷ Text modified per Modification 2 (Amendment 1)

²⁸ Text modified/added per Modification 2 (Amendment 1)

²⁹ Modified as per amendment 2

considered as seroconverted by both test results. The person time at risk was on average $44 * 4 = 176$ person years. The incidence rate estimate was 0% (0 / 176).

Superiority will be confirmed if the lower limit of the CI for the incidence rate of seronegative conversion for subjects in 60-day regimen is greater than the rate of historical placebo subjects with sero-conversion (i.e. 0 %).

Secondary efficacy analysis

The OD values from recombinant ELISA and total purified antigen ELISA will be analyzed descriptively. Changes from baseline will be summarized to show any serial reduction in OD. Baseline is defined as the OD values from the same ELISA tests measured at Visit 1 in part 1 of the study.

The proportion of subjects who show both seronegative conversion as confirmed by two assays (recombinant ELISA and IHA) and no evidence of established cardiomyopathy as measured by ECG will be calculated overall and by treatment regimen.

The same method for calculating incidence rate and its 2-sided 95% CI will be applied on seronegative conversion in subjects who were randomized and received at least one dose of 30-day nifurtimox treatment.

Protocol amendment summary of changes table

Amendment no. 10 (03 NOV 2020)

Overall rationale for the amendment

This amendment was prepared 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 impact the benefit-risk evaluation of the study.

Section # and name	Description of change
Section 1 Title page	Updated to indicate that this is an electronically generated document that does not bear any sponsor signatures. Removed page for Signature of the sponsor's medically responsible person.
Section 2 Synopsis Section 4.2 Objectives for part 2 (CHICO SECURE)	Exploratory objective added to assess the proportion of responders who show seronegative conversion and the incidence of seronegative conversion as confirmed by non-conventional serology in subjects who were randomized and received at least one dose of either 60- or 30-day nifurtimox treatment regimens.
Section 9.4 Efficacy Section 10.3.1.2 part 2 (CHICO SECURE)	Addition of efficacy variable: proportion of responders who show seronegative conversion and the incidence of seronegative conversion as confirmed by non-conventional ELISA in subjects who were randomized and received at least one dose of either 60- or 30-day nifurtimox treatment regimens.
Section 2 Methodology Section 5.2 Part 2 (CHICO SECURE) Section 9.1 Table 9-2: Schedule of procedures and assessments in Part 2 – Study 16027 Section 9.2.4.2 Annual study visits (FU Visits 1, 3, and 5) Section 9.4.2 Serological Tests	Addition of non-conventional serological test (ELISA F-29).
Section 10.3.2.2 Part 2 (CHICO SECURE)	Analysis of the secondary and exploratory efficacy variables was updated to include non-conventional serology.

Typographical, grammatical, and other minor edits are not identified.

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List of abbreviations³⁰

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
β-hCG	beta-human chorionic gonadotropin
BP	blood pressure
CI	confidence interval
C _{max}	maximum concentration
CCDS	company core data sheet
CRO	Contract Research Organization
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
ECG	electrocardiogram
eCRF	electronic case report form
EDTA	ethylenediaminetetra-acetic acid
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immune sorbent assay
EOT	end-of-treatment
FAS	full analysis set
FDA	US Food and Drug Administration
FU	follow up
GCP	Good Clinical Practice
GEB	Guanidine-EDTA-Blood
GMP	Good Manufacturing Practice
IAC	internal amplification control
ICH	International Conference on Harmonisation
IEC	independent ethics committee

³⁰ Abbreviations added or deleted based on changes to text throughout Synopsis and body of protocol; qualitative PCR changed to quantitative PCR (Amendment 1) and Amendment 6.

List of abbreviations³⁰

IHA	Indirect hemagglutination assay
IgG	Immunoglobulin G
INR	international normalized ratio
IRB	institutional review board
IVRS/IWRS	Interactive Voice Response System/Interactive Web Response System
LLN	lower limit of normal
LTFU	long-term follow-up
LSLV	Last subject last visit
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
OD	optical density
PAHO	Pan American Health Organization
PD	pharmacodynamics
PK	pharmacokinetics
PPS	Per protocol set
PT	prothrombin time
PTT	partial thromboplastin time
QC	quality control
qPCR	quantitative polymerase chain reaction
RBC	red blood cell
RPM	revolutions per minute
RR	respiratory rate per minute
SAE	serious adverse event
SAP	statistical analysis plan
SAS	Statistical Analysis System
SUSAR	suspected, unexpected, serious adverse reactions
<i>T. cruzi</i>	<i>Trypanosoma cruzi</i>
WBC	white blood cell
WHO	World Health Organization

3. Introduction

Background

American trypanosomiasis (Chagas' disease), first described by Carlos Chagas in 1909, is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) and is a vector-borne disease transmitted to humans by large, blood-sucking reduviid bugs (e.g., *Triatoma infestans*, *Rhodnius prolixus*, and *Triatoma dimidiata*) infected with the parasite. Other possible mechanisms of transmission include blood transfusions, congenitally-transmitted or acquired infection, infected solid organ or bone marrow transplants, ingestion of contaminated food or drink, or laboratory accidents.

In 2006, the Pan American Health Organization (PAHO) estimated the prevalence of Chagas' disease in 21 endemic Latin American countries to be approximately 8 million infected persons, with nearly 100,000 persons at risk for infection. The number of new cases per year was calculated to be 55,185, of which 40,800 (74%) were vector-related infections and 14,385 (26%) were congenitally acquired infection.[1] Long considered to be endemic in the Latin American and Caribbean region, there is increasing evidence to suggest that Chagas' disease is becoming a more global disease. Some estimate the prevalence of *T. cruzi* infection in Latin American-born persons living in the United States to be just over 300,000 infected individuals (1.31%)[2], while others suggest that the prevalence may be even higher (1 million cases) with an especially high burden of disease in Texas and along the Gulf of Mexico coast.[3] Further evidence of the globalization of Chagas' disease and confirmation that the *T. cruzi* parasite can travel from endemic to non-endemic countries is the data from PAHO that estimates the number of infected persons to be more than 80,000 in Europe, 5,500 in Canada, 3,000 in Japan, and 1,500 in Australia. In North America (which includes Canada, Mexico, and the United States), researchers have estimated the number of seropositive mothers and newborns infected with *T. cruzi* to be approximately 40,000 and 2,000, respectively.[4] Clearly, Chagas' disease can no longer be considered just a zoonotic disease that affects only poor rural communities in Latin America. It has become a worldwide concern with severe public health consequences over the long term.

The natural history of the disease following exposure to the parasite begins with an acute phase that may be completely asymptomatic. If symptoms occur, they are usually a self-limiting febrile illness that occurs 1 to 2 weeks after exposure to triatomine bugs, or up to a few months after transfusion of infected blood. Manifestations of the acute disease resolve spontaneously in about 90% of infected individuals even if the infection is not treated with trypanocidal drugs. Death may occur during the acute phase in 5% to 10% of symptomatic patients, usually the result of severe myocarditis and/or meningoencephalitis. About 60% to 70% of these individuals will never develop clinically apparent disease. These patients have the indeterminate form of chronic Chagas' disease. The remaining 30% to 40% of patients will go on to develop a determinate form of chronic disease manifested by cardiac disorders (e.g., conduction abnormalities, bradyarrhythmias and tachyarrhythmias, apical aneurysms, heart failure, thromboembolism and sudden death) or digestive disorders (e.g., megaesophagus, megacolon), or a combination of cardiodigestive disorders 10 to 30 years after the initial infection. Gastrointestinal dysfunction develops in 10% to 15% of chronically infected patients. The cardiac form of the disease is the most serious, and develops in about 20% to 30% of patients.

Most new infections occur in children younger than 15 years of age, either by vector or vertical transmission, with the highest frequency in young children aged 1 to 5 years.

Unfortunately, in most endemic areas, neither pregnant women nor newborns are routinely tested for *T. cruzi* infection at present. Congenital cases are frequently asymptomatic and, unless specific tests are ordered, the disease passes unnoticed. The acute phase of Chagas' disease can be particularly severe in children, with up to 10% of these patients dying of meningoencephalitis or myocarditis. Early diagnosis of congenital disease is essential for improving treatment outcomes, and research has shown that the disease is curable if treatment is initiated soon after infection.[5][6] Some of the clinical manifestations of congenital Chagas' disease are prematurity, low birth weight, low Apgar scores, hepatosplenomegaly, jaundice, edema, respiratory distress syndrome, and meningoencephalitis.

Comprehensive information on the study drug can be found in the latest available version of the Investigators' Brochure.³¹

Rationale of the study

Currently, only nifurtimox and benznidazole are approved in selected South American countries for the etiological treatment of Chagas' disease in children and adults. Nifurtimox was first introduced in 1965. It is approved in Argentina, Chile, El Salvador, Guatemala, Uruguay, and Honduras.

Recently, benznidazole has been approved in the United States for treatment of Chagas' disease in children aged 2-12 years. These medications were developed in the late 1960s (nifurtimox) and early 1970s (benznidazole). Nifurtimox, a nitrofurans compound, acts through production of reduced oxygen metabolites (e.g., superoxide and hydrogen peroxide), for which the parasites have lower detoxification capacity, as compared with vertebrate cells. Benznidazole, a nitroimidazole derivative, is thought to act through covalent binding of nitroreduction intermediates to parasite molecules. Based on clinical study data, anti-trypanosomal drug treatment in children with chronic *T. cruzi* infection was accepted as the standard of care throughout Latin America by the late 1990s, followed in recent years by a growing movement to offer treatment to older patients. Because treatment is expected to reduce the probability of congenital transmission, stronger consideration may be warranted for women of reproductive age.

Bayer was granted Orphan Drug Designation of nifurtimox for treatment of Chagas' disease (American Trypanosomiasis) caused by *T. cruzi* pursuant to Section 526 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360bb) on 05 AUG 2010.

Nifurtimox has been classified by the World Health Organization (WHO) as a vitally important medicine and is included on the WHO Model List of Essential Medicines for Chagas' disease.

This study consists of two parts:

- Part 1 comprises the treatment with nifurtimox including the 1-year follow up (CHICO)
- Part 2 comprises the 3-year long-term follow-up (LTFU) of all subjects who were randomized and received at least one dose of their assigned nifurtimox treatment regimen in CHICO (CHICO SECURE)

³¹ Revised since Investigators' Brochure became available to serve as the reference document for nifurtimox (Amendment 1)

Part 1 (CHICO) was designed to develop a better understanding of the efficacy, safety/tolerability, and pharmacokinetics (PK) (absorption, distribution, metabolism, and elimination) of nifurtimox in children with a diagnosis of Chagas' disease using pediatric formulations. Results from this study will demonstrate parasitological cure using conventional serological results to allow optimization of treatment of Chagas' disease in this most vulnerable population.³² In turn, this will improve the outlook for children by reducing mortality and long-term complications. This knowledge will allow for better and more appropriate approaches to the treatment of Chagas' disease. It will also provide more up-to-date information on the use of nifurtimox, and has the potential to increase our understanding of treatment response in children.

Part 2 (CHICO SECURE) was designed, at the request of the US Food and Drug Administration (FDA), to assess the incidence of seronegative conversion in subjects who were randomized and received at least one dose of their assigned 60- or 30-day nifurtimox treatment regimen. In part 2 (CHICO SECURE), subjects will be followed up for 3 years totaling to a follow-up period of 4 years after end of nifurtimox treatment, the first year of follow up is included in the design of part 1 (CHICO).

Conversion to negative serology is currently the only test available to assess parasitological cure. However, this seronegative conversion can take years to decades after treatment. There is evidence suggesting that the time to seronegative conversion is known to be inversely proportional to the pretreatment duration of infection. In the chronic stage serological conversion from positive to negative can be slow and can take years to decades [29, 30].

Benefit-risk assessment

Since 1991, the estimated global prevalence of *T. cruzi* infection has decreased from 18 million to 8 million due to intensive vector control and blood bank screening. The PAHO estimates that approximately 60,000 new *T. cruzi* infections occur each year [1]. As other transmission routes have diminished, the proportion of infections attributable to congenital infection has increased. An estimated 26% of incident infections now occur through mother-to-child transmission. Mortality among infected infants is significantly higher than in uninfected infants, ranging from less than 5% to 20% in published studies [7][8]. However, even severe congenital Chagas' disease may not be recognized because signs are often nonspecific, or because the diagnosis is not considered. Children younger than 2 years of age appear to be at higher risk of severe manifestations than older individuals. Severe acute Chagas' disease carries a substantial risk of mortality [8].

In *T. cruzi* infection, treatment with nifurtimox reduces the severity of symptoms, and shortens the clinical course and duration of detectable parasitemia. Parasitological cure is reported in 60% to 85% of patients treated in the early phase. Furthermore, published evidence suggests that if the duration of infection is less than 1 year, the percentage of patients cured (i.e., elimination of parasites) approaches 100% [5][6].

Nifurtimox is well tolerated in children, but its pharmacology in the pediatric population is still not well defined. The currently available marketed product was developed for adults, and there is a significant need for a pediatric formulation particularly for infants and newborns to support weight-adjusted dosing.³³ The safety profile of the drug is relatively well-

³² Text modified for clarity (Amendment 1)

³³ Text modified for clarity, and per changes as a result of Amendment 1

characterized. Adverse drug reactions are moderately common in adults, but significantly less in children. Nifurtimox causes gastrointestinal side effects in 50% to 75% of adult patients. These side effects include anorexia, nausea, vomiting, and abdominal discomfort. Mild neurologic toxicity is common (occurring in up to 50% of adult patients), including mainly headache, but also (less commonly) irritability, insomnia, disorientation, mood changes, paresthesias, and tremors. Peripheral neuropathy is a rare side effect in adults. Leukopenia is a very rare side effect [9].

Safety of nifurtimox in the pediatric population is well established. The drug has been used off-label in children for over 40 years. Adverse drug reactions are commonly milder and less frequent than those observed in adults. Studies performed to evaluate the effect of nifurtimox in pediatric oncological disease suggest safety even at super-therapeutic doses (e.g., 30 mg/kg/day) [10]. Case series of pediatric Chagas' patients support the safety of the drug [11].

Consensus documents from the WHO, and the majority of Latin American countries (i.e., Argentina, Brazil, Bolivia) [12][13][14] strongly recommend anti-trypanosomal treatment for acute, congenital, and reactivated *T. cruzi* infection, and for children with chronic infection. Given that Chagas' disease is an infection commonly acquired in childhood that leads to significant morbidity in adults, the benefits of treatment with nifurtimox in infants and children (to prevent long-term complications) far outweigh the small risks of AEs associated with the medication in children.

For the monitoring of efficacy of antitrypanosomal treatment, the criterion of cure usually depends on serologic conversion of anti-*T. cruzi* antibody response to negative. Sero-conversion in children and adolescents may occur several years after treatment, requiring long-term follow-up. Therefore, subjects who were randomized and received at least one dose of their assigned nifurtimox treatment and did not receive additional treatment for Chagas' disease after completion of nifurtimox treatment will be followed up for a total of 4 years after end of nifurtimox treatment.

The overall benefit-risk assessment for the LTFU is considered favorable based on the available data. It is expected that the information will help to gain further knowledge about the serological response after treatment with nifurtimox in Chagas' disease.

4. Study objectives

4.1 Objectives for part 1 (CHICO)

The primary objective of this Phase 3 clinical study is:

- 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 $\geq 20\%$ reduction in optical density [OD] measured by conventional enzyme-linked immune sorbent assay [ELISA]) compared to baseline in subjects ≥ 8 months to <18 years of age at randomization; or sero-conversion (defined as negative Immunoglobulin G [IgG] concentration) in all subjects³⁴

Secondary objectives of the study are:

³⁴ Text modified as per amendment 2

- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox as sero-reduction or sero-conversion at the 12-month follow-up (360 days from EOT)³⁵
- To evaluate the safety/tolerability profile of nifurtimox by laboratory parameters (hematology, blood chemistry, urinalysis), electrocardiogram (ECG) monitoring, vital sign measurements (blood pressure, heart rate, respiratory rate, temperature), adverse event (AE) monitoring, and physical examinations, including neurological examinations³⁶
- To evaluate the pharmacokinetics (PK)/pharmacodynamics (PD) of nifurtimox in children receiving the drug for treatment of Chagas' disease (to be described in a separate report)

Exploratory objectives of the study are:³⁷

- To assess the comparability of a 60-day regimen of nifurtimox to historical active control (benznidazole)³⁸ as sero-reduction or sero-conversion at the 12-month follow-up (360 days from EOT)³⁹
- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox using quantitative polymerase chain reaction (qPCR) at the 12-month follow-up (360 days from EOT)⁴⁰
- To evaluate the relationship of conventional serology (as sero-reduction or sero-conversion) to qPCR using frequencies of matches and mismatches to assess agreement⁴¹
- To evaluate the relationship of non-conventional serology to conventional serology⁴²
- To evaluate the relationship of conventional serology to IHA^{43,44}

4.2 Objectives for part 2 (CHICO SECURE)

The primary objective of the LTFU part of the study is:

- Assess the incidence of seronegative conversion as confirmed 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 untreated patients with Chagas' disease at the 4-year follow-up.

³⁵ Text modified per Modification 2 (Amendment 1)

³⁶ Text added to include neurological examinations per Modification 10 (Amendment 1)

³⁷ Exploratory objectives added as per Amendment 2

³⁸ Sero-conversion for the historical controls will be estimated from cure rates as presented in two publications.[15][16] See Section 5, Study Design, Justification of the design.

³⁹ Text modified per Modification 2 (Amendment 1)

⁴⁰ Text modified per Modification 2 (Amendment 1)

⁴¹ Text modified per Modification 2 (Amendment 1)

⁴² Fourth objective added per Modification 2 (Amendment 1)

⁴³ IFA test added per Amendment 5.

⁴⁴ Replaced IFA with IHA per Amendment 6.

The secondary objectives are:

- Assess the incidence of seronegative conversion, as confirmed by two types of assay (recombinant ELISA and IHA), in subjects who were randomized and received at least one dose of the 30-day nifurtimox treatment regimen at the 4-year follow-up.
- Evaluate the proportion of responders who show both seronegative conversion as confirmed by two types of assay (recombinant ELISA and IHA) and no evidence of established cardiomyopathy as evaluated in electrocardiogram (ECG) recordings.
- Assess serial reduction in OD measured by recombinant ELISA and total purified antigen ELISA, in subjects who were randomized and received at least one dose of either the 60- or 30-day nifurtimox treatment regimen compared to Visit 1 in part 1 of the study.

The exploratory objectives are:

- Assess the incidence of seronegative conversion as confirmed by two types of assay (recombinant ELISA and IHA) in subjects by age categories (≤ 2 years, > 2 years to ≤ 6 years, > 6 to ≤ 12 years, > 12 to < 18 years; age is defined as subject's age at randomization).
- Assess the proportion of responders who show seronegative conversion and the incidence of seronegative conversion as confirmed by non-conventional serology in subjects who were randomized and received at least one dose of either 60- or 30-day nifurtimox treatment regimens.
- Assess the occurrence of congenital infection with *T. cruzi* in children born of female subjects who were randomized and received at least one dose of 60- or 30-day nifurtimox treatment regimen. The congenital infection will be confirmed by parasitological method in children ≤ 8 months of age and by serological method in children > 8 months of age.

5. Study design

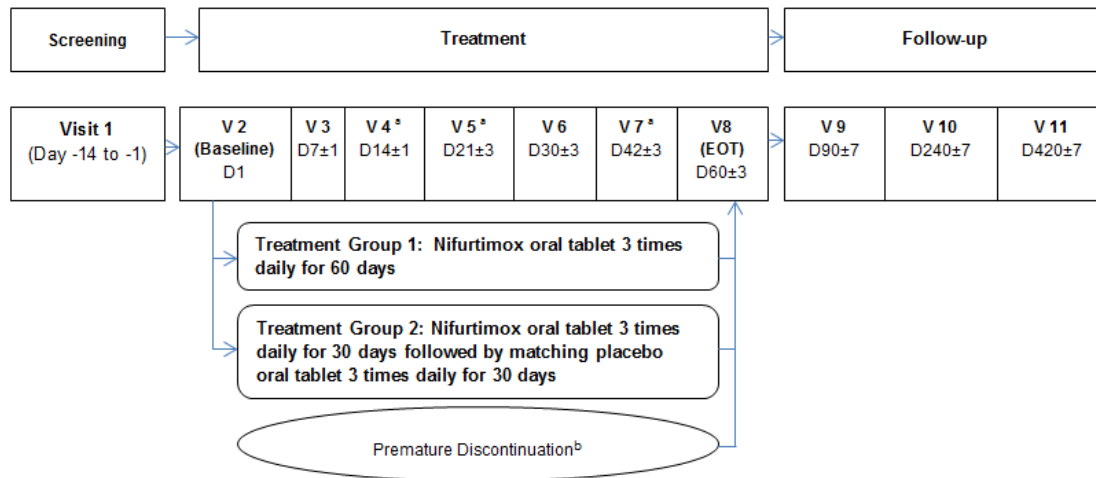
5.1 Part 1 (CHICO)

Design overview

This is a Phase 3, prospective, randomized (to dosing regimen), age-stratified, double-blind, parallel-group study to evaluate the efficacy, safety/tolerability, and PK of oral administration of nifurtimox in children with a diagnosis of Chagas' disease.

A schematic of part 1 (CHICO) of the study design is presented in [Figure 5–1](#).

Figure 5–1: Study Design of Part 1 – Protocol 16027⁴⁵



D = Day, EOT = end-of-treatment, V = Visit.

a At Visits 4, 5, and 7, study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, and compliance with study drug administration. If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

b Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visits 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (±3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments.

This study will enroll pediatric subjects aged 0 to younger than 18 years. Subjects < 8 months of age at randomization with a diagnosis of Chagas' disease must demonstrate direct observation of *T. cruzi* by concentration test, and subjects ≥ 8 months to < 18 years of age at randomization with a diagnosis of Chagas' disease must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:⁴⁶

- Recombinant ELISA
- Total purified antigen ELISA

Additionally, a non-conventional ELISA test and IHA ^{47,48} will be obtained; however, a positive result will not be required for diagnosis.

Subjects will also be eligible if there is acceptable documentation of positive Chagas' disease within three months prior to screening.⁴⁹

⁴⁵ Figure modified as a result of changes made to the protocol per Amendment 1; Visit 4 changed to 14 ± 1 day; Visit 5 was made a telephone assessment at 21 ± 3 days; visits renumbered from that point forward; footnote b deleted; footnote c re-lettered to footnote b; text to new footnote b changed per Modification 11 (Amendment 1)

⁴⁶ Text modified for clarity (Amendment 1)

⁴⁷ IFA test added per Amendment 5.

⁴⁸ Replaced IFA with IHA per Amendment 6.

⁴⁹ Text modified/added per Modification 2 (Amendment 1)

Approximately 300 pediatric subjects will be randomized (2:1 randomization, 60-day regimen vs. 30-day regimen).⁵⁰ Subjects will be stratified by age at randomization into four strata as specified below:⁵¹

Stratum 1: ⁵²	0 to 27 days
Stratum 2:	28 days to younger than 8 months
Stratum 3:	8 months to younger than 2 years
Stratum 4:	2 years to younger than 18 years

A minimum of 38 subjects in each age stratum is targeted, but not required, in order to be able to derive meaningful safety conclusions. Enrollment may⁵³ continue until this specification is met, unless it is determined that such a target would be unlikely to be reached in a reasonable time.⁵⁴

No study-specific procedures will be performed before the subject/legally authorized representative has signed the informed consent form, or a minor subject has provided assent, including asking a potential subject to fast (if applicable, depending on the age of the subject at the discretion of the investigator) prior to the screening blood samples. Informed consent/assent for optional PK blood sampling will also be obtained. After the consents/assents have been obtained, screening assessments will be performed from 1 to 14 days prior to randomization at Visit 2.⁵⁵

After study eligibility has been confirmed and safety assessments have been performed at Visit 2 (Day 1),⁵⁶ subjects will be randomized via Interactive Voice Response System/Interactive Web Response System (IVRS/IWRS) in a 2:1 ratio (60-day regimen vs. 30-day regimen) to one of two treatment groups:

- Nifurtimox tablets administered three times daily for 60 days (Days 1 – 60, active nifurtimox treatment, Treatment Group 1) or
- Nifurtimox tablets administered three times daily for 30 days, followed by nifurtimox placebo administered three times daily for 30 days (Days 1 – 30, active nifurtimox treatment; Days 31 – 60, placebo; Treatment Group 2).

The first dose of study drug will be administered at Visit 2. Pre- and post-dose PK blood samples will be obtained at specified time points from those subjects consenting to PK assessments (see [Table 9–1](#)). At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of maximum concentration [C_{max}]), an ECG (optional for subjects < 5 years of age at the discretion of the investigator) will be obtained to allow for PK/PD investigations. Study drug

⁵⁰ Modified as per amendment 2

⁵¹ Text modified for clarity (Amendment 1)

⁵² Cohort replaced with stratum throughout body of protocol as per amendment 2

⁵³ Text modified as per amendment 4

⁵⁴ Text added as per amendment 2

⁵⁵ Text added for clarity (Amendment 1)

⁵⁶ Text modified for clarity (Amendment 1)

will be dispensed, instructions for study drug administration will be provided, and diaries with instructions for completion will be given⁵⁷, to all subjects.⁵⁸

Subjects will return to the investigational site for efficacy and safety assessments at Visits 3 (Day 7±1) and 6 (Day 30). Subjects who have not consented to PK assessments will take that day's doses of study drug as instructed. Subjects consenting to PK assessments must hold the morning dose of study drug; for these subjects, a pre-dose PK blood sample will be obtained, then the morning dose of study drug will be administered with food and the PK diary completed.⁵⁹ Post-dose blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point (see [Table 9–1](#)). A pre-paid phone card will be provided to all subjects to facilitate contact between study site personnel and the subjects/subjects' authorized representatives.⁶⁰

At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment, along with subject diaries, will be dispensed.⁶¹

Visits 4 (Day 14), 5 (Day 21), and 7 (Day 42) will be telephone assessments where study site personnel will contact the subject/legally authorized representative via telephone to assess the occurrence of AEs, use of concomitant medications, and compliance with study drug administration, and completion of subject diary. A Phone Contact Form for the telephone assessments will be provided to all sites (see Section [16.1](#)).⁶²

Subjects will return to the investigational site for efficacy and safety assessments on Day 60 (Visit 8), which will be the EOT for both treatment groups. Subjects who have not consented to PK assessments will take that day's doses of study drug as instructed. Subjects consenting to PK assessments must hold the morning dose of study drug. A pre-dose PK blood sample will be collected, then the morning dose of study drug will be administered with food, and the subject will complete the PK diary. Post-dose PK blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point (see [Table 9–1](#)). Study drug will be collected, and no additional study drug will be dispensed.⁶³

After the EOT visit (Visit 8), subjects will return to the site on Days 90 (Visit 9), 240 (Visit 10), and 420 (Visit 11) for additional efficacy and safety assessments. The total duration of each subject's participation is expected to be approximately 14 months.⁶⁴

Assessments of efficacy and PK will be performed at specified time windows. In order to minimize the burden of blood loss due to PK sampling, age-appropriate sparse sampling strategies combined with (micro-)bioanalysis techniques will be applied (see [Table 9–5](#)). At

⁵⁷ Text modified as per amendment 4

⁵⁸ Text added for clarity and per Modification 1 (Amendment 1)

⁵⁹ Text modified as per amendment 4

⁶⁰ Text modified/added for clarity and per Modification 1 (Amendment 1)

⁶¹ Text added for clarity (Amendment 1)

⁶² Text added for clarity and per Modifications 6 and 8 (Amendment 1)

⁶³ Text modified/added for clarity and per Modification 1 (Amendment 1)

⁶⁴ Text modified for clarity (Amendment 1)

least 10 subjects per age stratum should ideally be recruited for PK assessments.⁶⁵
Recruitment of less than 10 subjects per age group is not considered as a protocol violation.⁶⁶

Safety will be assessed via laboratory parameters (hematology, blood chemistry, urinalysis), vital sign measurements (blood pressure [optional in subjects < 5 years of age at the discretion of the investigator], heart rate, respiratory rate, temperature), monitoring of ECGs (optional in subjects < 5 years of age at the discretion of the investigator), monitoring of AEs, and physical examinations, including a neurological examination (see Section 9.6.3.3).⁶⁷

Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visits 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (\pm 3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments.⁶⁸

Also, for reasons of patient safety and at the discretion of the investigator, a subject may be asked to take a “drug holiday” [interruption of study drug administration] or a reduced daily dosage may be requested by the investigator from the depot. A decision by the investigator for drug holiday or reduced dosage will be based on the occurrence and/or severity of adverse effects, and/or the clinical status of the subject. Any and all changes to study drug administration or daily dosage must be recorded within the subject’s study chart, as well as, the electronic case report form (eCRF).⁶⁹

Primary variable(s)

The primary efficacy variable will be sero-reduction or sero-conversion at 12 months post-treatment using two conventional ELISA serology tests as the measure of efficacy.⁷⁰

Justification of the design

This study uses an historical untreated-controlled design, as it is unethical to have a prospective placebo-controlled superiority study design. Only two benznidazole placebo-controlled studies have been conducted in children with Chagas’ disease.[15][16] Direct comparisons between these studies cannot be made due to differences in study populations (Argentina vs. Brazil), medication dosage (5 mg/kg vs. 7.5 mg/kg), outcome measures, and time of follow-up (4 years vs. 3 years). Data derived from these studies will, however, be used as the comparative historical placebo control to be utilized in the analysis of the study endpoints. Hence, a comparison of sero-reduction and/or sero-conversion from baseline to 12-month follow-up for both primary and secondary endpoint evaluations (versus

⁶⁵ Text deleted/added for clarity (Amendment 1)

⁶⁶ Modified as per amendment 4

⁶⁷ Text modified/deleted for clarity, and per Modifications 9 and 10 (Amendment 1)

⁶⁸ Text added per Modification 11 (Amendment 1)

⁶⁹ Text modified as per amendment 4

⁷⁰ Text modified per Modification 2 (Amendment 1)

historical placebo) will be made as well as for a comparison with benznidazole. Details are presented in Section 10.⁷¹

There are several choices for diagnosis of Chagas' disease and monitoring of drug efficacy after anti-parasitic treatment. Traditional parasitological methods, such as direct microscopic observation, xenodiagnoses, and hemoculture, show low sensitivities, limiting their usefulness. Microhematocrit is the method of choice to identify congenital infection because of its heightened sensitivity and the small amount of blood needed. Microscopic examination of cord blood or peripheral blood of the neonate by this technique is strongly recommended during the first month of life. For the monitoring of drug efficacy, the criterion of cure usually depends on serologic conversion of the anti-*T. cruzi* antibody response to negative. Sero-conversion in children and adolescents may occur several years after treatment, requiring long-term follow-up.

Sero-reduction (measured as a $\geq 20\%$ reduction of the ELISA absorbance [OD] value) at 12 months post-treatment will be considered equivalent to historical information on sero-conversion at subsequent time points after treatment.⁷²

Both primary and secondary study endpoints are based upon a target of $\geq 20\%$ decrease in antibody concentration (sero-reduction) to *T. cruzi* in conventional serological tests or negativization of serology. This is consistent with one previous study in children 6 to 12 years of age infected with *T. cruzi* in the intermediate (early chronic) phase of Chagas' disease [15]. Subjects were administered 5mg/kg benznidazole or placebo in a randomized fashion for 60 days, and followed for 48 months. At 12 months, a 21% reduction in concentration of Chagas' disease-specific antibodies compared to baseline (ELISA mean OD 0.467 [± 0.099] vs. 0.369 [± 0.107]) was observed in subjects who received benznidazole. For the subsequent time points of 18, 24, and 48 months, percent concentration reductions in antibody titer were reported as 23%, 29%, and 27%, respectively. After a 48-month follow-up, negative sero-conversion was detected in 11.3% ($P < 0.05$) of the benznidazole group and only 4.5% of the placebo group.⁷³

The above data are comparable to a study in early chronic Chagas' patients treated with 7.5 mg/kg benznidazole or placebo for 60 days, which also showed approximately 20% antibody concentration reduction at 12 months [16]. Analysis of concentration over time indicated a consistent decrease in antibody concentrations in the benznidazole group, and an initial decrease followed by a progressive increase in the placebo group. At the end of the 3-year follow-up, children who received benznidazole had a significantly lower mean antibody concentration than placebo-treated children ($P < 0.00001$). As persistence of positive results by conventional serology for years after treatment is common, these tests may or may not revert to sero-negative (sero-conversion) after many years or even decades. Thus, reduction in *T. cruzi* antibody concentration has been used in clinical practice as a surrogate indicator for parasitological cure (sero-conversion) following anti-trypanosomal treatment.⁷⁴

While a decrease $\geq 20\%$ in antibody concentration has been demonstrated across two independent clinical trials in pediatric patients with Chagas' disease, neither author has

⁷¹ Text modified for clarity (Amendment 1)

⁷² Text modified for clarity (Amendment 1)

⁷³ Text deleted for clarity as of amendment 2

⁷⁴ Text deleted as of amendment 2

published extended follow-up data which include serological results for the patients in these studies. Since signs and symptoms are usually lacking in early Chagas' disease, it is not possible to demonstrate immediate meaningful clinical benefit to active treatment. However, significant reductions in *T. cruzi* antibody concentration (sero-reduction) by conventional serology has been accepted as a predictor of future sero-conversion, and early treatment cure of infection will ultimately reduce the risk of developing visceral abnormalities, as well as contribute to the decrease in *T. cruzi* transmission.⁷⁵

Alternative and more rapid approaches as an early indicator of negative sero-conversion in treated patients have been suggested. Using non-conventional serological techniques, i.e., highly sensitive and specific chemiluminescent ELISA using a purified trypomastigote glycoconjugate antigen and an epimastigote complex [20], the time to negativization was significantly sooner for the non-conventional ELISA than for the conventional ELISA. The chemiluminescent ELISAs provide tests that are highly sensitive and specific for Chagas' disease diagnosis. They can be used in blood bank screening and to monitor the treatment of patients undergoing chemotherapy [21].⁷⁶

Non-conventional ELISA antigens, such as those used in the Sosa-Estani and deAndrade trials (i.e., F29 and F 2/3), [15][16] are often considered as early markers for effective Chagas' disease therapy. In the study by Altcheh, et al, the kinetics of disappearance of conventional serology and anti-F2/3 antibodies were compared in 21 patients with congenital Chagas' disease after receiving benznidazole treatment. Patients were divided into two groups: (A) age < 8 months at diagnosis and (B) age > 9 months at diagnosis. Group A presented negative outcome for conventional serology at 6.6 months (CI 95 3.4-9.8 months) and for anti-F2/3 at 4 months (CI 95 0.9-7.1 months, p = 0.18). Group B exhibited non-reactive conventional serology at 63.1 months (CI 95 42.1-84.2 months) whereas anti-F2/3 antibody determination became negative at 21.9 months (CI 95 5.7-38.1 months, p = 0.0025). In patients belonging to Group A, antibodies were undetectable by both conventional serology and anti-F2/3 ELISA soon after receiving chemotherapy. In infants included in Group B, a negative result for anti-F2/3 antibody detection occurred significantly in advance of negative conventional serology reactivity. Consequently, the anti-F2/3 antibody assay becoming negative should be considered as a surrogate endpoint for assessment of cure or positive response to treatment, particularly in those patients with prolonged time of infection [18].

A new perspective on Chagas' disease diagnosis was opened by the use of the PCR as a measure of treatment response. Over the past few years, the availability of PCR to detect *T. cruzi* DNA in blood samples has opened new possibilities for the evaluation of response to trypanocidal chemotherapy.⁷⁷

Clearance of parasitemia (evaluated most commonly by xenodiagnoses or PCR) combined with disappearance of antibodies (seronegative conversion) has been proposed as cure criteria by some authors.[19] The main limitations in evaluating treatment response for Chagas' disease using conventional serology stems from the need for long-term follow-up (years to decades) to observe negativization of conventional serological tests.⁷⁸

⁷⁵ Term "titer(s)" replaced with "concentration(s)"; text modified for clarity (Amendment 1)

⁷⁶ Paragraph added to include updated information (Amendment 1)

⁷⁷ Paragraphs added to include updated information (Amendment 1)

⁷⁸ Text deleted from end of paragraph and moved to appear earlier in this section (Amendment 1)

In the experience of Parasitology service, Ricardo Gutierrez Children's Hospital, Buenos Aires unpublished data (presented at European Society of Pediatric Infectious Disease 2014 and meeting of Food and Drug Administration) by PPD, a correlation between negativization of conventional serology (whole antibody against *T. cruzi* measured by ELISA or hemoagglutination inhibition) and PCR results in children 2 years of age and younger is fairly high; the correlation between these two tests in children older than 3 years is lower, as sero-conversion (i.e., becoming negative) may take several years, while PCR becomes negative within 60 days of treatment, and remains negative in this age group also. In the whole population, at least 20% sero-reduction of conventional serology was observed at 12 months. This percentage was higher in the younger children than in the older children.⁷⁹

By comparing 48 PCR methods from 26 laboratories, it was shown that the four best performing methods showed a high sensitivity (10 fg/μl DNA and 0.5 parasites/mL blood), and an overall accuracy of 87% to 90% [22]. More recent publications showed comparable sensitivity [23][24].

Several studies concluded that PCR has a higher sensitivity for detecting therapeutic failures compared to usual parasitological and conventional serologic methods. However, only two demonstrated specificity and showed that PCR could be used as a tool for early determination of cure [25][26].

Furthermore, real-time PCR (qPCR) protocols for detection of *T. cruzi*'s DNA were developed. The development of qPCR assays have several advantages compared with conventional PCR. First, the introduction of closed-tube qPCR systems has resulted in the development of rapid microbial diagnostics with low contamination risk. Also, qPCR has a shorter time-to-result, and allows quantitative assays and automation [27].⁸⁰

Studies have demonstrated the substantial value of qPCR, in terms of sensitivity and specificity, in the direct parasitological diagnosis of Chagas' disease and follow-up assessment of chemotherapy. However, in this Phase 3 study, parasitological cure based on serological tests for *T. cruzi* infection (ELISA) will be used as the primary efficacy endpoint to demonstrate efficacy of nifurtimox due to lack of historical PCR data from untreated children with Chagas' disease.⁸¹ Other parasitological methods (direct microscopic) and qPCR tests will comprise other variables to demonstrate drug efficacy.⁸²

The 30-day and 60-day treatment regimens were selected with the intention of providing the most effective treatment within the shortest possible duration. During the 1st Chagas Platform Technical meeting (September 1, 2009, Rio de Janeiro)[28] it was concluded that the minimum length of treatment for nifurtimox should be 30 days which is based on good results with fewer than 60 days of treatment. Hence, the 30-day arm is included to evaluate the potential of this shorter regimen.⁸³ In a recent study from Bianchi et al, 62 patients with Chagas' disease confirmed by different serological tests were treated with nifurtimox (Lampit), and followed for 30 months post-treatment. All children were treated during 60 days according to protocols established by the WHO. Monitoring was performed every 20

⁷⁹ Paragraph moved from previous page to here; text deleted (Amendment 1)

⁸⁰ Text modified for clarity (Amendment 1)

⁸¹ Text added for clarity (Amendment 1)

⁸² Text deleted for clarity as of amendment 2

⁸³ Text added as per amendment 2

days to evaluate treatment safety. Results indicated that both parasite load (measured through qPCR) and antibodies (ELISA absorbance) showed a significant median reduction 6 months after treatment from 6.2 to 0.2 parasite equivalents/mL, and from 0.6 to 0.2 absorbance units respectively ($p < 0.001$). Further reductions were evidenced by the 30-month post-treatment time point. Sixty days of treatment with nifurtimox was very well tolerated and successfully reduced parasite load and antibody titers. The results show for the first time the therapeutic response and safety of nifurtimox treatment for *T. cruzi* infection in a population of school-aged children in the asymptomatic chronic phase of Chagas' disease in Colombia [29].⁸⁴

End of part 1 of the study

The end of part 1 of the study as a whole will be reached as soon as the last visit of the last subject has been reached in all centers in all participating countries.⁸⁵

5.2 Part 2 (CHICO SECURE)

Design overview

This is a prospective, long-term follow-up of subjects, who were randomized and received at least one dose of their assigned 60- or 30-day nifurtimox treatment. The study is designed to evaluate seronegative conversion and serial reduction in OD measured by ELISA.

Subjects will be followed up for a total of 4 years after end of nifurtimox treatment. The first year of follow-up period is included in part 1 and 3 years of follow-up are included in part 2 of the study.

The following age categories will be considered for part 2 of the study: ≤ 2 years, > 2 years to ≤ 6 years, > 6 to ≤ 12 years, > 12 to < 18 years (age is defined as subject's age at randomization).

Subjects enrolled will be followed annually with visits to the investigational site. Seronegative conversion will be measured by two types of assay, recombinant ELISA, IHA, and by non-conventional ELISA.

For assessment of serial reduction compared to baseline defined as screening visit of part 1 (Visit 1), OD will be measured by recombinant ELISA and total purified antigen ELISA.

No study-specific procedures will be performed before the subject and/or parent(s) or legally authorized representative(s) has signed the informed consent form, or a minor subject has provided assent for part 2 of the study. After the consents/assents have been obtained, the assessments scheduled for FU Visit 1 including eligibility assessments will be performed. (FU Visit 1 according to Table 9–2; for details please refer to Section 9.2).

The first visit should be performed as soon as possible after availability of regulatory and ethics committee approval for all subjects who have already completed part 1 of the study for 1 year \pm 6 weeks or longer. Subjects who are entering the study beyond the time window for FU Visit 1 (2 years \pm 6 weeks) will have their eligibility assessments as soon as possible provided that consents/assents have been obtained (see Table 9–2; for details please refer to Section 9.2).

Subjects will have annual visits at the investigational site for efficacy and safety assessments for 3 years. Timing of study visits is relative to end of treatment in part 1 of the study.

⁸⁴ Text added for clarity (Amendment 1)

⁸⁵ Text modified as per amendment 2

At each investigational site visit, blood samples for serology tests (recombinant ELISA, total purified antigen ELISA, IHA, and non-conventional ELISA) will be collected. Blood samples will be taken under fasting conditions, if applicable, depending on the age of the subject, at the discretion of the investigator. Subjects will undergo a physical examination, body weight, height/length and vital signs will be measured and AEs considered at least possibly related to nifurtimox and AEs caused by protocol-required procedures will be recorded. A 12-lead electrocardiogram (ECG) will be obtained at each annual visit. The subject's concomitant medications will be recorded. Signs and symptoms of Chagas' disease will be assessed and blood will be taken for detection of *T. cruzi*'s deoxyribonucleic acid (DNA) using quantitative polymerase chain reaction (qPCR). If during the course of the study, OD measured by ELISA increases, the subject has positive qPCR results and shows signs or symptoms of Chagas' disease, it is up to the investigator's discretion to initiate antitrypanosomal treatment while the subject continues in the LTFU.

Whenever an AE considered at least possibly related to nifurtimox or caused by protocol-required procedures occurs, the subject should report it to the investigator.

Female subjects who give birth before end of part 2 of the study will be invited to test their child for the presence or absence of infection with *T. cruzi*.

Between the annual visits at the investigational site, subjects and/or his or her parent(s) or legally authorized representative(s) will be contacted by qualified personnel by a method agreed (e.g., phone call) about 6 months after a visit at the investigational site to identify the occurrence of potential clinical symptoms of Chagas' disease or any pathology mentioned in the exclusion criteria using a structured interview. In case of suspected findings, an unscheduled visit at the investigational site to initiate appropriate diagnostic evaluations will be requested.

The schedule of procedures/assessments to be performed during part 2 of the study is provided in Section 9.1.

Primary variable

The primary efficacy variable will be the incidence rate of seronegative conversion measured by two types of assay (recombinant ELISA and IHA) in subjects who were randomized and received at least one dose of the 60-day nifurtimox treatment regimen. Both test results should be negative for the subject to be considered as seroconverted.

End of part 2 of the study

The end of part 2 of the study as a whole will be reached as soon as the last visit of the last subject has been reached in all centers in all participating countries.

Primary completion

The primary completion event for part 2 of the study is last subject last visit (LSLV).

Justification of the design

For the monitoring of efficacy of antitrypanosomal treatment, the criterion of cure usually depends on serologic conversion of the anti-*T. cruzi* antibody response to negative. Seronegative conversion in children and adolescents may occur several years after treatment, requiring long-term follow-up. Conversion of serological responses to negative, demonstrated by more than one type of assay (ELISA and either IHA or IFA) is the FDA-accepted clinical criterion of cure.

Annual visits at the investigational site are planned for efficacy and safety measurements and subjects will be closely monitored for signs and symptoms of Chagas' disease. Criteria for withdrawal of individual subjects or termination of the entire study are described in Section 6.3 and Section 12, respectively.

It is well known, that mothers with Chagas' disease can transmit *T. cruzi* to their fetuses, who become carriers of the infection and are then at risk of developing severe cardiac disease later in the course of their lives. If identified early enough after birth, the infected newborns can be treated and cured.

6. Study population

6.1 Inclusion criteria

Inclusion criteria for part 1 (CHICO)

1. Male and female pediatric subjects aged 0 days to younger than 18 years
2. Chagas' disease diagnosed/confirmed by:
 - Subjects < 8 months of age at randomization must demonstrate direct observation of *T. cruzi* by concentration test⁸⁶
 - Subjects ≥ 8 months to < 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:
 - Recombinant ELISA
 - Total purified antigen ELISA⁸⁷

Additionally, a non-conventional ELISA test and IHA ^{88,89} will be obtained; however, a positive result will not be required for diagnosis.

Subjects will also be eligible if there is acceptable documentation (two ELISA tests) of positive Chagas' disease within three months prior to screening and they have not had prior anti-trypaenocidal or anti-parasitic treatment.⁹⁰ (The subject's parent or legally acceptable representative must agree to the collection of a baseline blood specimen for ELISA testing later during the study).⁹¹

3. Written informed consent by the subject and/or parent(s) or legally authorized representative(s) according to the age established per local regulations must be obtained from all subjects who are screened for the study. In addition, depending on the subject's age, subject's assent is required as applicable by local laws and regulations (varies in each country).
4. Females of childbearing potential (i.e., female subjects who have experienced menarche) and male subjects must agree to use adequate contraception if sexually active from the time of signing the informed consent/assent form until 3 months after the last study drug

⁸⁶ Text modified for clarity (Amendment 1)

⁸⁷ Text modified/added for clarity and per Modification 2 (Amendment 1)

⁸⁸ IFA test added per Amendment 5

⁸⁹ Replaced IFA with IHA per Amendment 6.

⁹⁰ Text added per Modification 2 (Amendment 1)

⁹¹ Text added as per amendment 2.

administration. Depending on the country, study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.

Inclusion criteria for part 2 (CHICO SECURE)

1. Male and female subjects who were randomized and received at least one dose of their assigned 60- or 30-day regimen of nifurtimox treatment
2. Written informed consent by the subject and/or parent(s) or legally authorized representative(s) according to the age established per local regulations must be obtained from all subjects who are screened for the study. In addition, depending on the subject's age, subject's assent is required as applicable by local laws and regulations (varies in each country)

6.2 Exclusion criteria

Exclusion criteria for part 1 (CHICO)

1. Subjects aged 0 to 27 days who, at birth, were pre-term (i.e., gestational age less than 37 weeks), weighed less than 2500 g, or had a maximum Apgar score <7 at 5 minutes
2. Subjects with any of the following conditions that is associated with Chagas' disease, such as:
 - Known evidence of Chagas' disease-related cardiomyopathy/Chagas' heart disease
 - Known evidence of Chagas' disease-related gastrointestinal dysfunction (e.g., megaesophagus, megacolon, or both) or Chagas' digestive disease
 - Serious manifestations of acute Chagas' disease, including myocarditis, meningoencephalitis, or pneumonitis
 - Known evidence of Chagas' disease-related damage to the peripheral nervous system or peripheral neuropathy (i.e., affected subjects show a combination of sensory impairment and diminished tendon reflexes that mainly involve the lower limbs)
 - Clinically significant psychiatric disorder (e.g., moderate to severe depression, severe anxiety, or psychosis) or epilepsy
3. Subjects with contraindications/warnings to nifurtimox administration, or with conditions that may increase the risk of the undesirable effects of nifurtimox, including:
 - Hypersensitivity to nifurtimox or any hydantoin, or to any of the excipients
 - Suspected or known porphyria
 - Severe renal impairment defined by the following:
 - For subjects < 1 year of age at randomization, estimated glomerular filtration rate (eGFR) < 100% of the lower limit of normal (LLN) appropriate for age (eGFR should be calculated according to Schwartz formula; see Section 16.2)⁹²

⁹² Text revised for clarity; cross-reference renumbered to 16.2 (Amendment 1)

- For subjects ≥ 1 year to < 18 years of age at randomization, eGFR $< 80\%$ of LLN appropriate for age
 - Severe hepatic impairment (alanine aminotransferase [ALT] or aspartate aminotransferase [AST] considered clinically significant by the investigator)
 - History of brain injury, predisposition to seizures or epilepsy, psychiatric disease, or serious behavioral alteration
 - Severe or significant gastrointestinal disorders, or metabolism and nutrition disorders
4. Subjects with a history of malignancy in the previous 5 years
 5. Subjects who are chronic abusers or current users of alcohol or recreational drugs, or the newborn infants of mothers who are chronic abusers or current users of alcohol or recreational drugs
 6. Subjects with any condition that would prevent him/her from taking oral medication
 7. Immuno-compromised subjects (e.g., those with human immunodeficiency virus infection, primary immunodeficiency, or prolonged treatment with corticosteroids or other immunosuppressive drugs)
 8. Subjects with any other acute or chronic health conditions or congenital disorders which, in the opinion of the investigator, would make them unsuitable for participation in a clinical study or may interfere with the efficacy, safety, and/or PK evaluation of the study drug
 9. Subjects who have had previous treatment with trypanocidal agents or an accepted indication for antiparasitic therapy (e.g., reactivation of Chagas' infection due to immunosuppression by several diseases or treatment with steroids)
 10. Subjects who have had treatment with any investigational medicinal product within 30 days before the first dose of study drug or⁹³ have previously received the study treatment
 11. Subjects who are pregnant or breastfeeding
 12. Subjects of breastfeeding mothers under treatment with trypanocidal agents
 13. Subjects who the investigator considers unlikely to adhere to the protocol, comply with study drug administration, or complete the clinical study and follow-up
 14. Subjects with close affiliation with the investigational site; e.g., a close relative of the investigator, dependent person (e.g., employee or student of the investigational site)
 15. Subjects living in housing conditions where there is no active or effective vector-control to *T. cruzi* reinfection as determined by Ministry of Health guidelines in each country

Exclusion criteria for part 2 (CHICO SECURE)

1. Subjects with acute or chronic health conditions or congenital disorders which, in the opinion of the investigator, would make them unsuitable for participation in the clinical study

⁹³ Clarified as per amendment 2

2. Subjects living in housing conditions where there is no active or effective vector-control to *T. cruzi* reinfection as determined by Ministry of Health guideline of the respective country and the judgement of the investigator
3. Subjects with clinical manifestations of Chagas' disease, such as:
 - Known evidence of Chagas' disease-related gastrointestinal dysfunction (e.g., megaesophagus, megacolon, or both) or Chagas' digestive disease
 - Serious manifestations of acute Chagas' disease, including myocarditis, meningoencephalitis, or pneumonitis
4. Immuno-compromised subjects (e.g., those with human immunodeficiency virus infection, primary immunodeficiency, or prolonged treatment with corticosteroids or other immunosuppressive drugs)
5. Subjects who the investigator considers unlikely to adhere to the protocol, or complete the long-term follow-up
6. Subjects with close affiliation with the investigational site; e.g., a close relative of the investigator, dependent person (e.g., employee or student of the investigational site)

Justification of selection criteria for part 2 (CHICO SECURE)

This LTFU has been designed at the request of the US FDA to assess the incidence of seronegative conversion in subjects who were randomized and received at least one dose of nifurtimox treatment for 4 years after end of nifurtimox treatment.

For assessment of conversion of serological response to negative, 2 distinct serologic tests are recommended that use different techniques and/or detect antibodies to different antigens. Two commonly used techniques are ELISA and IHA.

The exclusion criteria are valid for known or suspected conditions and are chosen to ensure that subjects with conditions which may have an effect on the aims of the follow-up are excluded.

Considering that the follow-up period is evaluating long-term seronegative conversion and OD reduction measured by ELISA after nifurtimox treatment, subjects who required antitrypanosomal treatment, including any investigational treatment for Chagas' disease after nifurtimox regimens will be analyzed separately.

To reduce the risk of reinfection as a confounding factor in the study, subjects residing in areas where vector control has not been instituted are excluded as well.

6.3 Withdrawal of subjects from study

In general, the criteria, rules, and procedures in this section apply to part 1 and part 2 of the study. Criteria, rules, and procedures not applicable to either part of the study are otherwise marked.

6.3.1 Withdrawal

Withdrawal criteria

Subjects *must* be withdrawn from the study if any of the following occurs:

- At their own request or at the request of their parents' or legally acceptable representative. At any time during the study and without giving reasons, a subject may

decline to participate further. The subject will not suffer any disadvantage as a result of this decision.

- Pregnancy. If any urine pregnancy test is positive, a serum pregnancy test must be performed to confirm the result. See Section 9.6.2.⁹⁴ (not applicable to part 2)
- Subjects, with positive parasitology test at the 30-day visit for whom (1) it has been determined that the drug was administered correctly and (2) the daily dosage given was correct, must be discontinued from the study and treated with an alternative trypanocidal medication, selected by the investigator.⁹⁵ (not applicable to part 2)

Subjects *may* be withdrawn from the study if any of the following occurs:

- If, in the investigator's opinion, continuation of the study would be harmful to the subject's well-being
- At the specific request of the sponsor and in liaison with the investigator (e.g., obvious non-compliance, safety concerns)
- For the following situations that are regarded by the investigator to be related to the use of study drug (nifurtimox) (not applicable to part 2):
 - Severe nervous system disorders such as seizure, vertigo/dizziness, polyneuropathy, or peripheral neuropathy
 - Severe gastrointestinal disorders, severe decreased appetite
 - Significant loss of body weight in the judgment of the investigator
 - Severe hepatic impairment
 - Severe renal impairment
 - Psychic disorders such as disorientation, psychotic behavior
 - Development of malignant disease
 - Hematological abnormalities such as severe leukopenia

Depending on the time point of withdrawal, a withdrawn subject is referred to as either “screening failure” or “dropout” as specified below:

Screening failure

A subject who, for any reason (e.g., failure to satisfy the selection criteria), terminates the study before the time point used for the definition of “dropout” (see below) is regarded as a “screening failure.”

Re-starting the defined set of screening procedures to enable the “screening failure” subject’s participation at a later time point is allowed only in the following situations (not applicable to part 2):

⁹⁴ Third bullet point and text re: positive microscopy deleted (Amendment 1)

⁹⁵ Text added as per amendment 2

- The subject had successfully passed the screening procedures, but could not start subsequent treatment on schedule.
- Initial screening occurred too early to complete the required washout period (Day -14 to Day -1) after prior therapy.
- The in-/exclusion criteria preventing the subject's initial attempt to participate have been changed (via protocol amendment).

Subjects not meeting inclusion/exclusion criteria at screening may be re-screened once. At the discretion of the investigator and in consultation with Bayer, the screening period (or re-screening period) may be extended up to 3 days to allow for receipt of test results, to evaluate changes in a subject's condition, or for reasons not under the subject's control.⁹⁶ In any case, the investigator has to ensure that the repeated screening procedures do not expose the subject to an unjustifiable health risk. Also, for re-screening, the subject has to re-sign the informed consent form, even if it was not changed after the subject's previous screening. Only the re-screening will be captured in the eCRF.⁹⁷ (Not applicable to part 2)

Dropout

A subject who discontinues study participation prematurely for any reason is defined as a "dropout" if the subject has already been randomized, assigned to treatment, and administered at least one dose of study drug.

General procedures

In all cases, the reason for withdrawal must be recorded in the electronic case report form (eCRF) and in the subject's medical records.

The subject may object to the generation and processing of post-withdrawal data as specified in Section 13.4.

Any subject removed from the study will remain under medical supervision until discharge or transfer is medically acceptable.

Details for the premature termination of the study as a whole (or components thereof) are provided in Section 12.

6.3.2 Replacement

A subject who is withdrawn from the study will not be replaced.

6.4 Subject identification

After documentation of consent/assent (informed consent/assent form) by the parent(s) and assent by the child in accordance with local regulations (varies in each country), each subject will be assigned a 9-digit number for unambiguous identification throughout the study constructed as follows:

⁹⁶ Text modified as per amendment 4

⁹⁷ Text regarding screening failure and re-screening modified/added for clarity (Amendment 1)

Digits 1 to 2 = Country code
Digits 3 to 5 = Center number within the country
(Digits 1 to 5 = Trial unit)
Digits 6 to 9 = Current subject number within the center

Subject identification numbers must be used in sequence, and no number should be skipped, substituted, or reused.

7. Treatment(s)

7.1 Treatments to be administered

Each subject will receive nifurtimox oral tablets three times daily for 60 days, or for 30 days followed by nifurtimox placebo administered three times daily for 30 days.⁹⁸

Each nifurtimox tablet contains either 30 mg or 120 mg of nifurtimox as the active ingredient, and the following excipients: calcium hydrogen phosphate dehydrate, maize starch, silica colloidal anhydrous, and sodium lauryl sulfate.

7.2 Identity of study treatment

Nifurtimox 30-mg and 120-mg oral tablets and matching placebo tablets for each dosage form will be provided by Bayer HealthCare AG. The tablets for both dosage forms are yellow, round, and biconvex. The matching placebo tablets for each dosage (30-mg and 120-mg formulations) will bear the same appearance and packaging as the active study drug tablets.⁹⁹

All study drugs will be labeled according to the requirements of local law and legislation. Label text will be approved according to the sponsor's agreed procedures, and a copy of the labels will be made available to the study site upon request.

For all study drugs, a system of numbering in accordance with all requirements of Good Manufacturing Practice (GMP) will be used, ensuring that each dose of study drug can be traced back to the respective bulk batch of the ingredients. Lists linking all numbering levels will be maintained by the sponsor's clinical supplies Quality Assurance group.

A complete record of batch numbers and expiry dates of all study treatment as well as the labels will be maintained in the sponsor's study file.

7.3 Treatment assignment

Subjects will be randomized via IVRS/IWRS to one of two treatment groups in a 2:1 ratio (60-day regimen vs. 30-day regimen) as follows:

- Nifurtimox tablets administered three times daily for 60 days (Days 1 – 60, active nifurtimox treatment, Treatment Group 1), or
- Nifurtimox tablets administered three times daily for 30 days, followed by nifurtimox placebo administered three times daily for 30 days (Days 1 – 30, active nifurtimox treatment; Days 31 – 60, placebo; Treatment Group 2).

⁹⁸ Last sentence deleted since it appears later in Section 7.4 (Amendment 1)

⁹⁹ Text modified for clarity (Amendment 1)

7.4 Dosage and administration

Nifurtimox oral tablets will be administered in recommended doses by body weight according to the company core data sheet (CCDS; Table 7-1). For infants and children weighing more than 6.0 kg and < 40kg, the daily dosage will be 10-20 mg/kg. Total daily dosages for adolescents weighing > 40 mg will be 8-10 mg/kg, slightly lower than that recommended in the CCDS, to minimize the likelihood of adverse experiences.

Table 7-1: Total daily dosage of nifurtimox oral tablets based on body weight

Body weight group	Total daily dose of nifurtimox [mg / kg body weight]
Adolescents (40 – 60 kg)	8-10
Infants and children (<40 kg)	10-20

Study drug will be dispensed at Visits 2 (Day 1) and 6 (Day 30), with instructions for administration.¹⁰⁰ Dosages of study drug dispensed at Visits 2 and 6 will be the same even if a different dosage is indicated based on a patient's weight change.¹⁰¹ Nifurtimox will be administered three times a day, in the morning, at noon, and at night, with food. The 30-mg and 120-mg tablets have score lines and can be divided into two equal halves to allow for 15-mg or 60 mg dose increments, respectively. The tablet is manufactured for quick disintegration in order to allow administration to subjects < 6 years old who are not able to swallow tablets. Before administration, the tablet should be dissolved in enough water to fill a teaspoon (approximately 5 mL) to form a soft slurry¹⁰² that should be given immediately with food. Nifurtimox will be dispensed based on body weight at randomization, as presented in Table 7-2.¹⁰³

Table 7-2: Individual dosages of nifurtimox oral tablets based on body weight¹⁰⁴

Age Group	Body Weight (kg) ¹⁰⁵	Number of 30-mg Tablets	Number of 120-mg Tablets
	2.5 – 4.5	3 x daily ½ tablet	
	4.6 - <9	3 x daily 1 tablet	---
	9 - <13	3 x daily 1 ½ tablets	---
	13 - <18	3 x daily 2 tablets	---
	18 - <22	3 x daily 2 ½ tablets	---
	22 - <27	3 x daily 3 tablets	---
	27 - <35	3 x daily 3 ½ tablets	---
	35 - <41	---	3 x daily 1 ½ tablets
≥12 to < 18 years	41 - <51	---	3 x daily 1 tablet
	51 - < 71	---	3 x daily 1 ½ tablets
	71 - < 91	---	3 x daily 2 tablets
	91 or greater	---	3 x daily 2 ½ tablets

¹⁰⁰ First sentence added for clarity (Amendment 1)

¹⁰¹ Text modified as per amendment 4

¹⁰² Bioequivalence testing of the slurry has determined it to be comparable to 4 x 30-mg tablets and the 120-mg tablet.

¹⁰³ Text modified for clarity (Amendment 1)

¹⁰⁴ Table 7-1, Table 7-2 and associated text in dosage and administration modified as of amendment 2

¹⁰⁵ In this column, upper limit of weight ranges in each category changed per the most current dosing information (Amendment 1)

At Visits 2 and 6, when study drug is dispensed, subjects/legally authorized representatives will be provided with a diary on which to document the date, exact time, and doses (morning, noon, and night) of study drug, and comments (see Section 1). Should a subject vomit or spit out study drug, the occurrence will be recorded on the diary along with the time of the event.¹⁰⁶ Subjects/legally authorized representatives will be instructed to bring their diaries with them to each in-clinic study visit. The diaries will be collected and reviewed at each study visit to perform drug accountability and assess treatment compliance.¹⁰⁷

Riboflavin is included as a colorant in the matching placebo tablets for study drug blinding purposes. A common side effect of riboflavin ingestion is bright yellow urine. Subjects and/or their legally authorized representatives will be advised of the possibility of change in the subject's urine color within the informed consent form, and when study drug is administered and dispensed at the investigational site.¹⁰⁸

Subjects consenting to optional PK assessments must withhold taking the morning dose of study drug on the day of Visits 3, 6, and 8, when pre- and post-dose PK blood samples will be obtained. Subjects not consenting to optional PK assessments may take their study drug on the mornings of Visits 3, 6, and 8 as instructed.¹⁰⁹

7.5 Blinding

In compliance with applicable regulations, in the event of a suspected, unexpected, serious adverse reaction (SUSAR; see Section 9.6.1.5) related to the blinded treatment, the subject's treatment code will usually be unblinded before reporting to the health authorities, ethic committees and investigators (see Section 9.6.1.4).

Emergency unblinding by the investigator¹¹⁰

In case of emergency unblinding for a serious adverse event (SAE), investigators will use the IVRS/IWRS to unblind a subject.

7.6 Drug logistics and accountability

At Visit 6 (Day 30), subjects will return their diaries, all remaining study drug, and empty packaging. The diaries will be reviewed to assess study drug accountability, and study drug for the remaining 30 days of treatment will be dispensed.

At Visit 8 (Day 60), subjects will return their diaries, all remaining study drug, and empty packaging. The diaries will be reviewed to assess study drug accountability, and no additional study drug will be dispensed.¹¹¹

Details regarding storage conditions for nifurtimox are provided in the Investigators' Brochure.¹¹² Additional details regarding storage conditions may be provided separately. Nifurtimox and the matching placebo are not to be stored above 25°Celsius.

¹⁰⁶ As per amendment 2

¹⁰⁷ Diaries capturing information on daily dosing to be provided to subjects; diary added as appendix in Section 16 (Amendment 1)

¹⁰⁸ Text re: possible discoloration of urine due to riboflavin added per Modification 4 (Amendment 1)

¹⁰⁹ Text added for clarity regarding study drug dosing in relation to PK assessments (Amendment 1)

¹¹⁰ Emergency unblinding added as of amendment 2

¹¹¹ Text added for clarity regarding diaries and return of study drug and packaging (Amendment 1)

¹¹² Revised since Investigators' Brochure became available to serve as the reference document for nifurtimox (Amendment 1)

All study drugs will be stored at the investigational site in accordance with Good Clinical Practice (GCP) and GMP requirements and the instructions given by the clinical supplies department of the sponsor (or its affiliate/Contract Research Organization [CRO]), and will be inaccessible to unauthorized personnel. Special storage conditions and a complete record of batch numbers and expiry dates can be found in the sponsor's study file; the site-relevant elements of this information will be available in the investigator site file. On the day of receipt, the responsible site personnel will confirm receipt of study drug via IVRS/IWRS. The site personnel will use the study drug only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return and destruction (if any) of the study drug must be properly documented according to the sponsor's agreed and specified procedures.

Written instructions on medication destruction will be made available to affected parties as applicable.

If performing drug accountability implies a potential risk of contamination, a safety process/guidance for handling returned drug will be provided.

7.7 Treatment compliance

Study site personnel will review diaries and¹¹³ perform a tablet count of all returned study medication to determine treatment compliance. Any discrepancies between actual and expected amount of returned study medication must be discussed with the subject at the time of the visit, and any explanation must be documented in the source records.

Treatment compliance will also be assessed during the telephone contacts at Visits 4, 5, and 7 (see Section 16.1).¹¹⁴

7.8 Treatment in part 2 (CHICO SECURE)

No study drug will be administered in the LTFU.

At their discretion, investigators may choose the appropriate drug for subjects assessed as requiring antitrypanosomal treatment during the course of LTFU. This treatment is not considered as study drug.

8. Non-study therapy

8.1 Prior and concomitant therapy

Information on prior and concomitant medications used during the study is useful to support PK evaluations as planned in this study in order to evaluate/exclude potential drug-drug interactions (part 1).

All prior and concomitant medications administered from the time the informed consent/assent is signed until the last follow-up visit will be recorded in the subject's source documentation file and reported in the eCRF. Information on concomitant medication use will also be recorded on the Phone Contact Form which will be completed during the telephone assessments at Visits 4, 5, and 7 in part 1 of the study and at FU Visits 2 and 4 in

¹¹³ Text added for clarity re: diaries (Amendment 1)

¹¹⁴ Sentence per Modification 12 (Amendment 1)

part 2 of the study (see Section 16.1).¹¹⁵ Antitrypanosomal treatment required during the course of LTFU at the discretion of the investigator is considered as concomitant medication. The following information will be entered in the eCRF: generic name of the medication, indication, dose, unit, frequency, route of administration, and start and stop date (if stopped before the end of study).

The ingestion of alcohol may increase the incidence and severity of undesirable effects of nifurtimox. Therefore, chronic abuse of alcohol and intake of alcohol or other alcohol-containing preparations during the study period are prohibited (applicable to part 1 only).

8.2 Post-study therapy

Study medication will not be continued after the study. Subsequent treatment should be discussed between the subject and/or the subject's legally authorized representative and the investigator during the final visit. At the end of this study, it is expected that the subject will receive standard treatment according to local clinical guidance.

9. Procedures and variables

9.1 Tabular schedule of evaluations

The schedule of procedures/assessments to be performed during part 1 (CHICO) of the study is presented in Table 9-1.¹¹⁶

The schedule of procedures/assessments to be performed during part 2 (CHICO SECURE) of the study is presented in Table 9-2.

¹¹⁵ Text modified for clarity, and sentence regarding concomitant medication assessment per Phone Contact Form added per Modifications 6 and 12 (Amendment 1)

¹¹⁶ Table revised to include a telephone assessment at Visit 4, and an assessment (Visit 5) prior to the Day 30 Visit as recommended (Modifications 6 and 8); wording of assessments expanded for clarity; diary dispense and collection added; coagulation added to serum laboratory assessments, and neurological assessment added per Modification 10; concomitant medications moved to appear earlier in table. Table footnotes revised and re-lettered according to changes made in text per Amendment 1 (Amendment 1). In addition, replaced IFA with IHA per Amendment 6.

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Table 9–1: Schedule of procedures and assessments in part 1 – Study 16027

Phase	Screening	Treatment Phase							Follow-up Phase		
Visit	1	2	3	4 ^a	5 ^a	6	7 ^a	8 (EOT) ^b	9	10	11
Day	-14 to -1	1 ^c	7 ± 1	14 ± 1	21 ± 3	30 ± 3	42 ± 3	60 ± 3	90 ± 7	240 ± 7	420 ± 7
Initiation procedures											
Informed consent/assent	●										
Demographics (sex, age)	●										
Medical/surgical history	●										
Inclusion/exclusion criteria	●	●									
Efficacy											
Assessment of Chagas' disease signs and symptoms ^d	●		●			●		●	●	●	●
Concentration test for <i>T. cruzi</i> (subjects < 8 months of age)	●		●			●		●	●		
Conventional and non-conventional serologic testing for Chagas' disease ^e	●		●			●		●		●	●
qPCR ^e	●		●			●		●		●	●
Indirect hemagglutination assay (IHA) [*]	●										●
Safety											
Adverse events	●	●	●	●	●	●	●	●	●	●	●
Concomitant medication	●	●	●	●	●	●	●	●	●	●	●
Physical examination ^f	●		●			●		●	●	●	●
Neurological examination ^g	●		●			●		●			
Vital signs (heart rate, RR, BP, temperature), height/length, and weight ^h	●	●	●			●		●	●	●	●
12-lead ECG ⁱ	●	●	●			●		●	●	●	●
Serum hematology, chemistry, and coagulation ^j	● ^k		●			●		●	●		
Urinalysis (if specimen can be obtained)	● ^k		●			●		●	●		
Urine pregnancy test ^l	●	●	●			●		●	●		
Pharmacokinetics											
PK blood sampling (optional) ^m		●	●			●		●			
Study Treatment											
Study drug administration ⁿ		●				●					
Study drug and diary dispensed ^o		●				● ^p					
Study drug and diary collected			●			● ^p		●			
Study drug accountability/review compliance			●	●	●	●	●	●			
Dispense pre-paid phone card ^q			●			●					
Collect pre-paid phone card						●		●			

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Table 9–1: Schedule of procedures and assessments in part 1 – Study 16027

Phase	Screening	Treatment Phase							Follow-up Phase		
Visit	1	2	3	4 ^a	5 ^a	6	7 ^a	8 (EOT) ^b	9	10	11
Day	-14 to -1	1 ^c	7 ± 1	14 ± 1	21 ± 3	30 ± 3	42 ± 3	60 ± 3	90 ± 7	240 ± 7	420 ± 7

BP = blood pressure, ECG = electrocardiogram, EOT = end-of-treatment, IHA = indirect hemagglutination assay, qPCR = quantitative polymerase chain reaction, PK = pharmacokinetic, RR = respiratory rate per minute.

- ^a At Days 14, 21, and 42, study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of adverse events, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.
- ^b Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visits 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (±3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments.
- ^c All assessments are to be obtained pre-treatment on Day 1 except for post-dose PK blood sampling.
- ^d To be performed during physical examinations; see Section 9.4.4
- ^e Specimen taken for non-conventional ELISA, qPCR and stored (as per amendment 2)
- ^f A complete physical examination of body systems will be performed at the Screening Visit. A brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and assessments for the presence of peripheral neuropathy will be performed at all subsequent designated time points. Abnormal findings on physical examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).
- ^g A neurological examination including assessments of mental status and cognition, cranial nerves, motor function, deep tendon reflexes, sensation, and coordination and gait will be performed. Abnormal findings on neurological examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).
- ^h Blood pressure is optional in subjects < 5 years of age at the discretion of the investigator. Height/length and weight will be obtained with the subject wearing minimal clothing and no shoes.
- ⁱ 12-lead ECG is optional in subjects < 5 years of age at the discretion of the investigator. At the 2 – 4 hour time point for PK blood sampling (i.e., at the time of maximum concentration), the ECG will be obtained to allow for PK/PD investigations.
- ^j Fasting of subjects for serum laboratory assessments is dependent on the age of the subject at the discretion of the investigator.
- ^k Test conducted by local laboratories (as per amendment 2)
- ^l Urine pregnancy tests will be performed on all females of childbearing potential (i.e., all female subjects who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be discontinued from the study and undergo all study assessments as described in the EOT Visit.
- ^m Blood samples for pharmacokinetic parameters will be obtained prior to administration of study drug, and at designated time points thereafter (see Table 9–1). They are optional and require consent/assent of the subject/subject's legally authorized representative(s).
- ⁿ Subjects consenting to optional PK assessments must withhold taking the morning dose of study drug on the day of Visits 3, 6, and 8. Subjects not consenting to optional PK assessments may take their study drug on the mornings of Visits 3, 6, and 8 as instructed.
- ^o Depending on the country, study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.

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Table 9–1: Schedule of procedures and assessments in part 1 – Study 16027

Phase	Screening	Treatment Phase							Follow-up Phase		
Visit	1	2	3	4 ^a	5 ^a	6	7 ^a	8 (EOT) ^b	9	10	11
Day	-14 to -1	1 ^c	7 ± 1	14 ± 1	21 ± 3	30 ± 3	42 ± 3	60 ± 3	90 ± 7	240 ± 7	420 ± 7

^a At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.

^a A pre-paid phone card will be provided at Visits 3 and 6 to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Visits 4, 5, and 7.

* IHA will be performed at Visit 1 and Visit 11 only, if adequate specimens are available

Table 9–2: Schedule of procedures and assessments in part 2 – Study 16027

	FU Visit 1 Year ^a 2 (± 6 weeks)	FU Visit 2 Phone Follow-up Year 2.5 (± 6 weeks)	FU Visit 3 Year ^a 3 (± 6 weeks)	FU Visit 4 Phone Follow-up Year 3.5 (± 6 weeks)	FU Visit 5 Year ^a 4 (± 6 weeks)
Informed consent/assent	●				
Inclusion/exclusion criteria	●				
Concomitant medications	●	●	●	●	●
Recombinant ELISA, total purified antigen ELISA, non-conventional ELISA	●		●		●
IHA	●		●		●
qPCR test	●	● ^d	●	● ^d	●
Physical examination	●		●		●
Clinical signs or symptoms of Chagas' disease	●	●	●	●	●
Body weight and height/length	●		●		●
Vital signs (systolic blood pressure, diastolic blood pressure, pulse)	●		●		●
12-lead ECG ^c	●		●		●
Adverse events ^b	●	●	●	●	●
Inquiry of housing conditions (exclusion criterion #3)	●	●	●	●	●

a The year of each visit will be relative to the end of treatment in part 1 (CHICO).

b Adverse events considered at least possibly related to nifurtimox and adverse events caused by protocol-required procedures.

c In case of abnormalities suspicious for cardiac involvement, further diagnostic examinations will be performed.

d At the discretion of the investigator in case of suspected reinfection or reactivation of Chagas' disease (unscheduled study visit).

9.2 Visit description

9.2.1 Screening

9.2.1.1 Visit 1 – screening visit (Days -14 to -1)

No study-specific procedures will be performed before the subject/legally authorized representative signs the informed consent form, or a minor subject provides assent, including asking a potential subject to fast (if applicable, depending on the age of the subject at the discretion of the investigator) prior to the screening blood samples. Informed consent/assent for optional PK blood sampling will also be obtained. After the consents/assents have been obtained, the following assessments will be performed:

- Collection of demographic data
- Medical and surgical history, including date of menarche for female subjects, and method of contraception
- Evaluation of inclusion and exclusion criteria
- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects <8 months of age at randomization) and conventional serologic testing for Chagas' disease (subjects \geq 8 months < 18 years of age) conducted in local laboratory (or acceptable documentation of positive Chagas' disease within 3 months prior to screening and no prior anti-trypanocidal or anti-parasitic treatment). Specimens taken for conventional serology, non-conventional serology, IHA, and qPCR will be frozen and stored at -20°C prior to shipment to the central laboratory where they will be stored at -70°C.^{117,118}
- Assessment of pretreatment AEs and concomitant medications
- A complete physical examination of body systems. Abnormal findings will be documented as medical history (see Section 9.6.1.1).
- Neurological examination (see Section 9.6.3.3). Abnormal findings will be documented as medical history (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator)
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.

¹¹⁷ Modified as per Amendment 2 and Amendment 5.

¹¹⁸ Replaced IFA with IHA per Amendment 6.

- Urine pregnancy test on all females of childbearing potential (i.e., those who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be excluded from study participation.¹¹⁹

9.2.2 Treatment phase

9.2.2.1 Visit 2 – baseline (treatment phase, Day 1)

Visit 2 will take place after all laboratory test results and assessments performed at the Screening Visit are available. The following assessments will be performed:

- Re-evaluation of inclusion and exclusion criteria
- Assessment of pretreatment AEs and concomitant medications
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects <5 years of age at the discretion of the investigator).
- Urine pregnancy test on all females of childbearing potential (i.e., those who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be excluded from study participation.
- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see [Table 9–1](#)).
- Administer first dose of study drug to all subjects
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see [Table 9–1](#)). At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG will be obtained to allow for PK/PD investigations.¹²⁰ Dispense study drug and diary with instructions for use. Subjects consenting to optional PK assessments will be instructed to withhold taking study drug on the morning of Visit 3 and to complete the PK subject diary, according to the instructions provided on the PK subject diary.¹²¹; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 3 as instructed. The PK subject diary has been added in order for the PK subjects to record all foods they consume when taking each dose of study drug, along with the times they eat each meal. Examples of these entries, based on foods typically consumed in South American countries, will be given to each subject at the PK study sites.¹²²

¹¹⁹ Text added/deleted/modified for clarity and to reflect changes made per Amendment 1

¹²⁰ Added as per amendment 2.

¹²¹ Text modified as per amendment 4

¹²² Text modified as per amendment 4

- Subjects and/or their legally authorized representatives will be advised of the possibility of change in the subject's urine color due to the ingredient riboflavin. Depending on the country, study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.¹²³

9.2.2.2 Visit 3 (Day 7 ± 1 days)

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age), conventional and non-conventional serologic testing for Chagas' disease, and qPCR
- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Neurological examination (see Section 9.6.3.3). Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator).
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.
- Urine pregnancy test on all females of childbearing potential (i.e., those who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be discontinued from the study and undergo all study assessments as described in the EOT Visit.
- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see Table 9-1).

¹²³ Text added/deleted/modified for clarity and to reflect changes made per Amendment 1

- Administer study drug with food to subjects consenting to PK assessments (who withheld their morning dose of study drug) and instruct subject on completion of PK diary, according to the instructions provided on the PK subject diary.¹²⁴
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9–1). At the 2 – 4 hour time point (i.e., at the time of C_{max}), an ECG will be obtained.
- Collect study drug and diary. Additional diaries may be provided if necessary.
- Drug accountability and review of diaries for study drug compliance
- Dispense pre-paid phone card to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Visit 4.¹²⁵

9.2.2.3 Visit 4 (Day 14 ± 1 days)

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, compliance with study drug administration, and completion of subject diary or PK subject diary¹²⁶. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.¹²⁷

9.2.2.4 Visit 5 (Day 21 ± 3 days)

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, compliance with study drug administration, and completion of subject diary or PK subject diary. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

Subjects consenting to optional PK assessments will be reminded to withhold taking the morning dose of study drug on the day of Visit 6; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 6 as instructed.¹²⁸

9.2.2.5 Visit 6 (Day 30 ± 3 days)

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age), conventional and non-conventional serologic testing for Chagas' disease and qPCR

¹²⁴ Text modified as per Amendment 4

¹²⁵ Text added/deleted/modified for clarity and to reflect changes made per Amendment 1

¹²⁶ Text modified as per amendment 4

¹²⁷ Text modified for clarity and per Modifications 6 and 8 (Amendment 1)

¹²⁸ Text modified for clarity and per Modifications 6 and 8 (Amendment 1)

- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, the skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Neurological examination (see Section 9.6.3.3). Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator)
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator).
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.
- Urine pregnancy test on all females of childbearing potential (i.e., those who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be discontinued from the study and undergo all study assessments as described in the EOT Visit.
- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see Table 9–1).
- Administer study drug with food to subjects consenting to PK assessments (who withheld their morning dose of study drug) and instruct subject on completion of PK diary, according to the instructions provided on the PK subject diary.¹²⁹
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9–1). At the 2 – 4 hour time point (i.e., at the time of C_{max}), an ECG will be obtained.
- Collect and dispense study drug and diary. At this visit, subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.
- Drug accountability and review of diaries for study drug compliance
- Collect and dispense pre-paid phone card to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Visit 7.¹³⁰

¹²⁹ Text modified as per amendment 4

¹³⁰ Visit 6 assessments added per changes made per Amendment 1

9.2.2.6 Visit 7 (Day 42 ± 3 days)

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, compliance with study drug administration, and completion of subject diary.¹³¹ A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

Subjects consenting to optional PK assessments will be reminded to withhold taking the morning dose of study drug on the day of Visit 8; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 8 as instructed.¹³²

9.2.2.7 Visit 8 (Day 60 ± 3 days)/End of Treatment Visit

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age), conventional and non-conventional serologic testing for Chagas' disease and qPCR
- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Neurological examination (see Section 9.6.3.3). Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator).
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.
- Urine pregnancy test on all females of childbearing potential (i.e., those who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be

¹³¹ Text modified as per amendment 4

¹³² Text modified/added for clarity and per Modification 6 (Amendment 1)

discontinued from the study and undergo all study assessments as described in the EOT Visit.

- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see [Table 9–1](#)).
- Administer study drug with food to subjects consenting to PK assessments (who withheld their morning dose of study drug), and instruct subject on completion of PK diary, according to the instructions provided on the PK subject diary. ¹³³
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see [Table 9–1](#)). At the 2 – 4 hour time point (i.e., at the time of C_{max}), an ECG will be obtained.
- Collect study drug, diary, and pre-paid phone card
- Drug accountability and review of diaries for study drug compliance and for PK subjects, documentation of meals during study drug administration. ^{134,135}

9.2.3 Follow-up phase

9.2.3.1 Visit 9 (Day 90 ± 7)

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section [9.4.4](#)
- Concentration test for *T. cruzi* (subjects < 8 months of age)
- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section [9.6.1.1](#)).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator)
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.

¹³³ Text modified as per amendment 4

¹³⁴ Text modified as per amendment 4

¹³⁵ Text added/deleted/modified for clarity and to reflect changes made per Amendment 1

- Urine pregnancy test on all females of childbearing potential (i.e., those who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results. See Section 9.6.2.¹³⁶

9.2.3.2 Visit 10 (Day 240 ± 7)

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator)¹³⁷
- Conventional and non-conventional serologic testing for Chagas' disease and qPCR¹³⁸
- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight¹³⁹

9.2.3.3 Visit 11 (Day 420 ± 7)

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator)¹⁴⁰
- Conventional and non-conventional serologic testing and IHA for Chagas' disease and qPCR^{141,142}
- Assessment of AEs
- Concomitant medications

¹³⁶ Text added/deleted/modified for clarity and to reflect changes made per Amendment 1

¹³⁷ Modified as per amendment 2.

¹³⁸ Modified as per amendment 2.

¹³⁹ Text added/deleted/modified for clarity and to reflect changes made per Amendment 1

¹⁴⁰ Modified as per amendment 2

¹⁴¹ IFA test added per amendment 5.

¹⁴² Replaced IFA with IHA per Amendment 6.

- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight¹⁴³

9.2.4 Long-Term Follow-up phase

Subjects will have an annual visit for 3 years (see Table 9–2).

Female subjects who give birth by the end of part 2 of the study will be invited to test their child for the absence or presence of infection with *T. cruzi*. The congenital infection will be confirmed by parasitological method in children ≤8 months of age and by serological method in children >8 months of age. Refusal to test the child will not influence the subject's enrollment and participation in the LTFU.

9.2.4.1 Eligibility procedures (FU Visit 1)

After the consent/assent has been obtained, eligibility assessments will be performed at the first visit. The first visit should be performed as soon as possible after availability of regulatory and ethics committee approval for all subjects who have already completed part 1 of the study for 1 year ± 6 weeks or longer. Subjects who completed part 1 of the study 10.5 months or less prior to the start of the LTFU will be informed about part 2 of the study and will have their eligibility assessments according to the visit schedule provided that consents/assents have been obtained.

The subject's update on medical history and concomitant medication will be recorded.

9.2.4.2 Annual study visits (FU Visits 1, 3, and 5)

At each investigational site visit, blood samples for serology tests (recombinant ELISA, total purified antigen ELISA, IHA, and non-conventional ELISA) and for detection of *T. cruzi*'s DNA using qPCR will be collected. Blood samples will be taken under fasting conditions, if applicable, depending on the age of the subject, at the discretion of the investigator.

Subjects will have a physical examination, including measurement of weight and height/length, measurement of vital signs and assessment of signs and symptoms of Chagas' disease. A 12-lead ECG will be obtained at each annual visit. AEs considered at least possibly related to nifurtimox and AEs caused by protocol-required procedures will be collected and any concomitant medications since their last visit will be recorded. The subjects will be asked about their housing conditions. Females of childbearing potential will be asked if the subject is pregnant or delivered a baby. In case of pregnancy, the pregnancy will be followed as described in Section 9.6.2.

¹⁴³ Text added/deleted/modified for clarity and to reflect changes made per Amendment 1

9.2.4.3 Interim phone contact visits (FU Visits 2 and 4)

As shown in [Table 9–2](#), between annual study visits, all subjects will be contacted to assess clinical signs and symptoms of Chagas' disease or any exclusion criteria and will be asked about their housing conditions. Any update in concomitant medications, and any protocol-specified AEs experienced since the last study visit will be recorded. Subjects may be asked to return prior to their next annual visit to the investigational site, if, in the opinion of the investigator, it is clinically necessary.

In addition, subjects will receive a reminder contact approximately 2 weeks prior to their scheduled next annual visit at the discretion of the investigator.

9.2.4.4 Unscheduled study visits

If in the opinion of the investigator, the subject needs to be evaluated clinically between the annual visits, the subject will be asked to return for an unscheduled study visit. At this visit, subjects will have a physical examination, including clinical signs and symptoms of Chagas' disease. Blood will be taken for detection of *T. cruzi*'s DNA using qPCR, if deemed necessary based on the judgement of the investigator.

Subjects in whom reinfection or reactivation of Chagas' disease is confirmed and, in the opinion of the investigator, require antitrypanosomal treatment will continue to be followed up.

9.3 Population characteristics

9.3.1 Demographic

At screening, each subject's date of birth¹⁴⁴, age, and sex will be recorded.

9.3.2 Medical history

At screening, medical history findings (i.e., previous diagnoses, diseases or surgeries) meeting all criteria listed below will be collected as available to the investigator:¹⁴⁵

- Not pertaining to the study indication
- Start before signing of the informed consent
- Considered relevant for the subject's study eligibility

Detailed instructions on the differentiation between (i) medical history and (ii) AEs can be found in Section [9.6.1.1](#).

¹⁴⁴ Text modified since full date of birth will be collected (rather than year of birth) (Amendment 1)

¹⁴⁵ Text modified for clarity (Amendment 1)

9.4 Efficacy

Part 1 (CHICO)

Efficacy assessments will be obtained at designated study visits (see [Table 9–1](#)). The primary efficacy variable will be sero-reduction or sero-conversion at 12 months post-treatment using two conventional ELISA serology tests as the measure of efficacy.

A quality control program will be implemented for all tests according to the laboratory QC/QA department SOPs.¹⁴⁶

Other efficacy variables include disease state determined by qPCR, concentration tests for *T. cruzi* (subjects < 8 months of age at randomization), conventional and non-conventional serologic testing, and clinical signs/symptoms of Chagas' disease.¹⁴⁷

The complete list of variables to be analyzed for this study will be provided in the statistical analysis plan (SAP).

Part 2 (CHICO SECURE)

Efficacy assessments will be obtained at designated study visits (see [Table 9–2](#)). The primary efficacy variable will be seronegative conversion as confirmed by two types of assay using recombinant ELISA and IHA, in subjects who were randomized and received at least one dose of the 60-day nifurtimox treatment regimen.

Other efficacy variables include:

- Incidence of seronegative conversion, as confirmed by two types of assays, recombinant ELISA and IHA, in subjects who were randomized and received at least one dose of the 30-day nifurtimox treatment regimen.
- Proportion of responders who show seronegative conversion and the incidence of seronegative conversion as confirmed by non-conventional ELISA in subjects who were randomized and received at least one dose of either 60- or 30-day nifurtimox treatment regimens.
- Proportion of responders who show both seronegative conversion as confirmed by two types of assay (recombinant ELISA and IHA) and no evidence of established cardiomyopathy as evaluated in ECG recordings.
- Serial reduction of OD measured by recombinant ELISA and total purified antigen ELISA, in subjects who were randomized and received at least one dose of either the 60- or 30-day nifurtimox treatment regimen compared to Visit 1 in part 1 of the study.

In the LTFU period, all assessments will follow the same procedures as in part 1 (CHICO) of the study. The same quality control program will also be implemented for all tests according to the laboratory QC/QA department SOPs.

¹⁴⁶ Modified as per amendment 2

¹⁴⁷ Text modified per Modification 2 and per changes made per Amendment 1

The complete list of variables for LTFU to be analyzed will be provided in the statistical analysis plan (SAP).

9.4.1 Polymerase chain reaction test

A qPCR test will be performed at the Screening Visit, Visits 3, 6, and 8, and Follow-up Visits 10 and 11 in part 1 of the study and at FU Visits 1, 3, and 5 in part 2 of the study. Additional qPCR test may be required for unscheduled visits, if applicable.

Details describing a suggested collection, processing, storage and shipment for qPCR blood samples, and a suggested qPCR test protocol, are described in Section 1. The study central laboratory procedures and qPCR protocol will be followed.¹⁴⁸

9.4.2 Serological tests

At screening, subjects must have the following serological tests to confirm a diagnosis of Chagas' disease:

- Subjects < 8 months of age at randomization must demonstrate direct observation of *T. cruzi* by concentration test¹⁴⁹
- Subjects ≥ 8 months to < 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:
 - Recombinant ELISA
 - Total purified antigen ELISA¹⁵⁰

Additionally, a non-conventional ELISA test and IHA will be obtained; however, a positive result will not be required for diagnosis^{151,152}.

Subjects will also be eligible if there is acceptable documentation of positive Chagas' disease within 3 months prior to screening and they have had no prior anti-trypanocidal or anti-parasitic treatment.¹⁵³

After randomization, conventional and non-conventional serological tests will be performed at Visits 3, 6, and 8 (EOT), and Visits 10 and 11 (Follow-up).¹⁵⁴

All subjects ≥ 8 months to < 18 years of age at randomization will have two baseline specimens collected for serologic testing. One sample will be used to determine the initial diagnosis of Chagas' disease while the other sample will be frozen and retained to be assayed later as an internal control. Subjects < 8 months of age at randomization will have one specimen obtained, which will be frozen and retained. Total blood volume to be taken for the

¹⁴⁸ Modified as per amendment 2

¹⁴⁹ Text modified for clarity (Amendment 1)

¹⁵⁰ Text modified for clarity and per Modification 2 (Amendment 1)

¹⁵¹ IFA test added per Amendment 5.

¹⁵² Replaced IFA with IHA per Amendment 6.

¹⁵³ Text modified/added for clarity and per Modification 2 (Amendment 1)

¹⁵⁴ Text modified for clarity and to reflect changes per Amendment 1

required three serological and PCR tests is 4.0-5.0 mL, depending on the subject's age. Care will be taken to minimize sample volumes by using a micro-sampling collection technique, when possible.¹⁵⁵

Details describing the collection, processing, storage and shipment of blood samples, and the serologic test protocols, will be described in a separate serologic test manual from the central laboratory.

For part 2 of the study, conventional serological tests (recombinant ELISA and total purified antigen ELISA), IHA, and a non-conventional serological test (ELISA F-29) will be performed at FU Visits 1, 3, and 5.

9.4.3 Concentration tests for *T. cruzi*

Concentration tests for *T. cruzi* will be performed on all subjects < 8 months of age at randomization at the screening visit, and at Visits 3, 6, 8 and 9.

Details describing the collection, processing, storage and shipment of blood samples, and the concentration test protocol, will be described in a separate manual. Concentration tests for *T. cruzi* will be performed by a local laboratory according to the standard of care for each country.¹⁵⁶

Concentration test for *T. cruzi* is not applicable for subjects in part 2 of the study. However, concentration test for *T. cruzi* may be performed in children born of female study subjects to detect congenital infection (see Section 9.6.2).

9.4.4 Assessment of Chagas' disease signs and symptoms

Assessments of the presence of Chagas' disease signs and symptoms will be performed during physical examinations at Screening, Visits 3, 6, and 8 (EOT), and Visits 9, 10, and 11 (follow-up) in part 1 and at FU Visits 1, 3, and 5 in part 2 of the study, and will be entered into the eCRF. Signs and symptoms of Chagas' disease include but are not limited to the following:¹⁵⁷

Acute Chagas' disease:

- Fever: usually prolonged
- malaise
- lymphadenopathy
- hepatomegaly
- splenomegaly
- subcutaneous edema (localized or generalized)

¹⁵⁵ Text added per Modification 3 (Amendment 1)

¹⁵⁶ Text modified for clarity and per Modification 2 (Amendment 1)

¹⁵⁷ Heading and text modified for clarity and reflect changes made per Amendment 1

- signs of portal of entry of *T. cruzi*:
 - through the skin - chagoma
 - via the ocular mucous membranes - Romaña sign
- hypotonicity
- anemia
- myocarditis
- meningoencephalitis
- pneumonitis
- ECG abnormality, may include but not limited to:
 - sinus tachycardia
 - first-degree atrioventricular block
 - low QRS voltage
 - primary T-wave changes
- Chest radiograph abnormality, include variable degrees of cardiomegaly

Chronic Chagas' disease:

Digestive manifestations:

- megaesophagus
- megacolon

Cardiac manifestations:

- abnormalities of the conduction system
- bradyarrhythmias and tachyarrhythmias
- apical aneurysms
- cardiac failure
- thromboembolism (systemic or pulmonary)
- sudden death
- ECG abnormalities, e.g.:
 - right bundle branch block
 - left anterior fascicular block
 - ventricular premature beats
 - ST-T changes
 - abnormal Q waves
 - low voltage of QRS

- various degrees of AV block
- sick sinus syndrome
- low QRS voltage
- others

In part 2, progression of clinical Chagas' disease will be defined as:

- presence of positive serological tests for *T. cruzi*
- clinical (e.g. progressive dyspnea, fatigue, asthenia) and electrocardiographic alterations that are characteristic of chronic Chagas' cardiomyopathy (e.g., new or worsening signs of cardiac conduction disorders, sustained ventricular tachycardia, etc.)
- alterations that are characteristic of chronic Chagas' gastrointestinal disease (e.g., megaesophagus, megacolon)

9.5 Pharmacokinetics/pharmacodynamics

If the subject/subject's legally authorized representative(s) provide(s) consent/assent to do so, plasma concentrations of nifurtimox will be evaluated at Visit 2 (baseline) and Visits 3, 6, and 8 (EOT) on at least 10 subjects per age stratum should be selected from all participating PK centers. Plasma nifurtimox concentrations will be determined using a sparse sampling approach.¹⁵⁸

At Visits 2, 3, 6, and 8, the subjects should present themselves to the investigational site without taking the morning dose of study medication. Blood samples for PK will be obtained in-clinic prior to the administration of study drug with food and at specific time points and time windows as presented in Table 9–1. At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG (optional for subjects < 5 years of age at the discretion of the investigator) will be obtained to allow for PK/PD investigations.¹⁵⁹ All subjects are to complete the PK subject diary according to instructions provided.¹⁶⁰

PK/PD investigation is not applicable in part 2 of the study.

¹⁵⁸ Text modified for clarity and per Amendment 1

¹⁵⁹ Text modified for clarity and per Modification 1 (Amendment 1)

¹⁶⁰ Text modified as per amendment 4

Table 9–3: Pharmacokinetic sampling time points and windows¹⁶¹

Visits (Days)	Time point	Time window
2, 3, 6, 8 (1, 7, 30, 60)	Pre-dose (pre-treatment) Post-dose	--- 5-10 minutes post-dose 10-120 minutes post-dose 2-4 hours post-dose ^a 4-8 hours post-dose

a An ECG will be obtained at this time point (optional in subjects < 5 years of age at the discretion of the investigator)

PK blood samples are to be obtained from a heel prick (subjects up to 1 month of age), finger stick, or ear lobe stick at the discretion of the investigator. For all subjects throughout the study, the selected specimen handling technique will be via microsampling in order to provide uniform analytical samples while maintaining total blood volumes within the limitations for safety.

Samples of a blood volume of $2 \times 75 = 150 \mu\text{L}$ at predefined time points should be taken according to Table 9–3. If samples cannot be taken due to other reasons (e.g., sampling deemed not feasible by the investigator due to safety) or if the samples are taken outside the defined time windows (e.g., > 8 hours post-dose), the subject will still remain valid for PK analysis. At least one sample within the specified timeframe should be taken during the course of the study.¹⁶² That means that, at the end of the study, at least one sample from each time window will be available, e.g., if the 4-8 hour post-dose sample was not collected until the last visit, then a sample at this time window should in any case be drawn at that time point. This must be documented by the investigator.

The PK samples should be obtained within the planned sampling time windows unless important or unforeseen mandatory interventions coincide (e.g., for medical reasons).¹⁶³ In these exceptional cases, the corresponding sample may be obtained outside the predefined windows without losing its validity for subsequent PK analysis. This will not be considered as a protocol violation. If a subject fails to provide the number of samples defined in the protocol, this will also not render him/her invalid for the subsequent PK analysis.

Details describing the procedures for obtaining and handling PK blood samples are provided in Section 16.5.

Analysis of PK samples

Nifurtimox concentrations in plasma will be measured by a validated assay using high performance liquid chromatography followed by tandem mass spectrometric detection. Quality control (QC) and calibration samples will be analyzed concurrently with study samples. The results of QC samples will be reported together with concentrations in the study samples in the clinical study report.

¹⁶¹ Visit numbers changed to reflect changes made per Amendment 1; footnote a added per Modification 1 (Amendment 1)

¹⁶² Text modified for clarity (Amendment 1)

¹⁶³ Text modified for clarity (Amendment 1)

The bioanalyst will remain unblinded for analysis of study samples. Concentrations are calculated from the chromatographic raw data in accordance with current Bayer guidelines [31].

Pharmacokinetic evaluation

Plasma concentration time courses will be analyzed and reported within the study report. In addition, a full PK evaluation of the data will be performed and reported in a separate study report. Population PK methods will be used to provide parameter estimates describing the PK behavior of nifurtimox and to identify possible covariates with significant impact on pharmacokinetics (e.g., age or body weight).^{164, 165}

9.6 Safety

9.6.1 Adverse events

9.6.1.1 Definitions

Definition of adverse event (AE)

In a clinical study, an AE is any untoward medical occurrence (i.e., any unfavorable and unintended sign [including abnormal laboratory findings], symptom, or disease) in a patient or clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

A surgical procedure that was planned prior to the start of the study by any physician treating the subject should not be recorded as an AE (however, the condition for which the surgery is required may be an AE).

In the following differentiation between medical history and AEs, the term “condition” may include abnormal physical examination findings, symptoms, diseases, laboratory, or ECG.

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present until signing of informed consent are recorded as medical history (e.g., seasonal allergy without acute complaints).
- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at unchanged intensity, are recorded as medical history (e.g., allergic pollinosis).
- Conditions that started or deteriorated after signing of informed consent will be documented as AEs. This includes intercurrent illnesses.

¹⁶⁴ Text modified for clarity (Amendment 1)

¹⁶⁵ Text modified as per amendment 4

Definition of serious adverse event (SAE)

An SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria (a – f):

- a. Results in death
- b. Is life-threatening

The term ‘life-threatening’ in the definition refers to an event in which the subject was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

- c. Requires inpatient hospitalization or prolongation of existing hospitalization

A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:

- The admission results in a hospital stay of less than 12 hours
- The admission is pre-planned
- (e.g., elective or scheduled surgery arranged prior to the start of the study; admission is part of the study procedures as described in [Section 9.2](#))
- The admission is not associated with an AE (e.g., social hospitalization for purposes of respite care).

However, it should be noted that invasive treatment during any hospitalization may fulfill the criterion of ‘medically important’ and as such may be reportable as an SAE dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedence.

- d. Results in persistent or significant disability/incapacity
Disability means a substantial disruption of a person’s ability to conduct normal life’s functions.
- e. Is a congenital anomaly/birth defect
- f. Is another serious or important medical event as judged by the investigator

9.6.1.2 Classifications for adverse event assessment

In part 1 of the study, all AEs will be assessed and documented by the investigator according to the categories detailed below.

In part 2 of the study, AEs considered at least possibly related to nifurtimox and AEs caused by protocol-required procedures will be assessed and documented by the investigator according to the categories detailed below.

9.6.1.2.1 Seriousness

For each AE, the seriousness must be determined according to the criteria given in [Section 9.6.1.1](#).

9.6.1.2.2 Intensity

The intensity of an AE is classified according to the following categories:

- Mild: a type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Moderate: a type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort, but poses no significant or permanent risk of harm to the subject.
- Severe: a type of AE that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects clinical status. The event possesses a significant risk of harm to the subject and hospitalization may be required.¹⁶⁶

9.6.1.2.3 Causal relationship

The assessment of the causal relationship between an AE and the administration of treatment is a decision to be made by the investigator, who is a qualified physician, based on all information available at the time of the completion of the eCRF.

Causality should be assessed separately for each study treatment as detailed in the eCRF. If the investigator feels that the event cannot be firmly attributed to one of the study treatments (e.g., owing to a suspected underlying interaction), the same assessment will be documented for each study treatment.

The assessment is based on the question whether there was a “reasonable causal relationship” to the study treatment in question.

Possible answers are “yes” or “no”

An assessment of “no” would include:

1. The existence of a highly likely alternative explanation, e.g., mechanical bleeding at surgical site.

or

2. Non-plausibility, e.g., the subject is struck by an automobile when there is no indication that the drug caused disorientation that may have caused the event; cancer developing a few days after the first drug administration.

An assessment of “yes” indicates that the AE is reasonably associated with the use of the study treatment.

Important factors to be considered in assessing the relationship of the AE to study treatment include:

¹⁶⁶ Text added per Modification 7 (Amendment 1)

- The temporal sequence from drug administration: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event.
- Recovery on drug discontinuation (de-challenge), recurrence on drug re-introduction (re-challenge): Subject's response after de-challenge or re-challenge should be considered in view of the usual clinical course of the event in question.
- Underlying, concomitant, intercurrent diseases:
Each event should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication or treatment:
The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them might have caused the event in question.
- Known response pattern for this class of drug: Clinical/preclinical
- Exposure to physical and/or mental stresses: The exposure to stress might induce adverse changes in the recipient and provide a logical and better explanation for the event
- The pharmacology and PK of the study treatment:
The PK properties (absorption, distribution, metabolism and excretion) of the study treatment, coupled with the individual subject's PD should be considered.
- The assessment is not possible

9.6.1.2.4 Action taken with study treatment

Any action on study treatment to resolve the AE is to be documented using the categories listed below.

The study treatment action should be recorded separately for each study treatment as detailed in the eCRF.

- Drug withdrawn
- Drug interrupted
- Dose reduced
- Dose not changed
- Dose increased
- Not applicable
- Unknown

9.6.1.2.5 Other specific treatment(s) of adverse events

- None
- Remedial drug therapy

- Other

9.6.1.2.6 Outcome

The outcome of the AE is to be documented as follows:

- Recovered/resolved
- Recovering/resolving
- Recovered/resolved with sequelae
- Not recovered/not resolved
- Fatal
- Unknown

9.6.1.3 Assessments and documentation of adverse events

Once informed consent/assent is obtained during part 1, all AEs occurring through the end of the part 1 follow-up phase will be recorded on the applicable eCRF pages. After the part 1 follow-up phase through the end of the part 2 follow-up phase, only AEs considered at least possibly related to nifurtimox and those caused by protocol-related procedures should be recorded.

No study drug will be administered during part 2. For subjects requiring further treatment with antitrypanosomal drugs, including retreatment with nifurtimox during part 2, the applicable national reporting system should be followed for any spontaneous non-serious and serious adverse reaction considered to be not related to the study medication administered in part 1 (nifurtimox and/or placebo).

After the end of the part 2 follow-up phase, there is no requirement to actively collect AEs, including those having an outcome of “death.” The type of information that should be assessed and recorded by the investigator for each AE is listed in Section [9.6.1.2](#).

“Death” should not be recorded as an AE on the AE page. Instead, “death” is the outcome of underlying AE(s).

For all SAEs, the sponsor has to carry out a separate assessment for expectedness, seriousness, and causal relationship to study drug.

9.6.1.4 Reporting of serious adverse events

The definition of SAEs is given in Section [9.6.1.1](#). Each SAE must be followed up until resolution or stabilization by submission of updated reports to the designated recipient in part 1 of the study. In part 2, only SAEs considered at least possibly related to nifurtimox or caused by protocol-related procedures must be reported. These events must be followed up until resolution or stabilization by the submission of updated reports to the designated recipient and in accordance with local regulations.

Investigator's notification of the sponsor

All investigators will be thoroughly instructed and trained on all relevant aspects of the investigator's reporting obligations for SAEs. This information, including all relevant contact details, is summarized in the investigator site file. This information will be updated as needed.

The investigator must report immediately (within 24 hours of the investigator's awareness) all SAEs occurring during the observation period defined in Section 9.6.1.3 to the recipient detailed in the instructions for SAE reporting included in the Investigator File. For this, an AE page in the eCRF as well as the complementary pages provided in the Investigator File must be completed for each SAE.

SAEs occurring after the protocol-defined observation period will be processed by the sponsor according to all applicable regulations.

Notification of the IECs/IRBs

Notification of the International Ethics Committees (IECs)/Institutional Review Boards (IRBs) about all relevant events (e.g., SAEs, SUSARs) will be performed by the sponsor and/or by the investigator according to all applicable regulations and IEC/IRB procedures.

Notification of the authorities

The processing and reporting of all relevant events (e.g., SAEs, SUSARs) to the authorities will be done by the sponsor according to all applicable regulations.

Sponsor's notification of the investigational site

The sponsor will inform all investigational sites about reported relevant events (e.g., SUSARs) according to all applicable regulations.

9.6.1.5 Expected adverse events

For this study, the applicable reference document is the most current Investigators' Brochure for nifurtimox.¹⁶⁷

If relevant new safety information is identified, the information will be distributed to all participating sites.

The expectedness of AEs will be determined by the sponsor according to the applicable reference document and according to all local regulations.

9.6.1.6 Adverse events of special safety interest

In this study, all AEs of weight loss of > 20%, severe rash, and severe polyneuropathy will be considered AEs of special safety interest.¹⁶⁸ Adverse events of special safety interest will be assessed, monitored, and reported as described in Section 9.6.1.4.

¹⁶⁷ Revised since Investigators' Brochure became available to serve as the reference document for nifurtimox (Amendment 1)

9.6.2 Pregnancies

Part 1 (CHICO)

Any subject who becomes pregnant during the course of the study will discontinue study treatment. Pregnancy must be confirmed by serum pregnancy test. The investigator must report to the sponsor any pregnancy occurring in a female study subject during her participation in this study. The outcome of the pregnancy should be followed up carefully, and any outcome of the mother and the child at delivery should be reported.

For a pregnancy in the partner of a male study subject, all efforts will be made to obtain similar information on course and outcome, subject to the partner's consent and any abnormal outcome of the child should be reported.

For all reports, the forms provided are to be used. The investigator should submit them within the same timelines as an SAE.

Part 2 (CHICO SECURE)

At each visit in part 2, female subjects will be asked if she is pregnant or gave birth to a baby after the first dose of nifurtimox treatment regimen or since the last visit.

The investigator must report to the sponsor any pregnancy occurring in a female study subject during her participation in this study. The outcome of the pregnancy should be followed up carefully, and any outcome of the mother and the child at delivery should be reported.

Female subjects who give birth by the end of part 2 of the study will be invited to test their child for the absence or presence of infection with *T. cruzi*, except if the child was already tested according to the standards of the country or has already received treatment for Chagas' disease. The congenital infection will be confirmed by parasitological method in children ≤ 8 months of age and by serological method in children > 8 months of age. Efforts should be made to obtain the results of congenital infection testing when already done according to the standards of the country. The result of congenital infection will be documented in the applicable eCRF form.

9.6.3 Further safety

9.6.3.1 Laboratory assessments

Safety laboratory assessments will be performed at screening, Visits 3, 6, 8 (EOT), and 9 (follow-up). Fasting is dependent on the age of the subject at the discretion of the investigator. If any abnormal laboratory values occur at the EOT Visit (Visit 8), these determinations will be followed until normal or stable.¹⁶⁹

Laboratory parameters will be analyzed by a local laboratory according to the schedule specified in [Table 9-4](#): .

¹⁶⁸ Sentence corrected as per amendment 3

¹⁶⁹ Heading 9.6.3.1 added; text modified for clarity and to reflect changes made per Amendment 1.

Table 9–4: Description of laboratory parameters (safety)

Hematology	Hemoglobin, hematocrit, RBC count, WBC count and differential (including lymphocytes, monocytes, eosinophils, basophils, neutrophils), MCV, MCH, MCHC, platelets
Blood chemistry	Albumin, total protein, BUN, creatinine, glucose, total bilirubin, ALP, AST, ALT, uric acid
Coagulation	INR, PT, PTT
Urinalysis (urine dipstick) ^a	RBCs, WBCs, pH, specific gravity, glucose, protein, bilirubin, urobilinogen, nitrite, ketones
Urine pregnancy test ^b	Urine β -hCG

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, β -hCG = beta-human chorionic gonadotropin, BUN = blood urea nitrogen, INR = international normalized ratio, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PT = prothrombin time, PTT = partial thromboplastin time, RBC = red blood cell, WBC = white blood cell.

- a If abnormalities are identified by the dipstick, the samples will be sent for confirmatory testing¹⁷⁰. Cell/sediment microscopy should be performed only if positive dipstick results are obtained for WBC, RBC/hemoglobin, or protein.
- b To be performed at Visits 1, 2, 3, 6, 8, and 9 on females of childbearing potential (i.e., female subjects who have experienced menarche). If any urine pregnancy test is positive, a serum pregnancy test will be performed to confirm the result.¹⁷¹

Safety laboratory assessments are not applicable in part 2 of the study.

9.6.3.2 Total volume of blood

In order to minimize the burden of blood loss due to PK sampling, age-appropriate sparse sampling strategies combined with (micro-)bioanalysis techniques will be applied. The total volume of blood obtained at each visit (for serology, qPCR, safety, and PK specimens) will be a maximum of approximately 8.1 mL 15.6 mL depending on the child's age and weight as per standards of Clinical Laboratory and Standards Institute; see Section 16.6 and Table 9–5 below.¹⁷²

Table 9–5: Total volume of blood by age

Age	Weight	Circulating blood volume	Maximum allowable every two weeks
Pre-term neonate	500 grams	40 mL	1.5 mL
Full-term neonate	4 kilograms	320 mL	12 mL
Infant (3 years)	15 kilograms	1.2 L	45 mL
Child (12 years)	40 kilograms	3.2 L	120 mL

¹⁷⁰ Modified as per amendment 2

¹⁷¹ Text modified to reflect changes made per Amendment 1

¹⁷² Text modified for clarity and to expand the maximum amount of blood required (Amendment 1)

9.6.3.3 Physical examination

Part 1 (CHICO)

Physical examinations will be performed by a medically qualified person. A complete physical examination of body systems will be performed at the Screening Visit. A brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and assessments for the presence of peripheral neuropathy will be performed at all subsequent designated time points (see [Table 9–1](#)). Abnormal findings on physical examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see [Section 9.6.1.1](#)).¹⁷³

A neurological examination will be performed at Screening, and Visits 3, 6, and 8 (EOT). The examination will include assessments of mental status and cognition, cranial nerves, motor function, deep tendon reflexes, sensation, and coordination and gait. Abnormal findings on neurological examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see [Section 9.6.1.1](#)).¹⁷⁴

Assessments of Chagas' disease signs and symptoms will be performed at the time of physical examinations (see [Section 9.4.4](#)).¹⁷⁵

Part 2 (CHICO SECURE)

Physical examinations will be performed by a medically qualified person. A complete physical examination of body systems will be performed at FU Visit 1, 3, and 5 (see [Table 9–2](#)). Abnormal findings on physical examination before signing informed consent will be documented as medical history; abnormal findings thereafter will be documented as AEs (see [Section 9.6.1.2](#)). Assessments of Chagas' disease signs and symptoms will be performed at the time of physical examinations.

9.6.3.4 Vitals signs, height/length, and weight

In part 1 of the study, vital signs (systolic and diastolic blood pressure [optional in subjects < 5 years of age at the discretion of the investigator], heart rate, respiratory rate, and oral or rectal body temperature) will be measured at screening and Visits 2, 3, 6, 8 (EOT), 9, 10, and 11. After a 5-minute rest in a sitting position, vital signs will be measured using non-invasive equipment. In part 2 of the study, vital signs (systolic blood pressure, diastolic blood pressure, pulse) will be performed at FU Visits 1, 3, and 5.

Height/length and weight will be obtained with the subject wearing minimal clothing and no shoes.¹⁷⁶

¹⁷³ Text modified/added for clarity (Amendment 1)

¹⁷⁴ Paragraph added per Modification 10 (Amendment 1)

¹⁷⁵ Text added for clarity (Amendment 1)

¹⁷⁶ Text modified to reflect changes made per Amendment 1; last sentence moved from previous section (9.6.3.3) to this section (Amendment 1)

9.6.3.5 12-lead electrocardiogram

A resting 12-lead ECG will be performed at screening and Visits 2, 3, 6, 8 (EOT), and 9, 10, 11 (Follow-up) on all subjects ≥ 5 years of age, and at the discretion of the investigator on all subjects < 5 years of age. For subjects consenting to PK assessments, an ECG will be obtained at the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), to allow for PK/PD investigations.

The ECG will be performed at the site according to procedure, and with the subject in the supine position after he/she has rested for 10 minutes.¹⁷⁷

A resting 12-lead ECG will be performed at FU Visits 1, 3, and 5 in part 2 of the study on all subjects. The ECG will be performed at the site according to standard procedures, and with the subject in the supine position after he/she has rested for at least 10 minutes.

ECG recordings will be evaluated by the investigator and a cardiologist associated with the site. In case of ECG abnormalities suspicious for cardiac involvement of Chagas' disease, further diagnostic examinations may be required at the discretion of the investigator, (e.g., echocardiography, chest X-ray, 24-hour ECG).

9.6.3.6 Urine pregnancy test

A urine pregnancy test on all female subjects of childbearing potential (i.e., all females who have experienced menarche) will be performed at screening and Visits 2, 3, 6, 8 (EOT), and 9 (Follow-up). If any urine pregnancy test is positive, a serum pregnancy test will be performed to confirm the result. If pregnancy is confirmed at Visits 1 or 2, the subject is to be excluded from study participation. If pregnancy is confirmed at Visits 3 or 6, the subject will be discontinued from the study (See Section 9.6.2) and undergo all study assessments as described for the EOT Visit (Section 9.2.2.7).¹⁷⁸

There will be no urine pregnancy test in part 2 of the study.

9.7 Other procedures and variables

In part 1 of the study, no other procedures will be performed, and no other variables will be measured.

In part 2 of the study, the housing conditions, especially with regard to active or effective vector-control to *T. cruzi* reinfection as determined by Ministry of Health guidelines in each country and the judgement of the investigator, will be inquired at FU Visits 1, 2, 3, 4 and 5.

9.8 Appropriateness of procedures/measurements

The efficacy and safety measurements scheduled for this study are widely used and generally accepted as reliable, accurate, and relevant.

The safety variables evaluated in this study are routine clinical parameters that allow risk assessment of the investigational drug.

¹⁷⁷ Text modified for clarity and per Modifications 1 and 9 (Amendment 1)

¹⁷⁸ Text modified/added for clarity and to reflect changes made per Amendment 1

10. Statistical methods and determination of sample size

10.1 General considerations

This study has been designed to focus on the efficacy and safety of treatment with nifurtimox in children diagnosed with Chagas' disease. In this study, approximately 300 pediatric subjects will be randomized (2:1 randomization, 60-day regimen vs. 30-day regimen). Subjects will be stratified by age into four strata. A minimum of 38 subjects in each age stratum is targeted, but not required, in order to be able to derive meaningful safety conclusions. Enrollment will continue until this specification is met, unless it is determined that such a target would be unlikely to be reached in a reasonable time.¹⁷⁹

Unless otherwise specified, all significance tests will be conducted using a 2-sided alpha level of 0.05, and CIs will be 2-sided 95% intervals. All variables collected in this study will be summarized with descriptive statistics at each assessment time. Subject disposition and demographic and baseline characteristics will be summarized descriptively for each treatment group. For continuous variables, descriptive statistics will include means, standard deviations, medians, minimums, and maximums. For categorical variables, frequency counts and percentages will be provided. These will be calculated for each age stratum by dose, as well as the overall set of study subjects.

No imputations will be made for missing values occurring in the safety and background variables. Treatment of missing values for efficacy variables is addressed later in this section.¹⁸⁰ Additional analyses may be specified in the SAP if missing data patterns observed during blinded review of the data suggest any possible bias.

Statistical analysis will be performed using Statistical Analysis System (SAS); the version used will be specified in the SAP.

10.2 Analysis sets

Part 1 (CHICO)

The primary efficacy analysis will be done using the full analysis set (FAS), which is the set of subjects who received at least one dose of study drug. Analyses will also be done using the per-protocol set, which is comprised of subjects treated with study drug who have no major protocol deviations. Complete specifications will be provided in the SAP.

Analyses of safety and background data will be performed on the FAS.

Part 2 (CHICO SECURE)

The primary efficacy analysis will be done using the full analysis set (FAS) as defined in part 1. Subjects will be analyzed as randomized in part 1 (CHICO) of the study. Complete specifications will be provided in the SAP.

¹⁷⁹ Modified as per amendment 2

¹⁸⁰ Text modified/added for clarity (Amendment 1)

Analyses of safety and background data will be performed on the FAS. For safety analyses, subjects will be analyzed as treated in part 1 of the study.

10.3 Variables and planned statistical analyses

10.3.1 Variables

10.3.1.1 Part 1 (CHICO)

The primary efficacy variable will be sero-reduction or sero-conversion at 12 months post-treatment using two conventional ELISA serology tests as the measure of efficacy. This sero-reduction or sero-conversion is considered cure, and the primary variable is binary (cure, no cure).

In the event of discordancy between the two conventional ELISA test results, the following will be considered:

- For sero-reduction, the average percentage of OD reductions will be used (e.g., Test #1 = 15% and Test #2 = 25%; average = 20% and, hence, cure).
- For sero-conversion, only when both test results are negative the subject is considered as negative (cure).¹⁸¹

Secondary efficacy variables include clinical signs/symptoms of Chagas' disease, concentration test for *T. cruzi* (subjects < 8 months of age at randomization), conventional and non-conventional serologic testing, and disease state determined by qPCR.

Background variables include demographics (sex, age), height/length, weight, medical/surgical history, and medication history.

Safety variables include AEs, physical examination abnormalities, vital signs, ECG abnormalities, hematology and blood chemistry, coagulation, and urinalysis.¹⁸²

10.3.1.2 Part 2 (CHICO SECURE)

Efficacy assessments will be obtained at designated study visits (see [Table 9–2](#)).

Primary efficacy variable

The primary efficacy variable will be the incidence rate of seronegative conversion measured and confirmed by two types of assay (recombinant ELISA and IHA) in subjects who were randomized and received at least one dose of the 60-day nifurtimox treatment regimen. Both test results should be negative for the subject to be considered as seroconverted. 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. [\[33\]](#)

Secondary and other exploratory efficacy variables

Secondary efficacy variables include:

¹⁸¹ Modified as per amendment 2

¹⁸² Text modified/added for clarity and to reflect changes per Amendment 1

- Incidence rate of seronegative conversion measured and confirmed by two types of assay (recombinant ELISA and IHA) in subjects who were randomized and received at least one dose of the 30-day nifurtimox treatment regimen
- Proportion of responders who show both seronegative conversion by two types of assays and no evidence of established cardiomyopathy as measured by ECG
- Serial reduction of OD measured by recombinant ELISA and total purified antigen ELISA

Other exploratory efficacy variables include:

- Incidence rate of seroconversion measured and confirmed by two types of assay (recombinant ELISA and IHA) in subjects by age categories (≤ 2 years, > 2 years to ≤ 6 years, > 6 to ≤ 12 years, > 12 to < 18 years; age is defined as subject's age at randomization)
- Proportion of responders who show seronegative conversion and the incidence rate of seronegative conversion as confirmed by non-conventional serology in subjects who were randomized and received at least one dose of either 60- or 30-day nifurtimox treatment regimens
- Proportion of presence of parasite or positive serology in babies born of mothers who were randomized and received at least one dose of either the 60- or 30-day nifurtimox treatment regimen

Safety variables

Safety variables include AEs and vital signs.

10.3.2 Statistical and analytical plans

10.3.2.1 Part 1 (CHICO)

Efficacy

Primary efficacy analysis

The difference in the proportion of nifurtimox subjects with sero-reduction¹⁸³ or sero-conversion (60-day regimen) and the proportion estimated from historical data will be tested using a 2-sided 95% CI for a single proportion.

The null hypothesis H_0 is $p_{\text{nifurtimox}} = p_{\text{placebo}}$

The alternative hypothesis H_1 is $p_{\text{nifurtimox}} \neq p_{\text{placebo}}$, where p_t is the sero-conversion rate for treatment t .

183 Text modified for clarity (Amendment 1)

Superiority will be confirmed if the lower limit of the CI for the proportion of nifurtimox subjects (60-day regimen) with sero-reduction or sero-conversion is greater than the proportion of historical placebo subjects with sero-conversion.¹⁸⁴

Sero-conversion for the historical placebo control is estimated as 16% from cure rates as presented in two publications [15][16], using a number greater than the larger of the upper limits of the 95% CIs from the two studies. Further details about the derivation of these rates are provided in Section 10.4.¹⁸⁵

Secondary and exploratory efficacy analyses¹⁸⁶

A secondary analysis will be done to compare the proportion of subjects with sero-reduction or sero-conversion for the 60-day and 30-day nifurtimox regimens. This will be performed using a 2-sided 95% CI for the difference of two independent proportions.

The analyses specified above will also be done using data from the EOT visit, and 3 and 6 months after EOT.

The relationship of conventional serology results to qPCR results will be done using frequencies of matches and mismatches on the determination of disease status, and phi-correlation and kappa coefficient to assess the degree of agreement. The relationship of non-conventional serology to conventional serology will be analyzed in the same way.¹⁸⁷ The results from IHA and conventional serology will also be compared. The details of secondary and exploratory efficacy analyses will be further specified in the SAP.^{188,189}

Demographics and baseline characteristics

Demographics and baseline characteristics will be summarized by treatment and total population, using descriptive statistics and frequency tables as appropriate.

Safety

Safety variables will be summarized by means of descriptive statistics and/or frequency tables as appropriate. Summaries will be given by treatment regimen. All post-treatment AEs and SAEs, as well as drug-related AEs and SAEs, will be summarized by MedDRA terms.¹⁹⁰ AEs occurring between the time of consent and the first dose of study drug will be summarized separately. Subjects experiencing urine discoloration from riboflavin ingestion will be listed also, although these incidences will not necessarily be considered AEs.

184 Text deleted per Modification 2 (Amendment 1)

185 Text modified as of amendment 2

186 “Exploratory” added per Amendment 5.

187 Text modified/added for clarity (Amendment 1)

188 Sentence added per Amendment 5.

189 Replaced IFA with IHA per Amendment 6.

190 Text modified as of amendment 2

Missing data/drop outs

No imputations will be made for missing data due to dropouts or other causes. For the primary efficacy analysis done on the FAS, a patient without an assessment at the primary 12 month time point will be considered a treatment failure. For the secondary analysis using the PPS, patients who do not have serology determinations to assess the primary endpoint will be excluded from the analysis.

The following sensitivity analyses for the primary efficacy variable will be performed:

- Missing values due to the following reasons treated as treatment failures and other missing values excluded from analysis:¹⁹¹
 1. the reason for discontinuation is known or suspected to be treatment ineffectiveness or an AE
 2. a subject has not had a negative serology at any visit and has no serology determination for the primary analysis visit
 3. an indeterminate or incorrectly performed serology determination occurs at the primary analysis visit¹⁹²
- Observed data only
- Analysis on PPS

Pharmacokinetic data

Specifications for PK data analysis will be provided in a separate document. Plasma concentration vs time data documented within the study report will be analyzed descriptively using univariate statistics.¹⁹³

10.3.2.2 Part 2 (CHICO SECURE)

Analysis of the primary efficacy variable

The difference in the incidence rate of nifurtimox subjects with seronegative conversion (60-day regimen) and the rate estimated from historical data will be tested using a Poisson two-sided 95% exact confidence interval (CI).

The incidence rate of seronegative conversion for the primary efficacy variable will be modelled using a Poisson distribution with a 2-sided 95% exact CI. The rate will be estimated as the number of seronegative conversion divided by the time at risk of event (person-year) during the study. The rate of seronegative conversion () and a 2-sided limits (,) of the 95% CI for the rate can be calculated using the following formula [34]:

¹⁹¹ Modified as per amendment 2

¹⁹² Text modified/added for clarity as per Modification 2 (Amendment 1)

¹⁹³ Modified as per amendment 4

where n is the number of seronegative conversion observed, N the total subjects at risk in annual visit, V_i the i th annual visit, the total number of visits, and l and u are lower and upper confidence limits for the rate of seronegative conversion respectively, χ^2_v is the chi-square quantile for upper tail probability on v degrees of freedom.

This study uses an external control group of untreated patients with Chagas' disease in the 4-year follow-up period presented in Sosa et al. [15, 35]. In the reference, seroconversion to a negative result in the placebo group after 4-year follow-up was detected in 2 subjects with conventional serology and in 0 subjects with IHA test. Thus, none of the subjects in the placebo group was considered as seroconverted by both test results. The person time at risk was on average $44 \times 4 = 176$ person years. The incidence rate estimate was 0% (0 / 176).

Superiority will be confirmed if the lower limit of the CI for the incidence rate of seronegative conversion for subjects in 60-day regimen is greater than the rate of historical placebo subjects with sero-conversion (i.e. 0 %).

No imputations will be made for missing data due to dropouts or other causes, as person-year is considered in the calculation of incidence rate.

Frequency counts and percentages of seronegative conversion will be provided by visit.

Missing data/drop outs

No imputations will be made for missing data due to dropouts or other causes. For the primary efficacy analysis done on the FAS, drop-outs will be included in the analysis using a Poisson distribution with person-year.

Analysis of the secondary and exploratory efficacy variables

The OD values from recombinant ELISA and total purified antigen ELISA will be analyzed descriptively. Changes from baseline will be summarized to show any serial reduction of OD. Baseline is defined as the OD values from the same ELISA tests measured at Visit 1 in part 1 of the study.

The proportion of subjects who show both seronegative conversion as confirmed by two assays (recombinant ELISA and IHA) and no evidence of established cardiomyopathy as measured by ECG will be calculated overall and by treatment regimen.

The proportion of subjects who show seronegative conversion as confirmed by non-conventional serology will be calculated overall and by treatment regimen.

The same method for calculating incidence rate and its 2-sided 95% CI will be applied on the following variables:

- Seronegative conversion as confirmed by two types of assay (recombinant ELISA and IHA) in subjects who were randomized and received at least one dose of the 30-day nifurtimox treatment regimen
- Seronegative conversion as confirmed by two types of assay (recombinant ELISA and IHA) in all subjects by age categories. The age categories are grouped as ≤ 2 years, >2

years to ≤ 6 years, >6 to ≤ 12 years, >12 to <18 ; age is defined as subject's age at randomization

- Seronegative conversion as confirmed by non-conventional serology in subjects who were randomized and received at least one dose of either 60- or 30-day nifurtimox treatment regimen

Analysis of the safety variables

Safety variables will be summarized by means of descriptive statistics and/or frequency tables as appropriate by treatment group.

AEs will be assessed by the investigator regarding their causal relationship to the administration of treatment and to any study procedure of the LTFU based on all information available. The results will be displayed in summary tables and listings as descriptive statistics.

10.4 Determination of sample size

Part 1 (CHICO)

This study uses historical controls estimated from cure rates presented in two publications [15][16]. The historical cure rates are as follows.

Publication	Age range	Sero-conversion rate (95%CI) in placebo patients
Andrade et al 1996	7-12	3/65 = 5% (1%,13%)
Sosa et al 1998	6 -12	2/44= 5% (1%,16%)

For the primary objective, superiority over placebo will be confirmed if the lower limit of the 95% CI for the nifurtimox (60-day regimen) cure rate is greater than 16%, the larger of the upper limits of the 95% CIs from the two publications.¹⁹⁴

According to Guhl et al [32], nifurtimox 12 month post treatment cure rate is about 55%, with a sample size of 200 for the 60-day regimen, the power is 99% for the lower limit of the 95% CI to be greater than 16%.¹⁹⁵

The number of subjects in the 30-day treatment regimen is based on the width of a 95% CI for the difference of two proportions. A sample size of 100 subjects for the 30-day subjects together with 200 subjects for the 60-day subjects will produce a CI with a half-width of approximately 0.12.

Part 2 (CHICO SECURE)

¹⁹⁴ Modified as per amendment 2

¹⁹⁵ Modified as per amendment 2

According to Sosa-Estani [15, 35], the 48 months post treatment cure rate for benznidazole is about 5% as confirmed by two types of assay. For the primary objective, assuming a drop-out rate of 15%, the expected number of patients to be observed at the end of Part 2 (i.e., 48 months post treatment) in the 60-day regimen would be 170 out of the 200 patients in Part 1. Using the assumptions of 170 nifurtimox 60-day regimen patients in Part 2, a 5% cure rate, and that seronegative conversion events follow Poisson distribution, the estimated incidence rate is calculated to be 1.32% and the 95% CI of the incidence rate is (0.61%, 1.51%).

10.5 Planned interim analyses

No interim analysis is planned for the entire study.

11. Data handling and quality assurance

In general, the criteria, rules, and procedures in this section apply to part 1 and part 2 of the study. Criteria, rules, and procedures not applicable to either part of the study are otherwise marked.

11.1 Data recording

The data collection tool for this study will be a validated electronic data capture system. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system (SAS).

Source documentation

It is the expectation of the sponsor that key data entered into the eCRF is supported by source documentation available at the site.

Study-specific data not needed for the subject's routine medical care (e.g., scores or questionnaires) may be entered directly into the eCRF, without availability of corresponding source documentation.

The site must implement processes to ensure availability of all required source documentation. A source document checklist (not part of this protocol) will be used at the site to identify the source data for key data points collected and the monitor will work with the site to complete this.

ECG tracings will be maintained with the subject's source documentation, and ECG findings will be entered into the eCRF.

Completed subject diaries will be collected by site personnel at specific study visits (see [Table 9-1](#)). Information entered onto the subject diaries will be used to assess drug accountability and treatment compliance, and entered into the eCRF. The completed diaries will be maintained with the subject's source documentation.¹⁹⁶ (Not applicable to part 2)

¹⁹⁶ Text added to describe that subject diaries will be considered as source documentation for this study (Amendment 1)

On the days of PK blood sampling, the investigator should document if the patient was on stable dose (stable dose = last 6 doses taken). The actual sampling date and sampling time should be documented. Failure to comply with the documented sampling windows or missing samples does not invalidate the subject or the sample for the PK evaluation. (Not applicable to part 2)

For subjects consenting to undergo PK blood sampling, blood samples will be sent to the sponsor, and the following data will be entered into the SAS after database lock for part 1 of the study.

Administered dose and exact times of two medication intakes during the visit, exact times of blood sampling, time of first dose after blood sampling, and samples collected within time window will be recorded in the eCRF.¹⁹⁷ (Not applicable to part 2)

Data recorded from screening failures

Data of 'only screened subjects' will be recorded at least as source data, as far as the reason for the premature discontinuation is identifiable. In addition to the applicable inclusion and/or exclusion criteria, the following data should be recorded in the eCRF:

- Demographic information (subject number; year of birth/age; sex; if applicable race/ethnicity)
- Date of informed consent
- Reason for premature discontinuation
- Date of last visit

These data will be transferred to the respective database.

For screening failures with an SAE, the following data should be collected in the eCRF in addition to the data specified above:

- All information related to the SAE such as:
 - Concomitant medication
 - Medical history
 - Other information needed for SAE complementary page

11.2 Monitoring

In accordance with applicable regulations, GCP, and sponsor's/CRO's procedures, monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and sponsor's requirements. When reviewing data collection procedures, the discussion will also include identification and documentation of source data items.

¹⁹⁷ Modified as per amendment 2

The sponsor/designee will monitor the site activity to verify that the:

- Data are authentic, accurate, and complete.
Supporting data may be requested (example: blood glucose readings to support a diagnosis of diabetes).
- Safety and rights of subjects are being protected
- Study is conducted in accordance with the currently approved protocol
(including study treatment being used in accordance with the protocol)
- Any other study agreements, GCP, and all applicable regulatory requirements are met.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

11.3 Data processing

Data will be collected as described in Section 11.1. Clinical data management will be performed in accordance with applicable sponsor's/CRO's standards and data cleaning procedures. This is applicable for data recorded on eCRF as well as for data from other sources (e.g., IVRS/IWRS, laboratory, ECG, ePRO, adjudication committees).

For data coding (AEs, medication), The Medical Dictionary for Regulatory Activities will be used for AEs, and the WHO Drug Dictionary will be used for prior and concomitant medications.¹⁹⁸

After its initial release for biometrical analysis, the clinical database is planned to be re-opened for the inclusion of the PK data.¹⁹⁹ (Not applicable to part 2)

11.4 Missing data

In general, no imputations will be made for missing data other than those described in Section 10.²⁰⁰ More details will be provided in the SAP.

11.5 Audit and inspection

To ensure compliance with GCP and regulatory requirements, a member of the sponsor's (or a designated CRO's) quality assurance unit may arrange to conduct an audit to assess the performance of the study at the study site and of the study documents originating there. The investigator/institution will be informed of the audit outcome.

In addition, inspections by regulatory health authority representatives and IEC(s)/IRB(s) are possible. The investigator should notify the sponsor immediately of any such inspection.

The investigator/institution agrees to allow the auditor or inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector to

¹⁹⁸ Text modified for clarity (Amendment 1)

¹⁹⁹ Text deleted for clarity (Amendment 1)

²⁰⁰ Text added for clarity (Amendment 1)

discuss findings and any issues. Audits and inspections may occur at any time during or after completion of the study.

11.6 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

Patient (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution, or private practice. Where the archiving procedures do not meet the minimum timelines required by the sponsor, alternative arrangements must be made to ensure the availability of the source documents for the required period.

The investigator/institution notifies the sponsor if the archival arrangements change (e.g., relocation or transfer of ownership).

The investigator site file is not to be destroyed without the sponsor's approval.

The contract with the investigator/institution will contain all regulations relevant for the study center.

12. Premature termination of the study

All criteria, rules, and procedures in this section apply to part 1 and part 2 of the study.

The sponsor has the right to close this study (or, if applicable, individual segments thereof [e.g., treatment arms; dose steps; centers]) at any time, which may be due but not limited to the following reasons:

- If risk-benefit ratio becomes unacceptable owing to, for example,
 - Safety findings from this study (e.g., SAEs)
 - Results of any interim analysis
 - Results of parallel clinical studies
 - Results of parallel animal studies (e.g., toxicity, teratogenicity, carcinogenicity or reproduction toxicity).
- If the study conduct (e.g., recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within a reasonable time frame.

The investigator has the right to close his/her center at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties. Final decision on the closure must be in writing.
- All affected institutions (e.g., IEC(s)/IRB(s); competent authority(ies); study center; head of study center) must be informed as applicable according to local law.

- All study materials (except documentation that has to remain stored at site) must be returned to the sponsor. The investigator will retain all other documents until notification is given by the sponsor for destruction.
- In the event of a partial study closure, ongoing subjects, including those in post study follow-up, must be taken care of in an ethical manner.

Details for individual subject's withdrawal can be found in Section [6.3.1](#).

13. Ethical and legal aspects

In general, the criteria, rules, and procedures in this section apply to part 1 and part 2 of the study. Criteria, rules, and procedures not applicable to either part of the study are otherwise marked.

13.1 Investigator(s) and other study personnel

This study will be conducted at investigational study centers in Latin America and Mexico.

The medical expert for this study is PPD 5F Citi Group Tower, No. 33 Huayuan Shiqiao Rd, Shanghai, 200120, P.R. China.

The Study Manager and the Medical Expert will assign the coordinating investigator responsible for signing the clinical study report.

All other study personnel not included in this section are identified in a separate personnel list (not part of this clinical study protocol) as appropriate. This list will be updated as needed; an abbreviated version with personnel relevant for the centers will be available in each center's investigator site file.

Whenever the term 'investigator' is noted in the protocol text, it may refer to either the principal investigator at the site, or an appropriately qualified, trained and delegated individual of the investigational site.

The principal investigator of each center must sign the protocol signature page and must receive all required external approvals (e.g., health authority, ethics committee, sponsor) before subject recruitment may start at the respective center. Likewise, all amendments to the protocol must be signed by the principal investigator and must have received all required external approvals before coming into effect at the respective center.

A complete list of all participating centers and their investigators, as well as all required signature documents, will be maintained in the sponsor's study file.

The global sponsor of this study is identified on the title page of this protocol. If required by local law, local co-sponsors will be nominated; they will be identified on the respective country-specific signature pages.

External data evaluation bodies

An independent Data Monitoring Committee (DMC) will meet periodically to review the safety data of enrolled subjects, as well as the continuing scientific merit of the trial.

The DMC may recommend stopping enrollment in the case of a negative risk/benefit assessment. The DMC will be comprised of a minimum of two clinicians with expertise in relevant clinical specialties and at least one statistician knowledgeable about statistical methods for clinical trials. Each committee member will be screened for evidence of an absence of serious conflicts of interest. The operation of the DMC will be governed by a charter that will describe the group's frequency of meetings, procedures (including, but not limited to, periodic safety monitoring), and requirements for reporting its observations to the Sponsor. (Not applicable to part 2)

13.2 Funding and financial disclosure

Funding

This study will be funded by its sponsor.

Financial disclosure

Each investigator (including principal and/or any sub investigators) who is directly involved in the treatment or evaluation of research subjects has to provide a financial disclosure according to all applicable legal requirements. All relevant documentation will be filed in the trial master file.

13.3 Ethical and legal conduct of the study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the sponsor and investigator abide by GCP guidelines and the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate IEC(s)/IRBs will be obtained for all participating centers/countries before start of the study, according to GCP, local laws, regulations, and organizations. When necessary, an extension, amendment, or renewal of the IEC/IRB approval must be obtained and also forwarded to the sponsor. The responsible unit (e.g., IEC/IRB, head of the study center/medical institution) must supply to the sponsor, upon request, a list of the IEC/IRB members involved in the vote and a statement to confirm that the IEC/IRB is organized and operates according to GCP and applicable laws and regulations.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by either the sponsor or the investigator without agreement by both parties. However, the investigator or the sponsor may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior IEC/IRB/sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution/sponsor. Any deviations from the protocol must be explained and documented by the investigator.

Details on discontinuation of the entire study or parts thereof can be found in Section [12](#).

13.4 Subject information and consent

All relevant information on the study will be summarized in an integrated subject information sheet and informed consent form provided by the sponsor or the study center. A sample subject information and informed consent form is provided as a document separate to this protocol.

Based on this subject information sheet, the investigator OR DESIGNEE will explain all relevant aspects of the study to each subject/legal representative or proxy consentor (if the subject is under legal protection) prior to his/her entry into the study (i.e., before any examinations and procedures associated with the selection for the study are performed or any study-specific data is recorded on study-specific forms).

The investigator OR DESIGNEE will also mention that written approval of the IRB/IEC has been obtained.

Each subject/legal representative or proxy consentor will be informed about the following aspects of premature withdrawal:

- Each subject has the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.
- The subject's consent covers end-of-study examinations as specified in the visit description described in Section 13.4 to be conducted after withdrawal of consent.
- The subject's data that have been collected until the time of withdrawal will be retained and statistically analyzed in accordance with the SAP.
- Subject-specific data on the basis of material obtained before withdrawal may be generated after withdrawal (e.g., image reading, analysis of biological specimen such as blood, urine, or tissues); these data would also be retained and statistically analyzed in accordance with the SAP. The subject has the right to object to the generation and processing of this post-withdrawal data. For this, he/she needs to sign a corresponding declaration of objection; alternatively, the subject's oral objection may be documented in the subject's source data.

Each subject/legal representative or proxy consentor will have ample time and opportunity to ask questions.

Only if the subject/legal representative or proxy consentor voluntarily agrees to sign the informed consent form and has done so, and assent is obtained from minor subjects, may he/she enter the study. Additionally, the investigator OR DESIGNEE will personally sign and date the form. The subject/legal representative or proxy consentor will receive a copy of the signed and dated form.

The signed informed consent statement is to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution.

In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject's clinical record must clearly show that informed consent was obtained prior to these procedures.

If the subject is not capable of providing a signature, a verbal statement of consent can also be given in the presence of an impartial witness (independent of the sponsor and the investigator). This is to be documented in the subject's source document and signed by the informing investigator OR DESIGNEE as well as the witness.

For minors or adults under legal protection, consent shall be given by the legal guardian(s). The consent of a minor or adult under legal protection shall also be requested where such a person is able to express his/her own will. His/her refusal or the withdrawal of his/her consent may not be disregarded.

The informed consent form and any other written information provided to subjects/legal representatives or proxy consenters will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and/or the written informed consent form. The investigator OR DESIGNEE will inform the subject/legal representative or proxy consenters of changes in a timely manner and will ask the subject to confirm his/her participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IEC/IRB's approval/favorable opinion in advance of use.

13.5 Publication policy and use of data

The sponsor has made the information regarding the study protocol publicly available on the internet at www.clinicaltrials.gov.

All data and results and all intellectual property rights in the data and results derived from the study will be the property of the sponsor who may utilize them in various ways, such as for submission to government regulatory authorities or disclosure to other investigators.

Regarding public disclosure of study results, the sponsor will fulfill its obligations according to all applicable laws and regulations. The sponsor is interested in the publication of the results of every study it performs.

The sponsor recognizes the right of the investigator to publish the results upon completion of the study. However, the investigator, whilst free to utilize study data derived from his/her center for scientific purposes, must obtain written consent of the sponsor on the intended publication manuscript before its submission. To this end, the investigator must send a draft of the publication manuscript to the sponsor within a time period specified in the contract. The sponsor will review the manuscript promptly and will discuss its content with the investigator to reach a mutually agreeable final manuscript.

13.6 Compensation for health damage of subjects/insurance

The sponsor maintains clinical trial insurance coverage for this study in accordance with the laws and regulations of the country in which the study is performed.

13.7 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Subject names will not be supplied to the sponsor. Only the subject number will be recorded in the eCRF, and if the subject name appears on any other document (e.g., pathologist report), it must be obliterated before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. As part of the informed consent process, the subjects will be informed in writing that representatives of the sponsor, IEC/IRB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

The investigator will maintain a list to enable subjects to be identified.

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²⁰¹ Reference 28 added as per amendment 2

²⁰² References 17, 19, 20, and 28 modified/added based on changes made to text in Section 5 (Amendment 1)

²⁰³ Reference added as per amendment 2

15. Protocol amendments

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents.

15.1 Amendment 1

Description of Amendment

On 30 DEC 2014, Bayer HealthCare Pharmaceuticals Inc. received a “study may proceed” letter for Investigational New Drug 109901 (BAY A2502 - nifurtimox), detailing recommended revisions to the protocol. The primary purpose of Amendment 1 is to update the protocol in response to these regulatory recommendations, as described below:

ECGs will be obtained at the time PK samples are collected when nifurtimox has reached C_{\max} or peak steady-state concentrations (i.e., at the 2 – 4 hour time point). The number of evaluable PK subjects will be at least 10 per age cohort.

The reduction in the ELISA OD value for subjects over 8 months of age at enrollment as an indicator of the decrease in antibody titer from study enrollment to 12 months post-treatment (percentage change over time in the study test sample baseline OD value minus the OD of the sample at 12 months divided by the baseline OD) was added to the protocol. A 20% or greater reduction in OD at 12 months is regarded as a clinically meaningful indicator of cure and will indicate positive treatment response (cure) for the primary endpoint. In subjects who are < 8 months old at study entry, an antibody titer below the level of detection of the ELISA (i.e., negative antibody titer) at 12-months post-treatment will be considered a positive treatment response. For placebo historical data, sero-reduction data from two publications will be used. For secondary endpoints, ELISA OD values will be measured at the 1 and 2 months during treatment, and at 3, 6, and 12 months post-treatment. To confirm the diagnosis of Chagas’ disease in subjects > 8 months old, a conventional ELISA test, plus another supplemental test as confirmation, will be utilized. The results from both tests must be positive in order for a patient to be considered eligible for study enrollment. For subjects under 8 months of age, direct microscopic observation of *T. cruzi* by concentration test will be required for diagnosis. New tests for confirming the diagnosis of Chagas’ disease were added: recombinant ELISA and total purified antigen ELISA. These changes were also reflected in the statistical methods section of the protocol (Section 10).

Since indirect immunofluorescence and indirect hemagglutination tests are not quantitative tests, which is required for evaluation of the primary endpoint, the ELISA tests for diagnosis were changed to recombinant ELISA and total purified antigen ELISA in the protocol. For evaluation of the primary endpoint at 12-months post-treatment, only one ELISA assay (the same one used for diagnosis) will be used. Additionally, two subject baseline specimens will be collected. One will be used for the initial diagnostic test; the other will be frozen and retained to assay as an internal subject control along with the subject’s 12-month specimen.

The use of riboflavin in the placebo tablets in the 30-day treatment group has the potential for unblinding subjects and investigators if coloring of the urine is observed. This is unlikely to significantly affect the safety evaluation or the primary efficacy endpoint; however, it may cause compliance issues and/or dropouts. Since the urine color change does not occur in all

subjects, this possibility will be reinforced to each subject during randomization and when study drug is dispensed.

Information about laboratory procedures, assay variability and cutoffs, and facilities to be used for all assays was added. Chemistry, hematology, and urinalysis will be done in the local labs of the selected sites. Relevant information and documents regarding laboratory procedures, assay variability and cutoffs, and facilities to be used, will be provided to the agencies in a separate IND information amendment after site selection in the participating countries. Serological testing will be done in a central lab.

A safety and compliance questionnaire/script (Phone Contact Form) for use by site staff when conducting the telephone assessments at Visits 4, 5, and 7 was developed and included in the protocol appendices.

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 to obtain safety and compliance information. A telephone assessment was selected so as not to impose the burden of a sixth study site visit on the subject and/or guardian, who typically resides in remote areas with transportation challenges.

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.

15.1.1 Overview of changes

This section provides a conceptual overview of all modifications to the amended protocol, as introduced by this amendment. The associated changes to the protocol text are detailed in Section [15.1.2](#).

Modification 1: Text added to indicate that ECGs will be obtained at the time PK samples are collected when nifurtimox has reached C_{max} or peak steady-state concentrations (i.e., at the 2 – 4 hour time point)

Rationale: Regulatory recommendation to assess efficacy and pharmacokinetics

- *Synopsis, Methodology*
- *Section 5, Study design*
- *Section 9.1, Tabular schedule of evaluations*
- *Table 9-1, Schedule of procedures and assessments – Study 16027*

- *Section 9.2.2, Treatment Phase*
- *Section 9.2.3, Follow-up Phase*
- *Section 9.5, Pharmacokinetic/pharmacodynamics assessments*
- *Table 9–2: Pharmacokinetic sampling time points and windows*
- *Section 9.6.3.5, 12-lead electrocardiogram*

Modification 2: 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)

Rationale: Regulatory recommendation for efficacy and inclusion criteria

- *Synopsis, Study objective(s), Diagnosis and main criteria for inclusion/exclusion, Primary efficacy variable(s), and Plan for statistical analysis*
- *Section 4, Study objectives*
- *Section 5, Study design*
- *Section 6.1, Inclusion criteria*
- *Section 9.1, Tabular schedule of evaluations*
- *Section 9.2.1, Screening*
- *Section 9.2.2, Treatment phase*
- *Section 9.2.3, Follow-up phase*
- *Section 9.4, Efficacy*
- *Section 9.4.2, Serological tests*
- *Section 9.4.3, Concentrations tests for *T. cruzi**
- *Section 10.3, Variables and planned statistical analyses*
- *Section 10.4, Determination of sample size*

Modification 3: Baseline specimens for serological tests to be collected for diagnosis, and frozen as a subject control.

Rationale: Regulatory recommendation

- *Serological tests, Section 9.4.2*

Modification 4: Change in urine color to be reinforced to subjects at the visits where study drug is dispensed

Rationale: Regulatory recommendation to avoid issues with treatment compliance

- *Section 7.4, Dosage and administration*
- *Section 9.2.2.1, Visit 2 – baseline (treatment phase, Day 1)*

Modification 5: Information about laboratory procedures, assay variability and cutoffs, and facilities to be used for all assays was added.

Rationale: Regulatory recommendation

- *Section 9.4.2, Serological tests*

Modification 6: A safety and compliance questionnaire/script (Phone Contact Form) for use by site staff when conducting the telephone assessments was added.

Rationale: Regulatory recommendation

- *Synopsis, Methodology*
- *Section 5, Study design*
- *Section 8.1, Prior and concomitant therapy*
- *Section 9.1, Tabular schedule of evaluations*
- *Table 9-1, Schedule of procedures and assessments – Study 16027*
- *Section 9.2.2.3, Visit 4 (Day 14 ± 3 days)*
- *Section 9.2.2.4, Visit 5 (Day 21 ± 3 days)*
- *Section 9.2.2.6, Visit 7 (Day 42 ± 3 days)*
- *Section 16.1, Phone Contact Form for telephone assessments*

Modification 7: For standardization of adverse event reporting, the terms “mild”, “moderate”, and “severe” were defined within the protocol.

Rationale: Regulatory recommendation for safety

- *Section 9.6.1.2.2, Intensity*

Modification 8: A telephone assessment at Day 14 was added.

Rationale: Regulatory recommendation to obtain safety and compliance information

- *Synopsis, Methodology*
- *Section 5, Study design*
- *Section 7.7, Treatment compliance*

- *Section 9.1, Tabular schedule of evaluations*
- *Table 9-1, Schedule of procedures and assessments – Study 16027*
- *Section 9.2.2.3, Visit 4 (Day 14 ± 3 days)*
- *Visit 5 (Day 30 ± 3 days), Section 9.2.2.4*

Modification 9: Measurement of ECGs will be optional assessments for subjects < 5 years of age, and required for subjects 5 years of age and older.

Rationale: Regulatory recommendation for safety

- *Synopsis, Methodology*
- *Section 5, Study design*
- *Section 9.1, Tabular schedule of evaluations*
- *Table 9-1, Schedule of procedures and assessments – Study 16027*
- *Section 9.2.1, Screening*
- *Section 9.2.2, Treatment phase*
- *Section 9.2.3, Follow-up phase*
- *Section 9.5, Pharmacokinetics/pharmacodynamics*
- *Section 9.6.3.4, Vital signs, height/length, and weight*
- *Section 9.6.3.5, 12-lead electrocardiogram*

Modification 10: A neurological examination to be performed at the time of each physical examination in the study was added.

Rationale: Regulatory recommendation for safety and efficacy

- *Synopsis, Methodology*
- *Section 4, Study objectives*
- *Section 5, Study design*
- *Section 9.1, Tabular schedule of evaluations*
- *Table 9-1, Schedule of procedures and assessments – Study 16027*
- *Section 9.2.1, Screening*
- *Section 9.2.2, Treatment phase*
- *Section 9.6.3.3, Physical examination*
- *Section 9.6.3, Further safety*

Modification 11: Detailed procedures regarding follow-up on all subjects who prematurely discontinue study drug was added.

Rationale: Regulatory recommendation for safety

- *Synopsis, Methodology*
- *Section 5, Study design*
- *Table 9-1, Schedule of procedures and assessments – Study 16027*
- *Section 9.1, Tabular schedule of evaluations*

Modification 12: 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.

Rationale: Regulatory recommendation for safety and efficacy

- *Synopsis, Methodology*
- *Section 5, Study design*
- *Section 7.7, Treatment compliance*
- *Section 8, Non-study therapy*
- *Section 9.2.2.5, Visit 6 (Day 30 ± 3 days)*
- *Section 16.1, Phone Contact Form for telephone assessments*

Modification 13: A Phone Contact Form was developed and added as an appendix (16.1) to the protocol.

Rationale: To assess incidence of adverse events, use of concomitant medications, and treatment compliance

- *Section 16.1*

Modification 14: A Subject Diary was developed and added as an appendix (16.3) to the protocol.

Rationale: To assess treatment compliance

- *Section 16.3*

15.1.2 Changes to the protocol text

Editorial note

In the sections on changes to the protocol text, all protocol sections affected by the respective amendment are detailed; the sequence of the sections follows the structure of the most recent protocol version. As applicable, changes to the protocol text are highlighted as follows:

- **Addition of a whole new portion** Brief identification of the new portion
- **Removal of a whole portion** Complete display of the removed portion, formatted as ~~crossed out~~
- **Editing of an existing portion** Comparative presentation of “old text” versus “new text”, with “old text” referring to the most recent previous protocol version. Deletions are crossed out in the “old text”. Additions are underlined in the “new text”.
- **Terminological changes** Brief specification of the terminological change

Editorial changes, correction of typographical errors, punctuation revisions, addition of references, updates to all cross-referenced field codes, and minor revisions of language were made to ensure clarity and consistency throughout the document.

The following terminological changes were made globally throughout the document:

- The term “titer(s)” was changed to “concentration(s)”
- Age groups in the study population were modified as follows: “subjects older than 8 months of age” to “subjects > 8 months of age at randomization”; “[subjects] younger than 18 years of age” to “[subjects] < 18 years of age at randomization”; “subjects 8 months of age and younger” to “subjects ≤ 8 months of age at randomization”
- “qualitative PCR” was changed to “quantitative PCR”

Synopsis, Study Objectives

This section was changed as a result of Modifications 2 and 10.

Old text:

The primary objective of this Phase 3 clinical study is:

- To assess the superiority of a 60-day regimen of nifurtimox to historical untreated control as seroreduction (~~≥20% sero-reduction~~ compared to baseline in subjects ~~older than 8 months of age and younger than 18 years of age~~), or sero-conversion (~~in subjects 8 months of age and younger~~) at the ~~12-month follow up (360 days from end of treatment [EOT])~~

New text:

The primary objective of this Phase 3 clinical study is:

- 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 seroreduction (defined as a $\geq 20\%$ reduction in optical density measured by conventional enzymelinked immune sorbent assay [ELISA]) compared to baseline in subjects ≥ 8 months to ≤ 18 years of age at randomization, or sero-conversion (defined as negative Immunoglobulin G concentration for all subjects).

Old text:

Secondary objectives of the study are:

- To assess the comparability of a 60-day regimen of nifurtimox to historical active control (benznidazole) as sero-reduction ($\geq 20\%$ ~~seroreduction compared to baseline in subjects older than 8 months of age and younger than 18 years of age~~) or seroconversion (~~in subjects 8 months of age and younger~~) and to qualitative polymerase chain reaction (qPCR) at the 12-month followup (360 days from EOT)
- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox as seroreduction ($\geq 20\%$ ~~sero reduction compared to baseline in subjects older than 8 months of age and younger than 18 years of age~~), or sero-conversion (~~in subjects 8 months of age and younger~~) and qualitative polymerase chain reaction (qPCR) at the 12-month follow-up (360 days from EOT)
- To evaluate the relationship of conventional serology ($\geq 20\%$ ~~seroreduction in subjects older than 8 months of age and younger than 18 years of age, or seroconversion in subjects 8 months of age and younger~~) to qPCR using frequencies of matches and mismatches to assess agreement
- To evaluate the safety/tolerability profile of nifurtimox by laboratory parameters (hematology, blood chemistry, urinalysis), electrocardiogram (ECG) monitoring (~~optional depending on the age of the subject, at the discretion of the investigator~~), vital sign measurements (blood pressure [~~optional depending on the age of the subject, at the discretion of the investigator~~], heart rate, respiratory rate, temperature), adverse event (AE) monitoring, and physical examinations
- ...

New text:

- To assess the comparability of a 60-day regimen of nifurtimox to historical active control (benznidazole) as sero-reduction or seroconversion at the 12-month followup (360 days from EOT)
- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox as seroreduction or sero-conversion and to quantitative polymerase chain reaction (qPCR) at the 12-month follow-up (360 days from EOT)
- To evaluate the relationship of conventional serology (as sero-reduction or sero-conversion) to qPCR using frequencies of matches and mismatches to assess agreement
- To evaluate the relationship of non-conventional serology to conventional serology
- To evaluate the safety/tolerability profile of nifurtimox by laboratory parameters (hematology, blood chemistry, urinalysis), electrocardiogram (ECG) monitoring, vital sign measurements (blood pressure, heart rate, respiratory rate, temperature), adverse event (AE) monitoring, and physical examinations, including neurological examinations
- ...

Synopsis, Diagnosis and main criteria for inclusion/exclusion

This section was changed for clarity and as a result of changes made per Modification 2.

Old text:

- Male and female pediatric subjects aged 0 days to younger than 18 years
- Chagas' disease diagnosed/confirmed by:
 - Subjects ~~younger than~~ 8 months of age must demonstrate direct observation of *Trypanosoma cruzi* by concentration test
 - Subjects 8 months of age ~~and older and younger than~~ 18 years must demonstrate a positive ~~enzyme-linked immune sorbent assay (ELISA) plus a positive~~ result for ~~one~~ of the following tests to confirm diagnosis:
 - ~~— Alternate ELISA (e.g., Chagatest ELISA recombinant v3.0)~~
 - ~~— Hemoagglutination (e.g., Chagatest hemoagglutination inhibition)~~
 - ~~— Direct Agglutination (e.g., SERODIA Chagas)~~

Written informed consent by the subject and/or parent(s) or legally authorized representative(s) ...

...

New text:

- Male and female pediatric subjects aged 0 days to younger than 18 years
- Chagas' disease diagnosed/confirmed by:
 - Subjects \leq 8 months of age at randomization must demonstrate direct observation of *Trypanosoma cruzi* by concentration test
 - Subjects \geq 8 months to \leq 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:
 - Recombinant ELISA
 - Total purified antigen ELISA

Additionally, a non-conventional ELISA test will be obtained; however, a positive result will not be required for diagnosis.

Subjects will also be eligible if there is acceptable documentation of positive Chagas' disease within three months prior to screening and they have not had prior anti-trypanocidal or anti-parasitic treatment.

Written informed consent by the subject and/or parent(s) or legally authorized representative(s) ...

...

Synopsis, Methodology

This section was changed for clarity and as a result of changes made per Modifications 1, 8, 10, and 11.

Old text:

~~After informed consent/assent has been obtained at screening and~~ study eligibility has been confirmed at Visit 2, subjects will be randomized via Interactive Voice Response System/Interactive Web Response System in a 2:1 ratio (60-day regimen vs. 30-day regimen) to one of two treatment groups:

...

The first dose of study drug will be administered at Visit 2.

Subjects will return to the investigational site for efficacy and safety assessments at Visits 3 (Day 7±1). A pre-paid phone card will be provided to facilitate contact between study site personnel and the subjects/subjects' authorized representatives.

Subjects will return to the investigational site for efficacy and safety assessments on Days 30 (±3 days) and 60 (±3 days). Day 60 will be the EOT for both treatment groups. On Days 21 (±3 days) and 42 (±3 days), study site personnel will contact the subject's parent or legal guardian via telephone to assess the occurrence of AEs and concomitant medications.

Subjects will then return to the site on Days 90, 240, and 420 (±7 days) after the start of treatment for the collection of additional efficacy and safety data. The total duration of each subject's participation is expected to be approximately 14.5 months.

Assessments of efficacy and PK will be performed at specified time windows. PK blood sampling will be optional on consent/assent of the subject/subject's legally authorized representative(s). In order to minimize the burden of blood loss due to PK sampling, age appropriate sparse sampling strategies combined with (micro-)bioanalysis techniques will be applied.

Safety will be assessed at specified time points via laboratory parameters (hematology, blood chemistry, urinalysis), vital sign measurements (blood pressure [optional depending on the age of the subject, at the discretion of the investigator], heart rate, respiratory rate, temperature), monitoring of ECGs (optional depending on the age of the subject, at the discretion of the investigator), monitoring of AEs, and physical examinations. As an additional safety precaution, all subjects with a positive microscopy or qPCR test result at Day 30 (±3 days) will be considered a treatment failure, discontinued from the study, and treated with an alternative antitrypanosomal therapy.

New text:

No study-specific procedures will be performed before the subject/legally authorized representative has signed the informed consent form, or a minor subject has provided assent, including asking a potential subject to fast (if applicable, depending on the age of the subject at the discretion of the investigator) prior to the screening blood samples. Informed consent/assent for optional PK blood sampling will also be obtained. After the consents/assents have been obtained, screening assessments will be performed from 1 to 14 days prior to randomization at Visit 2.

After study eligibility has been confirmed and safety assessments have been performed at Visit 2 (Day 1), subjects will be randomized via Interactive Voice Response System/Interactive Web Response System in a 2:1 ratio (60-day regimen vs. 30-day regimen) to one of two treatment groups:

...

The first dose of study drug will be administered at Visit 2. Pre- and post-dose PK blood samples will be obtained at specified time points from those subjects consenting to PK assessments. At the 2 – 4 hour post-dose time point of PK blood sampling (i.e., at the time of maximum concentration), an ECG (optional for subjects < 5 years of age at the discretion of the investigator) will be obtained to allow for PK/PD investigations. Study drug will be dispensed and instructions for study drug administration will be provided to all subjects.

Subjects will return to the investigational site for efficacy and safety assessments at Visits 3 (Day 7±1) and 6 (Day 30). Subjects who have not consented to PK assessments will take that day's doses of study drug as instructed. Subjects consenting to PK assessments must hold the morning dose of study drug; for these subjects, a pre-dose PK blood sample will be obtained, then the morning dose of study drug will be administered. Post-dose PK blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point. A pre-paid phone card will be provided to all subjects to facilitate contact between study site personnel and the subjects/subjects' authorized representatives.

At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.

Visits 4 (Day 14), 5 (Day 21), and 7 (Day 42) will be telephone assessments where study site personnel will contact the subject/legally authorized representative via telephone to assess the occurrence of AEs, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone assessments will be provided to all sites.

Subjects will return to the investigational site for efficacy and safety assessments on Day 60 (Visit 8), which will be the EOT for both treatment groups. Subjects who have not consented to PK assessments will take that day's doses of study drug as instructed. Subjects consenting to PK assessments must hold the morning dose of study drug. A pre-dose PK blood sample will be collected, then the morning dose of study drug will be administered. Post-dose PK blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point. Study drug will be collected, and no additional study drug will be dispensed.

After the EOT visit (Visit 8), subjects will return to the site on Days 90 (Visit 9), 240 (Visit 10), and 420 (Visit 11) for additional efficacy and safety assessments. The total duration of each subject's participation is expected to be approximately 14 months.

Safety will be assessed via laboratory parameters (hematology, blood chemistry, urinalysis), vital sign measurements (blood pressure [optional in subjects < 5 years of age at the discretion of the investigator], heart rate, respiratory rate, temperature), monitoring of ECGs (optional in subjects < 5 years of age at the discretion of the investigator), monitoring of AEs, and physical examinations, including neurological examinations.

Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visit 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (±3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments.

Synopsis, Primary variable(s)

This section was changed as a result of Modification 2.

Old text:

~~The primary efficacy variable will be a $\geq 20\%$ sero-reduction (subjects older than 8 months of age and younger than 18 years of age) or sero-conversion (subjects 8 months of age and younger) in antibody Immunoglobulin G titer at 12 months post-treatment using conventional ELISA serology as the measure of efficacy.~~

New text:

The primary efficacy variable will be sero-reduction or sero-conversion at 12 months post-treatment using two conventional ELISA serology tests as the measure of efficacy.

Synopsis, Plan for statistical analysis

This section was changed per Modification 2.

Old text:

Primary efficacy analysis

The difference in the proportion of nifurtimox subjects with $\geq 20\%$ seroreduction or sero-conversion (60-day regimen) and the proportion estimated from historical data will be tested using an exact 2-sided 95% confidence interval (CI) for a single proportion.

Superiority will be confirmed if the lower limit of the CI for the proportion of nifurtimox subjects (60day regimen) with $\geq 20\%$ sero-reduction or seroconversion is greater than the proportion of historical placebo subjects with $\geq 20\%$ sero-reduction or sero-conversion. ~~This same CI will also be used for comparison with the historical benznidazole subjects with $\geq 20\%$ seroreduction or sero conversion.~~

Secondary efficacy analysis

A secondary analysis will be done to compare the proportion of subjects with $\geq 20\%$ sero-reduction or sero-conversion for the 60-day and 30-day nifurtimox regimens. This will be performed using a standard, symmetric, asymptotic 2sided 95% CI for the difference of two independent proportions.

...

The relationship of ~~serological~~ results to qPCR results will be done using frequencies of matches and mismatches on the determination of disease status, and phicorrelation and kappa coefficient to assess the degree of agreement.

...

Sample size is based on the primary endpoint, the difference in the proportion of nifurtimox subjects with $\geq 20\%$ sero-reduction ~~(subjects older than 8 months of age and younger than 18 years of age)~~ or sero-conversion ~~(subjects 8 months of age and younger)~~ (60-day regimen) and the proportion of historical placebo subjects with $\geq 20\%$ seroreduction or sero-conversion for the two age groups. The test of superiority over placebo will be powered based on the detection of a difference in proportion of nifurtimox subjects with $\geq 20\%$ sero-reduction or seroconversion of 0.10. ~~This is considered a clinically meaningful difference according to several experts in the field.~~ Given that the nifurtimox (60day) proportion of subjects with $\geq 20\%$ seroreduction or seroconversion is 0.60, 220 evaluable subjects in this group will provide at least 80% power for an exact binomial test. This sample size will provide an exact 95% CI with a width of ≤ 0.15 (e.g., [0.53, 0.67]) for use in comparing nifurtimox to the proportion of historical benznidazole subjects with $\geq 20\%$ sero-reduction or sero-conversion.

New text:

Primary efficacy analysis

The difference in the proportion of nifurtimox subjects with seroreduction or sero-conversion (60-day regimen) and the proportion estimated from historical data will be tested using an exact 2-sided 95% confidence interval (CI) for a single proportion.

Superiority will be confirmed if the lower limit of the CI for the proportion of nifurtimox subjects (60day regimen) with sero-reduction or seroconversion is greater than the proportion of historical placebo subjects with seroconversion.

...

Secondary efficacy analysis

The same CI for the primary endpoint analysis will also be used for comparison with the historical benznidazole subjects with sero-conversion to assess whether or not their cure rates are comparable.

...

The relationship of conventional serology results to qPCR results will be done using frequencies of matches and mismatches on the determination of disease status, and phicorrelation and kappa coefficient to assess the degree of agreement. The relationship of non-conventional serology to conventional serology will be analyzed in the same way.

...

Sample size is based on the primary endpoint, the difference in the proportion of nifurtimox subjects with seroreduction or sero-conversion (60-day regimen) and the proportion of historical placebo subjects with sero-

conversion. The test of superiority over placebo will be powered based on the detection of a difference in proportion of 0.10. This quantity represents a difference between rates, such as 0.60-0.50=0.10, rather than the difference that would result from subtracting 10% of 0.60 (that is, 0.06) from 0.60. Given that the nifurtimox (60day) proportion of subjects with seroreduction or seroconversion is 0.60, 220 evaluable subjects in this group will provide at least 80% power for an exact binomial test. This sample size will provide an exact 95% CI with a width of ≤ 0.15 (e.g., [0.53, 0.67]) for use in comparing nifurtimox to the proportion of historical benznidazole subjects with sero-conversion.

...

List of abbreviations

This section was changed as a result of changes made to the body of the protocol.

Old text:

CCDS ~~Company Core Data Sheet~~

HAI ~~hemoagglutination inhibition~~

qPCR ~~qualitative~~ polymerase chain reaction

New text:

C_{max} maximum concentration

OD optical density

qPCR quantitative polymerase chain reaction

Introduction, Section 3

This section was changed since an Investigators' Brochure became available as a reference document for nifurtimox.

Background

Old text:

...

~~Further details can be found in the latest available versions of the Company Core Data Sheets (CCDS), which contain comprehensive information on the study drug.~~

New text:

...

Comprehensive information on the study drug can be found in the latest available version of the Investigators' Brochure.

Rationale of the study

This section was changed for clarity.

Old text:

...

This study was designed to develop a better understanding of the efficacy, safety/tolerability, and pharmacokinetics (PK) (absorption, distribution, metabolism, and elimination) of nifurtimox in children with a diagnosis of Chagas' disease. Results from this study will demonstrate parasitological cure (using serological results) to allow optimization of treatment of Chagas' disease in this most vulnerable population. In turn, this will improve the outlook for children by reducing mortality and longterm complications. This knowledge will allow for better and more appropriate approaches to the treatment of Chagas' disease. It will also provide more up-to-date information on the use of nifurtimox, and has the potential to increase our understanding of treatment response in children.

New text:

...

This study was designed to develop a better understanding of the efficacy, safety/tolerability, and pharmacokinetics (PK) (absorption, distribution, metabolism, and elimination) of nifurtimox in children with a diagnosis of Chagas' disease using pediatric formulations. Results from this study will demonstrate parasitological cure using conventional serological results to allow optimization of treatment of Chagas' disease in this most vulnerable population. In turn, this will improve the outlook for children by reducing mortality and long-term complications. This knowledge will allow for better and more appropriate approaches to the treatment of Chagas' disease. It will also provide more up-to-date information on the use of nifurtimox, and has the potential to increase our understanding of treatment response in children.

Benefit-risk assessment

This section was changed for clarity and as a result of Amendment 1.

Old text:

...

Nifurtimox is well tolerated in children, but its pharmacology in the pediatric population is still not well defined. The currently available ~~formulation~~ was developed for adults, and there is a significant need for a pediatric formulation particularly for infants and newborns. The safety profile of the drug is relatively well-characterized...

...

New text:

...

Nifurtimox is well tolerated in children, but its pharmacology in the pediatric population is still not well defined. The currently available marketed product was developed for adults, and there is a significant need for a pediatric formulation particularly for infants and newborns to support weightadjusted- dosing. The safety profile of the drug is relatively well-characterized...

...

Study objectives, Section 4

This section was changed as a result of Modifications 2 and 10.

Old text:

The primary objective of this Phase 3 clinical study is:

- To assess the superiority of a 60-day regimen of nifurtimox to historical untreated control as seroreduction (~~$\geq 20\%$ seroreduction compared to baseline in subjects older than 8 months of age and younger than 18 years of age~~), or sero-conversion (in subjects 8 months of age and younger) ~~at the 12month follow-up (360 days from end of treatment [EOT])~~

Secondary objectives of the study are:

- To assess the comparability of a 60-day regimen of nifurtimox to historical active control (benznidazole) as sero-reduction (~~$\geq 20\%$ seroreduction compared to baseline in subjects older than 8 months of age and younger than 18 years of age~~), or seroconversion (in subjects 8 months of age and younger) ~~and qualitative polymerase chain reaction (qPCR)~~ at the 12-month follow-up (360 days from EOT)
- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox as sero-reduction (~~$\geq 20\%$ sero reduction compared to baseline in subjects older than 8 months of age and younger than 18 years of age~~), or sero-conversion (in subjects 8 months of age and younger) and qPCR at the 12-month follow-up (360 days from EOT)
- To evaluate the relationship of conventional serology (~~$\geq 20\%$ sero reduction in subjects older than 8 months of age and younger than 18 years of age, or seroconversion in subjects 8 months of age and younger~~) to qPCR using frequencies of matches and mismatches to assess agreement
- To evaluate the safety/tolerability profile of nifurtimox by laboratory parameters (hematology, blood chemistry, urinalysis), electrocardiogram (ECG) monitoring (optional depending on the age of the subject and at the discretion of the investigator), vital sign measurements (blood pressure {optional depending on the age of the subject and at the discretion of the investigator}, heart rate, respiratory rate, temperature), adverse event (AE) monitoring, and physical examinations
- ...

New text:

The primary objective of this Phase 3 clinical study is:

- To assess the superiority of a 60-day regimen of nifurtimox to historical untreated control at the 12month follow-up (360 days from end of treatment [EOT]) as seroreduction (defined as a $\geq 20\%$ reduction in optical density [OD] measured by conventional enzymelinked immune sorbent assay [ELISA]) compared to baseline in subjects ≥ 8 months to ≤ 18 years of age at randomization or sero-conversion (defined

as negative Immunoglobulin G [IgG] concentration) in subjects \leq 8 months of age at randomization

Secondary objectives of the study are:

- To assess the comparability of a 60-day regimen of nifurtimox to historical active control (benznidazole)²⁷ as sero-reduction or sero-conversion at the 12-month follow-up (360 days from EOT)
- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox as sero-reduction or sero-conversion and to quantitative polymerase chain reaction (qPCR) at the 12-month follow-up (360 days from EOT)
- To evaluate the relationship of conventional serology (as sero-reduction or seroconversion) to qPCR using frequencies of matches and mismatches to assess agreement
- To evaluate the relationship of non-conventional serology to conventional serology
- To evaluate the safety/tolerability profile of nifurtimox by laboratory parameters (hematology, blood chemistry, urinalysis), electrocardiogram (ECG) monitoring, vital sign measurements (blood pressure, heart rate, respiratory rate, temperature), adverse event (AE) monitoring, and physical examinations, including neurological examinations
- ...

Footnote 27 was added to the bottom of the page for clarity, new text:

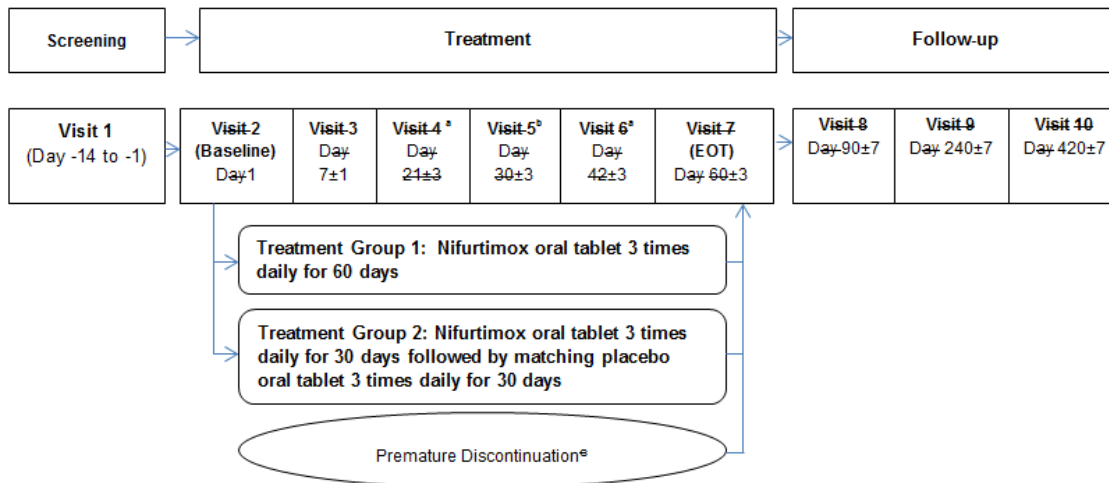
²⁷ Sero-conversion for the historical controls will be estimated from cure rates as presented in two publications.[15][16] See Section 5, Study Design, Justification of the design.

Study design, Section 5

This section was changed for clarity and as a result of Modifications 1, 2, 6, 8, 9, 10, 11, and 12.

Figure 5-1 was replaced with a new figure as a result of changes made per Amendment 1.

Old figure:



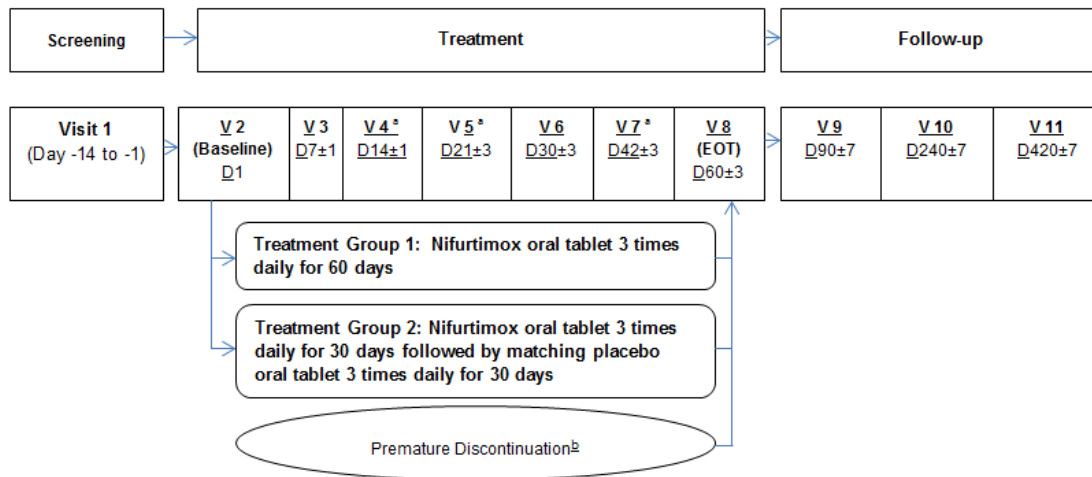
EOT = end-of-treatment.

a. At 21 (±3) days and 42 (±3) days, study site personnel will contact the subject's parent or legal guardian via telephone to assess for the occurrence of AEs and concomitant medications. If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

b. Subjects with a positive qualitative polymerase chain reaction result at Day 30 (±3 days) will be considered a treatment failure, discontinued from the study, and treated with an alternative anti-trypanosomal therapy.

c. Subjects discontinuing from the study early are to undergo all study assessments as described in the EOT visit.

New figure:



D = Day, EOT = end-of-treatment, V = Visit.

a. At Visits 4, 5, and 7, study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, and compliance with study drug administration. If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

b. Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visits 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (±3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments.

Old text:

This study will enroll pediatric subjects aged 0 to younger than 18 years. Subjects ~~younger than~~ ≤ 8 months of age with a diagnosis of Chagas' disease must demonstrate direct observation of *T. cruzi* by concentration test, and subjects ~~8 months of age and older and younger than~~ 18 years of age with a diagnosis of Chagas' disease must demonstrate a positive ~~enzymelinked-immune-sorbent assay (ELISA) plus a positive result for one of the following tests to confirm diagnosis:~~

- ~~— Alternate ELISA (e.g., Chagatest-ELISA-recombinate v3.0)~~
- ~~— Hemagglutination (e.g., Chagatest-hemoagglutination-inhibition [HAI])~~
- ~~— Direct Agglutination (e.g., SERODIA-Chagas)~~

Subjects will be stratified by age into four cohorts as specified below:

...

...

Subjects will return to the investigational site for efficacy and safety assessments on Days ~~30 (± 3 days) and 60 (± 3 days).~~ Day 60 will be the EOT for both treatment groups. ~~On Days 21 (± 3 days) and 42 (± 3 days), study site personnel will contact the subject's parent or legal guardian via telephone to assess the occurrence of AEs and concomitant medications.~~

Subjects will ~~then~~ return to the site on Days 90, 240, and 420 (± 7 days) ~~after the start of treatment for the collection of additional efficacy and safety data.~~ The total duration of each subject's participation is expected to be approximately 14.5 months.

Assessments of efficacy and PK will be performed at specified time windows. ~~PK blood sampling will be optional on consent/assent of the subject/subject's legally authorized representative(s).~~ In order to minimize the burden of blood loss due to PK sampling, ageappropriate sparse sampling strategies combined with (micro-)bioanalysis techniques will be applied.

Safety will be assessed ~~at specified time points~~ via laboratory parameters (hematology, blood chemistry, urinalysis), vital sign measurements (blood pressure [optional ~~depending on the age of the subject~~, at the discretion of the investigator], heart rate, respiratory rate, temperature), monitoring of ECGs (optional ~~depending on the age of the subject~~ and at the discretion of the investigator), monitoring of AEs, and physical examinations. ~~As an additional safety precaution, all subjects with a positive microscopy or qPCR test result at Day 30 (3 days) will be considered a treatment failure, discontinued from the study, and treated with an alternative antitrypanosomal therapy. This condition implies that the decision criteria for determining the presence of Chagas' disease use both serology and qPCR.~~

New text:

This study will enroll pediatric subjects aged 0 to younger than 18 years. Subjects ≤ 8 months of age at randomization with a diagnosis of Chagas' disease must demonstrate direct observation of *T. cruzi* by concentration test, and subjects ≥ 8 months to ≤ 18 years of age at

randomization with a diagnosis of Chagas' disease must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:

- Recombinant ELISA
- Total purified antigen ELISA

Additionally, a non-conventional ELISA test will be obtained; however, a positive result will not be required for diagnosis.

Subjects will also be eligible if there is acceptable documentation of positive Chagas' disease within three months prior to screening.

Subjects will be stratified by age at randomization into four cohorts as specified below:

...

No study-specific procedures will be performed before the subject/legally authorized representative has signed the informed consent form, or a minor subject has provided assent, including asking a potential subject to fast (if applicable, depending on the age of the subject at the discretion of the investigator) prior to the screening blood samples. Informed consent/assent for optional PK blood sampling will also be obtained. After the consents/assents have been obtained, screening assessments will be performed from 1 to 14 days prior to randomization at Visit 2.

After study eligibility has been confirmed and safety assessments have been performed at Visit 2 (Day 1), subjects will be randomized via Interactive Voice Response System/Interactive Web Response System (IVRS/IWRS) in a 2:1 ratio (60-day regimen vs. 30-day regimen) to one of two treatment groups:

...

The first dose of study drug will be administered at Visit 2. Pre- and post-dose PK blood samples will be obtained at specified time points from those subjects consenting to PK assessments (see Table 9-2). At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of maximum concentration [C_{max}]), an ECG (optional for subjects < 5 years of age at the discretion of the investigator) will be obtained to allow for PK/PD investigations. Study drug will be dispensed, and instructions for study drug administration will be provided to all subjects.

Subjects will return to the investigational site for efficacy and safety assessments at Visits 3 (Day 7±1) and 6 (Day 30). Subjects who have not consented to PK assessments will take that day's doses of study drug as instructed. Subjects consenting to PK assessments must hold the morning dose of study drug; for these subjects, a pre-dose PK blood sample will be obtained, then the morning dose of study drug will be administered. Post-dose blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point (see Table 9-2). A pre-paid phone card will be provided to all subjects to facilitate contact between study site personnel and the subjects/subjects' authorized representatives.

At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.

Visits 4 (Day 14), 5 (Day 21), and 7 (Day 42) will be telephone assessments where study site personnel will contact the subject/legally authorized representative via telephone to assess the occurrence of AEs, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone assessments will be provided to all sites (see Section 16.1).

Subjects will return to the investigational site for efficacy and safety assessments on Day 60 (Visit 8), which will be the EOT for both treatment groups. Subjects who have not consented to PK assessments will take that day's doses of study drug as instructed. Subjects consenting to PK assessments must hold the morning dose of study drug. A pre-dose PK blood sample will be collected, then the morning dose of study drug will be administered. Post-dose PK blood samples will be obtained at specified time points, and ECGs will be obtained at the 2-4 hour post-dose time point (see Table 9-2). Study drug will be collected, and no additional study drug will be dispensed.

After the EOT visit (Visit 8), subjects will return to the site on Days 90 (Visit 9), 240 (Visit 10), and 420 (Visit 11) for additional efficacy and safety assessments. The total duration of each subject's participation is expected to be approximately 14 months.

Assessments of efficacy and PK will be performed at specified time windows. In order to minimize the burden of blood loss due to PK sampling, ageappropriate sparse sampling strategies combined with (micro-)bioanalysis techniques will be applied (see Table 94). At least 10 subjects per age cohort will be recruited for PK assessments.

Safety will be assessed via laboratory parameters (hematology, blood chemistry, urinalysis), vital sign measurements (blood pressure [optional in subjects < 5 years of age at the discretion of the investigator], heart rate, respiratory rate, temperature), monitoring of ECGs (optional in subjects < 5 years of age at the discretion of the investigator), monitoring of AEs, and physical examinations, including a neurological examination (see Section 9.6.3.3).

Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visits 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (±3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments.

Primary variable(s)

This section was changed as a result of Modification 2.

Old text:

The primary efficacy variable will be a $\geq 20\%$ sero-reduction (~~subjects older than 8 months of age and younger than 18 years of age~~) or sero-conversion (~~subjects 8 months of age and younger~~) in antibody Immunoglobulin G (IgG) titer at 12 months posttreatment using conventional ELISA serology as the measure of efficacy.

New text:

The primary efficacy variable will be sero-reduction or sero-conversion at 12 months posttreatment using two conventional ELISA serology tests as the measure of efficacy.

Justification of the design

This section was changed for clarity and to provide updated study information.

Old text:

This study uses an historical untreated-controlled design as it is unethical to have a prospective placebo-controlled superiority study design. Only two benznidazole placebocontrolled studies have been conducted in children with Chagas' disease.[15][16] Direct comparisons between these studies cannot be made due to differences in study populations (Argentina vs. Brazil), medication dosage (5 mg/kg vs. 7.5 mg/kg), outcome measures, and time of follow-up (4 years vs. 3 years). Data derived from these studies will, however, be used as the comparative historical placebo control to be utilized in the analysis of the study endpoints. Hence, a comparison of sero-reduction from baseline to 12-month follow-up for both primary and secondary endpoint evaluations (versus historical placebo) will be made as well as for a ~~non-inferiority analysis versus benznidazole~~. Details are presented in Section 10.

...

Sero-reduction ~~of 20%~~ at 12 months post-treatment will be considered equivalent to historical information on seroconversion at subsequent time points.

Both primary and secondary study endpoints are based upon a target of $\geq 20\%$ decrease in ~~IgG-antibody titers~~ (sero-reduction) to *T. cruzi* in conventional serological tests or negativization of serology. This is consistent with one previous study, ~~which showed a 20% sero-reduction at 12 months~~ in children 6 to 12 years of age infected with *T. cruzi* in the intermediate (early chronic) phase of Chagas' disease.[15] Subjects were administered 5mg/kg benznidazole or placebo in a randomized fashion for 60 days, and followed for 48 months. Sero-conversion to a negative result after the 48-month post-treatment follow-up was detected by conventional serology in 11.3 % ($P < 0.05$) of the benznidazole groups and only 4.5% of the placebo group. In the benznidazole group, a significant decrease in the mean antibody ~~titer~~ against *T. cruzi* as measured by three different assays including ELISA was shown over the course of the study. No significant changes were observed in the placebo group. At 12 months, a 21% ~~sero-reduction titer~~ compared to baseline (ELISA mean OD 0.467 [± 0.099] vs. 0.369 [± 0.107]) was observed in subjects who received benznidazole. For the subsequent time points of 18, 24, and 48 months, percent ~~titer~~ reductions were 23%, 29%, and 27%, respectively. At the end of the 48-month follow-up period, nearly 62% of benznidazole-treated subjects and none of the placebotreated subjects were sero-negative for *T. cruzi*. Xenodiagnosis performed at that time was positive in 51.2% of the placebo group and 4.7% of the benznidazole group ($P < 0.001$). The low frequency of positive xenodiagnosis would be indicative of a low parasitemia and, therefore, low antigenic load which would prevent development of chronic disease.

...

While a decrease $\geq 20\%$ in antibody titer has been demonstrated across two independent clinical trials in pediatric patients with Chagas' disease, neither author has published extended follow-up data which include serological results for the patients in these studies. Since signs and symptoms are usually lacking in early Chagas' disease, it is not possible to demonstrate immediate meaningful clinical benefit to active treatment. However, it is presumed that significant reductions in *T. cruzi* antibody titer (seroreduction) by conventional serology is an early-predictor of future sero-conversion, and early treatment and cure of infection will ultimately reduce the risk of developing visceral abnormalities, as well as contribute to the decrease in *T. cruzi* transmission.

In the experience of Parasitology service, Ricardo Gutierrez Children's Hospital, Buenos Aires unpublished data (presented at European Society of Pediatric Infectious Disease 2014 and meeting of Food and Drug Administration) by PPD, a correlation between negativization of conventional serology (whole antibody against *T. cruzi* measured by ELISA or HAI) and PCR results in children 2 years of age and younger is fairly high; the correlation between these two tests in children older than 3 years is lower, as sero-conversion (i.e., becoming negative) may take several years, while PCR becomes negative within 60 days of treatment, and remains negative in this age group also. In the whole population, at least 20% sero-reduction of conventional serology was observed at 12 months. This percentage was higher in the younger children than in the older children.

Clearance of parasitemia (evaluated most commonly by xenodiagnoses or PCR) combined with disappearance of antibodies (seronegative conversion) has been proposed as cure criteria by some authors. The main limitations in evaluating treatment response for Chagas' disease using conventional serology stems from the need for long-term follow-up (years to decades) to observe negativization of conventional serological tests. (Iytic antibodies measured by chemiluminescent ELISA with purified trypomastigote), rapid serological response (i.e., negative titers) in a shorter time period were demonstrated.[16][18]

These non-conventional serological techniques are not standardized, have showed poor reproducibility, and are expensive and difficult to perform, and the preparation of the antigens is complex. Furthermore, there are no commercial reagents available for these tests, which means that the techniques must be prepared in-house with the consequent reproducibility issues among centers, and even within batches in the same center.

A new perspective on Chagas' disease diagnosis was opened by the use of the PCR as a measure of treatment response. Over the past few years, the availability of PCR to detect *T. cruzi* DNA in blood samples has opened new possibilities for the evaluation of response to trypanocidal chemotherapy. Furthermore, real-time PCR (qPCR) protocols for detection of *T. cruzi*'s DNA were developed.

There was no generally accepted and commercially available PCR method or kit available until recently. Many protocols were available differing with respect to 1) the method of DNA isolation, 2) the PCR conditions, i.e., conventional endpoint or real-time quantitative PCR, 3) use or lack of use of a Taqman probe, which increases the specificity of the test[19], and 4) inclusion of an internal standard, which can be employed to prove the validity of the data.

...

~~Subsequent~~ development of qPCR assays have several advantages compared with conventional PCR. First, the introduction of closed-tube qPCR systems has resulted in the development of rapid microbial diagnostics with low contamination risk. Also, qPCR has a shorter time-to-result, and allows quantitative assays and automation.[2725]

Studies have demonstrated the substantial value of qPCR, in terms of sensitivity and specificity, in the direct parasitological diagnosis of Chagas' disease and follow-up assessment of chemotherapy. However, in this Phase 3 study, parasitological cure based on serological tests for *T. cruzi* infection (ELISA) will be used as the primary efficacy endpoint to demonstrate efficacy of nifurtimox. Other parasitological methods (direct microscopic) and qPCR tests will be secondary efficacy variables...

The 30-day and 60day treatment regimens were selected with the intention of providing the most effective treatment within the shortest possible duration.

...

New text:

This study uses an historical untreated-controlled design as it is unethical to have a prospective placebo-controlled superiority study design. Only two benznidazole placebocontrolled studies have been conducted in children with Chagas' disease.[15][16] Direct comparisons between these studies cannot be made due to differences in study populations (Argentina vs. Brazil), medication dosage (5 mg/kg vs. 7.5 mg/kg), outcome measures, and time of follow-up (4 years vs. 3 years). Data derived from these studies will, however, be used as the comparative historical placebo control to be utilized in the analysis of the study endpoints. Hence, a comparison of sero-reduction and/or sero-conversion from baseline to 12-month follow-up for both primary and secondary endpoint evaluations (versus historical placebo) will be made as well as for a comparison with benznidazole. Details are presented in Section 10.

...

Sero-reduction (measured as a $\geq 20\%$ reduction of the ELISA absorbance [OD] value) at 12 months post-treatment will be considered equivalent to historical information on seroconversion at subsequent time points.

Both primary and secondary study endpoints are based upon a target of $\geq 20\%$ decrease in antibody concentration (sero-reduction) to *T. cruzi* in conventional serological tests or negativization of serology. This is consistent with one previous study in children 6 to 12 years of age infected with *T. cruzi* in the intermediate (early chronic) phase of Chagas' disease.[15] Subjects were administered 5mg/kg benznidazole or placebo in a randomized fashion for 60 days, and followed for 48 months. Sero-conversion to a negative result after the 48-month post-treatment follow-up was detected by conventional serology in 11.3 % ($P < 0.05$) of the benznidazole groups and only 4.5% of the placebo group. In the benznidazole group, a significant decrease in the mean antibody concentration against *T. cruzi* as measured by three different assays including ELISA was shown over the course of the study. No significant changes were observed in the placebo group. At 12 months, a 21% reduction in concentration of Chagas' diseasespecific antibodies compared to baseline

(ELISA mean OD 0.467 [\pm 0.099] vs. 0.369 [\pm 0.107]) was observed in subjects who received benznidazole. For the subsequent time points of 18, 24, and 48 months, percent concentration reductions were 23%, 29%, and 27%, respectively. At the end of the 48-month follow-up period, nearly 62% of benznidazole-treated subjects and none of the placebo-treated subjects were sero-negative for *T. cruzi*. Xenodiagnosis performed at that time was positive in 51.2% of the placebo group and 4.7% of the benznidazole group ($P < 0.001$). The low frequency of positive xenodiagnosis would be indicative of a low parasitemia and, therefore, low antigenic load which would prevent development of chronic disease.

...

While a decrease $\geq 20\%$ in antibody concentration has been demonstrated across two independent clinical trials in pediatric patients with Chagas' disease, neither author has published extended follow-up data which include serological results for the patients in these studies. Since signs and symptoms are usually lacking in early Chagas' disease, it is not possible to demonstrate immediate meaningful clinical benefit to active treatment. However, it is presumed that significant reductions in *T. cruzi* antibody concentration (seroreduction) by conventional serology is a predictor of future sero-conversion, and early treatment and cure of infection will ultimately reduce the risk of developing visceral abnormalities, as well as contribute to the decrease in *T. cruzi* transmission.

Alternative and more rapid approaches as an early indicator of negative sero-conversion in treated patients have been suggested. Using non-conventional serological techniques, i.e., highly sensitive and specific chemiluminescent ELISA using a purified trypomastigote glycoconjugate antigen and an epimastigote complex[20], the time to negativization was significantly sooner for the nonconventional ELISA than for the conventional ELISA. The chemiluminescent ELISAs provide tests that are highly sensitive and specific for Chagas' disease diagnosis. They can be used in blood bank screening and to monitor the treatment of patients undergoing chemotherapy.[21]

Non-conventional ELISA antigens, such as those used in the Sosa-Estani and deAndrade trials (i.e., F29 and F 2/3).[15][16] are often considered as early markers for effective Chagas' disease therapy. In the study by Altcheh, et al, the kinetics of disappearance of conventional serology and anti-F2/3 antibodies were compared in 21 patients with congenital Chagas' disease after receiving benznidazole treatment. Patients were divided into two groups: (A) age < 8 months at diagnosis and (B) age > 9 months at diagnosis. Group A presented negative outcome for conventional serology at 6.6 months (CI 95 3.4-9.8 months) and for anti-F2/3 at 4 months (CI 95 0.9-7.1 months, $p = 0.18$). Group B exhibited non-reactive conventional serology at 63.1 months (CI 95 42.1-84.2 months) whereas anti-F2/3 antibody determination became negative at 21.9 months (CI 95 5.7-38.1 months, $p = 0.0025$). In patients belonging to Group A, antibodies were undetectable by both conventional serology and anti F2/3 ELISA soon after receiving chemotherapy. In infants included in Group B, a negative result for anti-F2/3 antibody detection occurred significantly in advance of negative conventional serology reactivity. Consequently, the antiF2/3 antibody assay becoming negative should be considered as a surrogate endpoint for assessment of cure or positive response to treatment, particularly in those patients with prolonged time of infection.[18]

A new perspective on Chagas' disease diagnosis was opened by the use of the PCR as a measure of treatment response. Over the past few years, the availability of PCR to detect *T. cruzi* DNA in blood samples has opened new possibilities for the evaluation of response to trypanocidal chemotherapy.

Clearance of parasitemia (evaluated most commonly by xenodiagnoses or PCR) combined with disappearance of antibodies (seronegative conversion) has been proposed as cure criteria by some authors.[19] The main limitations in evaluating treatment response for Chagas' disease using conventional serology stems from the need for long-term follow-up (years to decades) to observe negativization of conventional serological tests.

In the experience of Parasitology service, Ricardo Gutierrez Children's Hospital, Buenos Aires unpublished data (presented at European Society of Pediatric Infectious Disease 2014 and meeting of Food and Drug Administration) by PPD, a correlation between negativization of conventional serology (whole antibody against *T. cruzi* measured by ELISA or hemoagglutination inhibition) and PCR results in children 2 years of age and younger is fairly high; the correlation between these two tests in children older than 3 years is lower, as sero-conversion (i.e., becoming negative) may take several years, while PCR becomes negative within 60 days of treatment, and remains negative in this age group also. In the whole population, at least 20% sero-reduction of conventional serology was observed at 12 months. This percentage was higher in the younger children than in the older children.

...

Furthermore, real-time PCR (qPCR) protocols for detection of *T. cruzi*'s DNA were developed. The development of qPCR assays have several advantages compared with conventional PCR. First, the introduction of closed-tube qPCR systems has resulted in the development of rapid microbial diagnostics with low contamination risk. Also, qPCR has a shorter time-to-result, and allows quantitative assays and automation.[27]

Studies have demonstrated the substantial value of qPCR, in terms of sensitivity and specificity, in the direct parasitological diagnosis of Chagas' disease and follow-up assessment of chemotherapy. However, in this Phase 3 study, parasitological cure based on serological tests for *T. cruzi* infection (ELISA) will be used as the primary efficacy endpoint to demonstrate efficacy of nifurtimox due to lack of historical PCR data from untreated children with Chagas' disease. Other parasitological methods (direct microscopic) and qPCR tests will be secondary efficacy variables...

The 30-day and 60day treatment regimens were selected with the intention of providing the most effective treatment within the shortest possible duration. In a recent study from Bianchi et al, 62 patients with Chagas' disease confirmed by different serological tests were treated with nifurtimox (Lampit), and followed for 30 months post-treatment. All children were treated during 60 days according to protocols established by the WHO. Monitoring was performed every 20 days to evaluate treatment safety. Results indicated that both parasite load (measured through qPCR) and antibodies (ELISA absorbance) showed a significant median reduction 6 months after treatment from 6.2 to 0.2 parasite equivalents/mL, and from 0.6 to 0.2 absorbance units respectively (p<0.001). Further reductions were evidenced by the 30-month post treatment time point. Sixty days of treatment with nifurtimox was very well

tolerated and successfully reduced parasite load and antibody titers. The results show for the first time the therapeutic response and safety of nifurtimox treatment for *T. cruzi* infection in a population of school-aged children in the asymptomatic chronic phase of Chagas' disease in Colombia.[28]

End of study

This section was changed to revise the template language.

Old text:

~~For this study, important data will be collected after last patient last visit; the end of the study as a whole will be the date when the clean database is available.~~

New text:

The end of the study as a whole will be reached as soon as the last visit of the last subject has been reached in all centers in all participating countries (EU and non-EU).

Study population, Section 6

This section was changed for clarity and as a result of Modification 2.

Inclusion criteria, Section 6.1

Old text:

1. Male and female pediatric subjects aged 0 days to younger than 18 years
2. Chagas' disease diagnosed/confirmed by:
 - Subjects ~~younger than~~ 8 months of age must demonstrate direct observation of *T. cruzi* by concentration test
 - Subjects ~~8 months of age and older and younger than~~ 18 years of age must demonstrate a positive ELISA ~~plus a positive result for one of the following tests to confirm diagnosis:~~
 - ~~Alternate ELISA (e.g., Chagatest ELISA recombinate v3.0)~~
 - ~~Hemoagglutination (e.g., Chagatest HAI)~~
 - ~~Direct Agglutination (e.g., SERODIA Chagas)~~

...

New text:

1. Male and female pediatric subjects aged 0 days to younger than 18 years
2. Chagas' disease diagnosed/confirmed by:
 - Subjects \leq 8 months of age at randomization must demonstrate direct observation of *T. cruzi* by concentration test

- Subjects ≥ 8 months to ≤ 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:
 - Recombinant ELISA
 - Total purified antigen ELISA

Additionally, a non-conventional ELISA test will be obtained; however, a positive result will not be required for diagnosis.

Subjects will also be eligible if there is acceptable documentation of positive Chagas' disease within three months prior to screening and they have not had prior anti-trypanocidal or anti-parasitic treatment.

...

Exclusion criteria, Section 6.2

This section was changed for clarity.

Old text:

3. Subjects with contraindications/warnings to nifurtimox administration, or with conditions that may increase the risk of the undesirable effects of nifurtimox, including:
 - ...
 - ...
 - Severe renal impairment defined by the following:
 - For subjects < 1 year of age, estimated glomerular filtration rate (eGFR) $< 100\%$ of the lower limit of normal (LLN) appropriate for age (eGFR should be calculated according to Schwartz formula; see Section ~~16.1~~)
 - For subjects ≥ 1 year to < 18 years of age, eGFR $< 80\%$ of LLN appropriate for age

New text:

3. Subjects with contraindications/warnings to nifurtimox administration, or with conditions that may increase the risk of the undesirable effects of nifurtimox, including:
 - ...
 - ...
 - Severe renal impairment defined by the following:
 - For subjects < 1 year of age at randomization, estimated glomerular filtration rate (eGFR) $< 100\%$ of the lower limit of normal (LLN) appropriate for age (eGFR should be calculated according to Schwartz formula; see Section 16.2)
 - For subjects ≥ 1 year to < 18 years of age at randomization, eGFR $< 80\%$ of LLN appropriate for age

...

Withdrawal of subjects from study, Section 6.3

This section was changed for clarity.

Withdrawal, Section 6.3.1

Withdrawal criteria

Old text:

Subjects *must* be withdrawn from the study if any of the following occurs:

- At their own request or at the request of their legally acceptable representative...
- Pregnancy. If any urine pregnancy test is positive, a serum pregnancy test must be performed to confirm the result. See Section 9.6.2.
- ~~Positive microscopy or qPCR at Visit 5 (Day 30). All subjects with a positive microscopy or qPCR test result at Visit 5 will be considered a treatment failure, discontinued from the study, and treated with an alternative antitrypanosomal therapy.~~

New text:

Subjects *must* be withdrawn from the study if any of the following occurs:

- At their own request or at the request of their legally acceptable representative...
- Pregnancy. If any urine pregnancy test is positive, a serum pregnancy test must be performed to confirm the result. See Section 9.6.2.

Screening failure

This section was changed for clarity.

Old text:

...

Re-starting the defined set of screening procedures to enable the “screening failure” subject’s participation at a later time point is ~~not allowed with the following exceptions:~~

- The subject had successfully passed the screening procedures, but could not start subsequent treatment on schedule.
- Initial screening occurred too early to complete the required washout period after prior therapy.
- The in-/exclusion criteria preventing the subject’s initial attempt to participate have been changed (via protocol amendment).

Subjects not meeting inclusion/exclusion criteria at screening may be re-screened once. In any case, the investigator has to ensure that the repeated screening procedures do not expose the subject to an unjustifiable health risk. Also, for re-screening, the subject has to re-sign the informed consent form, even if it was not changed after the subject’s previous screening.

...

New text:

...

Re-starting the defined set of screening procedures to enable the “screening failure” subject’s participation at a later time point is allowed only in the following situations:

- The subject had successfully passed the screening procedures, but could not start subsequent treatment on schedule.
- Initial screening occurred too early to complete the required washout period (Day -14 to Day -1) after prior therapy.
- The in-/exclusion criteria preventing the subject’s initial attempt to participate have been changed (via protocol amendment).

Subjects not meeting inclusion/exclusion criteria at screening may be re-screened once. At the discretion of the investigator and in consultation with Bayer, the screening period may be extended up to 3 days to allow for receipt of test results or to evaluate changes in a subject’s condition. In any case, the investigator has to ensure that the repeated screening procedures do not expose the subject to an unjustifiable health risk. Also, for re-screening, the subject has to re-sign the informed consent form, even if it was not changed after the subject’s previous screening. The second re-screening will be captured in the eCRF.

...

Treatment(s), Section 7

Treatments to be administered, Section 7.1

This section was changed for clarity.

Old text:

Each subject will receive nifurtimox oral tablets three times daily for 60 days, or for 30 days followed by nifurtimox placebo administered three times daily for 30 days. ~~Nifurtimox 30mg and 120-mg tablets have score lines and can be divided into two equal halves to allow for 15mg and 60-mg dose increments, respectively.~~

Each nifurtimox tablet...

New text:

Each subject will receive nifurtimox oral tablets three times daily for 60 days, or for 30 days followed by nifurtimox placebo administered three times daily for 30 days

Each nifurtimox tablet...

Identity of study treatment, Section 7.2

This section was changed for clarity.

Old text:

Nifurtimox 30-mg and 120-mg oral tablets and matching placebo tablets for each dosage form will be provided by Bayer HealthCare AG. The tablets for both dosage forms are yellow, round, and biconvex. The matching placebo tablets for the 30-mg and 120-mg formulations will bear the same appearance and packaging as the active study drug tablets.

~~Riboflavin is included as a colorant in the matching placebo tablets for study drug blinding purposes. A common side effect of riboflavin ingestion is bright yellow urine. Reports of urine color changes will not be considered as AEs but they will be entered in the eCRF.~~

...

New text:

Nifurtimox 30-mg and 120-mg oral tablets and matching placebo tablets for each dosage form will be provided by Bayer HealthCare AG. The tablets for both dosage forms are yellow, round, and biconvex. The matching placebo tablets for each dosage (30-mg and 120-mg formulations) will bear the same appearance and packaging as the active study drug tablets.

...

Dosage and administration, Section 7.4

This section was changed for clarity and per Modification 4.

Old text:

...

Nifurtimox will be administered three times a day, ~~preferably~~ in the morning, at noon, and at night, with food. The 30-mg and 120-mg tablets have score lines and can be divided into two equal halves to allow for 15-mg or 60 mg dose increments, respectively. The tablet ~~will be~~ manufactured for quick disintegration in order to allow administration to subjects < 6 years old who are not able to swallow tablets. Before administration, the tablet should be dissolved in ~~a small amount (5 mL) of water on a teaspoon~~ to form a soft slurry[†] that should be given immediately with food. Nifurtimox will be administered based on body weight as presented in Table 7-2.

New text:

...

Study drug will be dispensed at Visits 2 (Day 1) and 6 (Day 30), with instructions for administration. Nifurtimox will be administered three times a day, in the morning, at noon, and at night, with food. The 30-mg and 120-mg tablets have score lines and can be divided into two equal halves to allow for 15-mg or 60 mg dose increments, respectively. The tablet is manufactured for quick disintegration in order to allow administration to subjects < 6 years old who are not able to swallow tablets. Before administration, the tablet should be dissolved in enough water to fill a teaspoon (approximately 5 mL) to form a soft slurry⁶⁹ that should be given immediately with food. Nifurtimox will be administered based on body weight as presented in Table 7-2.

Footnote 1 was renumbered to 69, and revised for clarity as follows:

Old text:

¹ Bioequivalence testing of the slurry is being evaluated in an ongoing study. Preliminary results indicate PK comparable to a tablet; final results expected by end of 2014.

New text:

⁷⁰ Bioequivalence testing of the slurry has determined it to be comparable to 4 x 30mg tablets and the 120mg tablet.

Table 7-2 was modified to change upper limit of body weight in each category in Column 2.

Old Table 7-2:

Table 7-2: Dosage of nifurtimox oral tablets by body weight

Age Group	Body Weight (kg)	Number of 30-mg Tablets	Number of 120-mg Tablets
< 12 years	3 - 5	3 x daily ½ tablet	---
	6 - 9	3 x daily 1 tablet	---
	10 - 15	3 x daily 1 ½ tablets	---
	16 - 18	3 x daily 2 tablets	---
	19 - 24	3 x daily 2 ½ tablets	---
	22 - 26	3 x daily 3 tablets	---
	27 - 34	3 x daily 3 ½ tablets	---
	35 - 40	---	3 x daily 1 ½ tablets
12 to ≤18 years	41 - 45	---	3 x daily 1 tablet
	46 - 50	---	3 x daily 1 tablet
	51 - 55	---	3 x daily 1 ½ tablets
	56 - 60	---	3 x daily 1 ½ tablets
	61 - 70	---	3 x daily 1 ½ tablets
	71 - 80	---	3 x daily 2 tablets
	81 - 90	---	3 x daily 2 tablets
	> 90	---	3 x daily 2 ½ tablets

New Table 7-2:

Table 7-2: Dosage of nifurtimox oral tablets by body weight

Age Group	Body Weight (kg)	Number of 30-mg Tablets	Number of 120-mg Tablets
< 12 years	3 - <6	3 x daily ½ tablet	---
	6 - <10	3 x daily 1 tablet	---
	10 - <16	3 x daily 1 ½ tablets	---
	16 - <19	3 x daily 2 tablets	---
	19 - <22	3 x daily 2 ½ tablets	---
	22 - <27	3 x daily 3 tablets	---
	27 - <35	3 x daily 3 ½ tablets	---
	35 - <41	---	3 x daily 1 ½ tablets
≥12 to ≤ 18 years	41 - <46	---	3 x daily 1 tablet
	46 - <51	---	3 x daily 1 tablet
	51 - <56	---	3 x daily 1 ½ tablets
	56 - <61	---	3 x daily 1 ½ tablets
	61 - <71	---	3 x daily 1 ½ tablets
	71 - <81	---	3 x daily 2 tablets
	81 - <91	---	3 x daily 2 tablets
	91 or greater	---	3 x daily 2 ½ tablets

New text added beneath table:

At Visits 2 and 6, when study drug is dispensed, subjects/legally authorized representatives will be provided with a diary on which to document the date and doses (morning, noon, and night) of study drug, and comments (see Section 16.3). Subjects/legally authorized representatives will be instructed to bring their diaries with them to each in-clinic study visit. The diaries will be collected and reviewed at each study visit to perform drug accountability and assess treatment compliance.

Riboflavin is included as a colorant in the matching placebo tablets for study drug blinding purposes. A common side effect of riboflavin ingestion is bright yellow urine. Subjects and/or their legally authorized representatives will be advised of the possibility of change in the subject's urine color within the informed consent form, and when study drug is administered and dispensed at the investigational site.

Subjects consenting to optional PK assessments must withhold taking the morning dose of study drug on the day of Visits 3, 6, and 8, when pre- and post-dose PK blood samples will be obtained. Subjects not consenting to optional PK assessments may take their study drug on the mornings of Visits 3, 6, and 8 as instructed.

Drug logistics and accountability, Section 7.6

This section was changed for clarity.

Old text:

Details regarding storage conditions for nifurtimox are provided in the CCDSs. Additional details regarding storage conditions may be provided separately. Nifurtimox and the matching placebo are not to be stored above 25°Celsius.

...

New text:

At Visit 6 (Day 30), subjects will return their diaries, all remaining study drug, and empty packaging. The diaries will be reviewed to assess study drug accountability, and study drug for the remaining 30 days of treatment will be dispensed.

At Visit 8 (Day 60), subjects will return their diaries, all remaining study drug, and empty packaging. The diaries will be reviewed to assess study drug accountability, and no additional study drug will be dispensed.

Details regarding storage conditions for nifurtimox are provided in the Investigators' Brochure. Additional details regarding storage conditions may be provided separately. Nifurtimox and the matching placebo are not to be stored above 25°Celsius.

...

Treatment compliance, Section 7.7

This section was changed for clarity and per Modification 12.

Old text:

Study site personnel will perform a tablet count of all returned study medication to determine treatment compliance. Any discrepancies between actual and expected amount of returned study medication must be discussed with the subject at the time of the visit, and any explanation must be documented in the source records.

New text:

Study site personnel will review diaries and perform a tablet count of all returned study medication to determine treatment compliance. Any discrepancies between actual and expected amount of returned study medication must be discussed with the subject at the time of the visit, and any explanation must be documented in the source records.

Treatment compliance will also be assessed during the telephone contacts at Visits 4, 5, and 7 (see Section 16.1).

Non-study therapy, Section 8

This section was changed for clarity and per Modifications 6 and 12.

Prior and concomitant therapy, Section 8.1

Old text:

...

All prior and concomitant medications administered from the time the informed consent/assent is signed ~~during the study~~ will be recorded in the subject's source

documentation file and reported in the eCRF. The following information will be entered in the eCRF: generic name of the medication, indication, dose, unit, frequency, route of administration, and start and stop date (if stopped before the end of study).

...

New text:

...

All prior and concomitant medications administered from the time the informed consent/assent is signed until the last follow-up visit will be recorded in the subject's source documentation file and reported in the eCRF. Information on concomitant medication use will also be recorded on the Phone Contact Form which will be completed during the telephone assessments at Visits 4, 5, and 7 (see Section 16.1). The following information will be entered in the eCRF: generic name of the medication, indication, dose, unit, frequency, route of administration, and start and stop date (if stopped before the end of study).

...

Procedures and variables, Section 9

Tabular schedule of evaluations, Section 9.1

This section was changed as a result of Modifications 1, 2, 6, 8, 9, 10, and 11.

Table 9-1 was modified to reflect changes made per Amendment 1.

Old table:

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Table 9-1: Schedule of procedures and assessments – Study 16027

Phase	Screening	Treatment Phase						Follow-up Phase		
Visit	1	2	3	4 ^a	5	6 ^a	7 (EOT) ^b	8	9	10
Day	-14 to -1	1 ^c	7 ± 1	24 ± 3	30 ± 3	42 ± 3	60 ± 3	90 ± 7	240 ± 7	420 ± 7
Initiation procedures										
Informed consent/assent	●									
Demographics (sex, age)	●									
Medical/surgical history	●									
Inclusion/exclusion criteria	●	●								
Height/length ^d and weight	●	●	●		●		●	●	●	●
Efficacy										
Presence of Chagas' disease symptoms	●	●	●		●		●	●	●	●
Clinical evaluation			●		●		●	●	●	●
Microscopy (subjects < 8 months of age)	●	● ^e			● ^f					
Serologic testing for Chagas' disease	●	● ^e			●		●		●	●
qPCR	●	● ^e			● ^f		●		●	●
Safety										
Adverse events	●	●	●	● ^a	●	● ^a	●	●	●	●
Physical examination ^g	●	●	●		●		●	●	●	●
Vital signs (heart rate, RR, BP [optional], temperature) ^h	●	●	●		●		●	●	●	●
12-lead ECG (optional) ^h	●	● ^e	●		●		●	●		
Hematology and blood chemistry ⁱ	●	● ^e	●		●		●	●		
Urinalysis (local lab) (if specimen can be obtained)	●	● ^e	●		●		●	●		
Urine pregnancy test ^j	●	●	●		●		●	●		
Pharmacokinetics										
PK blood sampling (optional) ^k		●	●		●		●			
Medication										
Concomitant medication	●	●	●	● ^a	●	● ^a	●	●		
Study drug administration		●	●		●					
Study drug accountability			●		●		●			
Study drug dispensed and/or collected ^l		●	●		●		●			
Review of study drug compliance			●		●		●			
Dispense/collect pre-paid phone card ^m			●				●			

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BP = blood pressure, ECG = electrocardiogram, EOT = end-of-treatment, qPCR = qualitative polymerase chain reaction, RR = respiratory rate per minute.

^a At Days 21 and 42, study site personnel will contact the subject's parent or legal guardian via telephone to assess for the occurrence of adverse events and concomitant medications. If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

^b Subjects discontinuing from the study early are to undergo all study assessments as described in the EOT Visit.

^c All assessments are to be obtained pre-treatment on Day 1.

^d Height/length and weight will be obtained with the subject wearing minimal clothing and without shoes.

^e May be done at Visit 2 (Day 1) if not performed at screening or if the results are not available.

^f Subjects with a positive microscopy or qPCR test result will be considered a treatment failure, discontinued from the study, and treated with an alternative antitrypanosomal therapy.

^g A complete physical examination, including a complete review of body systems, will be performed at the Screening Visit. A directed physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and assessments for the presence of peripheral neuropathy will be performed at all subsequent visits.

^h Blood pressure and 12-lead ECG are optional depending on the age of the subject and at the discretion of the investigator. Whenever possible, ECGs should be obtained in close proximity to the 10- to 120-minute PK sample collection time window.

ⁱ Serum laboratory assessments are to be performed with the subject in a fasting state.

^j Urine pregnancy tests will be performed on all females of childbearing potential (i.e., all female subjects who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be discontinued from the study and undergo all study assessments as described in the EOT Visit.

^k Fasting blood samples for pharmacokinetic parameters are optional on consent/assent of the subject/subject's legally authorized representative(s).

^l Depending on the country, study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.

^m A pre-paid phone card will be provided to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Days 21 and 42.

[illegible]

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Dispense pre-paid phone card ^a			●			●					
Collect pre-paid phone card						●		●			

BP = blood pressure, ECG = electrocardiogram, EOT = end-of-treatment, qPCR = quantitative polymerase chain reaction, PK = pharmacokinetic, RR = respiratory rate per minute.

- ^a At Days 14, 21, and 42, study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of adverse events, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.
- ^b Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visits 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (±3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments.
- ^c All assessments are to be obtained pre-treatment on Day 1 except for post-dose PK blood sampling.
- ^d To be performed during physical examinations; see Section 9.4.4
- ^e A complete physical examination of body systems will be performed at the Screening Visit. A brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and assessments for the presence of peripheral neuropathy will be performed at all subsequent designated time points. Abnormal findings on physical examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).
- ^f A neurological examination including assessments of mental status and cognition, cranial nerves, motor function, deep tendon reflexes, sensation, and coordination and gait will be performed. Abnormal findings on neurological examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).
- ^g Blood pressure is optional in subjects < 5 years of age at the discretion of the investigator. Height/length and weight will be obtained with the subject wearing minimal clothing and no shoes.
- ^h 12-lead ECG is optional in subjects < 5 years of age at the discretion of the investigator. At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of maximum concentration), an ECG will be obtained to allow for PK/PD investigations.
- ⁱ Fasting of subjects for serum laboratory assessments is dependent on the age of the subject at the discretion of the investigator.
- ^j Urine pregnancy tests will be performed on all females of childbearing potential (i.e., all female subjects who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be discontinued from the study and undergo all study assessments as described in the EOT Visit.
- ^k Blood samples for pharmacokinetic parameters will be obtained prior to administration of study drug, and at designated time points thereafter (see Table 9-2). They are optional and require consent/assent of the subject/subject's legally authorized representative(s).
- ^l Subjects consenting to optional PK assessments must withhold taking the morning dose of study drug on the day of Visits 3, 6, and 8. Subjects not consenting to optional PK assessments may take their study drug on the mornings of Visits 3, 6, and 8 as instructed.
- ^m Depending on the country, study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.
- ⁿ At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.
- ^o A pre-paid phone card will be provided at Visits 3 and 6 to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Visits 4, 5, and 7.

Visit description, Section 9.2

Screening, Section 9.2.1

Visit 1 – screening visit (Days -14 to -1), Section 9.2.1.1

This section was changed for clarity and to reflect changes made Modifications 2, 9, and 10.

Old text:

No study-specific procedures, ~~including asking a potential subject to fast prior to the screening blood samples,~~ may be performed before the subject/legally authorized representative signs the informed consent form, or a minor subject provides assent. After the consent/assent is obtained, the following ~~evaluations~~ will be performed:

- ...
- ~~Presence~~ of Chagas' disease symptoms
- ~~Microscopy~~ (subjects ~~younger than~~ 8 months of age), serologic testing for Chagas' disease, and qPCR
- Assessment of pretreatment AEs
- Physical examination ~~including height/length and weight~~
- Vital signs (blood pressure ~~{optional depending on the age of the subject and at the discretion of the investigator}; heart rate, respiratory rate, and temperature~~)
- 12-lead ECG (optional ~~depending on the age of the subject and at the discretion of the investigator~~)
- ~~Fasting~~ serum hematology and chemistry, and urinalysis (if urine specimen can be obtained)
- ...
- ~~Concomitant medications~~

New text:

No study-specific procedures will be performed before the subject/legally authorized representative signs the informed consent form, or a minor subject provides assent, including asking a potential subject to fast (if applicable, depending on the age of the subject at the discretion of the investigator) prior to the screening blood samples. Informed consent/assent for optional PK blood sampling will also be obtained. After the consents/assents have been obtained, the following assessments will be performed:

- ...
- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age at randomization), conventional and non-conventional serologic testing for Chagas' disease (or acceptable documentation of positive Chagas' disease within 3 months prior to screening and no prior anti-trypanocidal or anti-parasitic treatment), and qPCR

- Assessment of pretreatment AEs and concomitant medications
- A complete physical examination of body systems. Abnormal findings will be documented as medical history (see Section 9.6.1.1).
- Neurological examination (see Section 9.6.3.3). Abnormal findings will be documented as medical history (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age), height/length, and weight
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator)
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.
- ...

Treatment Phase, Section 9.2.2

Visit 2 – baseline (treatment phase, Day 1), Section 9.2.2.1

This section was changed for clarity and to reflect changes made per Modifications 1, 2, 4, 9, and 10.

Old text:

Visit 2 will take place after all laboratory test results and assessments are available. The following assessments will be performed ~~prior to study drug administration~~:

- Re-evaluation of inclusion and exclusion criteria
- ~~• Presence of Chagas' disease symptoms~~
- ~~• Microscopy (subjects younger than 8 months of age), serologic testing for Chagas' disease, and qPCR (if not performed at screening or the results are not available)~~
- Assessment of pretreatment AEs
- ~~• Physical examination, and assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy~~
- Vital signs (blood pressure ~~[depending on the age of the subject and at the discretion of the investigator]~~, heart rate, respiratory rate, and temperature), height/length, and weight
- 12-lead ECG (if not performed at screening or the results are not available; optional ~~depending on the age of the subject and at the discretion of the investigator~~). ~~Whenever possible, ECGs should be obtained in close proximity to the 10- to 120-minute PK sample collection time window.~~
- ~~• Fasting serum hematology and chemistry, and urinalysis (if urine specimen can be obtained) (if not performed at screening or the results are not available)~~

- ...
- ~~Pharmacokinetic blood sampling (optional with consent/assent of the subject/subject's legally authorized representative)~~
- ~~Concomitant medications~~
- Administer first dose of study drug
- Dispense study drug with instructions for use. Depending on the country, study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.

New text:

Visit 2 will take place after all laboratory test results and assessments performed at the Screening Visit are available. The following assessments will be performed:

- Re-evaluation of inclusion and exclusion criteria
- Assessment of pretreatment AEs and concomitant medications
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator). At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG will be obtained to allow for PK/PD investigations.
- ...
- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see Table 9-2)
- Administer first dose of study drug to all subjects
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9-2). At the 2 – 4 hour time point (i.e., at the time of C_{max}), an ECG will be obtained.
- Dispense study drug and diary with instructions for use. Subjects consenting to optional PK assessments will be instructed to withhold taking study drug on the morning of Visit 3; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 3 as instructed.

Subjects and/or their legally authorized representatives will be advised of the possibility of change in the subject's urine color due to the ingredient riboflavin. Depending on the country, study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.

Visit 3 (Day 7 ± 1 days), Section 9.2.2.2

This section was changed for clarity and to reflect changes made per Amendment 1

Old text:

- ~~Presence~~ of Chagas' disease symptoms
- ~~Clinical evaluation~~
- Assessment of AEs
- Physical examination, ~~and~~ assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy
- Vital signs (blood pressure is optional ~~depending on the age of the subject and at the discretion of the investigator~~), height/length, and weight
- 12-lead ECG (optional ~~depending on the age of the subject and at the discretion of the investigator~~). ~~Whenever possible, ECGs should be obtained in close proximity to the 10- to 120-minute PK sample collection time window.~~
- ~~Fasting~~ serum hematology and chemistry, and urinalysis (if urine specimen can be obtained)
- Urine pregnancy test...
- ~~Pharmacokinetic blood sampling (optional on consent/assent of the subject/subject's legally authorized representative)~~
- ~~Concomitant medications~~
- ~~Drug accountability and review of study drug compliance~~
- Administer ~~and dispense~~ study drug
- ~~Provide~~ pre-paid phone card to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Visits 4 ~~and 6~~.

New text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age), conventional and non-conventional serologic testing for Chagas' disease, and qPCR
- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Neurological examination (see Section 9.6.3.3). Abnormal findings will be documented as AEs (see Section 9.6.1.1).

- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator). At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG will be obtained.
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.
- Urine pregnancy test...
- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see Table 9–2).
- Administer study drug to subjects consenting to PK assessments (who withheld their morning dose of study drug)
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9–2). At the 2 – 4 hour time point (i.e., at the time of C_{max}), an ECG will be obtained.
- Collect study drug and diary. Additional diaries may be provided if necessary.
- Drug accountability and review of diaries for study drug compliance
- Dispense pre-paid phone card to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Visit 4

Visit 4 (Day 21 ± 3 days), Section 9.2.2.3

This section was changed for clarity and per Modifications 6 and 8.

Old heading: Visit 4 (Day ~~21~~ ± 3 days)

New heading: Visit 4 (Day 14 ± 3 days)

Old text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs ~~and~~ concomitant medications. If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

New text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

Visit 5 (Day 30 ± 3 days), Section 9.2.2.4

This section was changed for clarity and per Modifications 6 and 8.

Old heading: Visit 5 (Day ~~30~~ ± 3 days)

New heading: Visit 5 (Day 21 ± 3 days)

Old text:

The bulleted list of assessments appearing in this section was deleted in its entirety.

New text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

Subjects consenting to optional PK assessments will be instructed to withhold taking the morning dose of study drug on the day of Visit 6; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 6 as instructed.

Visit 6 (Day 42 ± 3 days), Section 9.2.2.5

This section was changed as a result of Modification 12.

Old heading: Visit 6 (Day 42 ± 3 days)

New heading: Visit 6 (Day 30 ± 3 days)

Old text:

~~Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs and concomitant medications. If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.~~

New text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age), conventional and non-conventional serologic testing for Chagas' disease and qPCR.
- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1)
- Neurological examination (see Section 9.6.3.3). Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator)

- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator). At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG will be obtained.
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.
- Urine pregnancy test on all females of childbearing potential (i.e., those who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be discontinued from the study and undergo all study assessments as described in the EOT Visit.
- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see Table 9-2).
- Administer study drug to subjects consenting to PK assessments (who withheld their morning dose of study drug)
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9-2). At the 2 – 4 hour time point (i.e., at the time of C_{max}), an ECG will be obtained.
- Collect and dispense study drug and diary. At this visit, subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.
- Drug accountability and review of diaries for study drug compliance
- Collect and dispense pre-paid phone card to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Visit 7.

Visit 7 (Day 60 ± 3 days)/End of Treatment Visit, Section 9.2.2.6

This section was changed for clarity and per Modification 6.

Old heading: Visit 7 (Day 60 ± 3 days)/End of Treatment Visit

New heading: Visit 7 (Day 42 ± 3 days)

Old text:

The bulleted list of assessments appearing in this section was deleted in its entirety.

New text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

Subjects consenting to optional PK assessments will be instructed to withhold taking the morning dose of study drug on the day of Visit 8; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 8 as instructed.

Visit 7 (Day 60 ± 3 days)/End of Treatment Visit, Section 9.2.2.7

This section was revised for clarity and as a result of changes per Amendment 1.

Old heading: Visit 7 (Day 60 ± 3 days)/End of Treatment Visit

New heading: Visit 8 (Day 60 ± 3 days)/End of Treatment Visit

Old Text:

- ~~Presence~~ of Chagas' disease symptoms
- Serologic testing for Chagas' disease and qPCR
- Assessment of AEs
- Physical examination, ~~and~~ assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy.
- Vital signs (blood pressure is optional ~~depending on the age of the subject and~~ at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional ~~depending on the age of the subject and~~ at the discretion of the investigator).
- ~~Fasting~~ serum hematology and chemistry, and urinalysis (if urine specimen can be obtained).
- Urine pregnancy test ...
- ~~Pharmacokinetic blood sampling (optional with consent/assent of the subject/subject's legally authorized representative)~~
- ~~Concomitant medications~~
- Collect study drug and pre-paid phone card
- Drug accountability and review of study drug compliance

New Text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age), conventional and non-conventional serologic testing for Chagas' disease and qPCR
- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of

peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).

- Neurological examination (see Section 9.6.3.3). Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator). At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG will be obtained.
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.
- Urine pregnancy test ...
- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see Table 9-2).
- Administer study drug to subjects consenting to PK assessments (who withheld their morning dose of study drug).
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9-2). At the 2 – 4 hour time point (i.e., at the time of C_{max}), an ECG will be obtained.
- Collect study drug, diary, and pre-paid phone card
- Drug accountability and review of diaries for study drug compliance

Follow-up phase, Section 9.2.3

This section was changed for clarity and to reflect changes made per Modifications 1, 2, and 9.

Visit 8 (Day 90 ± 7), Section 9.2.3.1

This section was changed for clarity and to reflect changes made per Amendment 1.

Old heading: Visit 8 (Day 90 ± 7)

New heading: Visit 9 (Day 90 ± 7)

Old text:

- ~~Presence~~ of Chagas' disease symptoms
- ~~Clinical evaluation~~
- Assessment of AEs
- Physical examination, ~~and~~ assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy

- Vital signs (blood pressure is optional ~~depending on the age of the subject and~~ at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional ~~depending on the age of the subject and~~ at the discretion of the investigator)
- ~~Fasting~~ serum hematology and chemistry, and urinalysis (if urine specimen can be obtained).
- Urine pregnancy test...
- ~~Concomitant medications~~

New text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age)
- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator)
- Serum hematology and chemistry, coagulation, and urinalysis (if urine specimen can be obtained). Fasting is dependent on the age of the subject at the discretion of the investigator.
- Urine pregnancy test...

Visit 9 (Day 240 ± 7), Section 9.2.3.2

This section was changed for clarity and to reflect changes made per Amendment 1.

Old heading: Visit 9 (Day 240 ± 7)

New heading: Visit 10 (Day 240 ± 7)

Old text:

- ~~Presence~~ of Chagas' disease symptoms
- ~~Clinical evaluation~~
- Serologic testing for Chagas' disease and qPCR
- Assessment of AEs

- Physical examination, ~~and~~ assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy
- Vital signs (blood pressure is optional ~~depending on the age of the subject and~~ at the discretion of the investigator), height/length, and weight

New text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age), conventional and non-conventional serologic testing for Chagas' disease and qPCR
- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight

Visit 10 (Day 420 ± 7), Section 9.2.3.3

This section was changed for clarity and to reflect changes made per Amendment 1.

Old heading: Visit ~~10~~ (Day 420 ± 7)

New heading: Visit 11 (Day 420 ± 7)

Old text:

- ~~• Presence of Chagas' disease symptoms~~
- ~~• Clinical evaluation~~
- Serologic testing for Chagas' disease and qPCR
- Assessment of AEs
- Physical examination, ~~and~~ assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy
- Vital signs (blood pressure is optional ~~depending on the age of the subject and~~ at the discretion of the investigator), height/length, and weight

New text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Conventional and non-conventional serologic testing for Chagas' disease and qPCR

- Assessment of AEs
- Concomitant medications
- Brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and clinical assessments for the presence of peripheral neuropathy. Abnormal findings will be documented as AEs (see Section 9.6.1.1).
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight

Population characteristics, Section 9.3

Demographic, Section 9.3.1

This section was changed for clarity.

Old text: At screening, each subject's ~~year~~ of birth, age, and sex will be recorded.

New text: At screening, each subject's date of birth, age, and sex will be recorded.

Medical history, Section 9.3.2

This section was changed for clarity.

Old text:

Medical history findings (i.e., previous diagnoses, diseases or surgeries) meeting all criteria listed below will be collected as available to the investigator...

New text:

At screening, medical history findings (i.e., previous diagnoses, diseases or surgeries) meeting all criteria listed below will be collected as available to the investigator...

Efficacy, Section 9.4

This section was changed for clarity and to reflect changes made per Modification 2.

Old text:

The primary efficacy variable will be a $\geq 20\%$ sero-reduction (~~subjects older than 8 months of age and younger than 18 years of age~~) or sero-conversion (~~subjects 8 months of age and younger~~) in antibody IgG titer at 12 months posttreatment using conventional ELISA serology as the measure of efficacy.

A quality control program will be implemented for the primary efficacy variable (i.e., ELISA ~~titers~~). At least 20% of measurements, chosen at random, will be confirmed by a reference laboratory (Chagas Service, Buenos Aires Children's Hospital "Ricardo Gutierrez").

Other efficacy variables include disease state determined by qPCR, ~~microscopy~~ (subjects ~~younger than 8 months of age~~), clinical signs/symptoms of Chagas' disease, ~~and clinical evaluation~~.

...

New text:

Efficacy assessments will be obtained at designated study visits (see Table 9-1). The primary efficacy variable will be sero-reduction or sero-conversion at 12 months posttreatment using two conventional ELISA serology tests as the measure of efficacy.

A quality control program will be implemented for the primary efficacy variable (i.e., ELISA concentrations). At least 20% of measurements, chosen at random, will be confirmed by a reference laboratory (Chagas Service, Buenos Aires Children's Hospital "Ricardo Gutierrez").

Other efficacy variables include disease state determined by qPCR, concentrations tests for *T. cruzi* (subjects \leq 8 months of age at randomization), conventional and non-conventional serologic testing, and clinical signs/symptoms of Chagas' disease.

...

Polymerase chain reaction test, Section 9.4.1

This section was changed for clarity and to reflect changes made per Amendment 1.

Old text:

A qPCR test will be performed at the Screening Visit, ~~Visit 2 (if not done at screening or the results are not available), Visits 5 and 7, Visit 9 and 10 (Followup).~~

Details describing the collection, processing, storage and shipment of qPCR blood samples, and the qPCR test protocol, are described in Section ~~16.2~~.

New text:

A qPCR test will be performed at the Screening Visit, Visits 3, 6, and 8, and Follow-up Visits 10 and 11.

Details describing the collection, processing, storage and shipment of qPCR blood samples, and the qPCR test protocol, are described in Section 16.4.

Serological tests, Section 9.4.2

This section was changed for clarity and per Modifications 2, 3, and 5.

Old text:

At screening, subjects must have the following serological tests to confirm a diagnosis of Chagas' disease:

- Subjects ~~younger than~~ 8 months of age must demonstrate direct observation of *T. cruzi* by concentration test
- Subjects 8 months of age ~~and older and younger than~~ 18 years of age must demonstrate a positive ELISA ~~plus a positive~~ result for ~~one~~ of the following tests to confirm diagnosis:
 - ~~Alternate ELISA (e.g., Chagatest ELISA recombinant v3.0)~~
 - ~~Hemagglutination (e.g., Chagatest HAI)~~
 - ~~Direct Agglutination (e.g., SERODIA Chagas)~~

After ~~enrollment~~, serological tests will be performed at Visit ~~2 (if not done at screening, or the results are not available), 5, and 7,~~ and Visits ~~9 and 10 (Follow-up)~~.

Details describing the collection, processing, storage and shipment of blood samples, and the serologic test protocol, will be described in a separate serologic test manual. Serologic tests will be performed by a local laboratory according to the standard of care for each country.

New text:

At screening, subjects must have the following serological tests to confirm a diagnosis of Chagas' disease:

- Subjects \leq 8 months of age at randomization must demonstrate direct observation of *T. cruzi* by concentration test
- Subjects \geq 8 months of age to \leq 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:
 - Recombinant ELISA
 - Total purified antigen ELISA

Additionally, a non-conventional ELISA test will be obtained; however, a positive result will not be required for diagnosis.

Subjects will also be eligible if there is acceptable documentation of positive Chagas' disease within 3 months prior to screening and they have had no prior anti-trypanocidal or anti-parasitic treatment.

After randomization, conventional and non-conventional serological tests will be performed at Visits 3, 6, and 8, and Visits 10 and 11 (Follow-up).

All subjects \geq 8 months to $<$ 18 years of age at randomization will have two baseline specimens collected for serologic testing. One sample will be used to determine the initial diagnosis of Chagas' disease while the other sample will be frozen and retained to be assayed along with the 12-month post-treatment specimen as an internal control. Subjects $<$ 8 months of age at randomization will have one specimen obtained which will be frozen and retained. Total blood volume to be taken for the required for three serological and PCR tests is 4.0-5.0 mL, depending on the subject's age. Care will be taken to minimize sample volumes by using a micro-sampling collection technique.

Details describing the collection, processing, storage and shipment of blood samples, and the serologic test protocol, will be described in a separate serologic test manual. Initial serologic tests will be performed by a local laboratory according to the standard of care for each country. Subsequent tests will be performed by a central laboratory.

Microscopy, Section 9.4.3

This section was changed for clarity and per Modification 2.

Old heading: ~~Microscopy~~

New heading: Concentration tests for *T. cruzi*

Old text:

~~Microscopy~~ tests will be performed on all subjects ~~younger than~~ 8 months of age at the screening visit, and at Visits ~~2 (if not done at screening or the results are not available), and 5.~~

Details describing the collection, processing, storage and shipment of blood samples, and the ~~microscopy~~ test protocol, will be described in a separate ~~microscopy~~ test manual. ~~Microscopy~~ will be performed by a local laboratory according to the standard of care for each country.

New text:

Concentration tests for *T. cruzi* will be performed on all subjects \leq 8 months of age at randomization at the screening visit, and at Visits 3 and 6.

Details describing the collection, processing, storage and shipment of blood samples, and the concentration test protocol, will be described in a separate manual. Concentration tests for *T. cruzi* will be performed by a local laboratory according to the standard of care for each country.

Clinical manifestations of Chagas' disease, Section 9.4.4

This section was changed for clarity and per changes made per Amendment 1.

Old heading: ~~Clinical manifestations~~ of Chagas' disease

New heading: Assessment of Chagas' disease signs and symptoms

Old text:

Assessment of ~~clinical manifestations~~ of Chagas' disease will be performed at ~~designated study visits~~. ~~Subjects will be assessed for the following:~~

Acute Chagas' disease:

...

New text:

Assessments of the presence of Chagas' disease signs and symptoms will be performed during physical examinations at Screening, Visits 3, 6, and 8 (EOT), and Visits 9, 10, and 11 (follow-up), and will be entered into the eCRF. Signs and symptoms of Chagas' disease include but are not limited to the following:

Acute Chagas' disease:

...

Pharmacokinetics/pharmacodynamics, Section 9.5

This section was changed for clarity, per Amendment 1, and per Modifications 1 and 9.

Old text:

If the subject/subject's legally authorized representative(s) provide(s) consent/assent to do so, plasma concentrations of nifurtimox will be ~~performed~~ at Visit 2 (baseline) and Visits 3, ~~5~~, and 7 (EOT) on at least 10 subjects per cohort selected from all participating centers. Plasma nifurtimox concentrations will be determined using a sparse sampling approach.

~~The~~ time points and time windows for PK blood sampling ~~is~~ presented in Table 9-2.

New text:

If the subject/subject's legally authorized representative(s) provide(s) consent/assent to do so, plasma concentrations of nifurtimox will be evaluated at Visit 2 (baseline) and Visits 3, 6, and

8 (EOT) on at least 10 subjects per age cohort selected from all participating centers. Plasma nifurtimox concentrations will be determined using a sparse sampling approach.

At Visits 2, 3, 6, and 8, the subjects should present themselves to the investigational site without taking the morning dose of study medication. Blood samples for PK will be obtained in-clinic prior to the administration of study drug and at specific time points and time windows for PK blood sampling as presented in Table 9-2. At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG (optional for subjects < 5 years of age at the discretion of the investigator) will be obtained to allow for PK/PD investigations.

Table 9-2 was modified to clarify the visits at which PK sample will be drawn; footnote a added per Modification 1.

Old table:

Table 9-2: Pharmacokinetic sampling time points and windows

Visits (Days)	Time point	Time window
2, 3, 5 , 7 (1, 7, 30, 60)	Pre-dose (pre-treatment)	---
2, 3, 5 , 7 (1, 7, 30, 60)	Post-dose	5-10 minutes post-dose 10-120 minutes post-dose 2-4 hours post-dose 4-8 hours post-dose

New table:

Table 9-2: Pharmacokinetic sampling time points and windows

Visits (Days)	Time point	Time window
2, 3, <u>6</u> , <u>8</u> (1, 7, 30, 60)	Pre-dose (pre-treatment)	---
	Post-dose	5-10 minutes post-dose 10-120 minutes post-dose 2-4 hours post-dose ^a 4-8 hours post-dose

a An ECG will be obtained at this time point (optional in subjects < 5 years of age at the discretion of the investigator)

Old text:

Samples of a blood volume of $2 \times 75 = 150 \mu\text{L}$ at predefined time points should be taken according to Table 9-2. If samples cannot be taken due to other reasons (e.g., sampling deemed not feasible by the investigator due to safety) or if the samples are taken outside the defined time windows (e.g., > 8 hours post-dose), the ~~sample~~ will still remain valid for PK analysis. At least one sample ~~per period within the specified timeframe~~ should be taken during the course of the study. That means that, at the end of the study, at least one sample from each time window will be available, e.g., if the 4-8 hour post-dose sample was not collected until the last visit, then a sample at this time window should in any case be drawn at that time point. This must be documented by the investigator.

Details describing the procedures for obtaining and handling PK blood samples are provided in Section 16.3.

~~At these study visits, the subjects should present themselves to the hospital without taking the morning dose of study medication. During these visits, the PK samples should be obtained within the planned sampling time windows...~~

Analysis of PK samples

...

Pharmacokinetic evaluation

Plasma concentration time courses will be analyzed and reported within the study report. In addition, a full PK evaluation of the data will be performed and reported in a separate study report. ~~Nonparametric and parametric PK estimation~~ methods will be used to provide parameter estimates describing the PK behavior of nifurtimox and to identify possible covariates related to age.

New text:

Samples of a blood volume of $2 \times 75 = 150 \mu\text{L}$ at predefined time points should be taken according to Table 9-2. If samples cannot be taken due to other reasons (e.g., sampling deemed not feasible by the investigator due to safety) or if the samples are taken outside the defined time windows (e.g., > 8 hours post-dose), the subject will still remain valid for PK analysis. At least one sample should be taken during the course of the study. That means that, at the end of the study, at least one sample from each time window will be available, e.g., if the 4-8 hour post-dose sample was not collected until the last visit, then a sample at this time window should in any case be drawn at that time point. This must be documented by the investigator.

The PK samples should be obtained within the planned sampling time windows...

Details describing the procedures for obtaining and handling PK blood samples are provided in Section 16.3.

Analysis of PK samples

...

Pharmacokinetic evaluation

Plasma concentration time courses will be analyzed and reported within the study report. In addition, a full PK evaluation of the data will be performed and reported in a separate study report. Population PK methods will be used to provide parameter estimates describing the PK behavior of nifurtimox and to identify possible covariates related to age.

Intensity, Section 9.6.1.2.2

This section was changed as a result of Modification 7.

Old text:

The intensity of an AE is classified according to the following categories:

- Mild
- Moderate
- Severe

New text:

The intensity of an AE is classified according to the following categories:

- Mild: a type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Moderate: a type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort, but poses no significant or permanent risk of harm to the subject.
- Severe: a type of AE that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects clinical status. The event possesses a significant risk of harm to the subject and hospitalization may be required.

Expected adverse events, Section 9.6.1.5

This section was changed since an Investigators' Brochure for nifurtimox became available.

Old text:

For this study, the applicable reference documents ~~are the most current, locally approved versions of the CCDSs for nifurtimox.~~

New text:

For this study, the applicable reference documents is the most current Investigators' Brochure for nifurtimox.

Further safety, Section 9.6.3

This section was changed for clarity and to reflect changes made per Modification 10.

New section heading, 9.6.3.1 Laboratory assessments, was added. All Heading 4 titles in Section 9.6.3 were renumbered from this point forward.

Table 1 was revised to clarify visits at which urine pregnancy will be obtained per changes made via Amendment 1.

Old text and table:

Safety laboratory assessments will be performed ~~with the subject in a fasting state at screening, Visits 2 (if not done at screening or the results are not available), 3, 5, 7, 8, and 8 (follow-up), and the EOT Visit.~~ If any abnormal laboratory values occur at the EOT Visit (Visit 7), these determinations will be followed until normal or stable.

Laboratory parameters will be analyzed by a local laboratory according to the schedule specified in Table 9-3.

Table 9-3: Description of laboratory parameters (safety)

Hematology	Hemoglobin, hematocrit, RBC count, WBC count and differential (including lymphocytes, monocytes, eosinophils, basophils, neutrophils), MCV, MCH, MCHC, platelets
Blood chemistry	Albumin, total protein, BUN, creatinine, glucose, total bilirubin, ALP, AST, ALT, uric acid
Coagulation	INR, PT, PTT
Urinalysis (urine dipstick) ^a	RBCs, WBCs, pH, specific gravity, glucose, protein, bilirubin, urobilinogen, nitrite, ketones
Urine pregnancy test ^b	Urine β -hCG

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, β hCG = beta-human chorionic gonadotropin, INR = international normalized ratio, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PT = prothrombin time, PTT = partial thromboplastin time, RBC = red blood cell, WBC = white blood cell.

- a If abnormalities are identified by the local laboratory, the samples will be sent to a central laboratory. Cell/sediment microscopy should be performed only if positive dipstick results are obtained for WBC, RBC/hemoglobin, or protein.
- b To be performed at Visits 2 ~~(if not performed at screening)~~, 3, 5, 7, and 8 on females of childbearing potential (i.e., female subjects who have experienced menarche). If any urine pregnancy test is positive, a serum pregnancy test will be performed to confirm the result.

New text/table:

Safety laboratory assessments will be performed at screening, Visits 3, 6, 8 (EOT), and 9 (follow-up). Fasting is dependent on the age of the subject at the discretion of the investigator. If any abnormal laboratory values occur at the EOT Visit (Visit 8), these determinations will be followed until normal or stable.

Laboratory parameters will be analyzed by a local laboratory according to the schedule specified in Table 9-3.

Table 9-3: Description of laboratory parameters (safety)

Hematology	Hemoglobin, hematocrit, RBC count, WBC count and differential (including lymphocytes, monocytes, eosinophils, basophils, neutrophils), MCV, MCH, MCHC, platelets
Blood chemistry	Albumin, total protein, BUN, creatinine, glucose, total bilirubin, ALP, AST, ALT, uric acid
Coagulation	INR, PT, PTT
Urinalysis (urine dipstick) ^a	RBCs, WBCs, pH, specific gravity, glucose, protein, bilirubin, urobilinogen, nitrite, ketones
Urine pregnancy test ^b	Urine β -hCG

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, β hCG = beta-human chorionic gonadotropin, INR = international normalized ratio, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PT = prothrombin time, PTT = partial thromboplastin time, RBC = red blood cell, WBC = white blood cell.

- a If abnormalities are identified by the local laboratory, the samples will be sent to a central laboratory. Cell/sediment microscopy should be performed only if positive dipstick results are obtained for WBC, RBC/hemoglobin, or protein.
- b To be performed at Visits 1, 2, 3, 6, 8, and 9 on females of childbearing potential (i.e., female subjects who have experienced menarche). If any urine pregnancy test is positive, a serum pregnancy test will be performed to confirm the result.

Total volume of blood, Section 9.6.3.2

This section was changed for clarity.

Old text:

The total volume of blood obtained at each visit will be as per standards of Clinical Laboratory and Standards Institute; see Section ~~16.4~~ and Table 9-4 below.

New text:

In order to minimize the burden of blood loss due to PK sampling, ageappropriate- sparse sampling strategies combined with (micro-)bioanalysis techniques will be applied. The total volume of blood obtained at each visit will be a maximum of approximately 7.8 mL - 14.6 mL depending on the child's age and weight as per standards of Clinical Laboratory and Standards Institute; see Section 16.6 and Table 9-4 below.

Physical examination, Section 9.6.3.3

This section was changed for clarity and per Modification 10.

Old text:

Physical examinations will be performed by a medically qualified person. A complete physical examination, ~~including a complete review~~ of body systems will be performed at the screening visit. A brief physical examination, including ~~weight and~~ assessments of heart, lungs, and abdomen, ~~the skin for signs of severe dermatitis,~~ and assessments for the presence of peripheral neuropathy will be performed at designated time points (see Table 9-1). ~~Height/length and weight will be obtained with the subject wearing minimal clothing and without shoes.~~

New text:

Physical examinations will be performed by a medically qualified person. A complete physical examination of body systems will be performed at the screening visit. A brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and assessments for the presence of peripheral neuropathy will be performed at all subsequent designated time points (see Table 9-1). Abnormal findings on physical examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).

A neurological examination will be performed at Screening, and Visits 3, 6, and 8 (EOT). The examination will include assessments of mental status and cognition, cranial nerves, motor function, deep tendon reflexes, sensation, and coordination and gait. Abnormal findings on neurological examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).

Assessments of Chagas' disease signs and symptoms will be performed at the time of physical examinations (see Section 9.4.4).

Vitals signs, Section 9.6.3.4

This section was changed for clarity and as a result of changes made per Modification 9.

Heading 9.6.3.4 was revised: Vital signs, height/length, and weight

Old text:

Vital signs (systolic and diastolic blood pressure [~~optional depending on the age of the subject and at the discretion of the investigator~~], heart rate, respiratory rate, and oral or rectal body temperature) will be measured at screening and Visits 2, 3, ~~5~~, 7 (EOT), ~~8~~, 9, and ~~10~~. After a 5-minute rest in a sitting position, vital signs will be measured using non-invasive equipment.

New text:

Vital signs (systolic and diastolic blood pressure [optional in subjects < 5 years of age at the discretion of the investigator], heart rate, respiratory rate, and oral or rectal body temperature) will be measured at screening and Visits 2, 3, 6, 8 (EOT), 9, 10, and 11. After a 5-minute rest in a sitting position, vital signs will be measured using non-invasive equipment.

Height/length and weight will be obtained with the subject wearing minimal clothing and no shoes.

12-lead electrocardiogram, Section 9.6.3.5

This section was changed for clarity and per Modifications 1 and 9.

Old text:

~~Depending on the age of the subject and at the discretion of the investigator, a resting 12-lead ECG will be performed at screening and Visits 2, 3, 5, 7 (EOT), and 8 (Follow-up).~~

The ECG will be performed at the site according to procedure. ~~The ECG will be obtained with the subject in the supine position after he/she has rested for 10 minutes. Whenever possible, ECGs should be obtained in close proximity to the 10- to 120-minute PK sample collection time window.~~

New text:

A resting 12-lead ECG will be performed at screening and Visits 2, 3, 6, 8 (EOT), and 9 (Follow-up) on all subjects ≥ 5 years of age, and at the discretion of the investigator on all subjects < 5 years of age. For subjects consenting to PK assessments, an ECG will be obtained at the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), to allow for PK/PD investigations.

The ECG will be performed at the site according to procedure, and with the subject in the supine position after he/she has rested for 10 minutes.

Urine pregnancy test, Section 9.6.3.6

This section was changed for clarity and to reflect changes made per Amendment 1.

Old text:

A urine pregnancy test on all female subjects of childbearing potential (i.e., all females who have experienced menarche) will be performed at screening and Visits 2, 3, 5, 7 (EOT), and 8 (Follow-up). If any urine pregnancy test is positive, a serum pregnancy test will be performed to confirm the result.

New text:

A urine pregnancy test on all female subjects of childbearing potential (i.e., all females who have experienced menarche) will be performed at screening and Visits 2, 3, 6, 8 (EOT), and 9 (Follow-up). If any urine pregnancy test is positive, a serum pregnancy test will be performed to confirm the result. If pregnancy is confirmed at Visits 1 or 2, the subject is to be excluded from study participation. If pregnancy is confirmed at Visits 3 or 6, the subject will be discontinued from the study and undergo all study assessments as described for the EOT Visit. See Section 9.6.2.

Statistical methods and determination of sample size, Section 10

General considerations, Section 10.1

This section was changed for clarity.

Old text:

This study has been designed to focus on the efficacy and safety of treatment with nifurtimox in children diagnosed with Chagas' disease. In this study, approximately 390 pediatric subjects will be randomized to ensure 330 evaluable pediatric subjects (2:1 randomization, 60-day regimen vs. 30-day regimen). Subjects will be stratified by age into four cohorts...

...

No imputations will be made for missing values. Additional analyses may be specified in the SAP if missing data patterns observed during blinded review of the data suggest any possible bias.

...

New text:

This study has been designed to focus on the efficacy and safety of treatment with nifurtimox in children diagnosed with Chagas' disease. In this study, approximately 390 pediatric subjects will be randomized to ensure 330 evaluable pediatric subjects (2:1 randomization, 60-day regimen vs. 30-day regimen). A subject is considered evaluable if there is sufficient information to determine whether or not he or she is cured. Subjects will be stratified by age into four cohorts...

...

No imputations will be made for missing values occurring in the safety and background variables. Treatment of missing values for efficacy variables is addressed later in this section. Additional analyses may be specified in the SAP if missing data patterns observed during blinded review of the data suggest any possible bias.

...

Variables and planned statistical analyses, Section 10.3

This section was changed for clarity and to reflect changes made per Modification 2.

Old text:

Variables

The primary efficacy variable will be a $\geq 20\%$ sero-reduction (~~subjects older than 8 months of age and younger than 18 years of age~~) or seroconversion (~~subjects 8 months of age and younger~~) in antibody IgG titer at 12 months posttreatment using conventional ELISA serology as the measure of efficacy.

Secondary efficacy variables include clinical signs/symptoms of Chagas' disease, ~~clinical evaluation, microscopy~~ (subjects ~~younger than~~ 8 months of age), and disease state determined by qPCR.

...

Safety variables include AEs, physical examination abnormalities, vital signs, ECG abnormalities, hematology and blood chemistry, and urinalysis.

New text:

Variables

The primary efficacy variable will be sero-reduction or seroconversion at 12 months posttreatment using two conventional ELISA serology tests as the measure of efficacy. This sero-reduction or sero-conversion is considered cure, and the primary variable is binary (cure, no cure). In the event of discordancy between the two conventional ELISA test results for subjects ≥ 8 months of age, the average percentage sero-reduction will be used (e.g., Test #1 = 15% and Test #2 = 25%; average = 20% and, hence, cure). The methodology for calculating the average percentage sero-reduction will be specified in the SAP.

Secondary efficacy variables include clinical signs/symptoms of Chagas' disease, concentration test for *T. cruzi* (subjects ≤ 8 months of age at randomization), conventional and non-conventional serologic testing, and disease state determined by qPCR.

...

Safety variables include AEs, physical examination abnormalities, vital signs, ECG abnormalities, hematology and blood chemistry, coagulation, and urinalysis.

Statistical and analytical plans

Old text:

Efficacy

Primary efficacy analysis

The difference in the proportion of nifurtimox subjects with $\geq 20\%$ sero-reduction or seroconversion (60-day regimen) and the proportion estimated from historical data will be tested using an exact 2-sided 95% CI for a single proportion.

...

Superiority will be confirmed if the lower limit of the CI for the proportion of nifurtimox subjects (60day regimen) with $\geq 20\%$ sero-reduction or sero-conversion is greater than the proportion of historical placebo subjects with ~~$\geq 20\%$ sero-reduction or sero-conversion~~. ~~This same CI will also be used for comparison with the historical benznidazole subjects with $\geq 20\%$ sero-reduction or seroconversion.~~[15]

Sero-conversion for the historical controls will be estimated from cure rates as presented in two publications.[15][16] The sero-conversion rates used for placebo and benznidazole are 45% and 60%, respectively...

New text:

Efficacy

Primary efficacy analysis

The difference in the proportion of nifurtimox subjects with sero-reduction or seroconversion (60-day regimen) and the proportion estimated from historical data will be tested using an exact 2-sided 95% CI for a single proportion.

...

Superiority will be confirmed if the lower limit of the CI for the proportion of nifurtimox subjects (60day regimen) with sero-reduction or sero-conversion is greater than the proportion of historical placebo subjects with sero-conversion.

Sero-conversion for the historical controls will be estimated from cure rates as presented in two publications.[15][16] The sero-conversion rates used for placebo and benznidazole are 16% and 58%, respectively....

Old text:

Secondary efficacy analyses

A secondary analysis will be done to compare the proportion of subjects with $\geq 20\%$ seroreduction or sero-conversion for the 60-day and 30-day nifurtimox regimens. This will be

performed using a standard, symmetric, asymptotic 2-sided 95% CI for the difference of two independent proportions.

...

The relationship of ~~serological~~ results to qPCR results will be done using frequencies of matches and mismatches on the determination of disease status, and phicorrelation and kappa coefficient to assess the degree of agreement.

...

New text:

Secondary efficacy analyses

The same CI for the primary endpoint analysis will also be used for comparison with the historical benznidazole subjects with sero-conversion to assess whether or not their cure rates are comparable.

A secondary analysis will be done to compare the proportion of subjects with seroreduction or sero-conversion for the 60-day and 30-day nifurtimox regimens. This will be performed using a standard, symmetric, asymptotic 2-sided 95% CI for the difference of two independent proportions.

...

The relationship of conventional serology results to qPCR results will be done using frequencies of matches and mismatches on the determination of disease status, and phicorrelation and kappa coefficient to assess the degree of agreement. The relationship of non-conventional serology to conventional serology will be analyzed in the same way.

...

Missing data/drop outs

Old text:

No imputations will be made for missing data due to dropouts or other causes. Statistical analyses will be based on subjects with data necessary for the technique used.

...

New text:

No imputations will be made for missing data due to dropouts or other causes. For the primary efficacy analysis done on the FAS, a patient without an assessment at the primary 12 month time point will be considered a treatment failure. For the secondary analysis using the PPS, patients who do not have serology determinations to assess the primary endpoint will be excluded from the analysis.

Sensitivity analyses will be performed treating only missing values due to the following reasons as treatment failures:

1. the reason for discontinuation is known or suspected to be treatment ineffectiveness or an AE

2. a subject has not had a negative serology at any visit and has no serology determination for the primary analysis visit

3. an indeterminate or incorrectly performed serology determination occurs at the primary analysis visit.²⁰⁴

...

Determination of sample size, Section 10.4

This section was changed for clarity and per Modification 2.

Old text:

Sample size is based on the primary endpoint, the difference in the proportion of nifurtimox subjects with $\geq 20\%$ sero-reduction (~~subjects older than 8 months of age and younger than 18 years of age~~) or sero-conversion (~~subjects 8 months of age and younger~~) (60-day regimen) and the proportion of historical placebo subjects with $\geq 20\%$ sero-reduction or sero-conversion ~~for the two age groups~~. The test of superiority over placebo will be powered based on the detection of a difference in proportion of nifurtimox subjects with $\geq 20\%$ sero-reduction or sero-conversion of 0.10. ~~This is considered a clinically meaningful difference according to several experts in the field.~~ Given that the nifurtimox (60day) proportion of subjects with $\geq 20\%$ sero-reduction or sero-conversion is 0.60, 220 evaluable subjects in this group will provide at least 80% power for an exact binomial test. This sample size will provide an exact 95% CI with a width of ≤ 0.15 (e.g., [0.53,0.67]) for use in comparing nifurtimox to the proportion of historical benznidazole subjects with $\geq 20\%$ sero-reduction or sero-conversion.

...

New text:

Sample size is based on the primary endpoint, the difference in the proportion of nifurtimox subjects with sero-reduction or sero-conversion (60-day regimen) and the proportion of historical placebo subjects with sero-conversion. The test of superiority over placebo will be powered based on the detection of a difference in proportion of 0.10. This quantity represents a difference between rates, such as $0.60 - 0.50 = 0.10$, rather than the difference that would result from subtracting 10% of 0.60 (that is, 0.06) from 0.60. Given that the nifurtimox (60day) proportion of subjects with sero-reduction or sero-conversion is 0.60, 220 evaluable subjects in this group will provide at least 80% power for an exact binomial test. This sample size will provide an exact 95% CI with a width of ≤ 0.15 (e.g., [0.53,0.67]) for use in comparing nifurtimox to the proportion of historical benznidazole subjects with sero-conversion.

...

Data recording, Section 11.1

This section was changed for clarity.

²⁰⁴ Text modified/added for clarity as per Modification 2 (Amendment 1)

New paragraph added:

Completed subject diaries will be collected by site personnel at specific study visits (see Table 9-1). Information entered onto the subject diaries will be used to assess drug accountability and treatment compliance, and entered into the eCRF. The completed diaries will be maintained with the subject's source documentation.

Data processing, Section 11.3

This section was changed for clarity.

Old text:

...

For data coding (e.g., AEs, medication), ~~internationally recognized and accepted dictionaries will be used.~~

After its initial release for biometrical analysis, the clinical database is planned to be re-opened for the inclusion of the PK data. ~~If PK data are not available prior to database lock for this study, a planned database unlock/re-opening will be required to load the data.~~

New text:

...

For data coding (AEs, medication), The Medical Dictionary for Regulatory Activities will be used for AEs, and the WHO Drug Dictionary will be used for prior and concomitant medications.

After its initial release for biometrical analysis, the clinical database is planned to be re-opened for the inclusion of the PK data.

Missing data, Section 11.4

This section was changed for clarity.

Old text:

In general, no imputations will be made for missing data. More details will be provided in the SAP.

New text:

In general, no imputations will be made for missing data other than those described in Section 10. More details will be provided in the SAP.

Reference list, Section 14

The following references were added to the reference list per changes made as a result of Amendment 1, and the cross-references were renumbered in the list and within the protocol body accordingly.

18. Altcheh J, Corral R, Biancardi MA, Freilij H. Anti-F2/3 antibodies as cure marker in children with congenital *Trypanosoma cruzi* infection. Medicina (B Aires). 2003;63(1):37-40.

Old footnote 18

~~18. Almeida IC, Gazzinelli R, Ferguson MA, Travassos LR. Trypanosoma cruzi mucins: potential functions of a complex structure. Mem Inst Oswaldo Cruz. 1999;94 Suppl 1:173-6~~

New footnote 17

17. Almeida IC, Ferguson MA, Schenkman S, Travassos LR. Lytic antialpha--galactosyl antibodies from patients with chronic Chagas' disease recognize novel O-linked oligosaccharides on mucin-like glycosyl-phosphatidylinositol-anchored glycoproteins of Trypanosoma cruzi. Biochem J. 1994 Dec 15;304 (Pt 3):793-802.

20. Porcel BM, Bonrempi EJ, Henriksson J, Rydaker M, Aslund L, Segura EL, et al. Trypanosoma rangeli and Trypanosoma cruzi: molecular characterization of genes encoding putative calcium-binding proteins, highly conserved in trypanosomatids. Exp Parasitol. 1996 Dec;84(3):387-99.

28. Bianchi F, Cucunuba Z, Guhl F, Gonzalez NL, Freilij H, Nicholls RS, et al. Follow-up of an Asymptomatic Chagas' Disease Population of Children after Treatment with Nifurtimox (Lampit) in a Sylvatic Endemic Transmission Area of Colombia. PLoS Negl Trop Dis. 2015 Feb 27;10(2):e0003465.

Appendices, Section 16

Appendix 16.1, Phone Contact Form for telephone assessments was added per Modifications 6, 12, and 13.

Phone Contact Form

<p>A medically qualified site staff member should use this form during the phone calls with the study subject's parent/s at days 14, 21 and 42. This form is designed to find out how the treated child has been feeling and whether his/her parent/legal guardian has contacted a doctor.</p> <p>Please obtain information about any symptoms or medical events that are NEW or CHANGED and about any NEW concomitant medication or DOSE CHANGE since the last patient's visit.</p>			
Subject Number		Investigator Name	
Visit Number		Visit Date	
Form completion date			
Medical Care			
Has the health condition of the child changed since last study visit?			Yes / No
- If Yes, please specify all relevant information:			
Has your child experienced any Gastrointestinal, Metabolism and Nutrition problems since last study visit?			Yes / No
- If Yes, please specify the medical event, start/stop date, action taken and outcome:			
Has your child experienced any CNS problems since last study visit?			Yes / No
- If Yes, please specify the medical event, start/stop date, action taken and outcome:			
Has your child experienced any Skin and Subcutaneous Tissue problems since last study visit?			Yes / No
- If Yes, please specify the medical event, start/stop date, action taken and outcome:			
Has the child visited a Physician or attended to the emergency room due to a medical condition since last study visit?			Yes / No
- If yes, did the child's doctor provide any diagnose?			
Has your child experienced any weight loss since last study visit?			Yes / No
- If yes, please indicate the current weight:			

Other medical events (not listed above)	
1. If Yes, please specify the medical event, start/stop date, action taken and outcome:	
2. If Yes, please specify the medical event, start/stop date, action taken and outcome:	
Study Drug Intake	
Have your child taken the study drug three times a day as required?	Yes / No
- If not, please explain:	
Have your child taken the study drug with food?	Yes / No
- If not, please explain:	
Concomitants Medication	
Did your child start taking any NEW concomitant medication since last study visit?	Yes / No
If Yes, please specify the medication, start/stop date, dose and the reason:	
Did your child change the dose of or stop taking any of the concomitant medications since last study visit?	Yes / No
If Yes, please specify the medication, dose change/stop date, new dose and the reason:	
If any safety concern arises, subjects may return to the study site for an Unscheduled visit.	
Physician Name / Signature:	Date:

Appendix 16.1, Schwartz formula and mean (range) eGFR for infants and children of various ages was renumbered to Appendix 16.2.

The table of normal GFR in children up to 12 years of age was replaced with a table of normal GFR in Children and Young Adults as follows:

Old table:

Schwartz formula: $\text{CrCl (mL/min)} = (k * \text{Ht (cm)}) / \text{Cr (mg/dL)}$

	k
° Infant (low birth weight < 1 year)	° 0.33
° Infant (term < 1 year)	° 0.45
° Child or adolescent girl	° 0.55
° Adolescent boy	° 0.70

An online calculator can be found under:

<http://www-users.med.cornell.edu/~spon/picu/calc/crclschw.htm>

For further information, please refer to: Schwartz GJ, Gauthier B. ³³ [*J Pediatr* 1985 Mar;106(3):522-6] and Schwartz GJ, Feld LG, Langford DJ. ³⁴ [*J Pediatr* 1984 Jun;104(6):849-54]

Values of Mean (Range) eGFR for Infants and Children of Various Ages Using Classic Clearance Techniques

Age	eGFR (ml/min/1.73 m ²)
Preterm neonates at birth ^a	
28 weeks gestation	0.35
32 weeks gestation	0.50
36 weeks gestation	1.21
Full-term neonates at birth ^a	
2-8 days	39 (17-60)
4-28 days	47 (26-78)
37-95 days	58 (30-86)
1-6 months	77 (39-114)
6-12 months	103 (49-157)
12-19 months	127 (62-191)
2-12 years	127 (89-165)

^a: ml per minute uncorrected for body surface area

Table taken from the book "Pediatric Drug Development" from Mulberg, Silber and van den Anker, 2009, Wiley-Blackwell ³⁵

New table and text:

Normal GFR in Children and Young Adults

<u>Age (Sex)</u>	<u>Mean GFR ± SD (mL/min/1.73 m²)</u>
<u>1 week (males and females)</u>	<u>40.6 ± 14.8</u>
<u>2-8 weeks (males and females)</u>	<u>65.8 ± 24.8</u>
<u>>8weeks (males and females)</u>	<u>95.7 ± 21.7</u>
<u>2-12 years (males and females)</u>	<u>133.0 ± 27.0</u>
<u>13-21 years (males)</u>	<u>140.0 ± 30.0</u>
<u>13-21 years (females)</u>	<u>126.0 ± 22.0</u>

Data based on three studies.[0][5][6]

GFR, glomerular filtration rate; SD, standard deviation.

1. Heilbron DC, Holliday MA, al-Dahwi A, Kogan BA. Expressing glomerular filtration rate in children. Pediatr Nephrol. 1991 Jan;5(1):5-11.
2. Coulthard MG. Maturation of glomerular filtration in preterm and mature babies. Early Hum Dev. 1985 Sep;11(3-4):281-92.
3. Schwartz GJ, Feld LG, Lanford DJ. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. J Pediatr. 1984 Jun;104(6):849-54.

The estimated GFR may be calculated using serum creatinine results at screening, and using the calculator in the following link:

<http://www-users.med.cornell.edu/~spon/picu/calc/crclschw.htm>

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Appendix 16.3 Subject diary was added per Modification 14.



Patient Diary Nifurtimox

Month 1

Patient N°				Weight	
Start Date		Formulation assigned		Bottles dispensed	

Number of tablets for each administration
(Investigator feel the boxes)



Day of Treatment	Date	Morning	Noon	Night	Comments
1		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
2		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
3		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
4		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
5		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
6		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
7		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
8		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
9		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
10		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
11		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
12		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
13		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
14		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
15		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
16		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	



Patient Diary Nifurtimox

Month 1

17		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
18		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
19		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
20		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
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25		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
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29		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
30		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
31		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
32		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	
33		<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	

Dear patient: Please remember to return this diary with ALL bottles dispensed to the investigator.

Appendix 16.2 Procedure for multiplex real time polymerase chain reaction to *Trypanosoma cruzi* detection in patients with Chagas' disease was renumbered to 16.4.

Appendix 16.3 Pharmacokinetic blood sample handling was changed renumbered to 16.5.

Appendix 16.4 Maximum blood volumes for pediatric patients – Clinical and Laboratory Standards Institute was renumbered to 16.6.

15.2 Amendment 2

There have been 2 global amendments to the study protocol. Changes made to the study protocol by Amendment 1 are described in detail in Section 15.1 and by Amendment 2 in Section 15.2.

15.2.1 Overview of changes

This section provides a conceptual overview of all modifications to the amended protocol, as introduced by this amendment. The reasons for the protocol changes included in Amendment 2 are: 1) to address FDA comments regarding handling of the analyses of data for the primary efficacy endpoint, 2) to provide textual clarifications resulting from comments received from Ethics Committees and ex-US Health Authorities, and 3) overall editorial corrections and clarifications.

An important modification is that a minimum number of 38 subjects in each age stratum will be targeted, in order to obtain an adequate number of completed subjects per group for analyses. Enrollment will continue until this specification is met, unless it is determined that such a target would be unlikely to be reached in a reasonable time.

The associated changes to the protocol text are detailed in [Section 15.2.2](#).

Sections affected include:

- *Synopsis and Study objectives, Section 4*
- *Synopsis and Study design, Section 5*
- *Justification of the design, Section 5*
- *Inclusion criteria, Section 6.1*
- *Exclusion criteria, Section 6.2*
- *Withdrawal of subjects from study, Section 6.3*
- *Dosage and administration, Section 7.4*
- *Blinding, Section 7.5*
- *Procedures and variables, Section 9*
- *Tabular schedule of evaluations, Section 9.1*
- *Visit description, Section 9.2*
- *Screening, Section 9.2.1*
- *Treatment Phase, Section 9.2.2*
- *Visit 10 (Day 420 ± 7), Section 9.2.3.3*
- *Visit 11 (Day 420 ± 7), 9.3.2.2*
- *Efficacy, Section 9.4*
- *Polymerase chain reaction test, Section 9.4.1*

- *Serological tests, Section 9.4.2*
- *Statistical methods and determination of sample size, Section 10*
- *General considerations, Section 10.1*
- *Analysis sets, Section 10.2*
- *Variables and planned statistical analyses, Section 10.3*
- *Determination of sample size, Section 10.4*
- *Data recording, Section 11.1*
- *Reference list, Section 14*
- *Subject diary, Appendix 16.3*

15.2.2 Changes to the protocol text

In this section, deletions are ~~crossed out~~, while additions are underlined.

Synopsis and Section 4: Study Objectives

Old text:

The primary objective of this Phase 3 clinical study is:

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 $\geq 20\%$ reduction in optical density [OD] measured by conventional enzymelinked- immune sorbent assay [ELISA]) compared to baseline in subjects > 8 months to < 18 years of age at randomization; or sero-conversion (defined as negative Immunoglobulin G [IgG] concentration)²⁰⁵ in subjects ≤ 8 months of age at randomization.

Secondary objectives of the study are:

- ~~To assess the comparability of a 60-day regimen of nifurtimox to historical active control (benznidazole) as sero-reduction or seroconversion at the 12-month followup (360 days from EOT)~~
- ~~To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox as sero-reduction or sero-conversion and to quantitative polymerase chain reaction (qPCR) at the 12-month follow-up (360 days from EOT)~~
- ~~To evaluate the relationship of conventional serology (as sero-reduction or sero-conversion) to qPCR using frequencies of matches and mismatches to assess agreement~~
- ~~To evaluate the relationship of non-conventional serology to conventional serology~~
- To evaluate the safety/tolerability profile of nifurtimox by laboratory parameters (hematology, blood chemistry, urinalysis), electrocardiogram (ECG) monitoring, vital

²⁰⁵ Text modified per Modification 2 (Amendment 1)

sign measurements (blood pressure, heart rate, respiratory rate, temperature), adverse event (AE) monitoring, and physical examinations, including neurological examinations

- To evaluate the pharmacokinetics (PK)/pharmacodynamics of nifurtimox in children receiving the drug for treatment of Chagas' disease

New text:

The primary objective of this Phase 3 clinical study is:

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 $\geq 20\%$ reduction in optical density [OD] measured by conventional enzymelinked- immune sorbent assay [ELISA]) compared to baseline in subjects ≥ 8 months to <18 years of age at randomization; or sero-conversion (defined as negative Immunoglobulin G [IgG] concentration)²⁰⁶ in all subjects.

Secondary objectives of the study are:

- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox as sero-reduction or sero-conversion at the 12-month follow-up (360 days from EOT)
- To evaluate the safety/tolerability profile of nifurtimox by laboratory parameters (hematology, blood chemistry, urinalysis), electrocardiogram (ECG) monitoring, vital sign measurements (blood pressure, heart rate, respiratory rate, temperature), adverse event (AE) monitoring, and physical examinations, including neurological examinations
- To evaluate the pharmacokinetics (PK)/pharmacodynamics (PD) of nifurtimox in children receiving the drug for treatment of Chagas' disease

Exploratory objectives of the study are:

- To assess the comparability of a 60-day regimen of nifurtimox to historical active control (benznidazole) as sero-reduction or seroconversion at the 12-month follow-up (360 days from EOT)
- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox using quantitative polymerase chain reaction (qPCR) at the 12-month follow-up (360 days from EOT)
- To evaluate the relationship of conventional serology (as sero-reduction or seroconversion) to qPCR using frequencies of matches and mismatches to assess agreement
- To evaluate the relationship of non-conventional serology to conventional serology

²⁰⁶ Text modified per Modification 2 (Amendment 1)

Synopsis, and Study design, Section 5

Old text:

Subjects will be stratified by age at randomization into four ~~cohorts~~ as specified below:

- ~~Cohort~~ 1: 0 to 27 days
~~Cohort~~ 2: 28 days to younger than 8 months
~~Cohort~~ 3: 8 months to younger than 2 years
~~Cohort~~ 4: 2 years to younger than 18 years

New text:

Approximately 390 pediatric subjects will be randomized (2:1 randomization, 60-day regimen vs. 30-day regimen). Subjects will be stratified by age at randomization into four strata as specified below:

- Stratum 1: 0 to 27 days
Stratum 2: 28 days to younger than 8 months
Stratum 3: 8 months to younger than 2 years
Stratum 4: 2 years to younger than 18 years

A minimum of 38 subjects in each age stratum is targeted, but not required, in order to be able to derive meaningful safety conclusions. Enrollment will continue until this specification is met, unless it is determined that such a target would be unlikely to be reached in a reasonable time.

Justification of the design, Section 5

Old text:

...

At 12 months, a 21% reduction in concentration of Chagas' diseasespecific antibodies compared to baseline (ELISA mean OD 0.467 [\pm 0.099] vs. 0.369 [\pm 0.107]) was observed in subjects who received benznidazole. For the subsequent time points of 18, 24, and 48 months, ~~percent concentration~~ reductions were 23%, 29%, and 27%, respectively. ~~At the end of the 48-month follow-up period, nearly 62% of benznidazole-treated subjects and none of the placebo-treated subjects were sero-negative for *T. cruzi*. Xenodiagnosis performed at that time was positive in 51.2% of the placebo group and 4.7% of the benznidazole group ($P < 0.001$). The low frequency of positive xenodiagnosis would be indicative of a low~~

~~parasitemia and, therefore, low antigenic load which would prevent development of chronic disease.~~

The above data are comparable to a study in early chronic Chagas' patients treated with 7.5 mg/kg benznidazole or placebo for 60 days, which also showed approximately 20% antibody concentration reduction at 12 months.[16] Analysis of concentration over time indicated a consistent decrease in antibody concentrations in the benznidazole group, and an initial decrease followed by a progressive increase in the placebo group. ~~A significant difference between the groups was apparent 6 months after completion of treatment.~~ At the end of the 3-year follow-up, children who received benznidazole had ~~five-fold lower geometric mean concentration by indirect immunofluorescence~~ than placebo-treated children (196 [147-256] vs. 1068 [809-1408], $P < 0.00001$). ~~The intent-to-treat analysis based on ELISA showed that treatment was successful in 37 (58%) of 64 children in the benznidazole group compared with 3 (5%) of 65 children in the placebo group.~~ As persistence of positive results by conventional serology for years after treatment is common, these tests may or may not revert to seronegative (sero-conversion) after many years or even decades. Thus, reduction in *T. cruzi* antibody concentration has been used in clinical practice as a surrogate indicator for parasitological cure (sero-conversion) following anti-trypanosomal treatment.

While a decrease $\geq 20\%$ in antibody concentration has been demonstrated across two independent clinical trials in pediatric patients with Chagas' disease, neither author has published extended follow-up data which include serological results for the patients in these studies. Since signs and symptoms are usually lacking in early Chagas' disease, it is not possible to demonstrate immediate meaningful clinical benefit to active treatment. However, ~~it is presumed that~~ significant reductions in *T. cruzi* antibody concentration (sero-reduction) by conventional serology is a predictor of future sero-conversion, and early treatment ~~and~~ cure of infection will ultimately reduce the risk of developing visceral abnormalities, as well as contribute to the decrease in *T. cruzi* transmission.

...

Studies have demonstrated the substantial value of qPCR, in terms of sensitivity and specificity, in the direct parasitological diagnosis of Chagas' disease and follow-up assessment of chemotherapy. However, in this Phase 3 study, parasitological cure based on serological tests for *T. cruzi* infection (ELISA) will be used as the primary efficacy endpoint to demonstrate efficacy of nifurtimox due to lack of historical PCR data from untreated children with Chagas' disease. Other parasitological methods (direct microscopic) and qPCR tests will be ~~secondary efficacy variables to demonstrate drug efficacy.~~ ~~Significant progress has occurred over recent years on the standardization and validation of PCR method in Chagas' disease.~~ Studies in pediatric and adult Chagas' patients have been performed ~~demonstrating the high sensitivity and specificity of qPCR.~~ Meanwhile, it is well recognized as the method of choice for the evaluation of treatment response especially in children with acute or early chronic Chagas' disease. ~~Information on the correlation of PCR results in patients with Chagas' reactivation and also with micro-hematocrit findings in acute Chagas' disease provide evidence to support its use.~~

The 30-day and 60day treatment regimens were selected with the intention of providing the most effective treatment within the shortest possible duration. In a recent study from Bianchi et al, 62 patients with Chagas' disease confirmed by different serological tests were treated

with nifurtimox (Lampit), and followed for 30 months post-treatment. All children were treated during 60 days according to protocols established by the WHO.

...

New text:

...

At 12 months, a 21% reduction in concentration of Chagas' diseasespecific antibodies compared to baseline (ELISA mean OD 0.467 [\pm 0.099] vs. 0.369 [\pm 0.107]) was observed in subjects who received benznidazole. For the subsequent time points of 18, 24, and 48 months, further reductions in antibody titer were reported as 23%, 29%, and 27%, respectively. After a 48-month follow-up, negative sero-conversion was detected in 11.3% ($P<0.05$) of the benznidazole group and only 4.5% of the placebo group.

The above data are comparable to a study in early chronic Chagas' patients treated with 7.5 mg/kg benznidazole or placebo for 60 days, which also showed approximately 20% antibody concentration reduction at 12 months.[16] Analysis of concentration over time indicated a consistent decrease in antibody concentrations in the benznidazole group, and an initial decrease followed by a progressive increase in the placebo group. At the end of the 3-year follow-up, children who received benznidazole had a significantly lower mean antibody concentration than placebo-treated children ($P<0.00001$). As persistence of positive results by conventional serology for years after treatment is common, these tests may or may not revert to seronegative (sero--conversion) after many years or even decades. Thus, reduction in *T. cruzi* antibody concentration has been used in clinical practice as a surrogate indicator for parasitological cure (sero-conversion) following anti-trypanosomal treatment.

While a decrease $\geq 20\%$ in antibody concentration has been demonstrated across two independent clinical trials in pediatric patients with Chagas' disease, neither author has published extended follow-up data which include serological results for the patients in these studies. Since signs and symptoms are usually lacking in early Chagas' disease, it is not possible to demonstrate immediate meaningful clinical benefit to active treatment. However, significant reductions in *T. cruzi* antibody concentration (sero-reduction) by conventional serology has been accepted as a predictor of future sero-conversion, and early treatment with cure of infection will ultimately reduce the risk of developing visceral abnormalities, as well as contribute to the decrease in *T. cruzi* transmission.

...

Studies have demonstrated the substantial value of qPCR, in terms of sensitivity and specificity, in the direct parasitological diagnosis of Chagas' disease and follow-up assessment of chemotherapy. However, in this Phase 3 study, parasitological cure based on serological tests for *T. cruzi* infection (ELISA) will be used as the primary efficacy endpoint to demonstrate efficacy of nifurtimox due to lack of historical PCR data from untreated children with Chagas' disease. Other parasitological methods (direct microscopic) and qPCR tests will comprise other variables to demonstrate drug efficacy.

The 30-day and 60-day treatment regimens were selected with the intention of providing the most effective treatment within the shortest possible duration. During the 1st Chagas Platform Technical meeting (September 1, 2009, Rio de Janeiro) [28] it was concluded that the

minimum length of treatment for nifurtimox should be 30 days which is based on good results with fewer than 60 days of treatment. Hence, the 30-day arm is included to evaluate the potential of this shorter regimen. In a recent study from Bianchi et al, 62 patients with Chagas' disease confirmed by different serological tests were treated with nifurtimox (Lampit), and followed for 30 months post-treatment. All children were treated during 60 days according to protocols established by the WHO.

...

Old text:

End of study

The end of the study as a whole will be reached as soon as the last visit of the last subject has been reached in all centers in all participating countries (~~EU and non-EU~~).

New text:

End of study

The end of the study as a whole will be reached as soon as the last visit of the last subject has been reached in all centers in all participating countries.

Inclusion criteria, Section 6.1

Old text:

...

- Subjects \geq 8 months to $<$ 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:
 - Recombinant ELISA
 - Total purified antigen ELISA

Additionally, a non-conventional ELISA test will be obtained; however, a positive result will not be required for diagnosis.

Subjects will also be eligible if there is acceptable documentation of positive Chagas' disease within three months prior to screening and they have not had prior antitrypanocidal or anti-parasitic treatment.

...

New text:

...

- Subjects \geq 8 months to $<$ 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:
 - Recombinant ELISA

– Total purified antigen ELISA

Additionally, a non-conventional ELISA test will be obtained; however, a positive result will not be required for diagnosis.

Subjects will also be eligible if there is acceptable documentation (two ELISA tests) of positive Chagas' disease within three months prior to screening and they have not had prior antitrypanocidal or anti-parasitic treatment. (The subject's parent or legally acceptable representative must agree to the collection of a baseline blood specimen for ELISA testing later during the study).

...

Exclusion criteria, Section 6.2

Old text:

...

9. Subjects who have had previous treatment with trypanocidal agents or an accepted indication for antiparasitic therapy (e.g., reactivation of Chagas' infection due to immunosuppression by several diseases or treatment with steroids)
10. Subjects who have had treatment with any investigational medicinal product within 30 days before the first dose of study drug, ~~and~~ have previously received the study treatment
11. Subjects who are pregnant or breastfeeding

...

New text:

...

9. Subjects who have had previous treatment with trypanocidal agents or an accepted indication for antiparasitic therapy (e.g., reactivation of Chagas' infection due to immunosuppression by several diseases or treatment with steroids)
10. Subjects who have had treatment with any investigational medicinal product within 30 days before the first dose of study drug, or have previously received the study treatment
11. Subjects who are pregnant or breastfeeding

...

Withdrawal of subjects from study, Section 6.3

Old text:

Withdrawal criteria

Subjects *must* be withdrawn from the study if any of the following occurs:

- At their own request or at the request of their legally acceptable representative. At any time during the study and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result of this decision.
- Pregnancy. If any urine pregnancy test is positive, a serum pregnancy test must be performed to confirm the result. See Section 9.6.2.

New text:

Withdrawal criteria

Subjects *must* be withdrawn from the study if any of the following occurs:

- At their own request or at the request of their parents' or legally acceptable representative. At any time during the study and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result of this decision.
- Pregnancy. If any urine pregnancy test is positive, a serum pregnancy test must be performed to confirm the result. See Section 9.6.2.
- Subjects, with positive parasitology test at the 30-day visit for whom (1) it has been determined that the drug was administered correctly and (2) the daily dosage given was correct, must be discontinued from the study and treated with an alternative trypanocidal medication, selected by the investigator.

Dosage and administration, Section 7.4

Old text:

Nifurtimox oral tablets will be administered in recommended doses by body weight ~~as per WHO guidelines as presented in Table 7-1.~~

Table 7-1: Dosage of 30-mg nifurtimox oral tablets by body weight

Drug	Body weight	Dose and duration ^a
Nifurtimox	≤ 40 kilograms	10-12 mg/kilogram per day in 3 divided doses (maximum dosage 15 mg/kilogram per day)
	> 40 kilograms	8—10 mg/kilogram per day in 3 divided doses

^aThe planned mg/kg per day dosages as per WHO guidelines are less than the recommended daily dosages in the Company Core Data Sheet.

...

New text:

Nifurtimox oral tablets will be administered in recommended doses by body weight according to the CCDS (Table 7-1). For infants and children weighing more than 6.0 kg and < 40kg, the daily dosage will be 10 - 20 mg/kg. Total daily dosages for adolescents weighing > 40 mg will be 8-10mg/kg, slightly lower than that recommended in the CCDS, to minimize the likelihood of adverse experiences.

Table 7-1: Total daily dosage of nifurtimox oral tablets based on body weight

Body weight group	Total daily dose of nifurtimox [mg / kg body weight]
Adolescents (40 – 60 kg)	8-10
Infants and children (< 40 kg)	10-20

...

Old text:

Table 7-2: Dosage of nifurtimox oral tablets by body weight

Age Group	Body Weight (kg)	Number of 30-mg Tablets	Number of 120-mg Tablets
< 12 years	3 – <6	3 x daily ½ tablet	---
	6 – <10	3 x daily 1 tablet	---
	10 – <16	3 x daily 1 ½ tablets	---
	16 – <19	3 x daily 2 tablets	---
	19 – <22	3 x daily 2 ½ tablets	---
	22 - <27	3 x daily 3 tablets	---
	27 - <35	3 x daily 3 ½ tablets	---
	35 - <41	---	3 x daily 1 ½ tablets
≥12 to < 18 years	41 - <46	---	3 x daily 1 tablet
	46 – <51	---	3 x daily 1 tablet
	51 - <56	---	3 x daily 1 ½ tablets
	56 – <61	---	3 x daily 1 ½ tablets
	61 – <71	---	3 x daily 1 ½ tablets
	71 - <81	---	3 x daily 2 tablets
	81 – <91	---	3 x daily 2 tablets
	91 or greater	---	3 x daily 2 ½ tablets

At Visits 2 and 6, when study drug is dispensed, subjects/legally authorized representatives will be provided with a diary on which to document the date and doses (morning, noon, and night) of study drug, and comments (see Section 1). Subjects/legally authorized representatives will be instructed to bring their diaries with them to each in-clinic study visit. The diaries will be collected and reviewed at each study visit to perform drug accountability and assess treatment compliance.

...

New text:

...

Table 7-2: Individual dosages of nifurtimox oral tablets based on body weight

Age Group	Body Weight (kg) ²⁰⁷	Number of 30-mg Tablets	Number of 120-mg Tablets
	2.5 – 4.5	3 x daily ½ tablet	
	4.6 - <9	3 x daily 1 tablet	---
	9 - <13	3 x daily 1 ½ tablets	---
	13 - <18	3 x daily 2 tablets	---
	18 - <22	3 x daily 2 ½ tablets	---
	22 - <27	3 x daily 3 tablets	---
	27 - <35	3 x daily 3 ½ tablets	---
	35 - <41	---	3 x daily 1 ½ tablets
≥12 to < 18 years	41 - <51	---	3 x daily 1 tablet
	51 - < 71	---	3 x daily 1 ½ tablets
	71 - < 91	---	3 x daily 2 tablets
	91 or greater	---	3 x daily 2 ½ tablets

At Visits 2 and 6, when study drug is dispensed, subjects/legally authorized representatives will be provided with a diary on which to document the date, exact time, and doses (morning, noon, and night) of study drug, and comments (see Section 1). Should a subject vomit or spit out study drug, the occurrence will be recorded on the diary along with the time of the event. Subjects/legally authorized representatives will be instructed to bring their diaries with them to each in-clinic study visit. The diaries will be collected and reviewed at each study visit to perform drug accountability and assess treatment compliance.

...

Blinding, Section 7.5

Old text:

Blinding

In compliance with applicable regulations, in the event of a suspected, unexpected, serious adverse reaction (SUSAR) (see Section 9.6.1.5) related to the blinded treatment, the subject's treatment code will usually be unblinded before reporting to the health authorities, ethic committees and investigators (see Section 9.6.1.4).

New text:

²⁰⁷ In this column, upper limit of weight ranges in each category changed per the most current dosing information (Amendment 1)

Blinding

In compliance with applicable regulations, in the event of a suspected, unexpected, serious adverse reaction (SUSAR) (see Section 9.6.1.5) related to the blinded treatment, the subject's treatment code will usually be unblinded before reporting to the health authorities, ethic committees and investigators (see Section 9.6.1.4).

Emergency unblinding by the investigator

In case of emergency unblinding for a SAE investigators will use the IVRS/IWRS to unblind a subject.

Procedures and variables, Section 9

Tabular schedule of evaluations, Section 9.1

Old table:

Table 9-1: Schedule of procedures and assessments – Study 16027

	Phase	Screening	Treatment Phase							Follow-up Phase		
	Visit	1	2	3	4 ^a	5 ^a	6	7 ^a	8 (EOT) ^b	9	10	11
	Day	-14 to -1	1 ^c	7 ± 1	14 ± 1	21 ± 3	30 ± 3	42 ± 3	60 ± 3	90 ± 7	240 ± 7	420 ± 7
Initiation procedures												
Informed consent/assent		●										
Demographics (sex, age)		●										
Medical/surgical history		●										
Inclusion/exclusion criteria		●	●									
Efficacy												
Assessment of Chagas' disease signs and symptoms ^d		●		●			●		●	●	●	●
Concentration test for <i>T. cruzi</i> (subjects < 8 months of age)		●		●			●		●	●	●	●
Conventional and non-conventional serologic testing for Chagas' disease		●		●			●		●		●	●
qPCR		●		●			●		●		●	●
Safety												
Adverse events		●	●	●	●	●	●	●	●	●	●	●
Concomitant medication		●	●	●	●	●	●	●	●	●	●	●
Physical examination ^e		●		●			●		●	●	●	●
Neurological examination ^f		●		●			●		●			
Vital signs (heart rate, RR, BP, temperature), height/length, and weight ^g		●	●	●			●		●	●	●	●
12-lead ECG ^h		●	●	●			●		●	●		
Serum hematology, chemistry, and coagulation ⁱ		●		●			●		●	●		

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Urinalysis (if specimen can be obtained)	●		●			●		●	●		
Urine pregnancy test ^j	●	●	●			●		●	●		
Pharmacokinetics											
PK blood sampling (optional) ^k		●	●			●		●			
Study Treatment											
Study drug administration ^l		●	●			●					
Study drug <u>and diary</u> dispensed ^m		●	●			● ⁿ					
Study drug <u>and diary</u> collected			●			● ⁿ		●			
Study drug <u>accountability/review compliance</u>			●	●	●	●	●	●			
Dispense pre-paid phone card ^o			●			●					
Collect pre-paid phone card						●		●			

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BP = blood pressure, ECG = electrocardiogram, EOT = end-of-treatment, qPCR = quantitative polymerase chain reaction, PK = pharmacokinetic, RR = respiratory rate per minute.

- ^a At Days 14, 21, and 42, study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of adverse events, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.
- ^b Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visits 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (± 3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments.
- ^c All assessments are to be obtained pre-treatment on Day 1 except for post-dose PK blood sampling.
- ^d To be performed during physical examinations; see Section 9.4.4
- ^e A complete physical examination of body systems will be performed at the Screening Visit. A brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and assessments for the presence of peripheral neuropathy will be performed at all subsequent designated time points. Abnormal findings on physical examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).
- ^f A neurological examination including assessments of mental status and cognition, cranial nerves, motor function, deep tendon reflexes, sensation, and coordination and gait will be performed. Abnormal findings on neurological examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).
- ^g Blood pressure is optional in subjects < 5 years of age at the discretion of the investigator. Height/length and weight will be obtained with the subject wearing minimal clothing and no shoes.
- ^h 12-lead ECG is optional in subjects < 5 years of age at the discretion of the investigator. At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of maximum concentration), an ECG will be obtained to allow for PK/PD investigations.
- ⁱ Fasting of subjects for serum laboratory assessments is dependent on the age of the subject at the discretion of the investigator.
- ^j Urine pregnancy tests will be performed on all females of childbearing potential (i.e., all female subjects who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be discontinued from the study and undergo all study assessments as described in the EOT Visit.
- ^k Blood samples for pharmacokinetic parameters will be obtained prior to administration of study drug, and at designated time points thereafter (see Table 9-2). They are optional and require consent/assent of the subject/subject's legally authorized representative(s).
- ^l Subjects consenting to optional PK assessments must withhold taking the morning dose of study drug on the day of Visits 3, 6, and 8. Subjects not consenting to optional PK assessments may take their study drug on the mornings of Visits 3, 6, and 8 as instructed.
- ^m Depending on the country, study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.
- ⁿ At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.
- ^o A pre-paid phone card will be provided at Visits 3 and 6 to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Visits 4, 5, and 7.

New table:

Table 9-1: Schedule of procedures and assessments – Study 16027

[illegible]

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Dispense pre-paid phone card ^q			●			●					
Collect pre-paid phone card						●		●			

BP = blood pressure, ECG = electrocardiogram, EOT = end-of-treatment, qPCR = quantitative polymerase chain reaction, PK = pharmacokinetic, RR = respiratory rate per minute.

- ^a At Days 14, 21, and 42, study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of adverse events, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.
- ^b Subjects who discontinue prematurely from study drug administration will continue to return to the investigational site for study assessments at Visits 3, 6, and 8 (EOT), and undergo telephone assessments as described for Visits 4, 5, and 7. If subjects are unable/unwilling to do so, they must return to the investigational site 30 (±3) days after the last dose of study drug for EOT (Visit 8) assessments, and undergo telephone assessments as described for Visits 4, 5, and 7. If the subject is unable/unwilling to return to the clinic for the EOT Visit (Visit 8), then a telephone assessment as described for Visits 4, 5, and 7 may be performed in lieu of Visit 8 assessments..
- ^c All assessments are to be obtained pre-treatment on Day 1 except for post-dose PK blood sampling.
- ^d To be performed during physical examinations; see Section 9.4.4
- ^e Specimen taken for non-conventional ELISA, qPCR and stored (as per amendment 2)
- ^f A complete physical examination of body systems will be performed at the Screening Visit. A brief physical examination, including assessments of heart, lungs, and abdomen, skin for the presence of severe dermatitis, and assessments for the presence of peripheral neuropathy will be performed at all subsequent designated time points. Abnormal findings on physical examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).
- ^g A neurological examination including assessments of mental status and cognition, cranial nerves, motor function, deep tendon reflexes, sensation, and coordination and gait will be performed. Abnormal findings on neurological examination at Screening will be documented as medical history; abnormal findings thereafter will be documented as AEs (see Section 9.6.1.1).
- ^h Blood pressure is optional in subjects < 5 years of age at the discretion of the investigator. Height/length and weight will be obtained with the subject wearing minimal clothing and no shoes.
- ⁱ 12-lead ECG is optional in subjects < 5 years of age at the discretion of the investigator. At the 2 – 4 hour time point for PK blood sampling (i.e., at the time of maximum concentration), the ECG will be obtained to allow for PK/PD investigations.
- ^j Fasting of subjects for serum laboratory assessments is dependent on the age of the subject at the discretion of the investigator.
- ^k Test conducted by local laboratories (as per amendment 2)
- ^l Urine pregnancy tests will be performed on all females of childbearing potential (i.e., all female subjects who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be discontinued from the study and undergo all study assessments as described in the EOT Visit.
- ^m Blood samples for pharmacokinetic parameters will be obtained prior to administration of study drug, and at designated time points thereafter (see Table 9–2). They are optional and require consent/assent of the subject/subject's legally authorized representative(s).
- ⁿ Subjects consenting to optional PK assessments must withhold taking the morning dose of study drug on the day of Visits 3, 6, and 8. Subjects not consenting to optional PK assessments may take their study drug on the mornings of Visits 3, 6, and 8 as instructed.
- ^o Depending on the country, study site personnel may dispense condoms at the time that study drug is dispensed to either the subject or the subject's parent/legal guardian, with an explanation of rationale and risks.
- ^p At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.
- ^q A pre-paid phone card will be provided at Visits 3 and 6 to facilitate contact between study site personnel and the subjects/subjects' authorized representatives at Visits 4, 5, and 7.

Visit description, Section 9.2

Screening, Section 9.2.1

Visit 1 – screening visit (Days -14 to -1), Section 9.2.1.1

Old text:

...

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age at randomization), conventional and non-conventional serologic testing for Chagas' disease (or acceptable documentation of positive Chagas' disease within 3 months prior to screening and no prior anti-trypanocidal or anti-parasitic treatment), and qPCR
- Assessment of pretreatment AEs and concomitant medications

...

New text:

...

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Concentration test for *T. cruzi* (subjects < 8 months of age at randomization) and conventional serologic testing for Chagas' disease (subjects \geq 8 months < 18 years of age) conducted in local laboratory (or acceptable documentation of positive Chagas' disease within 3 months prior to screening and no prior anti-trypanocidal or anti-parasitic treatment). Specimens taken for conventional serology, non-conventional serology and qPCR will be frozen and stored at -20°C prior to shipment to the central laboratory where they will be stored at -70°C.
- Assessment of pretreatment AEs and concomitant medications

...

Treatment Phase, Section 9.2.2

Visit 2 – baseline (treatment phase, Day 1), Section 9.2.2.1

This section was changed for clarity and to reflect changes made per Modifications 1, 2, 4, 9, and 10.

Old text:

...

- Assessment of pretreatment AEs and concomitant medications
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight

- 12-lead ECG (optional in subjects <5 years of age at the discretion of the investigator). ~~At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG will be obtained to allow for PK/PD investigations.~~
- Urine pregnancy test on all females of childbearing potential (i.e., those who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be excluded from study participation.
- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see Table 9–1).
- Administer first dose of study drug to all subjects
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9–1). At the 2 – 4 hour time point (i.e., at the time of C_{max}), an ECG will be obtained.
- Dispense study drug and diary with instructions for use. Subjects consenting to optional PK assessments will be instructed to withhold taking study drug on the morning of Visit 3; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 3 as instructed.

...

New text:

...

- Assessment of pretreatment AEs and concomitant medications
- Vital signs (blood pressure is optional in subjects < 5 years of age at the discretion of the investigator), height/length, and weight
- 12-lead ECG (optional in subjects <5 years of age at the discretion of the investigator).
- Urine pregnancy test on all females of childbearing potential (i.e., those who have experienced menarche). Any subject with a positive urine pregnancy test will have a serum pregnancy test to confirm results; if pregnancy is confirmed, the subject is to be excluded from study participation.
- For subjects consenting to PK assessments, a pre-dose PK blood sample will be obtained (see Table 9–1).
- Administer first dose of study drug to all subjects
- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9–1) At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG will be obtained to allow for PK/PD investigations. Dispense study drug and diary with instructions for use. Subjects consenting to optional PK assessments will be instructed to withhold taking study drug on the morning of Visit 3; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 3 as instructed.

...

Section 9.2.2.3

Old text:

9.2.2.3 Visit 4 (Day 14 \pm 3 days)

New text:

9.2.2.3 Visit 4 (Day 14 \pm 1 days)

Visit 10 (Day 420 \pm 7), Section 9.2.3.2

Old text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- ~~Concentration test for *T. Cruzi* (subjects < 8 months of age)~~, conventional and non-conventional serologic testing for Chagas' disease and qPCR
- Assessment of AEs
- Concomitant medications

...

New text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator)
- Conventional and non-conventional serologic testing for Chagas' disease and qPCR
- Assessment of AEs
- Concomitant medications

...

9.3.2.2 Visit 11 (Day 420 \pm 7)

Old text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- Conventional and non-conventional serologic testing for Chagas' disease and qPCR
- Assessment of AEs

New text:

- Assessment of Chagas' disease signs and symptoms (to be performed during physical examinations); see Section 9.4.4
- 12-lead ECG (optional in subjects < 5 years of age at the discretion of the investigator)
- Conventional and non-conventional serologic testing for Chagas' disease and qPCR
- Assessment of AEs

...

Efficacy, Section 9.4

Old text:

Efficacy assessments will be obtained at designated study visits (see Table 9–1). The primary efficacy variable will be sero-reduction or sero-conversion at 12 months post-treatment using two conventional ELISA serology tests as the measure of efficacy.

A quality control program will be implemented for ~~the primary efficacy variable (i.e., ELISA concentration). At least 20% of measurements, chosen at random, will be confirmed by a reference laboratory (Chagas Service, Buenos Aires Children's Hospital "Ricardo Gutierrez").~~

Other efficacy variables include disease state determined by qPCR, concentration tests for *T. cruzi* (subjects < 8 months of age at randomization), conventional and non-conventional serologic testing, and clinical signs/symptoms of Chagas' disease.

...

New text:

Efficacy assessments will be obtained at designated study visits (see Table 9–1). The primary efficacy variable will be sero-reduction or sero-conversion at 12 months post-treatment using two conventional ELISA serology tests as the measure of efficacy.

A quality control program will be implemented for all tests according to the laboratory QC/QA department SOPs.

Other efficacy variables include disease state determined by qPCR, concentration tests for *T. cruzi* (subjects < 8 months of age at randomization), conventional and non-conventional serologic testing, and clinical signs/symptoms of Chagas' disease.

...

Polymerase chain reaction test, Section 9.4.1

Old text:

A qPCR test will be performed at the Screening Visit, Visits 3, 6, and 8, and Follow-up Visits 10 and 11.

Details describing the collection, processing, storage and shipment of qPCR blood samples, and the qPCR test protocol, are described in Section 1.

New text:

A qPCR test will be performed at the Screening Visit, Visits 3, 6, and 8, and Follow-up Visits 10 and 11.

Details describing a suggested collection, processing, storage and shipment for qPCR blood samples, and a suggested qPCR test protocol, are described in Section 1. The study central laboratory procedures and qPCR protocol will be followed.

Serological tests, Section 9.4.2

Old text:

...

All subjects ≥ 8 months to < 18 years of age at randomization will have two baseline specimens collected for serologic testing. One sample will be used to determine the initial diagnosis of Chagas' disease while the other sample will be frozen and retained to be assayed ~~along with the 12-month post-treatment specimen~~ as an internal control. Subjects < 8 months of age at randomization will have one specimen obtained which will be frozen and retained. Total blood volume to be taken for the required for three serological and PCR tests is 4.05.0 mL, depending on the subject's age. Care will be taken to minimize sample volumes by using a micro-sampling collection technique.

Details describing the collection, processing, storage and shipment of blood samples, and the serologic test protocol, will be described in a separate serologic test manual. Initial serologic tests will be performed by a local laboratory according to the standard of care for each country. Subsequent tests will be performed by a central laboratory.

...

New text:

...

All subjects ≥ 8 months to < 18 years of age at randomization will have two baseline specimens collected for serologic testing. One sample will be used to determine the initial diagnosis of Chagas' disease while the other sample will be frozen and retained to be assayed later as an internal control. Subjects < 8 months of age at randomization will have one specimen obtained which will be frozen and retained. Total blood volume to be taken for the required three serological and PCR tests is 4.05.0 mL, depending on the subject's age. Care will be taken to minimize sample volumes by using a micro-sampling collection technique, when possible.

Details describing the collection, processing, storage and shipment of blood samples, and the serologic test protocols, will be described in a separate serologic test manual from the central laboratory.

...

Statistical methods and determination of sample size, Section 10

General considerations, Section 10.1

Old text:

This study has been designed to focus on the efficacy and safety of treatment with nifurtimox in children diagnosed with Chagas' disease. In this study, approximately 390 pediatric subjects will be randomized to ensure 330 evaluable pediatric subjects (2:1 randomization, 60-day regimen vs. 30-day regimen). A subject is considered evaluable if there is sufficient information to determine whether or not he or she is cured. Subjects will be stratified by age into four cohorts. Enrollment will continue until there are a total of at least 330 evaluable subjects. A minimum of 38 subjects in each age stratum is targeted, as this would include an expected 25 subjects randomized to the 60-day dosing regimen. This is nearly 10% of the study sample size, and should provide a maximum CI half-width of approximately 0.20. Enrollment will continue until this specification is met, unless it is determined that such a target would be unlikely to be reached in a reasonable time.

...

New text:

This study has been designed to focus on the efficacy and safety of treatment with nifurtimox in children diagnosed with Chagas' disease. In this study, approximately 390 pediatric subjects will be randomized (2:1 randomization, 60-day regimen vs. 30-day regimen). Subjects will be stratified by age into four strata. A minimum of 38 subjects in each age stratum is targeted, but not required, in order to be able to derive meaningful safety conclusions. Enrollment will continue until this specification is met, unless it is determined that such a target would be unlikely to be reached in a reasonable time.

...

10.2 Analysis sets

Old text:

The primary efficacy analysis will be done using the full analysis set (FAS), which is the set of subjects who received at least one dose of study drug. ~~This set is sometimes referred to as the modified intent-to-treat set.~~ Analyses will also be done using the per-protocol set, which is comprised of subjects treated with study drug who have no major protocol deviations. Complete specifications will be provided in the SAP.

Analyses of safety and background data will be performed on the FAS.

New text:

The primary efficacy analysis will be done using the full analysis set (FAS), which is the set of subjects who received at least one dose of study drug. Analyses will also be done using the per-protocol set, which is comprised of subjects treated with study drug who have no major protocol deviations. Complete specifications will be provided in the SAP.

Analyses of safety and background data will be performed on the FAS.

Variables and planned statistical analyses, Section 10.3

Old text:

Variables

The primary efficacy variable will be sero-reduction or sero-conversion at 12 months posttreatment using two conventional ELISA serology tests as the measure of efficacy. This sero-reduction or sero-conversion is considered cure, and the primary variable is binary (cure, no cure). In the event of discordancy between the two conventional ELISA test results ~~for subjects ≥ 8 months of age, the average percentage sero-reduction will be used (e.g., Test #1 = 15% and Test #2 = 25%; average = 20% and, hence, cure). The methodology for calculating the average percentage sero-reduction will be specified in the SAP.~~

Secondary efficacy variables include clinical signs/symptoms of Chagas' disease, concentration test for *T. cruzi* (subjects < 8 months of age at randomization), conventional and nonconventional serologic testing, and disease state determined by qPCR.

...

New text:

Variables

The primary efficacy variable will be sero-reduction or sero-conversion at 12 months posttreatment using two conventional ELISA serology tests as the measure of efficacy. This sero-reduction or sero-conversion is considered cure, and the primary variable is binary (cure, no cure).

In the event of discordancy between the two conventional ELISA test results, the following will be considered:

- For sero-reduction, the average percentage of OD reductions will be used (e.g., Test #1 = 15% and Test #2 = 25%; average = 20% and, hence, cure).
- For sero-conversion, only when both test results are negative the subject is considered as negative (cure).

Secondary efficacy variables include clinical signs/symptoms of Chagas' disease, concentration test for *T. cruzi* (subjects < 8 months of age at randomization), conventional and nonconventional serologic testing, and disease state determined by qPCR.

...

Old text:

Statistical and analytical plans

...

Sero-conversion for the historical control ~~will be~~ estimated from cure rates as presented in two publications .[15][16 ~~The sero-conversion rates used for placebo and benznidazole are 16% and 45%, respectively. The placebo value was derived by using a number greater than the larger of the upper limits of the 95% CIs from the two studies. Further details about the derivation of these rates will be given in the SAP.~~

Secondary efficacy analyses

~~The same CI for the primary endpoint analysis will also be used for comparison with the historical benznidazole subjects with sero-conversion to assess whether or not their cure rates are comparable. Further details about the comparison will be given in the SAP.~~

A secondary analysis will be done to compare the proportion of subjects with seroreduction or sero-conversion for the 60-day and 30-day nifurtimox regimens. This will be performed using a standard, symmetric, asymptotic 2-sided 95% CI for the difference of two independent proportions.

New text:

Statistical and analytical plans

...

Sero-conversion for the historical placebo control is estimated as 16% from cure rates presented in two publications [15][16], taking a number greater than the larger of the upper limits of the 95% CIs from the two studies. Further details about the derivation of these rates are provided in Section10.4.

Secondary efficacy analyses

A secondary analysis will be done to compare the proportion of subjects with seroreduction or sero-conversion for the 60-day and 30-day nifurtimox regimens. This will be performed using a 2-sided 95% CI for the difference of two independent proportions.

...

Safety

Old text:

Safety variables will be summarized by means of descriptive statistics and/or frequency tables as appropriate. Summaries will be given by treatment regimen. All post-treatment AEs and ~~hematological/biochemical toxicities based on laboratory measurements~~, as well as drug-related AEs and SAEs, will be summarized by category ~~and worst grade~~. AEs occurring between the time of consent and the first dose of study drug will be summarized separately. Subjects experiencing urine discoloration from riboflavin ingestion will be summarized also, although these incidences will not necessarily be considered AEs.

New text:

Safety variables will be summarized by means of descriptive statistics and/or frequency tables as appropriate. Summaries will be given by treatment regimen. All post-treatment AEs and SAEs, as well as drugrelated AEs and SAEs, will be summarized by category. AEs occurring between the time of consent and the first dose of study drug will be summarized separately. Subjects experiencing urine discoloration from riboflavin ingestion will be summarized also, although these incidences will not necessarily be considered AEs.

Missing data/drop outs

Old text:

No imputations will be made for missing data due to dropouts or other causes. For the primary efficacy analysis done on the FAS, a patient without an assessment at the primary 12 month time point will be considered a treatment failure. For the secondary analysis using the PPS, patients who do not have serology determinations to assess the primary endpoint will be excluded from the analysis.

~~Sensitivity analyses will be performed, treating only missing values due to the following reasons as treatment failures:~~

1. the reason for discontinuation is known or suspected to be treatment ineffectiveness or an AE
2. a subject has not had a negative serology at any visit and has no serology determination for the primary analysis visit
3. an indeterminate or incorrectly performed serology determination occurs at the primary analysis visit.

...

New text:

No imputations will be made for missing data due to dropouts or other causes. For the primary efficacy analysis done on the FAS, a patient without an assessment at the primary 12 month time point will be considered a treatment failure. For the secondary analysis using the PPS, patients who do not have serology determinations to assess the primary endpoint will be excluded from the analysis.

The following sensitivity analyses for the primary efficacy variable will be performed:

- Missing values due to the following reasons treated as treatment failures and other missing values excluded from analysis:
 1. the reason for discontinuation is known or suspected to be treatment ineffectiveness or an AE
 2. a subject has not had a negative serology at any visit and has no serology determination for the primary analysis visit
 3. an indeterminate or incorrectly performed serology determination occurs at the primary analysis visit.
- Observed data only.
- Analysis on PPS.

Synopsis and Determination of sample size, Section 10.4

Old text:

~~Sample size is based on the primary endpoint, the difference in the proportion of nifurtimox subjects with sero-reduction or seroconversion (60-day regimen) and the proportion of historical placebo subjects with sero-conversion. The test of superiority over placebo will be~~

~~powered based on the detection of a difference in proportion of 0.10. This quantity represents a difference between rates, such as 0.60-0.50=0.10, rather than the difference that would result from subtracting 10% of 0.60 (that is, 0.06) from 0.60. Given that the nifurtimox (60day) proportion of subjects with sero-reduction or sero-conversion is 0.60, 220 evaluable subjects in this group will provide at least 80% power for an exact binomial test. This sample size will provide an exact 95% CI with a width of ≤ 0.15 (e.g., [0.53,0.67]) for use in comparing nifurtimox to the proportion of historical benznidazole subjects with seroconversion.~~

The number of subjects in the 30-day treatment regimen is based on the width of a 95% asymptotic symmetric CI for the difference of two proportions. A sample size of ~~440~~ subjects for the 30-day subjects together with ~~220~~ subjects for the 60-day subjects will produce a CI with a half-width of approximately 0.20.

...

New text:

This study uses historical controls estimated from cure rates presented in two publications [15][16]. The historical cure rates are as follows.

Publication	Age range	Sero-conversion rate (95%CI) in placebo patients
Andrade et al 1996	7-12	3/65 = 5% (1%,13%)
Sosa et al 1998	6 -12	2/44= 5% (1%,16%)

For the primary objective, superiority over placebo will be confirmed if the lower limit of the 95% CI for the nifurtimox (60day regimen) cure rate is greater than 16%, the larger of the upper limits of the 95% CIs from the two publications.

According to Guhl et al [31], nifurtimox 12 month post treatment cure rate is about 55%, with a sample size of 260 for the 60-day regimen, the power is 99% for the lower limit of the 95% CI to be greater than 16%.

The number of subjects in the 30-day treatment regimen is based on the width of a 95% CI for the difference of two proportions. A sample size of 130 subjects for the 30-day subjects together with 260 subjects for the 60-day subjects will produce a CI with a half-width of approximately 0.20.

...

Data recording, Section 11.1

Old text:

...

For subjects consenting to undergo PK blood sampling, blood samples will be sent to the sponsor, and the data will be entered into the SAS after database lock. ~~The following will be documented in the eCRF: Times of the two most recent medication intake prior to the visit, administered dose and exact time of dosing at the visit, exact time of blood sampling, time of first dose after blood sampling, samples sampled within time window.~~

New text:

...

For subjects consenting to undergo PK blood sampling, blood samples will be sent to the sponsor, and the data will be entered into the SAS after database lock.

Administered dose and exact times of two medication intakes during the visit, exact times of blood sampling, time of first dose after blood sampling, and samples collected within time window will be recorded in the eCRF.

Reference list, Section 14

The following references were added to the reference list per changes made as a result of Amendment 2, and the cross-references were renumbered in the list and within the protocol body accordingly.

28. First Chagas Platform Technical Meeting Report, September 1, 2009, Rio de Janeiro

31. Guhl F, Nicholls R, Montoya R, Rosas F, Velasco VM, et al. (2004) Rapid negativization of serology after treatment with benznidazole for chagas disease in a group of colombian schoolchildren. Medimond International Proceedings. Spain, Vol. 1. pp. 107–114.

Subject diary, Appendix 16.3

The subject diary was updated.

15.3 Amendment 3

There have been 3 global amendments to the study protocol. Changes made to the study protocol by Amendment 1 are described in detail in Section 15.1, by Amendment 2 in Section 15.2 and by Amendment 3 in Section 15.3.

15.3.1 Overview of changes

This section provides a conceptual overview of all modifications to the amended protocol, as introduced by this amendment. The reason for the protocol change included in Amendment 3 is to delete a phrase “and ECG abnormalities” which was added in error to amendment 2.

The associated changes to the protocol text are detailed in Section 15.3.2.

Sections affected include:

- *Adverse events of special safety interest, Section 9.6.1.6*

15.3.2 Changes to the protocol text

In this section, deletions are ~~crossed-out~~, while additions are underlined.

Section 9.6.1.6: Adverse events of special safety interest

Old text:

In this study, all AEs of weight loss of > 20%, severe rash, severe polyneuropathy, ~~and ECG abnormalities~~²⁰⁸ will be considered AEs of special safety interest. Adverse events of special safety interest will be assessed, monitored, and reported as described in Section 9.6.1.4

New text:

In this study, all AEs of weight loss of > 20%, severe rash and severe polyneuropathy will be considered AEs of special safety interest. Adverse events of special safety interest will be assessed, monitored, and reported as described in Section 9.6.1.4

15.4 Amendment 4

There have been 4 global amendments to the study protocol. Changes made to the study protocol by Amendment 1 are described in detail in Section 15.1, by Amendment 2 in Section 15.2, by Amendment 3 in Section 15.3 and by Amendment 4 in Section 15.4.

15.4.1 Overview of changes

This section provides a conceptual overview of all modifications to the amended protocol, as introduced by this amendment. The primary reason for amendment 4 is to implement a PK subject diary which will allow PK subjects to record the meals consumed with study drug administration. Additionally, a few clarifying editorial changes were made.

Modification 1: text was added to Section 5- Study design - “Also, for reasons of patient safety and at the discretion of the investigator, a subject may be asked to take a “drug holiday” [interruption of study drug administration] or a reduced daily dosage may be requested from the depot. A decision by the investigator for drug holiday or reduced dosage will be based on the occurrence and/or severity of adverse effects, and/or the clinical status of

²⁰⁸ Added as per amendment 2

the subject. Any and all changes to study drug administration or daily dosage must be recorded within the subject's study chart, as well as, the eCRF."

Rationale: Decision for "drug holiday" would be made by the investigator based on the nature and severity of AE's (eg, nausea, vomiting loss of appetite, etc) and clinical status of the subject. A "holiday" has been reported to allow the subject to resume and complete treatment.

Modification 2: text was added to 6.3 withdrawal of subjects from study. "Only the re-screening will be captured in the eCRF."

Rationale: This textural change was made to provide clarification regarding how data was captured for patients who are re-screened. In this study, only one number is assigned per patient. This same number is used for screening and randomization. If a subject fails initial screening and is re-screened, the same subject number is used for the re-screen and ultimately for the randomization. Hence, only the data from the re-screen visit will be captured in the eCRF. Data from the initial screening visit will be captured in the source document at the study site.

Modification 3: text was added to 7.4. "Dosages of study drug dispensed at Visits 2 and 6 will be the same even if a different dosage is indicated based on a patient's weight change."

Rationale: Modification was done as a clarification study drug was assigned. Keeping the dosages the same throughout the treatment period was a decision made for consistency.

The associated changes to the protocol text are detailed in Section 15.4.2.

Sections affected include:

- *Study design – Section 5*
- *Withdrawal of subjects from study – Section 6.3*
- *Dosage and administration – Section 7.4*
- *Treatment phase – Section 9.2.2*
- *Pharmacokinetics/pharmacodynamics – Section 9.5*
- *Statistical methods and determination of sample size – Section 10*
- *Subject diary, Appendix 16.3*

15.4.2 Changes to the protocol text

In this section, deletions are ~~crossed-out~~, while additions are underlined.

Section 5: Study design

Old text:

...

A minimum of 38 subjects in each age stratum is targeted, but not required, in order to be able to derive meaningful safety conclusions. Enrollment ~~will~~ continue until this specification is met, unless it is determined that such a target would be unlikely to be reached in a reasonable time.

...

Study drug will be dispensed, ~~and~~ instructions for study drug administration will be provided to all subject

...

Subjects consenting to PK assessments must hold the morning dose of study drug; for these subjects, a pre-dose PK blood sample will be obtained, then the morning dose of study drug will be administered. Post-dose blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point (see Table 9–1). A pre-paid phone card will be provided to all subjects to facilitate contact between study site personnel and the subjects/subjects' authorized representatives.

At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment will be dispensed.

Visits 4 (Day 14), 5 (Day 21), and 7 (Day 42) will be telephone assessments where study site personnel will contact the subject/legally authorized representative via telephone to assess the occurrence of AEs, use of concomitant medications, and compliance with study drug administration. A Phone Contact Form for the telephone assessments will be provided to all sites (see Section 16.1).

...

A pre-dose PK blood sample will be collected, then the morning dose of study drug will be administered. Post-dose PK blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point (see Table 9–1)

...

In order to minimize the burden of blood loss due to PK sampling, ageappropriate- sparse sampling strategies combined with (micro-)bioanalysis techniques will be applied (see Table 9–4). At least 10 subjects per age stratum ~~will~~ be recruited for PK assessments.

...

New text:

...

A minimum of 38 subjects in each age stratum is targeted, but not required, in order to be able to derive meaningful safety conclusions. Enrollment may continue until this specification is met, unless it is determined that such a target would be unlikely to be reached in a reasonable time.

...

Study drug will be dispensed, instructions for study drug administration will be provided, and diaries with instructions for completion will be given, to all subjects.

...

Subjects consenting to PK assessments must hold the morning dose of study drug; for these subjects, a pre-dose PK blood sample will be obtained, then the morning dose of study drug

will be administered with food and the PK diary completed. Post-dose blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point (see Table 9–1). A pre-paid phone card will be provided to all subjects to facilitate contact between study site personnel and the subjects/subjects' authorized representatives.

At Visit 6 (Day 30), subjects will return all remaining study drug and empty packaging, and study drug for the remaining 30 days of treatment, along with subject diaries, will be dispensed.

Visits 4 (Day 14), 5 (Day 21), and 7 (Day 42) will be telephone assessments where study site personnel will contact the subject/legally authorized representative via telephone to assess the occurrence of AEs, use of concomitant medications, and compliance with study drug administration, and completion of subject diary. A Phone Contact Form for the telephone assessments will be provided to all sites (see Section 16.1).

...

A pre-dose PK blood sample will be collected, then the morning dose of study drug will be administered with food, and the subject will complete the PK diary. Post-dose PK blood samples will be obtained at specified time points, and ECGs will be obtained at the 2 – 4 hour post-dose time point (see Table 9–1)

...

In order to minimize the burden of blood loss due to PK sampling, ageappropriate- sparse sampling strategies combined with (micro-)bioanalysis techniques will be applied (see Table 9–4). At least 10 subjects per age stratum should ideally be recruited for PK assessments. Recruitment of less than 10 subjects per age group is not considered as a protocol violation.

...

Also, for reasons of patient safety and at the discretion of the investigator, a subject may be asked to take a “drug holiday” [interruption of study drug administration] or a reduced daily dosage may be requested by the investigator from the depot. A decision by the investigator for drug holiday or reduced dosage will be based on the occurrence and/or severity of adverse effects, and/or the clinical status of the subject. Any and all changes to study drug administration or daily dosage must be recorded within the subject's study chart, as well as, the eCRF.

Section 6.3: Withdrawal of subjects from study

Screening failure:

Old text:

Subjects not meeting inclusion/exclusion criteria at screening may be re-screened once. At the discretion of the investigator and in consultation with Bayer, the screening period may be extended up to 3 days to allow for receipt of test results ~~or~~ to evaluate changes in a subject's condition. In any case, the investigator has to ensure that the repeated screening procedures do not expose the subject to an unjustifiable health risk. Also, for re-screening, the subject has to

resign the informed consent form, even if it was not changed after the subject's previous screening. ~~The second~~ re-screening will be captured in the eCRF.

New text:

Subjects not meeting inclusion/exclusion criteria at screening may be re-screened once. At the discretion of the investigator and in consultation with Bayer, the screening period (or re-screening period) may be extended up to 3 days to allow for receipt of test results, to evaluate changes in a subject's condition, or for reasons not under the subject's control. In any case, the investigator has to ensure that the repeated screening procedures do not expose the subject to an unjustifiable health risk. Also, for re-screening, the subject has to resign the informed consent form, even if it was not changed after the subject's previous screening. Only the re-screening will be captured in the eCRF.

Section 7.4: Dosage and administration

Old text:

Study drug will be dispensed at Visits 2 (Day 1) and 6 (Day 30), with instructions for administration. Nifurtimox will be administered three times a day, in the morning, at noon, and at night, with food. The 30-mg and 120-mg tablets have score lines and can be divided into two equal halves to allow for 15-mg or 60 mg dose increments, respectively. The tablet is manufactured for quick disintegration in order to allow administration to subjects < 6 years old who are not able to swallow tablets. Before administration, the tablet should be dissolved in enough water to fill a teaspoon (approximately 5 mL) to form a soft slurry that should be given immediately with food. Nifurtimox will be ~~administered~~ based on body weight as presented in Table 7-2.

New text:

Study drug will be dispensed at Visits 2 (Day 1) and 6 (Day 30), with instructions for administration. Dosages of study drug dispensed at Visits 2 and 6 will be the same even if a different dosage is indicated based on a patient's weight change. Nifurtimox will be administered three times a day, in the morning, at noon, and at night, with food. The 30-mg and 120-mg tablets have score lines and can be divided into two equal halves to allow for 15-mg or 60 mg dose increments, respectively. The tablet is manufactured for quick disintegration in order to allow administration to subjects < 6 years old who are not able to swallow tablets. Before administration, the tablet should be dissolved in enough water to fill a teaspoon (approximately 5 mL) to form a soft slurry that should be given immediately with food. Nifurtimox will be dispensed based on body weight at randomization, as presented in Table 7-2.

Section 9.2.2: Treatment phase

Old text:

...

- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9-1) At the 2 – 4 hour time point of PK blood sampling (i.e., at

the time of C_{max}), an ECG will be obtained to allow for PK/PD investigations. Dispense study drug and diary with instructions for use. Subjects consenting to optional PK assessments will be instructed to withhold taking study drug on the morning of Visit 3; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 3 as instructed.

New text:

- For subjects consenting to PK assessments, post-dose PK blood samples will be obtained (see Table 9–1) At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG will be obtained to allow for PK/PD investigations. Dispense study drug and diary with instructions for use. Subjects consenting to optional PK assessments will be instructed to withhold taking study drug on the morning of Visit 3 and to complete the PK subject diary, according to the instructions provided on the PK subject diary; subjects not consenting to optional PK assessments may take their study drug on the day of Visit 3 as instructed. The PK subject diary has been added in order for the PK subjects to record all foods they consume when taking each dose of study drug, along with the times they eat each meal. Examples of these entries, based on foods typically consumed in South American countries, will be given to each subject at the PK study sites.

Visit 3 (Day 7 \pm 1 days)

Old text:

...

Administer study drug to subjects consenting to PK assessments (who withheld their morning dose of study drug)

...

New text:

...

Administer study drug with food to subjects consenting to PK assessments (who withheld their morning dose of study drug) and instruct subject on completion of PK diary.

...

Visit 4 (Day 14 \pm 1 days)

Old text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, ~~and~~ compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and

compliance information will be provided to all sites (Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

New text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, compliance with study drug administration, and completion of subject diary. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (Section 16.1). If any safety concern arises, subjects may return to the study site for an unscheduled visit

Visit 5 (Day 21 ± 3 days)

Old text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, ~~and~~ compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

...

New text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, compliance with study drug administration, and completion of subject diary. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

...

Visit 6 (Day 30 ± 3 days)

Old text:

...

Administer study drug to subjects consenting to PK assessments (who withheld their morning dose of study drug)

New text:

...

Administer study drug with food to subjects consenting to PK assessments (who withheld their morning dose of study drug) and instruct subject on completion of PK diary.

Visit 7 (Day 42 ± 3 days)

Old text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, ~~and~~ compliance with study drug administration. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

...

New text:

Study site personnel will contact the subject/legally authorized representative via telephone to assess for the occurrence of AEs, use of concomitant medications, compliance with study drug administration, and completion of subject diary. A Phone Contact Form for the telephone contacts to obtain safety and compliance information will be provided to all sites (see Section 16.1). If any safety concern arises, subjects may return to the study site for an Unscheduled Visit.

...

Visit 8 (Day 60 ± 3 days)/End of Treatment Visit

Old text:

Administer study drug to subjects consenting to PK assessments (who withheld their morning dose of study drug).

...

Drug accountability and review of diaries for study drug compliance

New text:

Administer study drug to subjects consenting to PK assessments (who withheld their morning dose of study drug), and instruct subject on completion of PK diary.

...

Drug accountability and review of diaries for study drug compliance and for PK subjects, documentation of meals during study drug administration.

Section 9.5: Pharmacokinetics/pharmacodynamics

Old text:

If the subject/subject's legally authorized representative(s) provide(s) consent/assent to do so, plasma concentrations of nifurtimox will be evaluated at Visit 2 (baseline) and Visits 3, 6, and 8 (EOT) on at least 10 subjects per age stratum selected from all participating centers. Plasma nifurtimox concentrations will be determined using a sparse sampling approach.

At Visits 2, 3, 6, and 8, the subjects should present themselves to the investigational site without taking the morning dose of study medication. Blood samples for PK will be obtained in-clinic prior to the administration of study drug and at specific time points and time windows as presented in Table 9–1. At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG (optional for subjects < 5 years of age at the discretion of the investigator) will be obtained to allow for PK/PD investigations.

New text:

If the subject/subject's legally authorized representative(s) provide(s) consent/assent to do so, plasma concentrations of nifurtimox will be evaluated at Visit 2 (baseline) and Visits 3, 6, and 8 (EOT) on at least 10 subjects per age stratum should be selected from all participating PK centers. Plasma nifurtimox concentrations will be determined using a sparse sampling approach.

At Visits 2, 3, 6, and 8, the subjects should present themselves to the investigational site without taking the morning dose of study medication. Blood samples for PK will be obtained in-clinic prior to the administration of study drug with food and at specific time points and time windows as presented in Table 9–1. At the 2 – 4 hour time point of PK blood sampling (i.e., at the time of C_{max}), an ECG (optional for subjects < 5 years of age at the discretion of the investigator) will be obtained to allow for PK/PD investigations. All subjects are to complete the PK subject diary according to instructions provided.

Pharmacokinetic evaluation

Old text:

Plasma concentration time courses will be analyzed and reported within the study report. In addition, a full PK evaluation of the data will be performed and reported in a separate study report. Population PK methods will be used to provide parameter estimates describing the PK behavior of nifurtimox and to identify possible covariates ~~related to age~~.

New text:

Plasma concentration time courses will be analyzed and reported within the study report. In addition, a full PK evaluation of the data will be performed and reported in a separate study report. Population PK methods will be used to provide parameter estimates describing the PK behavior of nifurtimox and to identify possible covariates with significant impact on pharmacokinetics (e.g. age or body weight etc.).

Section 10: Statistical methods and determination of sample size

Pharmacokinetic data

Old text:

Specifications for PK data analysis will be provided in a separate document.

New text:

Specifications for PK data analysis will be provided in a separate document. Plasma concentration vs time data documented within the study report will be analyzed descriptively using univariate statistics.

Subject diary, Appendix 16.3

The subject diary was modified and PK subject diary was created from it.

15.5 Amendment 5

There have been 5 global amendments to the study protocol. Changes made to the study protocol by Amendment 1 are described in detail in Section 15.1, by Amendment 2 in Section 15.2, by Amendment 3 in Section 15.3, by Amendment 4 in Section 15.4 and by Amendment 5 in Section 15.5.

15.5.1 Overview of changes

The reason for Amendment 5 is 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.

The associated changes to the protocol text are detailed in Section 15.5.2.

Sections affected include:

- *Synopsis, Diagnosis and main criteria for inclusion/exclusion*
- *Study objectives, Section 4*
- *Study design, Section 5*
- *Inclusion criteria, Section 6.1*
- *Tabular schedule of evaluations, Section 9.1*
- *Visit 1 – screening visit (Days -14 to -1), Section 9.2.1.1*
- *Visit 11 (Day 420 ± 7), Section 9.2.3.3*
- *Serological tests (Section 9.4.2)*
- *Variables and planned statistical analyses, Section 10.3*

15.5.2 Changes to the protocol text

In this section, all additions are underlined.

Section 2: Synopsis

Added text:

Diagnosis and main criteria for inclusion /exclusion	<ul style="list-style-type: none">• Chagas' disease diagnosed/confirmed by: ...<ul style="list-style-type: none">○ Subjects \geq 8 months to < 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:<ul style="list-style-type: none">– Recombinant ELISA– Total purified antigen ELISA <p>Additionally, a non-conventional ELISA test <u>and Immunofluorescent antibody (IFA) test</u> will be obtained; however, a positive result will not be required for diagnosis.</p> <p>...</p>
---	---

List of abbreviations

Added text:

IFA Immunofluorescent antibody

Section 4: Study objectives

Added text:

Exploratory objectives of the study are:

...

- To evaluate the relationship of conventional serology to IFA test

Section 5: Study design

Added text:

... Additionally, a non-conventional ELISA test and IFA test will be obtained; however, a positive result will not be required for diagnosis.

Section 6.1: Inclusion criteria

Added text:

... Additionally, a non-conventional ELISA test and IFA test will be obtained; however, a positive result will not be required for diagnosis.

Section 9.1 Tabular schedule of evaluations

Added text (at Visits 1 and 11):

Immunofluorescent antibody (IFA) test *	●										●
---	---	--	--	--	--	--	--	--	--	--	---

[...] IFA = immunofluorescent antibody, [...]

* IFA will be performed at Visit 1 and Visit 11 only, if adequate specimens are available

Section 9.2.1.1 Visit 1 – screening visit (Days -14 to -1)

Added text:

[...] Specimens taken for conventional serology, non-conventional serology, IFA and qPCR will be frozen and stored at -20°C prior to shipment to the central laboratory where they will be stored at -70°C.

Section 9.2.3.3: Visit 11 (Day 420 ± 7)

Added text:

Conventional and non-conventional serologic testing and IFA for Chagas' disease and qPCR

Section 9.4.2: Serological tests

Added text:

[...] Additionally, a non-conventional ELISA test and IFA will be obtained; however, a positive result will not be required for diagnosis.

Section 10.3 Variables and planned statistical analyses

Added text:

Secondary and exploratory efficacy analyses

Added text:

The results from IFA test and conventional serology will also be compared. The details of secondary and exploratory efficacy analyses will be further specified in the SAP.

15.6 Amendment 6

There have been 6 global amendments to the study protocol. Changes made to the study protocol by Amendment 1 are described in detail in Section 15.1, by Amendment 2 in Section 15.2, by Amendment 3 in Section 15.3, by Amendment 4 in Section 15.4, by Amendment 5 in Section 15.5 and by Amendment 6 in Section 15.6.

15.6.1 Overview of changes

The reason for Amendment 6 is 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.

Rationale: Given that another type of assay has to be performed and the FDA recommended either IFA or IHA, the IHA is considered the more suitable assay compared to the IFA taking into account the number of samples to be analyzed and the time frame for performing the analyses.

Furthermore, the sponsor's responsible Global Clinical Leader has changed and the name was updated.

The associated changes to the protocol text are detailed in Section 15.6.2.

Sections affected include:

- *List of abbreviations*
- *Signature of the sponsor's medically responsible person*
- *Synopsis, Diagnosis and main criteria for inclusion/exclusion*
- *Study objectives, Section 4*
- *Study design, Section 5*
- *Inclusion criteria, Section 6.1*
- *Tabular schedule of evaluations, Section 9.1*
- *Visit 1 – screening visit (Days -14 to -1), Section 9.2.1.1*
- *Visit 11 (Day 420 ± 7), Section 9.2.3.3*
- *Serological tests (Section 9.4.2)*
- *Variables and planned statistical analyses, Section 10.3*

15.6.2 Changes to the protocol text

In this section, deletions are ~~crossed out~~, while additions are underlined.

Signature page of the sponsor's medically responsible person

Old text:

Name: PPD

New text:

Name: PPD

Section 2: Synopsis

Old text:

Diagnosis and main criteria for inclusion /exclusion

- Chagas' disease diagnosed/confirmed by:

...

- Subjects \geq 8 months to < 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:
 - Recombinant ELISA
 - Total purified antigen ELISA

Additionally, a non-conventional ELISA test and ~~Immunofluorescent antibody (IFA) test~~ will be obtained; however, a positive result will not be required for diagnosis.

...

New text:

Diagnosis and main criteria for inclusion /exclusion

- Chagas' disease diagnosed/confirmed by:

...

- Subjects \geq 8 months to < 18 years of age at randomization must demonstrate a positive conventional ELISA result for both of the following tests to confirm diagnosis:
 - Recombinant ELISA
 - Total purified antigen ELISA

Additionally, a non-conventional ELISA test and indirect hemagglutination assay (IHA) will be obtained; however, a positive result will not be required for diagnosis.

...

List of abbreviations

Old text:

~~IHA~~ ~~Immunofluorescent antibody~~

New text:

IHA Indirect hemagglutination assay

Section 4: Study objectives

Old text:

Exploratory objectives of the study are:

...

- To evaluate the relationship of conventional serology to ~~IHA~~ test

New text:

Exploratory objectives of the study are:

...

- To evaluate the relationship of conventional serology to IHA

Section 5: Study design

Old text:

... Additionally, a non-conventional ELISA test and ~~IHA-test~~ will be obtained; however, a positive result will not be required for diagnosis.

New text:

... Additionally, a non-conventional ELISA test and IHA will be obtained; however, a positive result will not be required for diagnosis.

Section 6.1: Inclusion criteria

Old text:

... Additionally, a non-conventional ELISA test and ~~IHA-test~~ will be obtained; however, a positive result will not be required for diagnosis.

New text:

... Additionally, a non-conventional ELISA test and IHA will be obtained; however, a positive result will not be required for diagnosis.

Section 9.1 Tabular schedule of evaluations

Old text (at Visits 1 and 11):

Immunofluorescent antibody (IFA) test *	●										●
---	---	--	--	--	--	--	--	--	--	--	---

[...] IFA = immunofluorescent antibody, [...]

* ~~IFA~~ will be performed at Visit 1 and Visit 11 only, if adequate specimens are available

New text (at Visits 1 and 11):

Indirect hemagglutination assay (IHA)*	●										●
--	---	--	--	--	--	--	--	--	--	--	---

[...] IHA = indirect hemagglutination assay [...]

* IHA will be performed at Visit 1 and Visit 11 only, if adequate specimens are available

Section 9.2.1.1 Visit 1 – screening visit (Days -14 to -1)

Old text:

... Specimens taken for conventional serology, non-conventional serology, ~~IFA~~ and qPCR will be frozen and stored at -20°C prior to shipment to the central laboratory where they will be stored at -70°C.

New text:

... Specimens taken for conventional serology, non-conventional serology, IHA and qPCR will be frozen and stored at -20°C prior to shipment to the central laboratory where they will be stored at -70°C.

Section 9.2.3.3: Visit 11 (Day 420 ± 7)

Old text:

Conventional and non-conventional serologic testing and ~~IFA~~ for Chagas' disease and qPCR

New text:

Conventional and non-conventional serologic testing and IHA for Chagas' disease and qPCR

Section 9.4.2: Serological tests

Old text:

... Additionally, a non-conventional ELISA test and ~~IFA~~ will be obtained; however, a positive result will not be required for diagnosis.

New text:

... Additionally, a non-conventional ELISA test and IHA will be obtained; however, a positive result will not be required for diagnosis.

Section 10.3 Variables and planned statistical analyses

Old text:

Secondary and exploratory efficacy analyses

...

The results from ~~IHA test~~ and conventional serology will also be compared. The details of secondary and exploratory efficacy analyses will be further specified in the SAP.

New text:

Secondary and exploratory efficacy analyses

...

The results from IHA and conventional serology will also be compared. The details of secondary and exploratory efficacy analyses will be further specified in the SAP.

15.7 Amendment 7

Integrated protocol Version 8.0, dated 11 MAY 2018.

15.7.1 Overview of changes to the study

Overall rationale for the amendment

This amendment is prepared 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.

Section # and name	Description of change	Brief rationale
Synopsis Section 3 Introduction	Defined the current ongoing study and the LTFU as part 1 and part 2; provided the rationale and benefit-risk assessment for the LTFU.	To satisfy FDA's request to "further describe and verify the clinical benefit of nifurtimox", and to implement FDA's recommendations to the detailed study concept for the LTFU
Synopsis Section 4 Study objectives	Provided study objectives for the LTFU Provided updated information on secondary objective pharmacokinetic (PK)/pharmacodynamic (PD) evaluation	To satisfy FDA's request to "further describe and verify the clinical benefit of nifurtimox", and to implement FDA's recommendations to the detailed study concept for the LTFU A pharmacokinetic model needs to be established to perform PK/PD evaluations.

Section # and name	Description of change	Brief rationale
Synopsis Section 5 Study design	Provided detailed study design for the LTFU	To satisfy FDA's request to "further describe and verify the clinical benefit of nifurtimox", and to implement FDA's recommendations to the detailed study concept for the LTFU
Synopsis Section 6.1 Inclusion criteria Section 6.2 Exclusion criteria	Provided selection criteria for the LTFU	
Synopsis Section 9.1 Tabular schedule of evaluations Section 9.2 Visit description	Provided the schedule of procedures/assessments and the detailed visits description for the LTFU	
Section 9.4 Efficacy	Provided efficacy assessments for the LTFU	
Section 9.6 Safety	Provided safety assessments for the LTFU	
Synopsis Section 10 Statistical methods and determination of sample size	Provided analysis sets, variables, statistical and analytical plan for the LTFU; Provided updated information on sample size	To satisfy FDA's request to "further describe and verify the clinical benefit of nifurtimox", and to implement FDA's recommendations to the detailed study concept for the LTFU; Sample size recalculation based on clarification of study objective in Amendment 2
Section 16.2 Form for interim phone contacts	Provided phone contact form for phone assessments in the LTFU	To satisfy FDA's request to "further describe and verify the clinical benefit of nifurtimox", and to implement FDA's recommendations to the detailed study concept for the LTFU

Typographical, grammatical, and other minor edits are not identified.

15.8 Amendment 8

Integrated protocol version 9.0, dated 23 JUL 2019.

15.8.1 Overview of changes to the study

Overall rationale for the amendment

This amendment was prepared 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.

Section # and name	Description of change	Brief rationale
--------------------	-----------------------	-----------------

Section # and name	Description of change	Brief rationale
Synopsis Section 4.2 Objectives for part 2 (CHICO SECURE) Section 5.2 Part 2 (CHICO SECURE) Section 6.2 Exclusion criteria Section 10.3 Variables and planned statistical analyses	Clarified measurement of optical density for recombinant ELISA and total purified antigen ELISA	Ensure all serology collected in part 2 (CHICO SECURE) will be the same as in part 1 (CHICO), as requested by FDA
Synopsis Section 6.2 Exclusion criteria Section 9.7 Other procedures and variables	Clarified evaluation of housing conditions as judged by the investigator	Consideration of clinical standards established in the study countries
Section 9.6.2 Pregnancies	Clarified evaluation of presence/absence of congenital infection with <i>T. cruzi</i> in babies born by female study participants	Consideration of clinical standards established in the study countries and to avoid unnecessary blood sampling in babies born by female study participants
Section 9.4.4 Assessment of Chagas' disease	Definition of progression of clinical Chagas' disease modified to include alterations that are characteristic of the chronic form of the disease	Allow for a more comprehensive evaluation of progression of clinical Chagas' disease in part 2 of the study
Section 9.6.1.4 Reporting of serious adverse events	Guidance updated to note that SAEs in part 2 must also be reported in accordance with local regulations	Consideration of clinical standards established in the study countries

Typographical, grammatical, and other minor edits are not identified.

15.9 Amendment 9

Integrated protocol version 10.0, dated 18 OCT 2019.

15.9.1 Overview of changes to the study

Overall rationale for the amendment

This amendment was prepared 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.

Section # and name	Description of change
--------------------	-----------------------

Section # and name	Description of change
Synopsis Section 5.2 Design overview of Part 2 Section 6.2 Exclusion criteria for Part 2	Part 2 exclusion criterion 1 removed
Synopsis Section 5.2 Design overview of Part 2 Section 6.3.1 Withdrawal Section 9.2.4.4 Unscheduled study visits	Subjects initiating antitrypanosomal treatment are no longer required to discontinue from the study
Section 7.8 Treatment in Part 2	Any antitrypanosomal treatment chosen by the investigator during LTFU is not considered as study drug.

Typographical, grammatical, and other minor edits are not identified.

16. Appendices

16.1 Phone Contact Form for telephone assessments²⁰⁹

16.1.1 Part 1 (CHICO)

<p>A medically qualified site staff member should use this form during the phone calls with the study subject's parent/s at days 14, 21 and 42. This form is designed to find out how the treated child has been feeling and whether his/her parent/legal guardian has contacted a doctor.</p> <p>Please obtain information about any symptoms or medical events that are NEW or CHANGED and about any NEW concomitant medication or DOSE CHANGE since the last patient's visit.</p>			
Visit Number		Visit Date	
Form completion date			
Medical Care			
Has the health condition of the child changed since last study visit?			Yes / No
- If Yes, please specify all relevant information:			
Has your child experienced any GI problem since last study visit?			Yes / No
- If Yes, please specify the medical event, start/stop date, action taken and outcome:			
Has your child experienced any CNS problems since last study visit?			Yes / No
- If Yes, please specify the medical event, start/stop date, action taken and outcome:			
Has your child experienced any respiratory problems since last study visit?			Yes / No
- If Yes, please specify the medical event, start/stop date, action taken and outcome:			
Has the child visited a Physician or attended to the emergency room due to a medical condition since last study visit?			Yes / No
- If yes, did the child's doctor provide any diagnose?			

²⁰⁹ Section heading and Form added per Amendment 1; Heading 2 renumbered from this point forward (Amendment 1)

Has your child experienced any weight loss since last study visit?	Yes / No
- If yes, please indicate the current weight:	
Other medical events (not listed above)	
- If Yes, please specify the medical event, start/stop date, action taken and outcome:	
- If Yes, please specify the medical event, start/stop date, action taken and outcome:	
Study Drug Intake	
Has your child taken the study drug three times a day as required?	Yes/No
- If not, please explain	
Has your child taken the study drug with food?	Yes/No
- If not, please explain	
Concomitants Medication	
Did your child start taking any NEW concomitant medication since last study visit?	Yes / No
<u>If Yes, please specify the medication, start/stop date, dose and the reason:</u>	
Did your child change the dose of or stop taking any of the concomitant medications since last study visit?	Yes / No
<u>If Yes, please specify the medication, dose change/stop date, new dose and the reason:</u>	
If any safety concern arises, subjects may return to the study site for an Unscheduled visit.	
Name / Signature:	Date:

16.1.2 Part 2 (CHICO SECURE)

Between annual study visits, all patients will be contacted to assess clinical signs and symptoms of potential reinfection or reactivation of Chagas disease or any exclusion criteria, to record any update in concomitant medications, and any AEs experienced since the last study visit. Patients may be asked to return prior to their next annual visit to the study center, if, in the opinion of the investigator, it is clinically necessary. A medically qualified site staff member will use this form when contacting the patient and/or his or her parent(s) or legally authorized representative about 6 months after the last annual site visit.

Visit 2 <input type="checkbox"/>	Form completion date:
----------------------------------	-----------------------

Visit 4 <input type="checkbox"/>	Form completion date:
----------------------------------	-----------------------

Has the health condition changed since last annual study visit? ☐ Yes
☐ No

- If YES, please specify:

Assessment of clinical signs and symptoms of potential Chagas' disease (mark all that apply)

- bug's bite
- localized swelling at the site of bug's bite
- eyelid swelling
- rash
- fever
- malaise
- fatigue
- body aches
- headache
- loss of appetite
- difficulty swallowing
- abdominal pain
- nausea
- vomiting
- diarrhea
- swollen glands
- irregular heartbeat
- other, please specify:

Only to be completed if any sign or symptom of Chagas' disease is ticked:

Was a physician visited?

☐ Yes

- If YES, did the physician provide any diagnose (please specify, if applicable)?

☐ No

Has any weight loss occurred since the last annual study visit?

☐ Yes

☐ No

- If YES, please indicate the current weight: _____ kg

Other acute or chronic health conditions (not listed above)

☐ Yes

☐ No

- If YES, please specify:

Health condition:

Start date:

Stop date:

Action taken:

Outcome:

Health condition:

Start date:

Stop date:

Action taken:

Outcome:

For female patients of childbearing age only

Is the patient currently pregnant?

☐ Yes

☐ No

If YES, are you willing to bring the baby to the next visit to have the baby tested for Chagas' disease?

☐ Yes

☐ No

If NO, please specify reason:

Have you moved your residence since the last annual study visit?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If YES, is your new residence included in enhanced domestic vector control?	<input type="checkbox"/> Yes <input type="checkbox"/> No
Are you currently taking any medication for treatment of Chagas' disease?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If YES, please specify:	
<ul style="list-style-type: none">• Nifurtimox Dose: Start date: Stop date: Reason:• Benznidazole Dose: Start date: Stop date: Reason:• Other, please specify: Dose: Start date: Stop date: Reason:	
Is there any change in concomitant medication since the last annual study visit?	<input type="checkbox"/> Yes <input type="checkbox"/> No
If YES, please specify:	
<u>Change in dose of concomitant medication:</u> <u>Drug:</u> <u>New dose:</u> <u>Reason:</u> <u>Drug:</u> <u>New dose:</u>	

Reason:

Stop taking concomitant medication:

Drug:

Dose:

Stop date:

Reason:

Drug:

Dose:

Stop date:

Reason:

New concomitant medication:

Drug:

Dose:

Start date:

Stop date (if applicable):

Reason:

Drug:

Dose:

Start date:

Stop date (if applicable):

Reason:

In case of suspected findings, an unscheduled visit at the study center has to be requested.

Unscheduled visit recommended?

☐ Yes

☐ No

Name / Signature:

Date:

16.2 Schwartz formula and mean (range) eGFR for infants and children of various ages²¹⁰

Normal GFR in Children and Young Adults

Age (Sex)	Mean GFR \pm SD (mL/min/1.73 m ²)
1 week (males and females)	40.6 \pm 14.8
2-8 weeks (males and females)	65.8 \pm 24.8
>8weeks (males and females)	95.7 \pm 21.7
2-12 years (males and females)	133.0 \pm 27.0
13-21 years (males)	140.0 \pm 30.0
13-21 years (females)	126.0 \pm 22.0

Data based on three studies.[0][5][6]

GFR, glomerular filtration rate; SD, standard deviation.

- Heilbron DC, Holliday MA, Al-Dahwi A, Kogan BA. Expressing glomerular filtration rate in children. *Pediatr Nephrol* 5:5-11, 1991.
- Coultard MG. Maturation of glomerular filtration in preterm and mature babies. *Early Hum Dev* 11:281-292, 1985.
- Schwartz GJ, Feld LG, Lanford DJ. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. *J Pediatr* 104:849-854, 1984.

The estimated GFR may be calculated using serum creatinine results at screening, and using the calculator in the following link: ²¹¹

<http://www-users.med.cornell.edu/~spon/picu/calc/crclschw.htm>

²¹⁰ National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. *Am J Kidney Dis* 39:S1-S266, 2002 (suppl 1)

²¹¹ Old table removed and new table/text added change in determination of eGFR per Amendment 1

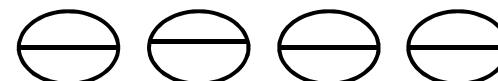
16.3 PK Subject diary and Subject diary ²¹²

PK Subject Diary

Patient N°				Weight at Randomization	
Treatment Start Date		Formulation assigned		Please return study drug at every study visit	

Number of tablets for each administration

(Investigator: fill the boxes according to the assigned dose)



Day of Treatment	Date	Time of Morning dose	Time of Noon dose	Time of Night dose	Meal Ingested	Comments
-1		N/A	N/A	N/A	<u>Night Meal:</u> Time__ : __ 	
1		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time__ : __ <u>Noon Meal:</u> Time__ : __ <u>Night Meal:</u> Time__ : __	

212 A subject diary and PK subject diary were created as per amendment 4.

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2		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
3		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
4		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
5		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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6		<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time <u> </u> : <u> </u> <u>Noon Meal:</u> Time <u> </u> : <u> </u> <u>Night Meal:</u> Time <u> </u> : <u> </u>	
7		<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time <u> </u> : <u> </u> <u>Noon Meal:</u> Time <u> </u> : <u> </u> <u>Night Meal:</u> Time <u> </u> : <u> </u>	
8		<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time <u> </u> : <u> </u> <u>Noon Meal:</u> Time <u> </u> : <u> </u> <u>Night Meal:</u> Time <u> </u> : <u> </u>	
9		<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time <u> </u> : <u> </u> <u>Noon Meal:</u> Time <u> </u> : <u> </u> <u>Night Meal:</u> Time <u> </u> : <u> </u>	

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10		<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time <u> </u> : <u> </u> <u>Noon Meal:</u> Time <u> </u> : <u> </u> <u>Night Meal:</u> Time <u> </u> : <u> </u>	
11		<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time <u> </u> : <u> </u> <u>Noon Meal:</u> Time <u> </u> : <u> </u> <u>Night Meal:</u> Time <u> </u> : <u> </u>	
12		<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time <u> </u> : <u> </u> <u>Noon Meal:</u> Time <u> </u> : <u> </u> <u>Night Meal:</u> Time <u> </u> : <u> </u>	
13		<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u> </u> : <u> </u> <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time <u> </u> : <u> </u> <u>Noon Meal:</u> Time <u> </u> : <u> </u> <u>Night Meal:</u> Time <u> </u> : <u> </u>	
14					<u>Morning Meal:</u> Time <u> </u> : <u> </u>	

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		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Noon Meal:</u>	Time ____ : ____	
					<u>Night Meal:</u>	Time ____ : ____	
15		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u>	Time ____ : ____	
					<u>Noon Meal:</u>	Time ____ : ____	
					<u>Night Meal:</u>	Time ____ : ____	
16		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u>	Time ____ : ____	
					<u>Noon Meal:</u>	Time ____ : ____	
					<u>Night Meal:</u>	Time ____ : ____	
17		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u>	Time ____ : ____	
					<u>Noon Meal:</u>	Time ____ : ____	
					<u>Night Meal:</u>	Time ____ : ____	
18		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u>	Time ____ : ____	

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		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<u>Noon Meal:</u> Time__ : __	
					<u>Night Meal:</u> Time__ : __	
19		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time__ : __ <u>Noon Meal:</u> Time__ : __ <u>Night Meal:</u> Time__ : __	
20		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time__ : __ <u>Noon Meal:</u> Time__ : __ <u>Night Meal:</u> Time__ : __	
21		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time__ : __ <u>Noon Meal:</u> Time__ : __ <u>Night Meal:</u> Time__ : __	

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22		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
23		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
24		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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25		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
26		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
27		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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28		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
29		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
30		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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31		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
32		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
33		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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34		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
35		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
36		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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37		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
38		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
39		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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40		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
41		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
42		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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43		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
44		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
45		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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46		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
47		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
48		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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49		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
50		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
51		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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52		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
53		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
54		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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55		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
56		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	
57		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____ <u>Noon Meal:</u> Time ____ : ____ <u>Night Meal:</u> Time ____ : ____	

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58		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____	
					<u>Noon Meal:</u> Time ____ : ____	
					<u>Night Meal:</u> Time ____ : ____	
59		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____	
					<u>Noon Meal:</u> Time ____ : ____	
					<u>Night Meal:</u> Time ____ : ____	
60		____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	____ : ____ <input type="checkbox"/> not taken	<u>Morning Meal:</u> Time ____ : ____	
					<u>Noon Meal:</u> Time ____ : ____	
					<u>Night Meal:</u> Time ____ : ____	

Instructions on how to use this Patient Diary:

- Please document the date and time of each study drug intake exactly. In case the dose was not taken, please check the corresponding box .
- If dose reduced, please mention in the comment field and provide the new dose the patient took.
- If dose not taken at all, please mention in the comment field with the reason.
- When taking the study drug, if the child vomits or spits out the medication, please mention in the comment field along with the time.

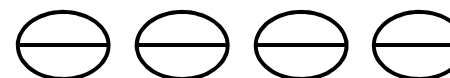
- If you have vomiting, please record it, along with the time, under the “Comments” column.
- Please record all foods ingested with the morning, noon and night doses of the study drug.
- Please remember to return this diary with ALL bottles of study drug dispensed (including empty ones) to the
- Investigator.

Subject Diary

Patient N°				Weight at Randomization	
Treatment Start Date		Formulation assigned		Please return study drug at every study visit	

Number of tablets for each administration

(Investigator: fill the boxes according to the assigned dose)



Day of Treatment	Date	Time of Morning dose	Time of Noon dose	Time of Night dose	Comments
1		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
2		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
3		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
4		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
5		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
6		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
7		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
8		__ : __	__ : __	__ : __	

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Day of Treatment	Date	Time of Morning dose <input type="checkbox"/> not taken	Time of Noon dose <input type="checkbox"/> not taken	Time of Night dose <input type="checkbox"/> not taken	Comments
		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
9		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
10		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
11		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
12		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
13		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
14		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
15		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
16		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
17		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
18		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
19		__ : __	__ : __	__ : __	

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Day of Treatment	Date	Time of Morning dose <input type="checkbox"/> not taken	Time of Noon dose <input type="checkbox"/> not taken	Time of Night dose <input type="checkbox"/> not taken	Comments
		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
20		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
21		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
22		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
23		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
24		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
25		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
26		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
27		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
28		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
29		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
30		__ : __	__ : __	__ : __	

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		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
31		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
32		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
33		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
34		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
35		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
36		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
37		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
38		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
39		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
40		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
41		__ : __	__ : __	__ : __	

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Day of Treatment	Date	Time of Morning dose	Time of Noon dose	Time of Night dose	Comments
		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
42		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
43		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
44		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
45		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
46		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
47		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
48		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
49		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
50		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
51		__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	__ : __ <input type="checkbox"/> not taken	
52		__ : __	__ : __	__ : __	

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Day of Treatment	Date	Time of Morning dose <input type="checkbox"/> not taken	Time of Noon dose <input type="checkbox"/> not taken	Time of Night dose <input type="checkbox"/> not taken	Comments
53		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
54		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
55		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
56		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
57		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
58		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
59		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	
60		<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	<input type="checkbox"/> not taken	

Instructions on how to use this Patient Diary:

- Please document the date and time of each study drug intake exactly. In case the dose was not taken, please check the corresponding box .
- If dose reduced, please mention in the comment field and provide the new dose the patient took.
- If dose not taken at all, please mention in the comment field with the reason.

- When taking the study drug, if the child vomits or spits out the medication, please mention in the comment field along with the time.
- If you have vomiting, please record it, along with the time, under the “Comments” column.
- Please remember to return this diary with ALL bottles of study drug dispensed (including empty ones) to the
- Investigator.

16.4 Procedure for multiplex real time polymerase chain reaction to *Trypanosoma cruzi* detection in patients with Chagas' disease

Blood samples collection

1. Material

- Ethanol 70%
- Latex gloves, without talc
- Disposable syringe and needle
- Conical tubes with the appropriate volume of Guanidine 6M-ethylenediaminetetraacetic acid (EDTA) 0.2M buffer
- Permanent marker
- Stretcher
- Container and bags for disposal of biological material

2. Implementation

Collected peripheral blood samples will be immediately mixed with an equal volume of Guanidine EDTA buffer pursuing the following volume consideration:

- Babies 1 day to 12 months of age: minimum 1 mL of blood – Use 15 mL tubes with red tag
- Children from 1 to 14 years old: minimum 2 mL of blood –Use 15 mL tubes with yellow tag
- Children ≥ 15 years of age: minimum 5 mL of blood – Use 50 mL tubes.

3. Sample collection procedure

NOTE: The collection of blood must be performed by personnel qualified by training to draw blood.

Label the conic tube with patient's full name and date of collection

Collect the appropriate volume of whole blood in the conic tube and close the screw cap.

Thoroughly mix conic tubes containing blood and buffer by gently inverting the tube 6-8 times.

The specimens can be stored at room temperature until and during shipment to the laboratory. Samples must be received in the laboratory within 1 month of collection.

NOTE: Excessive exposure to heat results in the breakdown of deoxyribonucleic acid (DNA). Guanidine-EDTA-Blood (GEB) specimens must be refrigerated at $5^{\circ} \pm 3^{\circ}\text{C}$ if room temperature could exceed 30°C .

Genetic material (DNA) extraction

1.1 Material

- Sodium hypochlorite 0.5%-1% freshly diluted
- Ethanol 70%
- Dry bath
- Automatic variable volume micropipette 0-10 µL
- Automatic variable volume micropipette 20-200 µL
- Automatic variable volume micropipette 100-1000 µL
- Latex gloves, without talc
- Racks (1.5/2 mL tubes)
- Racks (15/50 mL conical tubes)
- High Pure PCR Template Preparation Kit, Roche (catalogue number: 11796828001)
- Absolute ethanol
- Distilled water (PCR use)
- Microcentrifuge
- Vortex
- Permanent marker
- Container and bags for disposal of biological material
- Tubes eppendorf like (1,5 mL)
- Tubes (2 mL)
- Filter tips (10 µL, 200 µL y 1000 µL)

1.2 Sample reception

The sample reception will be conducted verifying samples codes, subject initials, date of birth and date of collection.

Samples are acceptable when they meet the requirements of homogeneity, absence of clots and adequate code.

Once received in the laboratory, and until analyzed, the specimens will be stored at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ or at room temperature if it does not exceed 30°C .

1. Pre-analytical procedure

Once collected, the samples will be preserved at least 24 hours before heating. GEB samples will be heated to 100°C during 15 minutes in a dry bath. Gently open the screw cap before heating.

Cool to room temperature and let stand at least 12 hours at 4°C before processing.

2. Implementation

The extraction procedure will be carried out with a maximum of 12 samples or controls each run. All runs must include one negative control (seronegative GEB sample) and one weak positive control. It will be performed up to 2 times a day (1 in the morning and 1 in the afternoon) to avoid pipetting mistakes and contaminations.

Before starting the DNA extraction procedure from GEB samples, pursue the following instructions:

- Disinfect work area, cleaning surfaces with damp cloth with sodium hypochlorite 0,51%: bench, pipettes, equipment, etc.
- Illuminate the work area with ultraviolet light (sterilization) for 15 minutes
- Verify samples code and the number of samples to be processed.
- Verify the selection of appropriate positive and negative controls.
- Aliquot the amount of extraction reagents and tubes that will be used during the procedure.
- Label tubes and columns with date and the relevant code seated in the workbook using permanent marker
- Turn on the dry bath at 70°C.

3. Extraction procedure

Before starting the purification procedure:

- Heat the required amount of elution buffer at 70°C in a dry bath
- Homogenize the samples by vortex during 15 to 30 seconds.
 1. Add 5 µL de IAC (40 pg/µL) and 40 µL of Proteinase K in a 2 mL tube DNase free
 2. Add 300 µL of GEB sample (blood – guanidine EDTA buffer).
 3. Add 100 µL of Binding Buffer and mix by pulse-vortexing for 15 seconds
 4. Mix by pulse-vortexing for 3 seconds, spin down tubes.
 5. Incubate for 10 min at 70°C in a dry bath, spin tubes before opening.
 6. Add 100 µL of isopropyl alcohol and mix by pulse-vortexing for 15 seconds. After mixing, briefly spin down tubes to remove drops from the inside of the lid spin tubes.
 7. Carefully apply the mixture from Step 6 to the High Pure Filter column (in a 2 mL collection tube) without wetting the rim, and close the cap.
 8. Centrifuge at 8,000 revolutions per minute (RPM) for 1 minute. Place the High Pure Filter column in a clean 2 mL collection tube, and discard the tube containing the filtrate.
 9. Carefully open the High Pure Filter column and add 500 µL of inhibitor removal buffer without wetting the rim, and close the cap.

10. Centrifuge at 8,000 RPM for 1 minute. Place the High Pure Filter column in a clean 2 mL collection tube, and discard the collection tube containing the filtrate.
11. Carefully open the High Pure Filter column and add 500 µL Wash Buffer without wetting the rim, and close the cap.
12. Centrifuge at 8,000 RPM for 1 minute. Place the High Pure Filter column in a clean 2 mL collection tube, and discard the collection tube containing the filtrate.
13. Repeat Steps 11 and 12.
14. Place the High Pure Filter column in a new 2 mL collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 10 seconds.
15. Place the High Pure Filter column in a clean 1.5 mL microcentrifuge tube labeled with the sample code seated in the workbook, and discard the collection tube containing the filtrate.
16. Carefully open the High Pure Filter column and add 100 µL of elution buffer (pre-incubate at 70°C) and centrifuge at 8,000 RPM for 1 minute.
17. For long-term storage of DNA, store at –20°C.

Procedure for multiplex real time polymerase chain reaction (qPCR)

1. Materials

- Automatic variable volume micropipette 0-10 µL
- Automatic variable volume micropipette 0-20 µL
- Automatic variable volume micropipette 20-200 µL
- Automatic variable volume micropipette 100-1000 µL
- Filter tips (10 µL, 20 µL, 200 µL y 1000 µL)
- Latex gloves without talc
- Racks (0.2 mL and 1.5 mL tubes)
- Microcentrifuge
- PCR cabinet with ultraviolet radiation
- Real time PCR thermo cycler Applied Biosystems, StepOne
- Vortex
- Permanent marker
- Container and bags for disposal of biological material
- Real time PCR reagents and master mix (Master mix Roche 2X: catalog number 04913949001; sterile distilled water PCR quality)
- Primers and probes to *T. cruzi*:

cruzi 1: 5'-ASTCGGCTGATCGTTTTCGA- 3'

cruzi 2: 5'-AATTCCTCCAAGCAGCGGATA- 3'

cruzi 3 (probe): 5'-CACACACTGGACACCAA- 3'

- Primers and probes IAC:

IAC Tq Fw: 5'-ACCGTCATGGAACAGCACGTA- 3'

IAC Tq Rv: 5'-CTCCCGCAACAAACCCTATAAAT- 3'

IAC (sonda): 5'-AGCATCTGTTCTTGAAGGT- 3'

- Plates and adhesive films to qPCR (Applied biosystems catalog number 4375816 and 4375928)

2. Implementation

The qPCR may be performed twice a day, making a run in the morning and one in the afternoon, taking into account the time required for each run is 4 hours (reagents pipetting and cycles)

The number of samples per run may be at most 43, plus 5 quality controls, making a total of 48 wells, maximum capacity of qPCR thermo cycler.

Before start the mix preparation pursue the following instructions:

- Defrost DNA samples, controls and reagents, and hold at 4°C until use. Will be use on each plate two positive controls in duplicate (CL Brener 10 fg/μL, 1 fg/μL) and 1 white control (distilled water used to mix preparation)
- Prepare the reaction protocol (see related documents) with the amount and order of samples and controls in the PCR plate.
- Turn on ultraviolet light in the PCR cabinet during 15 minutes
- Enter reaction protocol data in the StepOne software using the template "POE qPCRMtq Chagas" and save the file.

3. qPCR procedure

- Pipette the reaction mixture according to the following detail (example for N reactions):

Reagents	Final concentration	Volume (x 1 reaction)	Volume (x N reactions)
Roche Fast Start Universal Probe Master Mix (Rox) (2X)	1X	10 µL	N x 10
cruzi 1 (Fw) (50 µM)	0,75 µM	0,3 µL	N x 0,3
cruzi 2 (Rv) (50 µM)	0,75 µM	0,3 µL	N x 0,3
cruzi 3 (probe) (5 µM)	0,05 µM	0,2 µL	N x 0,2
IAC Tq Fw (5 µM)	0,1 µM	0,4 µL	N x 0,4
IAC Tq Rv (5 µM)	0,1 µM	0,4 µL	N x 0,4
IAC probe (5 µM)	0,05 µM	0,2 µL	N x 0,2
H ₂ O PCR quality	-	3,2 µL	N x 3,2
Total volume (mix) x well	-	15 µL	15 µL
DNA sample volume		5 µL	5 µL
Final volume		20 µL	20 µL

- Distribute 15 µL of the reaction mixture per well.
- Add 5 µL of DNA sample or controls in the wells following the reaction protocol.
- Seal the plate with the adhesive film without touch the surface.
- Confirm that the samples have been applied in the reaction mixture and not in the edges of the tube.
- Start the reaction

4. Cycles and reaction conditions

First step: 10 min at 95°C.

Second step: 40 cycles of 15 sec at 95°C and 1 min at 58°C, fluorescence data collection in the yellow (IAC-VIC) and green (*T. cruzi*-FAM) channel.

5. Interpretation of results

Establish the threshold in 0.02 to *T. cruzi* and 0.01 to the internal amplification control (IAC).

Analyze the results of PCR negative and positive controls

Run valid:

- Positive controls are detectable to *T. cruzi* analysis (green channel). The fluorescent curve crosses the threshold leading to a Ct appropriate in relation to the concentration of *T. cruzi* DNA in the respective positive control (assess with control chart).
- White controls are not detectable to *T. cruzi* analysis. The fluorescent curve does not cross the threshold leading to absence of Ct.

Valid sample:

- The internal amplification control (yellow channel) amplified with and efficient signal

Positive sample or detectable to *T. cruzi*:

- The fluorescent curve in the green channel crosses the threshold leading to a Ct value.

Negative sample or not detectable to *T. cruzi*:

- The fluorescent curve does not cross the threshold leading to absence of Ct value.

16.5 Pharmacokinetic blood sample handling

Sample Handling Sheet

Version 1.0

Study Identifier: BAY a2502 (Nifurtimox) / 16027

Analyte Name: BAY a2502 Nifurtimox

Author:

Name:	PPD
Department:	GDD-GED-DMPK-BANP-BAW
Date:	2014-10-06

Matrix: Plasma for bioanalysis of PK samples

Precautions:

- Samples degrade in the presence of light. Light protection measures have to be considered during all steps of sample handling. Avoid exposure to direct daylight or neon light. Please transfer the samples immediately into the refrigerator after blood sampling and after centrifugation. Reduce the time when samples are exposed to light as much as possible.

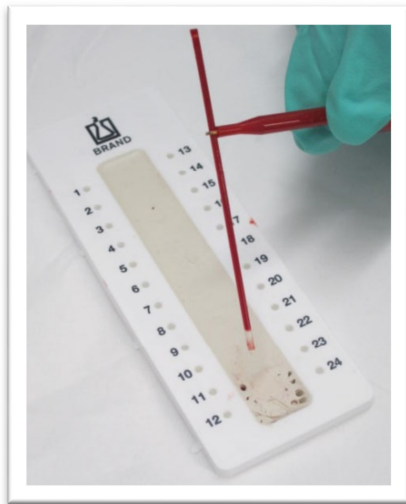
Materials:

- Polypropylentube with a length of at least 90 mm, e.g., Sarstedt 100 x 15.7 mm REF 55.539
- Push caps to close the polypropylentube mentioned above, e.g., Sarstedt REF 65.803.512
- Micro Haematocrit tubes, lithium heparinized, ca.75 µL, e.g., Vitrex Medical (DK) REF 161813
- Haematocrit sealing wax, e.g. Brand GmbH (Germany) REF 749510

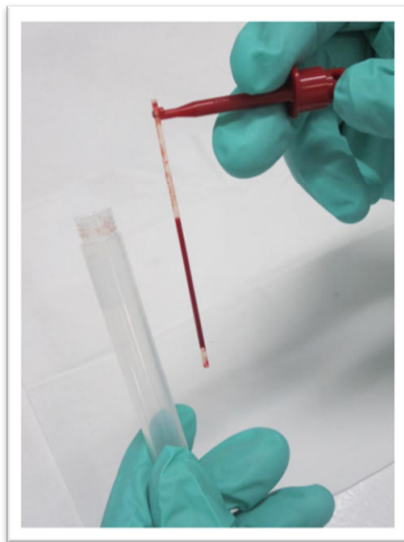
Sample collection, handling and storage:

→ The total duration between blood sample collection at room temperature and final storage of the plasma sample must not exceed 90 minutes.

- Please avoid exposure to direct daylight or neon light during sample processing.
- Please refer to study protocol for sampling time points.
- The blood samples have to be collected into two Micro Haematocrit tubes from the heel prick (subjects up to 1 month of age), fingerstick or ear lobe stick at the discretion of the investigator.
- Seal the lower side of the Micro Haematocrit tubes with wax.



- Transfer every Micro Haematocrit tube into one labelled polypropylene tube and lock with push cap.
Caution: Please put the wax sealed lower side downwards.



- Transfer the blood samples immediately into the refrigerator (protected from light) until centrifugation or proceed with centrifugation immediately as described below.

- Centrifuge blood samples at 2000 g (not higher) for approximately 10 minutes at room temperature.
- Immediately after centrifugation transfer the centrifuged samples into the freezer. In case samples cannot be frozen immediately, please store the samples temporarily in the refrigerator. Please sort samples according to subject ID
- Freeze the samples in an upright position until shipment at $\leq -15^{\circ}\text{C}$

Sample stability

- Analyte of interest is stable at $\leq -15^{\circ}\text{C}$ for at least 557 days

Sample shipment:

- Please inform the responsible Analytical Laboratory and Biosample Operations Manager in time (2 to 3 days before shipment) regarding sample shipment.
- Packaging of samples on sufficient dry ice
- Preferably use World Courier as courier service or use the central lab preferred provider
- Sample arrival is preferred on Monday to Thursday from 8:00 am to 16:00 pm CET.

Shipment Address:

Laboratory PPD

Bayer Pharma AG

GDD-GED-DMPK-BANP

Aprather Weg, Geb. 468

42096 Wuppertal

Phone: PPD

Fax: PPD

Email: PPD

16.6 Maximum blood volumes for pediatric patients – Clinical and Laboratory Standards Institute

Patient's Weight (lbs)	Patient's weight (kg)	Maximum amount to be drawn at any one time (mL)	Maximum cumulative amount to be drawn during a hospital stay of 1 month or less (mL)
6-8	2.7-3.6	2.5	23
8-10	3.6-4.5	3.5	30
11-15	4.5-6.8	5	40
16-20	7.3-9.1	10	60
21-25	9.5-11.4	10	70
26-30	11.8-13.6	10	80
31-35	14.1-15.9	10	100
36-40	16.4-18.2	10	130
41-45	18.6-20.5	20	140
46-50	20.9-22.7	20	160
51-55	23.2-25.0	20	180
56-60	25.5-27.3	20	200
61-65	27.7-29.5	25	220
66-70	30.0-31.8	30	240
71-75	32.3-34.1	30	250
76-80	34.5-36.4	30	270
81-85	36.8-38.6	30	290
86-90	39.1-40.9	30	310
91-95	41.1-43.2	30	330
96-100	43.6-45.5	30	350

* For patients that weigh less than 6 lbs, consult physician