CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

213464Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Cross Discipline Team Leader Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

NDA Multidisciplinary Review and Evaluation

Application Type	NDA
Application Number	213464
Priority or Standard	Priority
Submit Date	December 6, 2019
Received Date	December 6, 2019
PDUFA Goal Date	August 6, 2020
Division/Office	Division of Anti-Infectives (DAI) /Office of Infectious Diseases (OID)
Review Completion Date	August 5, 2020
Established/Proper Name	Nifurtimox
(Proposed) Trade Name	Lampit
Pharmacologic Class	Antiprotozoal
Code name	BAY A2502
Applicant	Bayer Healthcare Pharmaceuticals, Inc.
Dosage form	Oral tablets; 30 mg and 120 mg
Applicant proposed Dosing Regimen	The total daily dose: (b) (4) (≥40 kg): 8–10 mg/kg orally (<40 kg): 10-20 mg/kg orally
Applicant Proposed Indication(s)/Population(s)	Treatment of Chagas disease in term newborns, infants, children and adolescents less than 18 years of age
Applicant Proposed SNOMED CT Indication Disease Term for each Proposed Indication	77506005 | Infection caused by *Trypanosoma cruzi* (disorder)
Regulatory Action	Approval
Indication(s)/Population(s) (if applicable)	Treatment of Chagas disease in pediatric patients (birth to less than 18 years of age and weighing at least 2.5 kg)
Dosing Regimen	The total daily dose (regardless of age): ≥40 kg: 8–10 mg/kg orally <40 kg: 10-20 mg/kg orally

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CDRH= Center for Devices and Radiological Health
OPQ = Office of Pharmaceutical Quality
QT-IRT = QT Interdisciplinary Review Team
OB = Office of Biostatistics
OCP = Office of Clinical Pharmacology
OPDP = Office of Prescription Drug Promotion
OSI = Office of Scientific Investigations
OSIS = Office of Study Integrity and Surveillence
OSE = Office of Surveillance and Epidemiology
DMPP = Patient Labeling Team
DAI = Division of Anti-Infectives
DEPI = Division of Epidemiology
DMEPA = Division of Medication Error Prevention and Analysis
DMPP = Division of Medical Policy Programs
DPMH = Division of Pediatric and Maternal Health
DRISK = Division of Risk Management
TL = Team Leader

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	Signature: ***{See appended electronic signature page}***			
Pharmacometrics Reviewer	Jason Moore, PharmD	OCP/DIDP	Sections: 6, 20.4	**Select one:** _X_ Authored ___ Approved
	Signature: ***{See appended electronic signature page}***			
Pharmacometrics Team Leader	Justin Earp, PhD	OCP/DPM	Sections: 6, 20.4	**Select one:** ___ Authored _X_ Approved
	Signature: ***{See appended electronic signature page}***			
Clinical Microbiology Reviewer	Shukal Bala, PhD	OID/DAI	Sections: 9, 20.6	**Select one:** _X_ Authored ___ Approved
	Signature: ***{See appended electronic signature page}***			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Clinical Microbiology Team Leader	Avery Goodwin, PhD	OID/DAI	Sections: 9, 20.6	**Select one:** ___ Authored _X_ Approved
	Signature: ***{See appended electronic signature page}***			
Clinical Reviewer	Mukil Natarajan, MD	OID/DAI	Sections: 1, 2, 3, 4.1, 7, 8, 10, 11, 12, 13, 14, 15, 20.5	**Select one:** _X_ Authored ___ Approved
	Signature: ***{See appended electronic signature page}***			
Clinical Team Leader/Cross Discipline Team Leader	Peter Kim, MD, MS	OID/DAI	Sections: 1, 2, 3, 4.1, 7, 8, 10, 11, 12, 13, 14, 15, 20.5	**Select one:** ___ Authored _X_ Approved
	Signature: ***{See appended electronic signature page}***			
Statistical Reviewer	Jie Cong, PhD	OB/DBIV	Sections: 7, 8	**Select one:** _X_ Authored ___ Approved
	Signature: ***{See appended electronic signature page}***			
Statistical Team Leader	Karen M Higgins, ScD	OB/DBIV	Sections: 7, 8	**Select one:** ___ Authored _X_ Approved
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DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Deputy Division Director (OB)	Daphne Lin, PhD	OB/DBIV	Sections: 7, 8	**Select one:** ___ Authored _X_ Approved
	Signature: ***{See appended electronic signature page}***			
Associate Director for Labeling	Abimbola Adebowale, PhD	OID/DAI	Section: 13	**Select one:** ___ Authored _X_ Approved
	Signature: ***{See appended electronic signature page}***			
Regulatory Project Manager	Gregory F. DiBernardo	DRO-ID/DAI	Section: 3	**Select one:** _X_ Authored ___ Approved
	Signature: ***{See appended electronic signature page}***			
Chief, Regulatory Project Management Staff	Maureen Dillon-Parker, MS, RAC	DRO-ID/DAI	Section: 3	**Select one:** ___ Authored _X_ Approved
	Signature: ***{See appended electronic signature page}***			
Division Director (Clinical)	Sumathi Nambiar, MD, MPH	OID/DAI	Section: 1	**Select one:** ___ Authored _X_ Approved
	Signature: ***{See appended electronic signature page}***			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Office Director	John Farley, MD, MPH	OID	Sections: 1	**Select one:** ___ Authored _X_ Approved
	Signature: ***{See appended electronic signature page}***			

Glossary

AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AR	adverse reaction
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
DMC	data monitoring committee
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
ICH	International Conference on Harmonisation

IND	investigational new drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NME	new molecular entity
NFX	nifurtimox
OCS	Office of Computational Science
OND	Office of New Drugs
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert (also known as Patient Information)
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee

SOC	standard of care
TEAE	treatment emergent adverse event
ULN	upper limit of normal

1. Executive Summary

1.1. Product Introduction

Nifurtimox (Lampit) is a nitrofuran antiprotozoal drug available in two oral tablet formulations, 30 and 120 mg. The proposed indication is for the treatment of children aged 0 to <18 years with Chagas disease. The dose is 8 to 10 mg/kg/day for pediatric patients weighing ≥40 kg and 10 to 20 mg/kg/day for pediatric patients weighing <40 kg. The daily dose is divided in three doses for all ages. Nifurtimox is a new molecular entity, but has been marketed outside of the United States for several decades.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The Applicant has provided substantial evidence of effectiveness for the treatment of Chagas disease in pediatric patients from birth to <18 years of age based on a surrogate endpoint of change in serologic test results. In a Phase 3 trial of nifurtimox (NFX) in pediatric patients with Chagas disease (study 16027), seroconversion or a 20% decrease in optical density was demonstrated for the lysate and recombinant ELISA tests.

In Study 16027, both the lysate and the recombinant ELISA test results demonstrated a dose response relationship between the 60-day and 30-day treatment arms using the proportion of subjects responding by obtaining either seroconversion or a 20% decrease in optical density. For the lysate ELISA test, the difference [(60 day – 30 day), 95% CI], was 13% (3.5%, 22.6%), p=0.007, and for the recombinant ELISA test, the difference was 13% (3.2%, 23.0%), p=0.010. This difference was driven primarily by subjects aged 6 to <18 years who accounted for most (77%) of the study population. For subjects <8 months of age, the response only included seroconversion, while in all other age groups, the endpoint was at least a 20% decrease in optical density.

There is some uncertainty with the endpoint of 20% decrease in optical density as it is not known how much reduction in optical density corresponds to a specific reduction in antibody titer. However, a 20% decrease in optical density at 1 year post-treatment was considered an acceptable surrogate endpoint based on the following: (1) an analysis of data from a prior study of the treatment of Chagas disease patients 6 to 12 years of age showed a 21% decrease in mean optical density (using an ELISA similar to the lysate ELISA used by the Applicant) in benznidazole-treated subjects versus no change in the mean optical density for placebo subjects at 12 months post-treatment (Sosa Estani et al. 1998); (2) data from this study also showed that a 20% reduction in optical density in the ELISA at 1 year correlated with seroconversion in the same ELISA at 4 years; (3) the statistically significant differences observed in the randomized comparisons (60-day versus 30-day NFX treated groups) for the lysate and recombinant ELISA tests would rule out a chance event, i.e., variability associated with random error in the assays; and (4) seroconversion in the youngest age group (<8 months old) supports

the hypothesis that patients who have had Chagas disease for the shortest duration would be the first to seroconvert to negative.

Additional supportive evidence of efficacy was obtained from the F29 ELISA test (thought to detect antibody response to live parasites) which was positive at baseline in 214/330 (65%) of the study population. These data showed a significant increase in the seroconversion rate on the F29 ELISA test compared to a historical placebo control in Chagas disease patients 6 to 12 years of age (Sosa Estani et al. 1998).

The Applicant will continue to follow the subjects in Study 16027 for an additional 3 years as a required confirmatory study for the Subpart H approval. The follow up study will provide confirmation that the 20% decrease in optical density at 1 year post-treatment in children from 8 months to less than 18 years old is predictive of seroconversion at 4 years post-treatment.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

In NDA 213464, the Applicant is seeking approval of nifurtimox for the treatment of Chagas disease in pediatric patients from birth to less than 18 years of age. Chagas disease is a serious disease affecting 8 million individuals worldwide and at least 300,000 in the United States. Although patients can be asymptomatic for years, disease manifestations of Chagas disease can be life-threatening and include sudden cardiac death and heart failure. Chagas disease is caused by *Trypanosoma cruzi* and is transmitted through a triatomine insect vector. The diagnosis of chronic Chagas disease relies on antibody-based tests which can take years to become negative after treatment. Benznidazole is the only approved treatment for Chagas disease in the United States. However, there is no approved option for children less than 2 years of age or greater than 12 years of age. Having additional approved treatment options would be beneficial to patients.

Study 16027 was a randomized double-blind study of pediatric subjects from birth to <18 years old with asymptomatic Chagas disease diagnosed by direct observation of *T. cruzi* by concentration test for subjects <8 months of age and positive serology for subjects ≥8 months of age. A total of 330 subjects were randomized 2:1 to receive either 60 days or 30 days of nifurtimox and were followed for 12 months. An additional 3 years of follow-up is ongoing. The Applicant defined "cure" or positive response as either seroconversion or a ≥20% decrease in optical density on two different ELISA tests, the lysate ELISA (referred to as total purified antigen ELISA by the Applicant) and the recombinant ELISA. For subjects <8 months of age, only seroconversion was counted as a response. Using this endpoint for the lysate ELISA, the positive response rate was significantly higher in the 60-day arm (70/219, 32%) compared to the 30-day arm (21/111, 19%) with a p-value of 0.007. Similarly, for the recombinant ELISA, the positive response rate was significantly higher in the 60-day arm (76/219, 35%) compared to the 30-day arm (24/111, 22%) with a p-value of 0.01. These data show that there was a significant dose response between the 60-day and 30-day arms indicating an effect of the drug on *T. cruzi* antibody levels.

This dose response between the 60-day and 30-day arms was seen in subjects 6 to <18 years of age who accounted for 77% of the study population. In the subjects 0 to <6 years of age, the rate of positive response was high in both arms, but there was not a notable difference between the treatment arms. For example, using the lysate ELISA, the positive response rate for subjects 8 months to <2 years of age was 88% in both the 60-day (15/17) and 30-day arms (7/8). Similarly, for subjects <8 months of age the response rate, based on seroconversion to negative, was 83% (10/12) in the 60-day arm. Given the high positive response rate in subjects 0 to <2 years of age, and that in subjects <8 months of age the response was based solely on seroconversion, it is reasonable to extrapolate the finding of efficacy from subjects 6 to <18 years of age to the younger age groups.

It should be noted that the clinical meaningfulness of a 20% decrease in optical density in the two ELISA tests, which accounted for the difference between the arms in subjects >8 months of age, is not well established. The lysate and recombinant ELISA tests were designed as qualitative rather than quantitative tests. The relationship between the optical density values from the ELISA tests and antibody titers is not linear. Therefore it is not known how much a reduction in the optical density values in an ELISA test corresponds to antibody levels in the blood. However, a 20% decrease in optical density at 1 year post-treatment was considered an acceptable surrogate endpoint likely to predict seroconversion to negative, and would therefore be reasonably likely to predict clinical benefit in this population, based on the following: (1) analysis of data from a prior study of the treatment of Chagas disease patients 6 to 12 years of age that showed a 21% decrease in mean optical density (using an ELISA similar to the lysate ELISA used by the Applicant) in benznidazole-treated subjects versus no change in the mean optical density for placebo subjects at 12 months post-treatment (Sosa Estani et al. 1998); (2) data from this study also showed that a 20% reduction in optical density in the ELISA at 1 year correlated with seroconversion in the same ELISA at 4 years; (3) the statistically significant differences observed in the randomized comparisons (60-day versus 30-day NFX-treated groups) for the lysate and recombinant ELISA tests would rule out a chance event, i.e., variability associated with random error in the assays; and (4) seroconversion in the youngest age group (<8 months old) supports the hypothesis that patients who have had Chagas disease for the shortest duration would be the first to seroconvert to negative.

Additional evidence of effectiveness was obtained using the results of the F29 ELISA test (thought to detect antibody response to live parasites). At baseline, there were 142 subjects in the 60-day arm who were seropositive using the F29 ELISA. At 12 months, 32% (46/142) of these subjects seroconverted using the F29 ELISA. The Applicant provided data from the Sosa Estani study showing the placebo seroconversion rate at 12 months in subjects aged 6 to 12 years was 2.8% (1/36). In Study 16027, the seroconversion rate using the F29 ELISA in subjects 6 to 12 years old in the 60-day NFX arm was 34% (20/59) which was significantly greater than the Sosa Estani placebo rate ($p<0.001$).

In summary, the effectiveness of nifurtimox for pediatric patients with Chagas disease was established by the observed dose response between the 60-day and 30-day arms on the endpoint of ≥20% decrease in optical density or seroconversion using the lysate and recombinant ELISAs in children from birth to <18 years of age. While the findings of dose response were driven by subjects in the subgroup 6 to <18 years of age, efficacy could be extrapolated to subjects <6 years of age given the high response rate in subjects 0 to <2 years of age, and the seroconversion seen in subjects <8 months of age. Additional evidence of effectiveness was obtained using the F29 ELISA response compared to a historical placebo control. Subjects in Study 16027 will continued to be followed up for an additional 3 years as a requirement of the Subpart H approval. This will provide confirmation that the 20% decrease in optical density at 1 year post-treatment in children from 8 months to less than 18 years of age is predictive of seroconversion at 4 years post-treatment.

The safety information used to determine the risk for nifurtimox comes from Study 16027 as well as from animal studies, the literature, and postmarketing reports. Gastrointestinal adverse reactions were commonly reported and include nausea, vomiting, abdominal pain, diarrhea,

poor appetite, and weight loss. Hypersensitivity reactions include rash, urticaria, DRESS, eosinophilia, and anaphylaxis. Rash was noted in 5.5% of subjects in the 60-day arm of the study. DRESS and anaphylaxis were not observed in Study 16027, but were reported in the literature and postmarketing data. Headache was reported in 12.8% of 60-day arm subjects. Other neurological adverse reactions were uncommon and include seizure, paresthesia, tremor, and amnesia. Psychiatric adverse reactions were uncommon and include insomnia, irritability, and anxiety. Hematological adverse reactions were uncommon and include anemia, leukopenia, neutropenia, and thrombocytopenia. Animal studies indicate a risk for embryo-fetal toxicity. Labeling includes a warning with a recommendation for contraception. In addition, there is a postmarketing requirement (PMR) for a postmarketing single-arm pregnancy safety study. The available safety information is sufficient to describe the risk profile of nifurtimox in the treatment of Chagas disease. The identified safety issues do not preclude approval and can be managed through labeling.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Chagas disease is a serious disease affecting 8 million individuals worldwide and at least 300,000 in the United States. It is caused by *Trypanosoma cruzi* and is transmitted through a triatomine insect vector. • Chagas disease has an acute and chronic phase. In the acute phase, most patients are asymptomatic, but myositis, meningitis, or encephalitis can occur. In the chronic phase, which can be lifelong, 20% to 30% of individuals will develop disease manifestations including cardiac arrhythmia which can lead to sudden cardiac death and dilated cardiomyopathy which can result in thromboembolic disease, heart failure, stroke, and death. Gastrointestinal manifestations are less common, but are associated with significant morbidity. • The diagnosis of acute Chagas can be made by direct observation of the parasite in blood while chronic Chagas is diagnosed using antibody-based tests. Multiple tests are needed to confirm the diagnosis. After treatment, it can take years for the antibody tests to become negative.	Chagas disease is a serious infection with life-threatening manifestations including sudden cardiac death and heart failure. The diagnosis of chronic Chagas disease relies on antibody-based tests which can take years to become negative after treatment.
Current Treatment Options	• Benznidazole and nifurtimox are recommended for the treatment of Chagas disease by the WHO and CDC. • Benznidazole was approved in 2017 for the treatment of Chagas disease in patients aged 2 to 12 years old. The drug was approved under the accelerated approval pathway and clinical benefit has not yet been confirmed. • Nifurtimox is only available in the United States through an IND held by CDC.	Benznidazole is the only approved treatment for Chagas disease in the United States. However, there is no approved option for children less than 2 years of age or greater than 12 years of age. Having additional treatment options would be beneficial to patients.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	• The efficacy of nifurtimox in the treatment of Chagas disease was primarily obtained from Study 16027 which enrolled pediatric subjects from birth to <18 years of age with positive serology. • Subjects were randomized 2:1 to receive either 60 days or 30 days of nifurtimox and were followed for 12 months. An additional 3 years of follow-up is ongoing. • The rate of reaching the endpoint of seroconversion or a ≥20% decrease in optical density using the lysate ELISA was significantly higher in the 60-day arm (70/219, 32%) compared to the 30-day arm (21/111, 19%). • The rate of reaching the endpoint of seroconversion or a ≥20% decrease in optical density using the recombinant ELISA was also significantly higher in the 60-day arm (76/219, 35%) compared to the 30-day arm (24/111, 22%). • Among nifurtimox-treated subjects aged 6 to 12 years with positive F29 ELISA at baseline, 34% seroconverted to negative (20/59 in the 60-day arm; 10/29 in the 30-day arm). In comparison, the historical placebo seroconversion rate on F29 ELISA from a pediatric population of the same age was 2.8% (1/36) which was significantly lower than nifurtimox-treated subjects. • Published literature studies provided supportive evidence for the efficacy of nifurtimox in the treatment of Chagas disease in the pediatric patient population.	Study 16027 showed the effectiveness of nifurtimox for the treatment of Chagas disease through the significantly higher rates of seroconversion or ≥20% decrease in optical density at 12 months in the 60-day arm compared to the 30-day arm using two different ELISAs. As it not known how much a reduction in the optical density values in an ELISA corresponds to antibody levels in the blood, additional evidence of effectiveness was obtained that showed a significantly higher rate of seroconversion of the F29 ELISA in nifurtimox-treated subjects compared to a historical placebo control. Longer follow-up of subjects in Study 16027 is ongoing and should provide confirmation of the clinical benefit of nifurtimox for the treatment of Chagas disease. Additional supportive evidence was obtained from several studies from the published literature.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and Risk Management	• The safety information used to determine the risk for nifurtimox comes from a study of 330 children with Chagas disease (Study 16027) as well as from animal studies, the literature, and postmarketing reports. • Gastrointestinal adverse reactions were commonly reported and include nausea, vomiting, abdominal pain, diarrhea, poor appetite, and weight loss. • Hypersensitivity reactions include rash, urticaria, DRESS, eosinophilia, and anaphylaxis. Rash was noted in 5.5% of subjects in the 60-day arm. DRESS and anaphylaxis were not observed in Study 16027, but were reported in the literature and postmarketing data. • Headache was reported in 12.8% of 60-day arm subjects. Other neurological adverse reactions were uncommon and include seizure, paresthesia, tremor, and amnesia. • Psychiatric adverse reactions were uncommon and include insomnia, irritability, and anxiety. • Hematological adverse reactions were uncommon and include anemia, leukopenia, neutropenia, and thrombocytopenia. • Animal studies indicate a risk for embryo-fetal toxicity.	The available safety information is sufficient to describe the risk profile of nifurtimox in the treatment of Chagas disease. The identified safety issues do not preclude approval and can be managed through labeling.

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

☐	**The patient experience data that were submitted as part of the application include:**			Section of review where discussed, if applicable
	☐	Clinical outcome assessment (COA) data, such as		
		☐	Patient reported outcome (PRO)	
		☐	Observer reported outcome (ObsRO)	
		☐	Clinician reported outcome (ClinRO)	
		☐	Performance outcome (PerfO)	
	☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Natural history studies		
	☐	Patient preference studies (e.g., submitted studies or scientific publications)		
	☐	Other: (Please specify):		
X	**Patient experience data that were not submitted in the application, but were considered in this review:**			
	☐	Input informed from participation in meetings with patient stakeholders		
	X	Patient-focused drug development or other stakeholder meeting summary reports (Apr 28, 2015 on Chagas Disease)		Section 2.1
	☐	Observational survey studies designed to capture patient experience data		
	☐	Other: (Please specify):		
☐	**Patient experience data was not submitted as part of this application.**			

2. Therapeutic Context

2.1. Analysis of Condition

Chagas disease or American trypanosomiasis is endemic to parts of Mexico, Central America, and South America where it is estimated that 8 million people are affected. However, because of migration its prevalence is growing in the United States and Europe. CDC estimates at least 300,000 individuals in the United States are infected with *Trypanosoma cruzi*, a flagellated protozoan, which is the causative parasite. Transmission usually occurs through a triatomine insect vector, commonly referred to as the "kissing bug." The vector is present in rural areas and in poor housing conditions, such as homes with thatched roofs. An infected insect takes a blood meal and deposits the trypomastigote form of the parasite in its feces near the bite wound. The parasite enters the human host, invades cells, and differentiates into the intracellular amastigote form. The amastigotes multiply by binary fission and differentiate into the trypomastigote form which are released into the bloodstream. These trypomastigotes can infect other cells or get ingested by the insect vector which continues the lifecycle. The parasite can also be transmitted through blood transfusions, organ transplantation, and transplacentally.

Chagas disease has an acute and chronic phase. In the acute phase, which lasts weeks to months, the parasite can be found in the peripheral circulation. Most patients are asymptomatic, but there may be fever or an inflammatory nodule at the bite site, referred to as a chagoma. Rarely, patients may have myositis, meningitis, or encephalitis in the acute phase. In the chronic phase, which can be lifelong without treatment, the parasite is usually not present in the bloodstream. Many patients remain asymptomatic for life. However, between 20% and 30% of chronically-infected patients develop disease manifestations after many years. The most commonly observed manifestation is cardiac arrhythmia, including AV block, ventricular tachyarrhythmia, and ventricular fibrillation, which can lead to sudden cardiac death. Another cardiac manifestation is dilated cardiomyopathy which can result in thromboembolic disease, heart failure, stroke, and death. Megacolon and achalasia are less commonly observed, but can also cause significant morbidity. These symptoms can have significant physical and emotional impact on patients' quality of life.

The diagnosis of acute Chagas disease can be made by directly observing the parasites in the blood using a variety of methods (microhematocrit, Strout method, quantitative buffy coat, thick blood smear, Giemsa stain). The diagnosis of chronic Chagas disease relies on the detection of antibodies produced in response to the parasitic infection. The more commonly used methods to detect antibodies measure IgG antibodies against lysates of whole parasites. These methods are referred to as "conventional" serological assays and include complement fixation, indirect hemagglutination assay (IHA), immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA or EIA). Two or more tests that use different techniques to detect different parasitic antigens are usually used to confirm the diagnosis. Patients who are treated continue to have positive conventional serological assays for several years, which is

thought to result from persistent parasitic antigen in macrophages. Nonconventional serological assays which use recombinant parasitic antigens are being developed as an alternative diagnostic method. These assays may reduce false positive test results that arise from cross-reactivity of antibodies to *Leishmania* species.

2.2. Analysis of Current Treatment Options

In endemic countries, there are two commonly used drugs for the treatment of Chagas disease, benznidazole and nifurtimox. Both drugs have been used for several decades and are included in the WHO Model List of Essential Medicines. Several published guidelines, including those from the WHO and CDC recommend the use of these drugs for the treatment of Chagas disease. In the United States, benznidazole was approved in 2017 for the treatment of Chagas disease in pediatric patients 2 to 12 years of age. This approval was under accelerated approval and clinical benefit has not yet been confirmed. Nifurtimox is not approved in the United States, but can be obtained through an IND held by CDC. Of note, per their annual report, CDC released nifurtimox to 12 subjects between January 1, 2018 and September 15, 2019. As a reference, the CDC-recommended treatment regimens for both drugs are listed in Table 1.

Table 1. CDC-Recommended Treatment Options for Chagas Disease

Drug	Age Group	Dose and Duration
Benznidazole	2 to 12 years[a]	5–8 mg/kg per day orally in 2 divided doses for 60 days
Nifurtimox	≤10 years	15–20 mg/kg per day orally in 3 or 4 divided doses for 90 days
	11 to 16 years	12.5–15 mg/kg per day orally in 3 or 4 divided doses for 90 days
	≥17 years	8–10 mg/kg per day orally in 3 or 4 divided doses for 90 days

Source: CDC (Centers for Disease Control and Prevention 2019)
[a] Benznidazole is FDA-approved for the treatment of Chagas disease (American trypanosomiasis) caused by *Trypanosoma cruzi* in pediatric patients 2–12 years of age. Use of benznidazole to treat a patient outside of the FDA-approved age range of 2–12 years is based on clinical diagnosis and decision by a treating physician under practice of medicine.

M.O. Comment: *Although nifurtimox can be obtained in the United States through CDC under IND, having this drug available in pharmacies would be expected to improve access for treating physicians and patients.*

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Lampit (nifurtimox) tablets has not been approved or marketed in the United States and is considered a New Molecular Entity (NME). The development program was conducted under IND 109901.

3.2. Summary of Presubmission/Submission Regulatory Activity

On May 24, 2010, Bayer Healthcare Pharmaceuticals, Inc. (Bayer) submitted a request for Orphan Drug Designation for nifurtimox (trade name: Lampit) for: "treatment of Chagas Disease (*American Trypanosomiasis*) caused by *T. cruzi*." On August 5, 2010, the FDA Office of Orphan Products Development granted Orphan Product Designation to nifurtimox for treatment of Chagas Disease (*American Trypanosomiasis*) caused by *T cruzi*.

Pre-Investigation New Drug (PIND) History

Pre-Investigation New Drug (PIND) file (PIND 109901) was established on October 10, 2010, for nifurtimox for the treatment of Chagas disease.

A type B Pre-Investigation New Drug (PIND) meeting was scheduled for December 17, 2010, but was cancelled by Bayer. The purpose of the meeting was to discuss the nonclinical and clinical data, as well as data from other clinical trials and literature in support of both the efficacy and safety of nifurtimox in the treatment of Chagas disease for nifurtimox tablets.

On May 1, 2012, a meeting was held to discuss FDA's December 13, 2010 Preliminary Meeting Comments and to discuss the proposed clinical development plan outlined in a January 4, 2012, submission. FDA emphasized the need for a single dose administration of radio-labeled nifurtimox in healthy volunteers to characterize the elimination pathways for the parent compound and metabolites.

The May 2012 meeting was followed by a January 28, 2013, meeting that was held to discuss and gain guidance on the CMC development issues associated with nifurtimox to support the future filing of nifurtimox tablets.

On September 9, 2013, Type B PIND meeting was held to discuss a Phase 3 clinical development program.

Investigation New Drug (IND) History

Bayer submitted the Investigation New Drug (IND) 109901, to FDA on November 21, 2014, for nifurtimox tablets for the treatment of Chagas' disease (American Trypanosomiasis) caused by Trypanosoma cruzi in pediatric populations.

The IND was determined safe to proceed on December 18, 2014. FDA issued a Study May Proceed letter with comments from the CMC, Biopharmaceutics, Biostatistics, Pharmacology/Toxicology, Clinical Pharmacology, and Clinical Teams on December 30, 2014.

Bayer submitted a request for the proposed proprietary name, Lampit, on April 5, 2016. On June 22, 2016, FDA issued to Bayer a Conditionally Acceptable letter for the proprietary name Lampit.

A type C IND meeting was held on August 14, 2017 to discuss the clinical development program for Lampit (nifurtimox) tablet. FDA noted that, if successful, the ongoing clinical trial, supported

by additional available preclinical and clinical data, could adequately support the proposed indication of treatment of Chagas disease (American trypanosomiasis) in pediatric patients ages 0 to 18 years. It was noted that, if the trial was successful, this would likely result in a subpart H approval with a requirement for a study to confirm clinical benefit. (b) (4) FDA recommended Bayer outline their plan for organizing the NDA in their pre-NDA meeting package.

On August 13, 2019, a Pre-New Drug Application (NDA) meeting was held to discuss the planned NDA submission for Lampit (nifurtimox) tablet. The meeting was canceled as the Preliminary Meeting Comments addressed Bayer's concerns and stand a the record of this meeting. No agreements for late submissions were made.

On October 22, 2019, a Pre-NDA CMC Only meeting was held to discuss and reach agreement on the CMC specific content and organization of Bayer's proposed NDA for Lampit (nifurtimox) for the treatment of Chagas disease. The meeting was canceled as the Preliminary Meeting Comments addressed all of Bayer's concerns and stand a the record of this meeting. No agreements for late submissions were made.

Bayer submitted NDA 213464 for Lampit (nifurtimox) tablets, 30 mg and 120 mg on December 6, 2019, for the treatment of Chagas disease. This submission included a request for priority review and for consideration for a Tropical Disease Priority Review Voucher (TDPRV) upon approval. Bayer is also seeking both New Chemical Entity and Orphan Product Exclusivity for their NDA upon approval.

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

4.1. Office of Scientific Investigations (OSI)

Office of Regulatory Affairs conducted remote inspections of two clinical sites from Study 16027, site 48001 in Colombia and site 43005 in Argentina. These sites were chosen because they each enrolled a large number of subjects. The inspections were conducted remotely at the Applicant's office in New Jersey because of FDA travel restrictions related to the COVID-19 pandemic (Argentina) and preexisting travel restrictions to Colombia. There was no evidence of under-reporting of adverse events or GCP compliance issues noted at either site. An additional investigation was conducted of the source records for the qualitative and quantitative serology testing results from the (b) (4). These records were supplied to the field investigator at the Applicant's office in New Jersey. No issues or discrepancies were noted between the source records and data listings for the recombinant and lysate (total purified antigen) ELISAs. Certified copies of the source records for the qPCR, IHA, and F29 nonconventional serology test results were unable to be provided by

the Applicant. However, these data were reviewed and verified against screen shots of the data that had been transcribed into an electronic database from paper source records and no discrepancies were noted. The OSI memo notes that, "the study appears to have been conducted adequately, and the primary efficacy endpoint data generated by these sites appear acceptable in support of the respective indication."

4.2. Product Quality

Summary

This NDA provides for two tablet amounts of nifurtimox (30 and 120-mg tablets). The NDA, as amended, has provided adequate CMC information to assure the identity, strength, purity, and quality of the proposed drug product. Therefore, this NDA is currently recommended for Approval by the Office of Pharmaceutical Quality (OPQ).

The overall Manufacturing Inspection recommendation was entered as Approve on August 5, 2020.

Comments on Novel Excipients

The 30 and 120 mg nifurtimox tablets do not contain any novel excipients. The excipients included in the nifurtimox drug product, dibasic calcium phosphate dihydrate, corn starch, magnesium stearate, colloidal silicon dioxide, and sodium lauryl sulfate are all used in amounts that are lower than the amounts used in previously approved oral products (Table 2).

Table 2. Excipient Amounts in Nifurtimox Tablets and the Maximum Daily Dose of Nifurtimox and Qualified Limits

Excipient	30 mg Nifurtimox Tablet	120 mg Nifurtimox Tablet	900 mg Nifurtimox[a]	Maximum Amount/Day in a Previously Approved Oral Product[b]
Dibasic calcium phosphate dihydrate			(b) (4)	1036 mg (Horizant; NDA 22399)
Corn starch				1928 mg (Ibuprofen; ANDA 72169)
Magnesium stearate				256 mg (Dalmane; NDA 16721)
Colloidal silicon dioxide				300 mg (Benzonatate; ANDA 40851)
Sodium lauryl sulfate				123 mg (Zytiga, NDA 202379)

Source: Compiled based upon the information available in FDA Inactive Ingredient Search for Approved Drug Products
[a] 900 mg is the maximum approved daily clinical dose of nifurtimox.
[b] Based on values listed in the "FDA Inactive Ingredient Search for Approved Drug Products."

Comments on Impurities/Degradants of Concern

Drug Substance Impurities

The specified impurities in the nifurtimox drug substance are [redacted] (b) (4) These impurities were initially specified at [redacted] (b) (4)% in the drug substance, but the specifications were later adjusted by the Applicant (in response to an information request from the Division) to [redacted] (b) (4)% to meet the qualification threshold of 0.15% or 1 mg (whichever is less) for impurities in drugs administered in amounts of 2 g/day or less as specified in the ICH Q3A Guidance. With specifications of [redacted] (b) (4)%, the maximum amount of each specified impurity in the maximum daily dose of nifurtimox (900 mg/day) is [redacted] (b) (4) mg. Analysis of 3 clinical batches found levels of all three specified impurities to be [redacted] (b) (4)%. Other potential impurities were identified, but none of these impurities was detected in batches of nifurtimox drug substance at measurable levels. The drug substance specification for any unspecified impurity is [redacted] (b) (4)% and the sum of all organic impurities is ≤1.0% in compliance with the thresholds in the ICH Q3A Guidance.

Drug Product Degradants

No drug product degradants were identified or specified. The limit of [redacted] (b) (4)% for unspecified degradation products is based on the ICH Q3B(R2) Guidance for Industry and applies for the release and shelf life of the nifurtimox drug product.

Potential Mutagenic Impurities

The organic impurities in nifurtimox are potentially genotoxic. However, because the weight of experimental evidence indicates that nifurtimox is mutagenic and clastogenic, the nifurtimox impurities can be controlled at acceptable levels for nonmutagenic impurities. This control strategy is in agreement with the ICH M7 Guidance for Industry which includes the following statement: “Additionally, there may be some cases where a drug substance intended for other indications is itself genotoxic at therapeutic concentrations and may be expected to be associated with an increased cancer risk. Exposure to a mutagenic impurity in these cases would not significantly add to the cancer risk of the drug substance. Therefore, impurities could be controlled at acceptable levels for nonmutagenic impurities.” One known carcinogenic impurity, [redacted] (b) (4), is included and controlled [redacted] (b) (4) for nifurtimox at a maximum concentration of [redacted] (b) (4) mg/kg. [redacted] (b) (4) analyses of batches of nifurtimox drug substance consistently measured [redacted] (b) (4) in maximal concentrations of [redacted] (b) (4) mg/kg which corresponds to a maximal daily exposure of [redacted] (b) (4)mcg [redacted] (b) (4) in a 900 mg maximum daily dose of nifurtimox. The lifetime acceptable intake of [redacted] (b) (4) designated in the ICH M7 Guidance is [redacted] (b) (4)mcg/day which substantially exceeds the [redacted] (b) (4)mcg/day intake of [redacted] (b) (4) in the nifurtimox drug substance.

Residual Solvents and Elemental Impurities

No residual solvents were used in the synthesis of nifurtimox drug substance in amounts exceeding the thresholds specified in the ICH Q3C Guideline. Similarly, (b) (4) elemental impurities including heavy metals were monitored and shown to occur in amounts below the oral concentration limits specified in the ICH Q3D Guidance of Industry.

4.3. Devices and Companion Diagnostic Issues

CDRH Review Comments

In general, qualitative positive results from at least two *T. cruzi* serological assays have been considered evidence of exposure to *T. cruzi*, and the seroconversion from antibody positive to antibody negative status has been used as a surrogate marker to predict parasite reduction and potential clearance (i.e., cure). Potential challenges with the interpretation of such serological assays have been discussed in the literature and are common to serological assays that are used for other infectious diseases, such as the persistence of some antibodies after infection resolution, slow changes in antibody response, and potential cross reactivity with antibodies against other pathogens.

Polymerase chain reaction (PCR) testing for *T. cruzi* DNA is used less frequently than serological testing, as the sensitivity varies depending on the specific methods, sampled tissue, and stage of disease. Positive PCR results are meaningful evidence of infection; however, negative PCR results are not conclusive of parasite clearance. Due to the biology of the disease, higher numbers of parasites circulate in peripheral blood during acute disease, while in later disease parasite stages are primarily sequestered in target tissues (e.g., cardiac, gastrointestinal), so that blood concentrations may be below the limit of detection of PCR.

The evidence presented in the NDA included results from several *T. cruzi* serological assays: recombinant ELISA, lysate ELISA, F29 ELISA, and an IHA. Consistent with current clinical practice, these serological assays were validated for qualitative use (positive or negative results) only. The NDA also included results from PCR testing to detect *T. cruzi* DNA in peripheral blood. (See section 9.2).

The numerical output value of qualitative ELISAs are interpreted as a positive or negative result per the cut off value that is established for each assay. For many colorimetric ELISAs that are read in a spectrophotometer, the output value is expressed as an optical density (OD) value. ELISA results can also be reported as index signal-to-cutoff (S/CO) values in which the OD value for each sample is divided by an assay cut-off value. Importantly, although ELISA OD and S/CO outputs are numeric values, it is not appropriate to assume that these numeric outputs convey absolute quantitative results. A quantitative assay would need additional supporting validation for aspects such as the Limit of Quantitation, Limit of Blank, Limit of Detection and linearity/variability across the assay dynamic range before it could be determined to be

accurate for a stated quantitative use. The Applicant has not provided validation to support the quantitative use of the assays used in the trial, nor is the use of these assays, based on OD, for quantitation an accepted practice. The information provided in the FDA cleared package insert for the recombinant ELISA indicates that the underlying OD values do not change linearly with serial dilutions of known positive samples. This is not consistent with a quantitative use of the assays. Since the clinical relevance of a change in ELISA OD value without conversion from seropositive to seronegative is not known and since the assays were only validated for qualitative use, the recommendation is to use the qualitative assay results in primary analyses.

Qualitative serological assays can also be used to provide antibody titer results, which are accepted measures of relative antibody levels. Titer results are produced when qualitative assays are performed on serially diluted samples, and so provide a relative assessment of antibody levels by the same test over time. Each dilution is tested individually, and the most dilute sample that is positive by the assay is reported as the titer (e.g., 1:16 or 1:256). Since the measures are not absolute quantitations, it is understood that titers cannot be compared between different assay platforms, nor are they necessarily comparable for results of the same assay performed at different labs. The recommendation is to assess titer changes over time per subject as measures of relative antibody levels.

In the NDA submission, trial results were only available for Month 12 follow-up after treatment, which is a relatively early time point for assessing *T. cruzi* serological assay endpoints. In previous studies that evaluated potential Chagas disease treatments (including studies used for the historical control cohorts of the current trial), seroconversion endpoints were assessed at 36 – 48 month follow-ups. Seroconversion of F29 ELISA results from positive to negative has been used in a previous NDA as a surrogate endpoint. The current trial is ongoing, and so it is expected that the serological test results will continue to be monitored for the study subjects over time to parallel historical control cohorts and provide additional data for analysis.

CDRH assessments of qualitative assay results at Month 12 follow-up are provided below. Note: calculations were based on the number of patients tested at the Month 12 follow-up visit without adjustments for intention to treat or imputing missing values.

Qualitative Test Seroconversions to Negative by Month 12 Follow-up

Tmt. Arm	Recomb ELISA	IHA	Lysate ELISA	F29 ELISA
30-Day	5.6% (6/107)	4.6% (5/108)	4.7% (5/107)	28.2% (20/71)
60-Day	5.2% (11/210)	4.3% (9/207)	5.2% (11/210)	32.9% (46/140)

IHA Titer Change by Month 12 Follow-up

Tmt. Arm	4-fold or more Decrease	No Change, or less than 4-fold Change	4-fold or more Increase	Cannot Determine Titer Change
30-Day	23.1% (25/108)	47.2% (51/108)	0	29.6% (32/108)
60-Day	28.2% (59/209)	46.4% (97/209)	0	25.4% (53/209)

PCR Positive Results at Visit 1 and Visit 11 (Month 12 Follow-up)

Tmt. Arm	PCR Detected V1	PCR Detected V11
30-Day	51.8% (56/108)	4.6% (5/108)
60-Day	53.2% (109/205)	1.5% (3/205)

5. Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

Nifurtimox (Lampit) is a nitrofuran antiprotozoal drug that is administered in oral tablets and is intended for the treatment of pediatric patients with Chagas disease. Nonclinical studies that were submitted in support of the NDA for nifurtimox include: in vitro and in vivo cardiovascular safety studies; numerous in vitro and in vivo nonclinical pharmacokinetic studies evaluating the absorption, distribution, metabolism, and excretion of nifurtimox; repeated-dose toxicology studies in rats (28-day and 13-week studies) and dogs (28-day and 52-week studies); genetic toxicology studies (Ames, in vitro micronucleus, and in vivo micronucleus, chromosomal aberration and sister chromatid-exchange assays); fertility and pre-postnatal studies in rats; and embryo-fetal studies in mice, rats, and rabbits. Many of the submitted studies were conducted decades ago, without GLP compliance, and are reported in literature publications.

An important nonclinical toxicity of nifurtimox with clinical relevance is dose-dependent CNS toxicity including seizures that correlated with histopathology in several brain structures in rats. Neurological clinical signs included asymmetric posture, staggering gait, dyspnea, and seizures in the 13-week toxicology study in rats and stiff limbs, ataxia, nystagmus, hind-limb weakness, and seizures in the 1-year toxicology study in dogs. In rats, brain histopathology in the form of spongiosis, isolated nerve degeneration, and fresh perivascular bleeding was observed to varying degrees in the midbrain, cerebellum, pons, and medulla oblongata regions of the brain. Similar clinical signs were also observed in 28-day toxicology studies in rats and dogs.

The other major nifurtimox-related toxicity in rats was testicular atrophy occurring at doses ≥60 mg/kg/day which appeared to be only partially reversible. The primary histopathology was degeneration or an absence of germ cells in seminiferous tubules. Consistent with the testicular toxicity, epididymal lumens contained very few spermatozoa, germ cells in varying degrees of degeneration, giant cells, and cellular debris. Similar testicular and epididymal effects occurred in mice. Similar findings were not observed in dogs, but significant dose-dependent reductions in prostate weight were observed in the 1-year toxicology study in dogs and deciduous spermatoblasts in seminiferous tubules and no long sperm in epididymides were reported in male dogs treated with nifurtimox in the 28-day toxicology study.

Consistent with the testicular atrophy observed in repeated-dose toxicology studies in rats, nifurtimox in a fertility study reported in the literature produced infertility in male rats with an approximate oral dose of 60 mg/kg/day. Following a recovery period of 11 weeks, 75% of male rats treated for 32 weeks with 60 mg/kg/day nifurtimox still had impaired fertility indicating incomplete reversibility. Although the data are limited, nifurtimox does not appear to impair female fertility. In addition to its description in section 13.1 of the Lampit product label, nifurtimox-related inhibition of fertility in male rats will be described in Section 8.3.

Cardiovascular safety pharmacology studies suggest nifurtimox has the potential to alter cardiac function including slight QTc prolongation at high doses. Nifurtimox at high concentrations was shown to inhibit hERG channels with an estimated IC_{50} value of 98 mcM (28.2 mcg/ml). In a cardiovascular study in conscious telemetered dogs, QTc intervals were slightly increased at a nifurtimox dose of 80 mg/kg. In the same study, nifurtimox produced 10-20% dose-dependent reductions in systolic blood pressure, and compensatory changes in heart rate. The nifurtimox plasma C_{max} associated with the 80 mg/kg dose in the dog cardiovascular study (5.4 mcg/ml) and the 28.2 mcg/ml IC_{50} value for nifurtimox inhibition of hERG channels have respective exposure margins of approximately 7- and 38-times the clinical C_{max} value of 751 ng/ml measured in healthy human subjects administered a single dose of 15 mg/kg nifurtimox (Paulos et al. 1989) Also, changes in QT/QTc interval were assessed in the Phase 3 Study 16027 in which over 300 pediatric patients with Chagas disease received doses of 8-20 mg/kg/day for 30 or 60 days. In this study, nifurtimox administration did not result in large mean increases (>20 ms) in the QTc interval. These results suggest relative safety for clinical nifurtimox administration with respect to QTc prolongation.

Other toxicities included swelling of liver cells with clumping of cytoplasm, fat-free vacuoles, and distension of cell nuclei to varying degrees in the livers of rats from all dose groups.

However, liver enzymes were generally unaltered, suggesting the toxicological significance of this finding is unclear. The NOAEL values for nifurtimox in rats and dogs in the 13-week and 1-year toxicology studies were considered to be 25 and 30 mg/kg/day, respectively. Because toxicokinetic evaluations were not performed in the 13-week study in rats or the 1-year study in dogs, comparisons to human exposures based on AUC measurements cannot be determined. The human equivalent dose (HED) values for the rat and dog NOAEL values based on body surface area comparison provide only low safety margins of 0.4 for rats and 1.7 for dogs with respect to a 10 mg/kg/day clinical dose and 0.2 for rats and 0.8 for dogs with respect to a 20 mg/kg/day clinical dose. Although, the animal toxicity results do not strongly support the clinical safety of nifurtimox in doses of 10-20 mg/kg/day, decades of past clinical use of nifurtimox for the treatment of Chagas disease in Central and South America provides important information for assessing the clinical importance of the nifurtimox-related toxicities observed in repeated-dose toxicology studies in rats and dogs, including neurological effects. Other toxicities including inhibition of male fertility as well as the potential for genotoxicity and carcinogenicity and embryo-fetal effects in pregnant females are largely informed by nonclinical study results.

Genetic toxicity assays predominantly indicate that nifurtimox is genotoxic with positive results in both in vitro and in vivo studies as well as one negative result for the frequency of sister chromatid exchange in blood lymphocytes from Chagas patients. In Ames assays, nifurtimox produced dose-dependent increases in the number of mutants with and without S9 activation, and, in an in vitro micronucleus assay, nifurtimox was clastogenic in human lymphocytes. In vivo, nifurtimox was shown to be positive for clastogenesis in a mouse micronucleus assay, a mouse sister chromatid exchange assay, and a chromosome aberration assay in Chagas patients. Based on the weight of evidence from these studies, nifurtimox is considered to be genotoxic and the product label for Lampit will include a Warning for genotoxicity. Consistent with recommendations in the ICH M7 Guidance, the impurities in the nifurtimox drug substance are not expected to add to the genotoxicity potential of nifurtimox and can be controlled at acceptable levels for nonmutagenic impurities.

Because the proposed treatment duration of nifurtimox is less than 6 months, a carcinogenicity assessment is not required. However, three carcinogenicity studies for nifurtimox were submitted in NDA 213464, two in limited brief study reports and one in a published manuscript. Both positive and negative carcinogenicity results were reported in the submitted studies. In one study in mice, nifurtimox administered intraperitoneally for 60 days was associated with an increased prevalence of lymphoblastic lymphomas in lymph nodes, spleen, and liver. In contrast, in a two-year rat carcinogenicity study of atypical design (variable oral or subcutaneous weekly doses contributing to a cumulative dose of 11,700 mg/kg), nifurtimox-treated rats lived as long as control rats receiving vehicle and did not exhibit an increased frequency of malignant tumors although benign tumors were increased. Similarly, in an accelerated cancer bioassay, nifurtimox administered alone or as an initiator followed by promoters either did not produce tumors or did not produce tumors above the frequencies produced by the promoters alone. Because all the carcinogenicity studies and the study reports

submitted to NDA 213464 have significant deficiencies that limit a comprehensive review and diminish the adequacy of the studies, the results of the studies are not recommended for inclusion in the product label for Lampit. However, other findings including the genotoxicity findings for nifurtimox and carcinogenicity findings for other nitrofuran antimicrobials more definitively suggest a carcinogenicity potential for nifurtimox in humans. The product label for an approved nitrofuran antimicrobial, FURADANTIN® (nitrofurantoin) describes both positive and negative carcinogenicity findings in nonclinical studies in Section 13.1 of its product label. Also, nitrofurazone, a nitrofuran antimicrobial previously used as a feed additive was shown to increase the incidence of tumors in rats and mice in 2-year carcinogenicity studies (Kari et al. 1989). These results and similar carcinogenicity findings for other nitrofuran antimicrobials, supported a 1991 decision by the FDA to withdraw several nitrofuran products used in animal feed due to carcinogenicity concerns and complete prohibition of nitrofurans for livestock production by the European Union in 1995. Based on the genotoxicity findings for nifurtimox and the carcinogenicity potential associated with nitrofurans as a chemical class, the Lampit product label will identify nifurtimox as a potential carcinogen, and the label will also include a Warning for carcinogenicity.

Embryo-fetal studies in mice and rats described in a 1972 publication are considered to be preliminary due to deficiencies in study methods and the detail of the published report. In these studies, maternal body weights were significantly reduced in mid- and high-dose rats, but not in mice, and mean fetal weights were significantly reduced in high-dose mice and mid- and high-dose rats. No fetal malformations were reported for any group in either study, but the lack of findings may have been related to the use of low numbers of pregnant females in each group and deficiencies in the methods and criteria used to evaluate malformations in fetuses. The respective NOAEL values for maternal toxicity in mice and rats were considered to be 125 mg/kg/day and 20 mg/kg/day which are, respectively, approximately equal to the maximum recommended human dose (MRHD) of 10 mg/kg/day in females of childbearing potential or 0.3 times the MRHD based on body surface area comparison. The respective NOAEL values for fetal toxicity in mice and rats were considered to be 50 mg/kg/day and 20 mg/kg/day which are, respectively, equivalent to 0.4-times or 0.3-times the MRHD based on body surface area comparison.

In a GLP-compliant embryo-fetal study in rabbits, maternal toxicity included abortions in 8/20 high-dose females as well as significantly reduced mean values for body weight gain and food consumption in the same group. Fetal effects included significantly reduced numbers of live fetuses/litter and a lower percentage of fetuses per total implantations in the mid- and high-dose groups compared to control values. Also, increased fetal and litter incidences of a skeletal malformation, fusion of caudal vertebral bodies, was observed in fetuses in the low-dose group. The NOAEL value for maternal toxicity in the rabbit study was considered to be the mid-dose of 15 mg/kg/day which is approximately equivalent to the 0.5 times the MRHD of 10 mg/kg/day in females of childbearing potential based on body surface area comparison. Because low-dose fetuses exhibited a nifurtimox-related malformation, a NOAEL for fetal toxicity was not determined in this study, but the LOAEL value was the low dose of 5 mg/kg/day which is

approximately equal to 0.2 times the MRHD. Based on the maternal toxicity findings in rats and rabbits, fetal toxicity in mice, rats, and rabbits, and increased fetal malformations in rabbits, the Lampit product label will include a Warning for embryo-fetal toxicity. Because of the deficiencies in the embryo-fetal studies in rats and mice, a new embryo-fetal study of acceptable design in rats will be conducted as a postmarketing requirement.

In a pre-postnatal study in rats, maternal findings included reduced maternal body weights in mid- and high-dose dams during dosing, but no changes in uterine content parameters. In F1 offspring, body weights were significantly reduced in high-dose males and females during the lactation and postlactation periods, but physical development, neurological function, and reproduction of F1 offspring were not substantially changed. However, male F1 offspring in the low-, mid- and high-dose groups exhibited slightly small testes with incidences of 5%, 20% and 15%, respectively. The NOAEL value for maternal toxicity and fetal development of female F1 offspring was considered to be the mid-dose of 30 mg/kg/day which is approximately equivalent to 0.5 times the MRHD. The NOAEL value for fetal development of male F1 offspring was considered to be the low dose of 15 mg/kg/day which is approximately equivalent to 0.2 times the MRHD based on body surface area comparison.

Nifurtimox was shown to be 80-90% orally bioavailable in rats with maximum plasma concentrations generally occurring in ≤3 hours in rats and dogs. In the 28-day toxicology studies in rats and dogs, nifurtimox plasma exposures increased in an approximately dose-proportional manner and did not greatly accumulate with repeated dosing. The plasma $t_{1/2}$ values for nifurtimox in rats was approximately 5 hours. Nifurtimox bound at a low level to plasma proteins with the percent nifurtimox bound ranging from 28% to 43% across species (rat, dog, mouse, monkey, and human). In a mass-balance study, [^{35}S]nifurtimox was shown to extensively distribute to tissues, with the highest concentrations of radioactivity in the kidneys and liver followed by lung, adrenal and thyroid glands, testes, brain, and spinal cord. The metabolism of nifurtimox and the nifurtimox metabolic profile were extensively characterized in rats and humans. In both species approximately 20 metabolites were identified in plasma and/or urine. Metabolism of nifurtimox is primarily mediated by tissue nitroreductases with little metabolism occurring in liver microsomes via CYP450 enzymatic activity. Consistent with this finding, nifurtimox did not inhibit a panel of CYP450 isozymes and did not induce the activity of CYP1A2 or CYP3A4 in cultured human hepatocytes. One metabolite, M-6 occurred in high abundance in both rat plasma (29-35% of total AUC exposure) and human plasma (69.4% of total AUC exposure) following a single oral dose of nifurtimox. M-6 is not qualified for safety in nonclinical studies, but like nifurtimox itself, the clinical safety of the M-6 metabolite is to some degree qualified by the decades of past clinical use of nifurtimox. Also, nonclinical evaluations of the M-6 metabolite for genetic toxicology, carcinogenicity, and reproductive and developmental toxicology are not expected to add to the concerns already identified for nifurtimox for these categories of toxicity. In rats and dogs, 40% to 60% of a single IV or oral dose of nifurtimox was shown to be excreted via urine with 30% to 40% eliminated via feces within 2-3 days of administration. The extensive excretion of nifurtimox in urine in rats and dogs suggests that nifurtimox may undergo altered pharmacokinetics in patients with renal

impairment. Currently, the effect of renal impairment on the pharmacokinetics of nifurtimox in humans has not been elucidated.

In summary, in nonclinical studies nifurtimox was associated with serious toxicities including seizures in rats and dogs and testicular atrophy and impaired fertility in male rats at near therapeutic exposures. Both findings will be addressed on the Lampit product label with nifurtimox-related neurological and psychiatric conditions the subject of a Warning and impaired male fertility in rats reported in Sections 8.3 and 13.1. A limited potential for QTc prolongation noted in safety pharmacology studies is not expected to be a clinical concern. Decades of nifurtimox clinical use in countries outside the United States is informative in assessing the clinical safety of nifurtimox, but some toxicities including effects on male fertility, maternal and fetal toxicity in pregnant women, and the potential for genotoxicity and carcinogenicity are largely informed by nonclinical study results. Nifurtimox is considered to be genotoxic, and based on nitrofuran class effects, potentially carcinogenic as well. These potential issues will be addressed as Warnings in the Lampit product label. In preliminary embryo-fetal studies in rats and mice and a comprehensive study in rabbits, nifurtimox produced dose-related maternal and/or fetal toxicity in all test species as well as a fetal malformation in rabbits. Based on these findings, nifurtimox-related embryo-fetal toxicity will be addressed in a Warning in the Lampit label, and a new embryo-fetal study in rats will be conducted as a postmarketing requirement to more fully elucidate the effects of nifurtimox in pregnant animals. Referenced NDAs, BLAs, DMFs

None.

5.2. Pharmacology

5.2.1. Safety Pharmacology

Study Title: Assessment of Potential Effects of Nifurtimox on hERG K+ Current Expressed in HEK293 Cells (Study No.: T100770-5; Study Report No.: PH-37446)

Methods

This GLP-compliant study was conduct in Germany by Bayer Pharma AG in 2013. Briefly, HEK292 cells stably transfected with the hERG channel gene were successfully incubated with 0, 1, 10, and 50 mcmol/L nifurtimox for periods of approximately 6 minutes with washout periods between each exposure. The vehicle used was extracellular salt solution and the positive control was E-4031. Potassium currents controlled by hERG channels were measured using a single electrode, whole-cell, voltage-clamp method. Measurements were conducted on 5 cells for nifurtimox and 3 cells for E-4031.

Results

Actual concentrations of nifurtimox were shown to be within a ±20% range of nominal concentrations. Nifurtimox produced a concentration-dependent inhibition of hERG-mediated

tail current amplitude. For the highest nifurtimox concentration tested, 50 mcmol/L (14.4 mcg/ml), the inhibitory effect on K+ channels was less than 40%. However, statistically significant reductions in K+ conduction occurred at nifurtimox concentrations ≥1 mcmol/L (287.3 ng/ml) versus predrug control levels. The IC_{20} for nifurtimox was calculated to be 13 mcmol/L (3.7 mcg/ml), and the IC_{50} was extrapolated to be 98 mcmol/L (28.2 mcg/ml). In comparison, the positive control, E-4031, at a concentration of 1 mcmol/L reduced hERG mediated tail current amplitude by approximately 96% with an IC_{50} of approximately 33 nmol/L. The 28.2 mcg/ml IC_{50} value for nifurtimox inhibition of hERG channels is approximately 38-fold higher than the clinical C_{max} value of 751 ng/ml measured in healthy human subjects administered a single dose of 15 mg/kg nifurtimox (Paulos et al. 1989). These results suggest nifurtimox-related hERG inhibition may not be a major concern with clinical administration of nifurtimox.

Study Title: Assessment of Potential Effects of Nifurtimox on the Action Potential in Rabbit Cardiac Purkinje Fibers (Study No.: T100818-8; Study Report No.: PH-37575)

Methods

This non-GLP study was conducted in Germany by Bayer Pharma AG in 2013. Isolated rabbit purkinje fibers were incubated in an organ bath apparatus with vehicle (modified Tyrode recording solution), nifurtimox (Batch #BXA4AX5, purity of 99.7%) in concentrations of 1, 10, and 50 mcmol/L (287.3 ng/ml, 2.9 mcg/ml and 14.4 mcg/ml respectively), and a positive control substance (E-4031, 10 nmol/L). Incubations were of approximately 30 minutes duration with washouts between different exposure conditions. Action potentials in the purkinje fibers were elicited by electrical stimulation and parameters including membrane potential, AP amplitude, maximal upstroke velocity, maximal repolarization velocity, arrhythmia markers, and early after depolarization were measured.

Results

Nifurtimox at concentrations of ≤50 mcmol/L did not alter resting membrane potential (RMP), action potential amplitude (APA), maximal upstroke velocity, maximal repolarization velocity or arrhythmia markers including triangulation and after depolarization. However, action potential duration (APD) at 50% and 90% repolarization was prolonged in a concentration-dependent, but frequency-independent manner (only tested for APD_{90}). Also the action potential plateau was shifted to more positive potentials. The positive control, E-4031 reduced maximal repolarization velocity, prolonged action potential duration at 50% and 90% membrane repolarization, and induced triangulation. The results suggest that nifurtimox may interact with cardiac ion channels other than the hERG channel. The clinical relevance of the study results are not clear.

Study Title: Effects on Cardiovascular Function in Conscious Telemetered Beagle Dogs. (Study No.: T100590-5; Study Report No.: PH-37571)

Methods

This GLP-compliant study was conducted by Bayer Pharma AG in Germany in 2013. Nifurtimox (Batch #BXA4AX5, purity of 99.7%) was administered by single oral gavage to two male and two female Beagle dogs (4/dose) in a Latin square design at doses of 0, 20, 40, and 80 mg/kg followed by a 16-hour observation period. Dogs were conscious and telemetered, and blood pressure, pulse, and ECG intervals were evaluated. In addition, blood samples were collected from a satellite group of four male and two female dogs with samples collected predose and at 1, 3, 7, and 24 hours from 3 animals/dose.

Results

Systolic blood pressure was reduced in a dose-dependent manner by as much as 10%, 13%, and 18% at 20, 40, and 80 mg/kg nifurtimox, respectively, for approximately 11 hours after administration compared to control values. Diastolic blood pressure was not changed at any nifurtimox dose. Heart rate increased up to 35% at the highest nifurtimox dose. Nifurtimox produced dose-dependent reductions in the PQ interval and a dose-independent reduction in QT interval (8-9%), but did not substantially affect the QRS interval. The QTc interval determined using three different correction formulas (Fridericia, van de Water, and Matsunaga) was only slightly increased (2-9%) at any nifurtimox dose. The plasma C_{max} values associated with the nifurtimox doses of 20, 40, and 80 mg/kg were 2670, 2290, and 5440 mcg/ml respectively. The plasma C_{max} value (5440 mcg/L) associated with the 80 mg/kg dose in this study is approximately 7-times higher than the clinical C_{max} value of 751 ng/ml measured in healthy human subjects administered a single dose of 15 mg/kg nifurtimox (Paulos et al. 1989). These results suggest a limited potential for QTc prolongation to occur with clinical administration of nifurtimox.

5.3. ADME/PK

Table 3. Nifurtimox ADME and PK Findings

Type of Study	Major Findings		
Absorption			
Nifurtimox: Quantification of Nifurtimox and Metabolites in Rat Plasma Study no.: KINM 190173-ELB Study report no.: PH-41040	**Plasma Pharmacokinetic Parameters for Nifurtimox Following a Single Oral Dose of 200 mg/kg in Male and Female Rats**		
	PK Parameter	**Male**	**Female**
	$AUC_{(0\text{-last})}$ (mcg•h/L)	52,300	34,700
	C_{max} (mcg/L)	2,910	5,260
	t_{max} (h)	2.00	1.00
	$t_{1/2}$ (h)	5.01	5.00

Type of Study	Major Findings
Toxicokinetics of Nifurtimox Following Oral Administration in the Female Rat Study no.: 8323550 Study report no.: R-12147	**Plasma Pharmacokinetic Parameters for Nifurtimox Following a Single Oral Dose of 25, 50, or 100 mg/kg in Female Rats** **Nifurtimox Doses (mg/kg)** **PK Parameter** / **25** / **50** / **100** $AUC_{(0\text{-last})}$ (mcg•h/L) / 13700 / 22000 / 40300 C_{max} (mcg/L) / 4340 / 6480 / 7550 t_{max} (h) / 0.5 / 1.0 / 1.0 Abbreviations: PK, pharmacokinetic
Effects on Cardiovascular Function in Conscious Telemetered Beagle Dogs Study no.: T100590-5 Study report no.: PH-37571)	**Plasma Toxicokinetic Parameters for Nifurtimox Following Single Oral Administrations of 20, 40, and 80 mg/kg in Dogs** **Nifurtimox Doses (mg/kg)** **PK Parameter** / **20** / **40** / **80** $AUC_{(0\text{-last})}$ (mcg•h/L) / 8,350 / 6,150 / 20,100 C_{max} (mcg/L) / 2,670 / 2,290 / 5,440 t_{max} (h) / 1.44 / 1.44 / 3.98 Abbreviations: PK, pharmacokinetic
Distribution	
Nifurtimox: Investigations on Binding to Plasma Proteins in Different Species and Determination of the Blood/Plasma Partitioning of Nifurtimox In Vitro Study nos.: I 5471-7,I 5475-1 Study report no.: PH-39990	• Nifurtimox plasma-protein binding was low in all species tested with 57.1%, 57.6%, 62.1%, 62.3%, and 71.7% binding in plasma from dogs, humans, rats, monkeys, and mice respectively. • Nifurtimox partitioned 15-20% more in plasma compared to blood in dogs and humans, but portioned about evenly in plasma and blood from rats.
BAY 2502: Pharmacokinetics and Metabolism in Rats and Dogs Using the ^{35}S-Labeled Substance Study report no.: PH-1569)	In rats, radioactivity associated with ^{35}S-BAY 2502 (nifurtimox) distributed primarily to the liver, kidneys, heart, lungs, brain, and testes with faster distribution following IV administration compared to oral administration. Radioactivity greatly concentrated in the lung following IV but not oral administration. After 2.4 days, similar low concentrations of radioactivity were present in most tissues.
Metabolism	
Nifurtimox: Metabolite Identification and Structure Elucidation from In Vitro and In Vivo Metabolism Studies	The following nifurtimox metabolites were identified in different in vitro incubation conditions including incubations of nifurtimox with primary hepatocytes from rats and humans, human urine and feces, nitroreductase, cysteine and acetylcysteine, S9 fraction from rat liver, rat liver cytosol, human kidney S9, human whole blood, and sodium dithionite as a reducing agent. In addition, metabolites were identified in plasma, urine, and feces samples from

Type of Study	Major Findings
Study no.: KINM 130154-ELB Study report no.: PH-40552	rats and plasma and urine samples from humans administered oral nifurtimox in vivo (see table below). The structures for each of the listed metabolites are shown in Table 83 in Section 20.3.

In Vitro and In Vivo[a] Occurrence of Nifurtimox Metabolites

Metabolite	Occurrence
M-1	• In plasma and urine in vivo samples from rats and humans. • Incubations with bacterial nitroreductase and in chemical reductions.
M-2	• In plasma and urine in vivo samples from rats and humans. • Incubations with bacterial nitroreductase and in chemical reductions.
M-3	• In plasma and urine in vivo samples from rats and humans. • Incubations with bacterial nitroreductase and in chemical reductions.
M-4	• In plasma in vivo samples from rats and plasma and urine in vivo samples from humans. • Incubations with cysteine.
M-5	• In plasma and urine in vivo samples from rats and humans. • Incubations with N-acetylcysteine.
M-6	In plasma in vivo samples from rats and plasma and urine in vivo samples from humans.
M-7	Predominantly in rat and human urine in vivo samples.
M-8	Predominantly in rat and human urine in vivo samples.
M-9	Predominantly in rat and human urine in vivo samples.
M-10a/b	Predominantly in rat and human urine in vivo samples.
M-11	Predominantly in rat and human urine in vivo samples.
M-12	Predominantly in rat and human urine in vivo samples.
M-13a/b	Only in rat feces in vivo samples.
M-14	Only in rat feces in vivo samples.
M-15	Only in rat and human urine in vivo samples
M-16	Only in rat and human urine in vivo samples.
M-17a/b	Only in incubations with bacterial nitroreductase.
M-18a/b	• Low amounts in human plasma and urine in vivo samples. • In incubations in vitro with human blood.

[a] Data for metabolites in rat and human in vivo samples was obtained from Study Report Nos.: PH-41040, PH-40556, PH-40569, and PH-40959

Type of Study	Major Findings
Nifurtimox: Quantification of Nifurtimox and Metabolites in Rat Plasma Study no.: KINM 190173-ELB Study report no.: PH-41040	Based on plasma AUC calculations in units of nmol•hr/ml, nifurtimox exhibited the highest AUC exposure (43.5% in females and 43.8% in males of total AUC exposure) followed by M-6 with 29.8% in females and 35.2% in males of total AUC exposure, and M-1 with 13.7% in females and 11.1% in males of total AUC exposure. Metabolites M-2, M-3, M-4 and M-5 exhibited similar low AUC exposures in the range of 1% to 3% of the total AUC exposure. Plasma C_{max} values followed the same rank order as the plasma AUC values in both sexes, and plasma T_{max} and $t_{1/2}$ values varied for nifurtimox and the different metabolites with some differences between sexes (see the two tables below).

PK Parameters of Nifurtimox and Its Major Metabolites in Plasma Pooled From 4 Female Wistar Rats Administered a Single Oral Dose of 200 mg/kg Nifurtimox

Parameter	NFX	M-1	M-2	M-3	M-4	M-5	M-6
AUC	121,000	44,200	3,630	3,700	7,410	2,430	96,100
% of total AUC	**43.5**	**13.7**	**1.1**	**1.1**	**2.3**	**0.8**	**29.8**
C_{max}	18,300	2,050	447	547	568	186	3,920
t_{max}	1.00	8.00	2.00	1.00	3.00	3.00	3.00
$t_{1/2}$	5.00	4.59	8.46	5.78	18.6	25.3	13.5

Source: Study report no. PH-41040
The total AUC for nifurtimox and the major metabolites is: 278470 nmol•hr/ml
Units: AUC, nmol·hr/mL; $C_{max,}$ nmol/L; t_{max} and $t_{1/2}$, hr
Abbreviations: NFX, nifurtimox; PK, pharmacokinetic

PK Parameters of Nifurtimox and Its Major Metabolites in Plasma Pooled From 4 Male Wistar Rats Administered a Single Oral Dose of 200 mg/kg Nifurtimox

Parameter	NFX	M-1	M-2	M-3	M-4	M-5	M-6
AUC	18,4000	46,600	13,400	10,100	9,770	8,020	148,000
% of total AUC	**43.8**	**11.1**	**3.2**	**2.4**	**2.3**	**1.9**	**35.2**
C_{max}	10,100	1390	220	255	338	328	3,650
t_{max}	2.00	24.0	2.00	1.00	2.00	3.00	24.0
$t_{1/2}$	5.01	4.20	63.3	39.6	18.3	8.23	10.6

Source: Study report no. PH-41040
The total AUC for nifurtimox and the major metabolites is: 419890 nmol•hr/ml
Units: AUC, nmol·hr/mL; $C_{max,}$ nmol/L; t_{max} and $t_{1/2}$, hr
Abbreviations: NFX, nifurtimox; PK, pharmacokinetic

Type of Study	Major Findings
Nifurtimox: Quantification of Nifurtimox and Metabolites in Human Plasma Study no.: KINM 190010-ELB Study report no.: PH-40959	Based on AUC calculations in units of nmol•hr/ml, the M-6 metabolite had the highest AUC accounting for 69.4% of the total quantified AUC followed in decreasing order by nifurtimox (16.9% of the total AUC), M-4 (8.87% of the total AUC), M-1 (1.78% of the total AUC), M-2 (1.35% of the total AUC), M-3 (1.20% of the total AUC) and M-5 (0.499% of the total AUC). Plasma C_{max} values followed the same rank order as the plasma AUC values and plasma T_{max} and $t_{1/2}$ values varied for nifurtimox and the different metabolites (see table below).

PK Parameters of Nifurtimox and Its Major Metabolites in Plasma Pooled From 6 Male Chagas Patients Administered a Single Oral Dose of 120 mg Nifurtimox

Parameter	NFX	M-1	M-2	M-3	M-4	M-5	M-6
AUC	8,680	913	691	616	4,550	256	35,600
% of total AUC	**16.9**	**1.78**	**1.35**	**1.20**	**8.87**	**0.50**	**69.4**
C_{max}	1,730	113	85.4	28.4	207	30.0	2,250
t_{max}	3.0	4.0	6.0	4.0	4.0	4.0	4.0
$t_{1/2}$	3.14	3.16	3.30	28.9	27.5	3.80	10.1

Source: Data from Study report no.: PH 40959
The total AUC for nifurtimox and the major metabolites is: 51306 nmol•hr/ml
Units: AUC, nmol·hr/mL; C_{max}, nmol/L; t_{max} and $t_{1/2}$, hr
Abbreviations: NFX, nifurtimox; PK, pharmacokinetic

Nifurtimox: Metabolite Scouting and Quantification of Nifurtimox and Metabolites in Human Urine Study no.: KINM 180071-ELB Study report no.: PH-40569	In the urine of six Chagas patients, the largest component of the total dose of nifurtimox was the metabolite M-6, followed in descending order by M-4, M-5, M-1, M-2, M-3, and nifurtimox when the patients were fasted before dosing. When the patients were dosed after being fed, the urine concentrations of all the metabolites and nifurtimox were approximately 2-fold higher than the values associated with fasted dosing except for M-1 which appeared in urine in higher concentrations after fasting (see table below).

Mean Percent of Total Dose of Nifurtimox and Its Metabolites in Human Urine Collected for 24 Hours After a Single Oral Administration of 120 mg Nifurtimox

Parameter	NFX	M-1	M-2	M-3	M-4	M-5	M-6
Fasted pts	0.025	3.23	1.04	0.060	5.91	3.41	13.7
Fed pts	0.075	2.02	2.36	0.149	12.3	6.59	20.8

Source: Data from Study report no.: PH 40959
Abbreviations: NFX, nifurtimox; pts, patients

Type of Study	Major Findings
BAY A 2502: Determination of the Inhibitory Potency Towards Human CYP Isoforms In Vitro Study no.: KINM 130083-ELB Study report no.: PH-37686. Note: BAY A 2502 is another name for nifurtimox	When incubated in vitro with human liver microsomes and specific substrates for CYP-450 isozymes, none of the metabolic reactions mediated by a large panel of CYP-450 isozymes (CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) was inhibited by nifurtimox in concentrations up to 50 mcM.
Nifurtimox: In Vitro Evaluation of Nifurtimox and its Metabolites M-4 and M-6 as Inducers of Cytochrome P450 Expression Study no.: KINM 190191-ELB Study report no.: PH-41133	Nifurtimox and its M-4 and M-6 metabolites did not induce the expression or activity of the CYP 450 enzymes, CYP1A2, CYP2B6, CYP2C19, and CYP3A4 in vitro in incubations with human liver hepatocytes from three separate donors.
Excretion	
BAY 2502: Pharmacokinetics and Metabolism in Rats and Dogs Using the ^{35}S-Labeled Substance Study report no.: PH-1569 Note: BAY 2502 is nifurtimox	**Rats** • Following single oral and IV administration of 25 mg/kg [^{35}S]nifurtimox, 50-60% of radioactivity was excreted in urine and 30-35% in feces. Two days after administration by either route only 4% of the administered radioactivity remained in animals. • In rats with a biliary fistula after intraduodenal dosing of 25 mg/kg [^{35}S]nifurtimox, 35% of the dose was excreted via bile 4 hours after administration. **Dogs** • Oral administration of 25 mg/kg [^{35}S]nifurtimox resulted in a different excretion pattern than what was seen in rats. Approximately 15% of the radioactivity was excreted in urine with 60-80% in feces in a three day period following administration.
TK data from general toxicology studies	
A Comparison of Toxicity and Toxicokinetics in Rats and Dogs Following Twenty-Eight-Day, Repeat-Dose Oral Administration of Nifurtimox	**Rat** (see table below) $t_{1/2}$: Not reported Accumulation: After repeated dosing for 28 days, plasma C_{max} values ranged from 1.1- to 2.2-fold higher than Day 1 values and plasma AUC values ranged from 1.1 to 2.3 fold higher than Day 1 values indicating nifurtimox tended to accumulate with repeated dosing.

Type of Study	Major Findings
(Li et al. 2017)	Dose proportionality: For both sexes on Day 28, plasma C_{max} values tended to increase in a less than dose-proportional manner, and plasma AUC values increased in a more than dose-proportional manner.

Plasma Toxicokinetics in Rats After 1 and 28 Days of Oral Dosing

		C_{max} (ng/ml)		AUC_{0-t} (ng•hr/ml)	
Dose	**Sex**	**Day 1**	**Day 28**	**Day 1**	**Day 28**
25 mg/kg/day	M	5,330	5,786	19,604	22,135
	F	2,206	4,916	8,909	14,881
75 mg/kg/day	M	5,012	11,068	23,697	46,340
	F	4,826	8,308	23,983	42,573
150 mg/kg/day	M	11,632	12,100	74,010	100,484
	F	7,542	11,950	43,319	99,375

Dog (see table below)

$t_{1/2}$: Not reported

Accumulation: For the low-and mid-dose groups, plasma C_{max} and AUC values tended to decrease with repeated dosing for 28 days compared to values on Day 1 of dosing. In contrast, in the high-dose groups, in both sexes, plasma C_{max} increased 2.0 to 2.8 fold and plasma AUC values increased approximately 4 fold with repeated dosing indicating plasma accumulation.

Dose proportionality: For both sexes, on Day 28, plasma C_{max} and AUC values generally increased in a less than dose-proportional manner between the 25 and 75 mg/kg/day doses and in a more than dose-proportional manner between the 75 and 150 mg/kg/day doses.

Plasma Toxicokinetics in Dogs After 1 and 28 Days of Oral Dosing

		C_{max} (ng/ml)		AUC_{0-t} (ng•hr/ml)	
Dose	**Sex**	**Day 1**	**Day 28**	**Day 1**	**Day 28**
25 mg/kg/day	M	1,043	599	3,530	2,785
	F	970	375	3,747	1,196
75 mg/kg/day	M	1,062	1,181	3,922	3,738
	F	1,108	857	3,747	5,406
150 mg/kg/day	M	634	1,785	2,401	9,812
	F	1,308	2,620	4,651	18,491

Type of Study	Major Findings
TK data from reproductive toxicology studies	
Embryo-fetal Study in Rabbits Study no.: T6063160 Study report no.: PH-33192	**Toxicokinetic Parameters for BAY 2502 (Nifurtimox) in the Rabbit Embryo-Fetal Study**

Dose [mg/kg]		5[a]		15[b]		60[c]	
		Mean geom.	S.D. geom.	Mean geom.	S.D. geom.	Mean geom.	S.D. geom.
Day 6 p.c.							
$AUC(0\text{-}t_n)$	[µg·h/L]	323	1.21	1332	1.21	5035	1.26
$AUC(0\text{-}t_n)_{norm}$	[kg·h/L]	0.0646	1.21	0.0888	1.21	0.0839	1.26
C_{max}	[µg/L]	293	1.21	893	1.07	2410	1.35
$C_{max,norm}$	[kg/L]	0.0586	1.21	0.0596	1.07	0.0402	1.35
t_{max}	[h]	1.00	1.00	1.00	1.00	1.00	1.00
Day 20 p.c.							
$AUC(0\text{-}t_n)$	[µg·h/L]	262	1.34	1635	1.15	9475	1.57
$AUC(0\text{-}t_n)_{norm}$	[kg·h/L]	0.0524	1.34	0.109	1.15	0.158	1.57
C_{max}	[µg/L]	232	1.32	1020	1.06	2568	1.56
$C_{max,norm}$	[kg/L]	0.0464	1.32	0.0680	1.06	0.0428	1.56
t_{max}	[h]	1.00	1.00	1.00	1.00	1.00	1.00
R_{A1}	[%]	79.2	1.53	114	1.10	107	1.28
R_{A3}	[%]	81.1	1.59	123	1.05	188	1.51

$R_{A1} = C_{max,ss} / C_{max,Day\ 1}$ summary t6063160.xls \ Tabelle1 \ Thuß \ 21.08.03
$R_{A3} = AUC_{ss} / AUC_{Day\ 1}$
n.c. = not calculated
[a] : $t_n = 2$ h
[b] : $t_n = 4$ h
[c] : $t_n = 7$ h

Source: Study report

Pre-postanatal Study in Rats

Study no.: T102196-9

Study report no.: R-11265

Toxicokinetic Parameters of Nifurtimox Exposure in Maternal Plasma on GD 6 in the Pre-postnatal Study

Dose Parameter	[mg/kg] Unit	15	30	60
$AUC(0\text{-}t_{last})$	µg·h/L	5930	17800	16500
$AUC(0\text{-}t_{last})_{norm}$	(µg·h/L)/(mg/kg)	396	593	275
t_{last}	h	7.0	7.0	7.0
C_{max}	µg/L	2500	5030	4950
$C_{max,norm}$	(µg/L)/(mg/kg)	166	168	82.6
t_{max}	h	0.5	0.5	1.0
C_{last}	µg/L	27.6	1190	1670
C_{last}/C_{max}	%	1.1	23.7	33.7

Source: Study report

Toxicokinetic Parameters of Nifurtimox Exposure in Maternal Plasma on GD 20 in the Pre-postnatal Study

Dose Parameter	[mg/kg] Unit	15	30	60
$AUC(0\text{-}t_{last})$	µg·h/L	8770	20600	27100
$AUC(0\text{-}t_{last})_{norm}$	(µg·h/L)/(mg/kg)	585	688	451
t_{last}	h	7.0	7.0	7.0
C_{max}	µg/L	3010	5990	5730
$C_{max,norm}$	(µg/L)/(mg/kg)	201	200	95.4
t_{max}	h	1.0	1.0	2.0
C_{last}	µg/L	299	1150	2490
C_{last}/C_{max}	%	9.9	19.2	43.5
$RA\text{-}AUC(0\text{-}t_{last})$	%	148	116	164
$RA\text{-}C_{max}$	%	121	119	116

Source: Study report

Type of Study	Major Findings
TK data from carcinogenicity studies	Toxicokinetic measurements were not performed in any of the carcinogenicity studies.

5.4. Toxicology

5.4.1. General Toxicology

None of the repeated-dose toxicology studies were GLP-compliant. Pivotal studies included a 13-week study in rats and dog studies of 8, 16, 30, and 52 weeks. In addition to the 13-week rat study, the 52-week study in dogs is evaluated below but not the shorter-term dog studies because they appear to represent earlier time-point measurements in the 52-week study, and histopathology was evaluated only in dogs euthanized at 52-weeks. Neither the 13-week rat study nor the 52-week dog study included toxicokinetic measurements. Two other nonpivotal studies, 28-day toxicology studies in rats and dogs were reported in the same literature manuscript and included toxicokinetic measurements.

Study Title: BAY 2502 - Subchronic Toxicity in Rats After Oral Administration (13-Week Study)/Study Report No. PH-2048

Note: BAY 2502 is another name for nifurtimox.

Also described in: Toxicological Investigations on the Tolerability of Nifurtimox (Hoffmann 1972).

Reviewer Comment: *This non-GLP study was conducted in 1970 and included several deficiencies relative to current recommendations. Animals were dosed for only 5 days of each week and the study did not include a recovery period for high-dose animals thus eliminating the ability to assess reversibility of the multiple histopathology findings. Also, the study report for this study was only 43 pages in length and did not included details that are normally included, such as individual animal data for many measurements. One exceptional and positive attribute of this study is that it included an extensive histopathology examination of brain and CNS tissues at different timepoints during the study from animals in each group.*

Key Study Findings:

- Several (6/25) high-dose (400 mg/kg/day) females died in the third week of treatment in conjunction with severe neurological signs including asymmetric posture and staggering gait. By the third week of dosing, all high-dose females and 16/25 high-dose males displayed neurological signs. The neurological signs correlated with widespread brain histopathology including spongiosis, perivascular bleeding, and nerve degeneration.
- Absolute and relative testicular weights were significantly lower in mid- (100 mg/kg/day) and high-dose males. The reduced weights correlated with organ atrophy, and seminiferous tubule and germinal epithelium degeneration with severely

depleted germ cells. Consistent with these findings lumen of epididymides contained very few spermatozoa.

- The NOAEL was considered to be the low dose of 25 mg/kg/day which is approximately equivalent to 0.2 to 0.4 times the maximum recommended dose of 10 to 20 mg/kg/day based on body surface area comparison.

Conducting laboratory and location: Farbenfabriken Bayer AG, Institute of Toxicology, Institute of Histopathology, Wuppertal, Germany

GLP compliance: No

Table 4. Methods, 13-Week Subchronic Toxicity Study, Study report no.: PH-2048

Method	Details
Dose and frequency of dosing:	0 (Group IV; vehicle control group), 25 mg/kg (Group III; low-dose group), 100 mg/kg (Group II; mid-dose group), 400 mg/kg (Group 1, high-dose group) nifurtimox Daily dosing 5 days per week for 13 weeks (the treatment with 400 mg/kg nifurtimox had to be stopped due to severe toxicity after 3 weeks of dosing).
Route of administration:	Oral gavage
Formulation/vehicle:	1% tragacanth solution, pH 6.0 was used as the suspension vehicle.
Species/strain:	SPF Wistar rats
Number/sex/group:	25/sex/group
Age:	Not identified
Satellite groups/unique design:	Control animals (Group IV) and low-dose (Group III) and mid-dose (Group II) were dosed for 13 weeks, but due to pronounced clinical signs, dosing for high-dose animals (Group 1) was ended after 3 weeks. All animals were dosed 5 days per week. In all groups, 5/sex/group of the surviving animals were necropsied at the end of dosing and tissues (except for brain tissue) from these animals were examined for histopathology. Additional animals were euthanized and extensively examined for brain and CNS histopathology in Weeks 3, 7, and 13. In the low- and mid-dose groups, 5 animals/sex/group were maintained for 4 weeks after treatment in a recovery period then euthanized and examined macroscopically.
Deviation from study protocol affecting interpretation of results:	Study deviations were not identified in the study report. No deviations were thought to have affected interpretation of the results.

Table 5. Observations and Results - Changes From Control, 13-Week Subchronic Toxicity Study, Study report no.: PH-2048

Parameters	Major Findings
Mortality	Six females in Group I (400 mg/kg, HD) died in the third week of treatment in conjunction with severe neurological signs (seizures and signs of dyspnea). Two other HD females died (one in the second week due to gavage error) and another during the 12th week during blood collection. One control male died in the 10th week of the experiment.

Parameters	Major Findings
Clinical signs	In the third week of treatment, a few HD females exhibited a slightly asymmetric posture, a staggering gait, partially paralyzed hind limbs, seizures and signs of dyspnea. By the end of the third week these clinical signs were apparent in all Group I females and 16/25 Group I males. Clinical signs dissipated upon cessation of dosing (beginning in Week 4) and Group 1 animals were reportedly unremarkable three weeks after the end of dosing. Similar clinical signs did not occur in Group II animals.
Body weights	Body weights of both males and females in Group I (HD animals) were markedly lower (particularly in females) after the first week of treatment. Relative to control values, body weights were reduced by approximately 13% and 14% for HD males and females respectively after one week of treatment. Treatment was stopped after 3 weeks for HD animals due to severe clinical signs. Subsequently, mean body weights rose again until HD body weights were roughly the same as those of comparable control animals by the 8th week. Mean body weights in MD and LD animals were only slightly lower than the corresponding weights in the control group throughout the study, but MD male and female body weights were significantly lower than control values by approximately 7% and 16% respectively at the end of the 12-week dosing period.
Hematology	No significant nifurtimox-related changes in any of the measured hematology parameters (hematocrit, hemoglobin and hemoglobin E, red and white cell numbers, differential blood counts, platelet counts) were reported.
Clinical chemistry	Plasma alkaline phosphatase was significantly elevated in HD male (+27% compared to control values) and female animals (+35%) and in LD males (+29%) at the 4-week measurement, but not at the 12-week measurement. These results may have been related to reduced food intake (implied by the observed weight loss) particularly in HD animals. No other significant changes were observed in the serum chemistry parameters (plasma urea, blood glucose, glutamate pyruvate transaminase, and alkaline phosphatase) that were measured.
Urinalysis	No changes in urinalysis parameters were noted at the 4-week measurement. At the 12-week measurement, protein in urine was observed in 1 male each in the MD and HD groups, but also in 1 control male. The urine of all MD animals was intensely yellow in coloration.
Gross pathology	All of the prematurely deceased animals and an additional 5 animals/sex/group were necropsied and examined for gross pathology and histopathology. Additional animals, 7 HD animals (3 males and 4 females) and 3 control animals (1/sex) were necropsied after 3 weeks of dosing, 1 MD female and 2 LD animals (1/sex) were necropsied after 6 weeks of dosing, and 2 control (1/sex), 2 LD animals (1/sex), 3 MD (2 males and 1 female) and 3 HD males were necropsied after 13 weeks of dosing. At the end of treatment in Week 13, a clear reduction in testes size was noted in all 5 MD males and 2/5 HD males (note: dosing was stopped for HD animals after 3 weeks). Also in 5/13, 4/13, and 2/12 animals in the HD, MD, and LD groups respectively, the edges of the liver appeared slightly swollen, and reportedly one animal in each dose group exhibited uneven kidney edges. In the LD and MD animals that were maintained for 4 weeks after the end of dosing then sacrificed, no gross pathology was reported.

Parameters	Major Findings
Organ weights	Absolute and relative weights (based on terminal body weights) of thymus, thyroid, heart, lung, liver, spleen, kidneys, adrenal glands, and testes or ovaries were determined at the necropsy. Absolute and relative testicular weights were significantly reduced in HD males by 13% and 12% respectively and in MD males by 52% and 46% respectively. In addition, relative liver weights were significantly increased in MD males by approximately 9% and relative spleen weights were increased in MD females by approximately 17% compared to control values.
Histopathology Adequate battery: The battery was adequate but multifaceted. See the description in the major findings section.	Tissue Collection for Histopathology: In a subset of animals in all groups (5/sex/group), an extensive panel of tissues (excluding the brain) were examined for histopathology at the end of 13 weeks of dosing. In addition, some animals in each group were euthanized and examined for histopathology in multiple regions of the brain and the CNS in Weeks 3, 7 and 13. These included: all of the animals that died during the study, 7 HD animals (3 males and 4 females) and 3 control animals (1/sex) necropsied after 3 weeks of dosing, 1 MD female and 2 LD animals (1/sex) necropsied after 6 weeks of dosing, and 2 control (1/sex), 2 LD animals (1/sex), 3 MD (2 males and 1 female) and 3 HD males necropsied after 13 weeks of dosing. The brain and CNS tissues that were examined for histopathology included: cerebrum, interbrain, midbrain, cerebellum, pons, medulla oblongata, spinal cord, Sciatic nerve, red nucleus, nucleus of the posterior colliculi, fastigial nucleus, globose and emboliform nuclei, dentate nucleus, vestigial nucleus, cochlear nucleus, and superior olivary nucleus. Brain sections were stained with H&E, luxol fast blue and in some sections Marchi to stain degenerated myelin. Liver: In all MD animals, 8/10 HD animals, and in 5/10 LD animals, swelling of liver cells with clumping of cytoplasm, fat-free vacuoles, and distension of cell nuclei were present to varying degrees. The changes were most prominent in MD males. Also, lymphocyte and neutrophil infiltration into liver lobes and necrosis of individual parenchymal cells were observed in all groups including control animals. Kidney: Proximal and distal tubule epithelial cells exhibited fine PAS-positive droplets with the greatest numbers in HD and MD animals and more marked presentation in female animals. A few animals in nifurtimox-dose groups demonstrated isolated calcifications in protein casts in degenerated tubule epithelia (1/10 HD animals, 2/10 MD animals, and 1/10 LD animals), isolated small regenerated uriniferous tubules in the renal cortex (1/10 MD and 1/10 LD animals), and small interstitial infiltrates (1/10 HD, 1/10 MD, and 1/10 LD animals). Spleen: In all groups including the control group, the spleen was engorged with blood and iron pigment deposits in the red pulp and focally in the trabeculae. Iron pigment deposits were more marked in females and more severe in the females in Groups II and III than in females in Group I and the control group. Pituitary: Pituitary findings beyond that seen in control animals included: slightly increased pyknotic acidophilic cells in Group HD males, enlarged basophilic cells containing accumulation of droplets primarily in MD males, eosinophilic material in the neurohypophysis of 3/10 LD animals, and pyknosis in the region of the pars intermedia in 2/10 LD animals.

Parameters	Major Findings
	Adrenal gland: in 2/10 MD animals, small lymphocytic infiltrates were visible in the region of the fascicular zone. Testes: The testes of all MD males were clearly atrophied at the end of dosing. In 2/5 MD males, no germ cells were present and only Sertoli's cells (swollen and increased in number) were present in seminiferous tubules. In the 3 other MD males, the germinal epithelium was clearly degenerated and seminiferous tubules were highly degenerated and contained no germ cells. A slight increase in Leydig's cells was observed in 3/5 MD males. Similar effects were not noted in control or LD males or surviving HD males. Epididymis: Consistent with the testicular findings, the lumen of the epididymis in all MD males contained very few spermatozoa, germ cells in varying degrees of degeneration, giant cells, and cellular debris. A similar milder pattern was also noted in 2 HD males. Ovary: In three females in Group II and in one female each in Group I and Group III, a slight vacuolation of the lutein cells in the peripheral layer of the corpus luteum was observed. Central nervous system: In the HD animals that demonstrated neurological clinical signs, histopathology was noted to varying degrees in the region of the midbrain, the cerebellum, the pons, and the medulla oblongata. In the 7 HD animals (3 males and 4 females) that were sacrificed at the time of occurrence of the neurological symptoms after three weeks of treatment, spongiosis was observed in places, as well as fresh perivascular bleeding to a modest extent. Also observed were isolated nerve degeneration such as pyknosis, vacuolation and swelling in specific brain nuclei including red nuclei, nuclei of the posterior colliculi of the midbrain, the globose, emboliform, fastigial and dentate nuclei of the cerebellum, the vestigial and cochlear nuclei of the pons, and the superior olivary nuclei of the medulla oblongata. Also, occasional isolated very small homogenized necrotic lesions were noted. No peripheral histopathology in the sciatic nerve or femoral biceps muscle was observed in the animals that had demonstrated neurological clinical signs. In the 6 HD animals (3 males and 3 females) that were euthanized in Week 7 (after 4 weeks without dosing), glial cell multiplication, spongiosis, dilation of capillaries and isolated slight deposition of brown pigment were found in the brain nuclei to varying degrees. Also in the same animals, edematous rarefaction of the fiber systems was observed in cerebellar white matter and the pons without signs of demyelination. Similar findings were not observed in LD or MD animals or in the three HD males that did not exhibit neurological signs. In the animals that were euthanized after 13 weeks of dosing, there was no brain histopathology in the 3 HD males that did not display neurological clinical signs nor in the 3 MD and 2 LD animals from which brain and CNS tissue was collected.

Abbreviations: LD: low dose; MD: mid dose; HD: high dose.

Study Title: Oral Toxicity Study in Purebred Dogs Repeated Dosage for One Year/Study No. 3048/69/474; Study Report No.: 3048-69-474

Also described in: Toxicological Investigations on the Tolerability of Nifurtimox (Hoffmann 1972)

Reviewer Comment: *This non-GLP study was conducted in 1970, and did not include a recovery period. Consequently the reversibility of the multiple histopathology findings could not be assessed. Toxicokinetics measurements were not conducted. Also, the study report for this study was only 99 pages in length and did not included normal details including individual animal data for most measurements.*

Key Study Findings:

- Beagle dogs receiving nifurtimox suffered dose-dependent clinical signs including stiff limbs, ataxia, nystagmus, and convulsions. Histopathology including neurological histopathology was not reported except for one high-dose male euthanized in extremis with neurological clinical signs where histopathology included changes in the pontive and cerebellar portions of the brain and occasional degenerative fingers in the spinal cord and peripheral nerves.
- Organ weight changes included: increased absolute and relative spleen weights in Group 1 (240 mg/kg/day) and 2 (120 mg/kg/day) animals and decreased absolute and relative prostate weights and increased relative thymus weights in high-dose animals.
- The NOAEL was considered to be the low dose of 30 mg/kg/day which is approximately equivalent to 0.8 to 1.7-times the maximum recommended dose of 10 to 20 mg/kg/day based on body surface area comparison.

Conducting laboratory and location: (b) (4)

GLP compliance: No

Table 6. Methods, Study 3048/69/474

Method	Details
Dose and frequency of dosing:	0 (Group 5, not dosed; control group) 30, (Group 4), 60 (Group 3), 120 (Group 2) and 240 (Group 1) mg/kg/day nifurtimox. Animals were dosed once per day, 6 days a week for 1 year.
Route of administration:	Oral dosing in a gelatin capsule
Formulation/vehicle:	None (nifurtimox powder in capsule)
Species/strain:	Beagle dogs
Number/sex/group:	4/sex/group
Age:	Not identified
Satellite groups/unique design:	No satellite groups. Beagle dogs (n=4/sex/group) were dosed with vehicle, or 30, 60, 120, or 240 mg/kg/day BAY 2502 (nifurtimox) administered orally in gelatin capsules for 52 weeks.
Deviation from study protocol affecting interpretation of results:	Study deviations were not described, but the results were not considered to have been affected by study deviations

Table 7. Observations and Results - Changes From Control, Study 3048/69/474

Parameters	Major Findings
Mortality	Two deaths occurred during the dosing period. Both animals were HD males receiving 240 mg/kg/day, one on Day 128 (humane euthanasia) and one on Day 182 (found dead).
Clinical signs	Both of the animals that died prematurely exhibited neurological signs in the days and weeks before death. Signs included stiff limbs, altered gait, ataxia, nystagmus and, in the animal that died on Day 182, convulsions. Clinical signs that were observed in animals that survived until the scheduled euthanasia included: convulsive episodes, and abnormalities of gait and hind limb weakness, sedation, yellow discoloration of urine, discoloration of coat, yellowish discoloration of the legs and abdomen (all dosed animals). Repeated short-duration convulsions were observed in 4 animals receiving 240 mg/kg/day, 2 animals receiving 120 mg/kg/day and 1 animal receiving 60 mg/kg/day.
Body weights	Nifurtimox did not significantly reduce body weights or body weight gains at any dose relative to control animals.
Ophthalmoscopy	No nifurtimox-related ophthalmoscopy findings were observed.
ECG	ECG measurements were not performed
Hematology	No nifurtimox-related changes in any hematology parameters were observed 4-weeks, 8-weeks, 16-weeks, 6-months, 9-months, and 1-year after dosing.
Clinical chemistry	No nifurtimox-related changes in any serum chemistry parameters were observed 4-weeks, 8-weeks, 16-weeks, 6-months, 9-months, and 1-year after dosing. One HD female receiving 240 mg/kg/day exhibited higher than normal serum glutamic-pyruvic transaminase (SGPT) levels. However, this trend was not apparent in other HD animals, and not considered to be clearly related to nifurtimox administration.
Urinalysis	No changes in any urinalysis parameters were observed 4-weeks, 8-weeks, 16-weeks, and 6-months, 9-months, and 1-year after dosing. Blood in urine was only noted in females in estrus.
Gross pathology	No gross pathology findings related to nifurtimox were noted.
Organ weights	The following organs were weighed upon necropsy: brain, liver, thymus, thyroids, pituitary, spleen, prostate/uterus, adrenals, heart, pancreas, kidneys, gonads, and lungs. Organ weight changes included: significantly increased absolute and relative spleen weights in HD animals receiving 240 mg/kg/day (absolute and relative increases of 49% and 60%, respectively, compared to control values) and animals receiving 120 mg/kg/day (absolute and relative increases of 65% and 53%, respectively, compared to control values). Also, absolute and relative prostate weights in HD males were decreased by 38% and 39%, respectively, compared to control values.

Parameters	Major Findings
Histopathology Adequate battery: Yes	In the one male animal that was euthanized in extremis after exhibiting neurological clinical signs, histopathology including status spongiosa in the pontive and cerebellar portions of the brain with some generalized malacia, glial proliferation, and vascular hyperplasia. Also, occasional degenerative fibers in the spinal cord and peripheral nerves were observed in the same animal. In the majority of animals that survived until the scheduled euthanasia after 1 year, no nifurtimox-specific histopathology was reported.

Abbreviations: LD: low dose; MD: mid dose; HD: high dose.

5.4.2. General Toxicology—Additional Studies

Study Title: A Comparison of Toxicity and Toxicokinetics in Rats and Dogs Following Twenty-Eight-Day, Repeat-Dose Oral Administration of Nifurtimox (Li et al. 2017)

Reviewer Comment: *The methods and results for this study were reported in a brief literature report. The results in the rat study suggest a more comprehensive evaluation than that conducted with dogs. However, for some assessments including histopathology, the limited results suggest an inexpert evaluation.*

Key Study Findings:

- One high-dose female rat was euthanized on Day 21 due to nifurtimox-related clinical signs including neurological signs (hind leg weakness and paralysis). The neurological signs correlated with significantly elevated relative brain weights in mid- and high-dose males and females and cerebral tissue softening in high-dose animals.
- Mean body weights were significantly decreased from Day 8 throughout dosing in mid- and high-dose male and female rats and until the end of the recovery period in males but not females. The body weight losses correlated with significantly reduced food consumption for the first two weeks of dosing in high-dose males and the first three weeks of dosing in high-dose females.
- The primary histopathology finding was testicular atrophy and reduced numbers of sperm in epididymal tissue, with loss of necrotic cells. The findings correlated with decreased testicular and epididymal weights and occurred in male rats in all the nifurtimox treatment groups with severity increasing with dose.

Conducting laboratory and location: New Drug Safety Evaluation Center, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

GLP compliance: No

Table 8. Methods, Rats, Li et al. (2017)

Method	Details
Dose and frequency of dosing:	0, 30, 60, 120 mg/kg/day administered once per day for 28 days.
Route of administration:	Oral gavage
Formulation/vehicle:	1% hydroxypropyl-methylcellulose (HPMC)
Species/strain:	Sprague Dawley rats
Number/sex/group:	15/sex/group
Age:	Approximately 7 weeks of age
Satellite groups/unique design:	10/sex/group were used in the Main Study and euthanized after 28 days of dosing and 5/sex/group were used as recovery animals and euthanized on Day 56.
Deviation from study protocol affecting interpretation of results:	Study deviations were not identified

Table 9. Observations and Results - Changes From Control, Rats, Li et al. (2017)

Parameters	Major Findings
Mortality	One HD female was euthanized on Day 21 due to nifurtimox-related clinical signs.
Clinical signs	In the prematurely euthanized HD female, the following clinical signs were observed beginning on Day 18: thin appearance and hunched posture, poor food consumption, eyes partially closed, cold to the touch, and rough haircoat. On Day 21 before euthanasia, the female exhibited hind leg weakness and paralysis.
Body weights	A dose-dependent decrease in mean body weight was observed in rats following the administration of nifurtimox when compared to control group animals and significant decreases in mean body weight were observed from Day 8 in MD and HD male and female rats throughout dosing. Also, weight reductions persisted through the recovery period in males, but in females the weight reductions were reversed after the recovery period.
Hematology	No changes in hematology or coagulation parameters were reported
Clinical chemistry	A significant difference in total bilirubin was observed on Day 28 in males in all the treatment groups with increases of 1.2, 1.3, and 1.5 times for the LD, MD, and HD groups and in HD females by 1.4 times compared to control values. Also, urea levels were significantly increased in MD and HD males by 1.2 and 1.4 times compared to control values.
Urinalysis	No nifurtimox-related changes in any urinalysis parameters were observed.
Gross pathology	One gross pathology finding was softening of cerebral tissue in HD males and females.
Organ weights	Organ weight changes are shown in the table below. <u>Brain</u>: In MD and HD rats of both sexes, relative brain weights were significantly increased compared to control animals. This finding correlated with cerebral tissue softening in high-dose animals. <u>Heart</u>: In MD and HD female rats, absolute heart weights were significantly decreased, and in male rats in all the nifurtimox treatment groups, mean absolute and relative heart weights were significantly decreased. <u>Thymus</u>: Mean absolute and relative thymus weights in HD male and female rats were decreased. The thymus weight changes were not accompanied by correlating histopathology.

Parameters	Major Findings
	Kidneys: Relative kidney weights were significantly increased in MD and HD males and HD females but no correlating histopathology was reported. Testes and epididymides: In MD and HD male rats, mean absolute and relative weights of the testes and epididymides were significantly decreased and associated with histopathologic changes.

Significant Absolute (Abs) and Relative (Rel) Organ Weight Changes in the 28-Day Toxicology Study in Rats

	Weight	Control		LD		MD		HD	
Organ	**Type**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Brain	Abs (g)	1.98	1.88	2.06	1.96	2.01	1.94	2.00	1.92
	Rel (%)	0.56	0.77	0.58	0.79	**0.62**	**0.86**	**0.63**	**0.90**
Heart	Abs (g)	1.42	0.91	**1.28**	0.93	**1.16**	**0.80**	**1.12**	**0.79**
	Rel (%)	0.40	0.37	**0.36**	0.38	**0.36**	0.35	**0.35**	0.37
Thymus	Abs (g)	0.48	0.43	0.49	0.39	0.39	0.37	**0.37**	**0.29**
	Rel (%)	0.14	0.18	0.14	0.16	0.12	0.16	**0.11**	**0.13**
Kidney	Abs (g)	2.49	1.79	2.63	1.85	2.57	1.74	2.71	1.80
	Rel (%)	0.70	0.73	0.74	0.75	**0.79**	0.77	**0.84**	**0.84**
Testis	Abs (g)	3.05	----	3.24	----	**1.69**	----	**1.11**	----
	Rel (%)	0.86	----	0.92	----	**0.52**	----	**0.35**	----
Epididymis	Abs (g)	1.08	----	1.01	----	**0.83**	----	**0.72**	----
	Rel (%)	0.31	----	0.28	----	**0.26**	----	**0.22**	----

Mean absolute and relative organ to body weight ratios are shown.
Statistically significant differences relative to control values ($p<0.05$) are shown in bold
Dosage: Control: 0 mg/kg/day; LD: 30 mg/kg/day; MD: 60 mg/kg/day; HD: 120 mg/kg/day

Histopathology Adequate battery: Yes	Brain: At the end of the dosing period, the brains of HD rats revealed cerebral tissue softening, which remained after the recovery period. Brain tissue was examined by electron microscopy, but histopathology findings were only described as cerebral tissue softening. Testes and epididymides: In male rats in all nifurtimox dosing groups histopathology findings included: testicular atrophy and the death of sperm in epididymal tissue, with loss of necrotic cells. The findings were dose-related for severity and remained after the 28-day recovery period.
[Other evaluations]	Toxicokinetic analysis was performed and the results are summarized in Section 5.4 of this review.

Abbreviations: LD: low dose; MD: mid dose; HD: high dose.

Study Title: A Comparison of Toxicity and Toxicokinetics in Rats and Dogs Following Twenty-Eight-Day, Repeat-Dose Oral Administration of Nifurtimox (Li et al. 2017)

Reviewer Comment: *The methods and results for this study were reported in a brief literature report. For some assessments including clinical chemistry and histopathology, the limited results suggest an inexpert and potentially incomplete evaluation.*

Key Study Findings:

- One high-dose male dog died on Day 18 after exhibiting tremor and paralysis of the hind legs and high muscular tension.

- Histopathology findings included deciduous spermatoblasts in the seminiferous tubules and no long sperm in the epididymides in males in all the nifurtimox treatment groups. These changes did not reverse during the 28-day recovery period.

Conducting laboratory and location: New Drug Safety Evaluation Center, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

GLP compliance: No

Table 10. Methods, Dogs, Li et al. (2017)

Method	Details
Dose and frequency of dosing:	0, 30, 60, and 120 mg/kg/day administered once per day for 28 days.
Route of administration:	Oral gavage
Formulation/vehicle:	1% hydroxypropyl-methylcellulose (HPMC)
Species/strain:	Beagle dogs
Number/sex/group:	5/sex/group
Age:	Approximately 6-9 months of age
Satellite groups/ unique design:	3/sex/group were used in the Main Study and euthanized after 28 days of dosing and 2/sex/group were used as recovery animals and euthanized on Day 56.
Deviation from study protocol affecting interpretation of results:	Study deviations were not identified

Table 11. Observations and Results—Changes From Control, Dogs, Li et al. (2017)

Parameters	Major Findings
Mortality	One HD male dog died on Day 18 after exhibiting tremor and paralysis of the hind legs and high muscular tension.
Clinical signs	Clinical signs were only reported for the HD male that died as noted above.
Body weights	Reportedly no nifurtimox-related changes in body weight were observed.
ECG	Not performed
Hematology	No nifurtimox-related changes in any hematology or coagulation parameters were reported.
Clinical chemistry	No nifurtimox-related changes in any serum chemistry parameters were reported.
Urinalysis [delete the row if not evaluated]	No nifurtimox-related changes in any urinalysis parameters were reported.
Gross pathology	Gross pathology findings were not reported.
Organ weights	No changes in organ weights were reported.
Histopathology Adequate battery: Yes; the literature report listed an extensive battery, but the limited results that were reported are consistent with a more limited evaluation.	Microscopic examination revealed that there were deciduous spermatoblasts in the seminiferous tubules and no long sperm in the epididymides in males in all the nifurtimox treatment groups and these changes did not reverse during the 28-day recovery period. No other histopathology findings were reported.
[Other evaluations]	Toxicokinetic analysis was performed and the results are summarized in Section 5.4 of this review.

Abbreviations: LD: low dose; MD: mid dose; HD: high dose.

5.4.3. Genetic Toxicology

<u>In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)</u>

Study Title: Salmonella/Microsome Test to Investigate Point Mutagenic Effect (Study Report No.: PH-9657, Study Conducted in 1980)

Key Study Findings:

- In three tester strains TA 100, TA 1537, and TA98, BAY 2052 (nifurtimox) tested positive for mutagenicity and produced an increase in the numbers of mutants with and without S9 activation up to more than twice the negative control values. In the TA 1535 strain, nifurtimox was not associated with significantly increased numbers of mutants.

GLP compliance: No

Test system: *Salmonella typhimurium* strains: TA 1535, TA 1537, TA 100, and TA 98

Study is valid: Study validity was not directly addressed in the study report. However, based on the low negative control values for revertants, and the significantly higher revertant counts produced by the positive control agents, the study appeared valid. Also the tested concentrations of nifurtimox extended to substantially cytotoxic concentrations indicating the concentration range was sufficiently high. While cytotoxicity occurred with the highest test concentrations of nifurtimox, revertants occurred with lower concentrations confirming that mutagenicity did not occur as a result of cytotoxicity.

Study Title: EPL BS891, EPL BS2459, EPL BS2460: Mini-Ames Assay (Study No.: 167-0014-GT; Study Report No.: R-9411, Study Initiated on May 22, 2013)

Reviewer Comment: *Historical control data were not provided in the study report, but it was noted that: "In both tester strains, the mean number of his+ revertant colonies observed for the negative/solvent control was comparable to the data from previous studies performed in the Testing facility, and the positive controls…induced the expected increased (3-fold or greater) in the mean number of revertant colonies when compared to the concurrent solvent control in the presence or absence of the S9 mix."*

Key Study Findings:

- Racemic nifurtimox (EPL BS891) produced a dose-dependent increase in revertant counts in excess of 2-fold above solvent control levels with and without S9 activation in two *Salmonella typhimurium* strains, TA98 and TA100 in a mini-Ames assay. These results indicate a positive mutagenic effect for nifurtimox.

GLP compliance: No

Test system: *Salmonella typhimurium* strains TA98 and TA100.

Study is valid: All the following study validity criteria were shown to be fulfilled and the study was considered to be valid.

1) The tester strain integrity must be verified. The presence of the rfa and uvrB mutations must be verified by showing that cultures of both strains are sensitive to crystal violet. The presence of the pKM101 plasmid R-factor must be verified in both tester strain cultures by their resistance to ampicillin. To demonstrate the requirement for histidine, both tester strain cultures must exhibit a characteristic number of spontaneous revertants per plate, consistent with historical control data.
2) Each positive control value must exhibit at least a 3-fold increase over the respective mean negative control value (solvent) for each tester strain and should be comparable to historical control data.
3) While cytotoxicity occurred with the highest test concentrations of nifurtimox, revertants occurred with lower concentrations confirming that mutagenicity did not occur as a result of cytotoxicity.

<u>In Vitro Assays in Mammalian Cells</u>

Study Title: Genotoxicity Revaluation of Three Commercial Nitroheterocyclic Drugs: Nifurtimox, Benznidazole, and Metronidazole (Buschini et al. 2009)

Key Study Findings:

- Human lymphocytes incubated with nifurtimox for 24 hours exhibited a concentration-dependent increase in micronuclei formation and a significant 6-fold increase in micronuclei formation with the highest concentration of nifurtimox compared to the negative control. The results are consistent with a positive result for clastogenesis.

GLP compliance: No

Test system: Lymphocytes from healthy, nonsmoking human males. Metabolic activation was not included in the assay.

Study is valid: Study validity was not directly addressed in the manuscript. However, based on the low negative control values for micronuclei, and the significantly higher micronuclei counts produced by the positive control agents, the study appeared valid. Also, the two highest concentrations of nifurtimox produced significant 20-30% reductions in nuclear division of the lymphocytes consistent with reduced cell proliferation and cytotoxicity suggesting sufficiently high concentrations were included for testing.

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study Title: Micronucleus Formation in Bone Marrow of Mice Treated With Nifurtimox or Benznidazole (Gorla and Castro 1985)

Key Study Findings:

- Treatment with nifurtimox in two doses 12 hours apart in oral gavage dosages of 600, 1200, and 2000 mg/kg significantly increased bone-marrow micronucleus formation by at least 2-fold at all the dosage levels. However nifurtimox administered by the intraperitoneal route in two administrations of 1200 mg/kg did not increased micronucleus formation.

GLP compliance: No

Test system: Swiss mice, male, 9-11 weeks old administered nifurtimox in two doses 24 hours apart by oral gavage or intraperitoneal injection.

Study is valid: The validity of the study was not addressed in the literature report. However, the percent incidence of micronuclei in the four different negative control groups used in the study (2 different negative control groups for oral and intraperitoneal dosing in the nifurtimox and benznidazole experiments, respectively) ranged from 1.6% to 3.3% suggesting reasonable consistency. The micronuclei results for the three doses of oral nifurtimox were significantly higher than control values with values of 6.9%, 7.2%, and 7.2% for the 600, 1200, and 2000 mg/kg doses respectively indicating consistent results in excess of control values and supporting the sensitivity of the system.

Study Title: Thirteenfold Increase of Chromosomal Aberrations Nonrandomly Distributed in Chagasic Children Treated With Nifurtimox (Gorla et al. 1989)

Key Study Findings:

- In 6 children (ages 7 months to 5 years) with Chagas disease treated with 12-15 or 60 mg/kg/day nifurtimox for 60 days (note the dose was listed as 12-15 or 60 mg/kg/day in different sections of the report), the mean frequency of chromosomal aberrations (23.5±9.5 aberrations per 100 cells) in peripheral lymphocytes, was significantly higher than the mean frequency of chromosomal aberrations in 8 chagasic children (ages 1-14 years) before treatment with nifurtimox (1.7±1.3 aberrations per 100 cells).

GLP compliance: No

Test system: Peripheral lymphocytes in human chagasic children 7-months to 14-years old.

Study is valid: The validity of the study was not directly addressed in the literature report. Negative control subjects (chagasic children before treatment with nifurtimox) were included for comparison with the effects of nifurtimox orally administered for 60 days to chagasic children. Notably the negative control subjects did not receive treatment with a placebo control. Also, it is not clear how the degree of Chagas infection (blood titers, length of infection)

compared between the groups. However, two subjects (Subjects 1 and 2) were included in both groups and served as their own controls.

Study Title: Sister-Chromatid Exchange in Splenic Lymphocytes of Mice After Exposure to Nifurtimox or Benznidazole (Gorla 1987)

Key Study Findings:

- Sister chromatid exchange is a measurement of genotoxicity in the form of chromatid DNA breakage and recombination.
- A single dose of nifurtimox administered to adult male mice by oral gavage produced a dose-dependent increase in the mean number of sister chromatid exchanges (SCE)/splenic lymphocyte, and the highest dose of 2000 mg/kg nifurtimox produced a significantly higher mean value (7.7±1.4 SCE/cell) compared to the mean value for the negative control group (4.3±1.3 SCE/cell).

GLP compliance: No

Test system: Splenic lymphocytes from male Swiss mice, 2-3 months old

Study is valid: The study validity was not directly assessed in the literature manuscript. Negative control animals received a single oral dose of vehicle (1% sodium carboxymethyl cellulose), and the resulting values for the number of sister chromatid exchanges per splenic lymphocyte were very similar for all the negative control animals indicative of a consistent baseline. Also, the positive control agent, nitrogen mustard administered intravenously in a dose of 2.5 mg/kg, increased the incidence of SCE to 12.0±1.4 SCE/cell approximately 3-fold compared to negative control value (4.3±1.3 SCE/cell) confirming the sensitivity of the test system.

Study Title: Lack of Sensitivity of Sister-Chromatid Exchange for Lymphocyte Chromosomal Damage Detection Caused by Antichagasic Treatment (Gorla et al. 1991)

Key Study Findings:

- Daily oral treatment of chagasic children with 12-15 mg/kg nifurtimox for 60 days did not result in a significant increase in sister chromatid exchange (SCE) formation in peripheral lymphocytes. The mean incidence of SCE/lymphocyte analyzed in 30-35 cells per patient was not different in the negative control (5 untreated chagasic children) and nifurtimox treatment (3 chagasic children treated with nifurtimox) groups with values of 6.7±1.9 and 8.6±2.9 respectively.

GLP compliance: No

Test system: Peripheral lymphocytes obtained from chagasic children before or after treatment with nifurtimox.

Study is valid: The study validity was not directly assessed in the literature manuscript. Negative control subjects (chagasic children before treatment with nifurtimox) were included for

comparison with the effects of 60 days of nifurtimox in chagasic children. Notably, the negative control subjects did not receive a placebo control. Also, it is not clear how the degree of Chagas infection (blood titers, length of infection) compared between the groups. One limitation of the study was that the nifurtimox treatment group included only 3 patients.

Other Genetic Toxicity Studies

Study: Genotoxicity Testing of Antiparasitic Nitrofurans in the Drosophila Wing Somatic Mutation and Recombination Test (Moraga and Graf 1989)

Key Study Findings:

- Nifurtimox and other structurally related nitrofuran compounds were tested for genotoxicity in the somatic mutation and recombination test in Drosophila wing cells. This assay has been reported to detect somatic mutation and mitotic recombination.
- Three-day-old Drosophila larvae that received nifurtimox in liquid feed over 48 hours exhibited concentration-dependent increases (up to 4-times higher than negative control values) in total wing spots in adult flies consistent with a positive finding for genotoxicity in the form of mitotic recombination and somatic mutation.

GLP compliance: No

Test system: Drosophila fruit flies

Study is valid: The literature report did not directly assess study validity criteria. In the manuscript, it was noted the spontaneous mutation frequency of 0.28 for total wing spots in the negative control group compares favorably with other control data obtained with the same solvent as published in a cited paper (Würgler et al. 1985). One deficiency in the study was the absence of a positive control. However, another nitrofuran, Compound ada, produced similarly positive results in the assay suggesting the sensitivity of the assay. Also, the highest test concentrations of nifurtimox and Compound ada were toxic leading to delayed development of treated flies and more than 50% mortality in each group suggesting sufficiently high concentrations were included for testing.

5.4.4. Carcinogenicity

Because the proposed clinical duration of treatment with nifurtimox is less than 6 months, nonclinical carcinogenicity assessments are not recommended. However, three carcinogenicity studies for nifurtimox were submitted in NDA 213464, two in brief study reports and one in a published manuscript. One of the studies was a 2-year study in rats, another was a shorter-term study in rats (variable durations of treatment up to 40 weeks) involving the use of initiators and promoters, and the third was a 60-day study in mice. The reports present mixed positive and negative results for nifurtimox-related carcinogenicity. While the submitted carcinogenicity studies except the 60-day mouse study fall within the general categories of studies recommended in the ICH S1B Guidance, all the studies as well as the submitted study reports

have deficiencies that limit a comprehensive review and diminish the adequacy of the studies to serve as acceptable carcinogenicity studies. Because the carcinogenicity studies and the study reports submitted to NDA 213464 have significant deficiencies, the results of the studies are not recommended for inclusion in the product label for Lampit. This has been communicated to the Applicant. However, other findings including the largely positive genotoxicity findings for nifurtimox and carcinogenicity findings for other nitrofuran antimicrobials more definitively suggest a carcinogenicity potential for nifurtimox in humans. Based on these factors, nifurtimox is considered to have the potential to be carcinogenic in humans and will be appropriately identified as such in the labeling.

5.4.5. Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Study Title: Histological Investigations on Mice Testes After Feeding With BAYER 2502 or Furacin (Study Report No.: PH-1025, study conducted in 1968)

Also described in: Toxicological Investigations on the Tolerability of Nifurtimox (Hoffmann 1972)

Reviewer Comment: *This study was conducted in 1968 and did not include fertility assessments. The study report was just 6 pages long and had limited evaluation of data presented in a summary format. Mortality, clinical signs, and body weights were not assessed or reported. Only the results for BAYER 2502 (nifurtimox) are summarized below; the results for furacin are not summarized.*

Key Study Findings

- Male mice fed 0.08% or 0.16% BAY 2502 (nifurtimox) for 14 days experienced dose-dependent testicular toxicity including complete inhibition of spermatogenesis with the high nifurtimox exposure, evidence of arrested mitosis, signs of pyknosis, and no mature sperm. However, interstitial cells were unchanged, and fibrosis and inflammatory infiltrates were not observed. Nine weeks after the end of nifurtimox exposure, all testicular effects were almost entirely reversed.

Conducting laboratory and location: Farbenfabriken Bayer AG, Institute of Exp. Pathology

GLP compliance: No

Table 12. Methods, Histological Investigations on Mice Testes After Feeding With BAYER 2502 or Furacin

Method	Details
Dose and frequency of dosing:	0, 0.08% and 0.16% BAY 2502 (nifurtimox) and 0.08% furacin in the feed for 14 days.
Route of administration:	Oral in feed
Formulation/vehicle:	Mouse feed
Species/strain:	Male CF_1 mice, body weight 18-24 grams.
Number/sex/group:	6-10 animals per timepoint for BAY 2502 and 1-4 animals per timepoint for furacin
Satellite groups:	None
Study design:	Male mice were fed 0.08% or 0.16% BAY 2502 (nifurtimox) or furacin for 14 days, then groups of animals (2-10 animals per timepoint for BAY 2502 and negative control animals and 1-8 animals per timepoint for furacin) were sacrificed on the last day of dosing, one day after the end of dosing, 2 weeks after the end of dosing, 4 weeks after the end of dosing, or 9 weeks after the end of dosing.
Deviation from study protocol affecting interpretation of results:	The study report did not address deviations from the study protocol, but deviations are not expected to have altered the study results.

Table 13. Observations and Results, Histological Investigations on Mice Testes After Feeding With BAYER 2502 or Furacin

Parameters	Major Findings
Mortality	Not reported
Clinical signs	Not reported
Body weights	Not reported
Necropsy findings *[Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc]*	BAY 2502 (nifurtimox) Testes from animals treated with 0.16% nifurtimox for 14 days then sacrificed on the last day of treatment (n=6) or one day after the end of treatment (n=10) exhibited no mature sperm and complete inhibition of spermatogenesis. Other findings included: serial appearance of defective and pyknotic mitotic figures and repeated appearance of giant cells in the upper layers of tubular epithelium. In individual tubules only the layer of primary spermatogonia were observed, but no fibrosis or inflammatory infiltrates were observed, and the interstitial cells appeared unchanged. In animals (n=10) treated with 0.08% BAY 2502 for 14 days then sacrificed one day after the end of treatment, the histological appearance showed inhibition of spermatogenesis as described above. However, a few morphologically intact mitotic figures were repeatedly observed in tubular epithelium, and in some tubules the layering of the epithelium was normal and fully mature with morphologically intact sperm. In untreated control animals (n=10) sacrificed one day after the end of treatment, testicular findings were essentially normal

Parameters	Major Findings
	except there were isolated pyknoses and giant cells in tubular epithelium. Animals fed 0.08% or 0.16% nifurtimox (total of 13 animals) for 14 days then sacrificed 2 weeks after the end of treatment showed nuclear degeneration in tubular epithelium with giant cells as well as only the lower layer of spermatogonia in some tubules. But in most tubules, triple or quadruple cell layers with numerous pyknotic mitotic figures interspersed with normal cell layers were noted with normally formed sperm in several tubules. Germinal epithelium remained thinned overall. In testes from animals fed 0.08% (n=10) or 0.16% (n=2) nifurtimox then sacrificed four weeks after the end of treatment, germinal epithelium was essentially normal in all testes, but with a somewhat reduced sperm count. Other adverse findings included isolated pyknotic spermatogonia in individual tubules and occasional giant cells. In testes from animals (n=2) fed 0.08% nifurtimox then sacrificed nine weeks after the end of treatment, the testicular tissue appeared normal and not distinguishable from control tissues. In testes from animals (n=2) fed 0.16% nifurtimox then sacrificed nine weeks after the end of treatment, the testicular tissue appeared normal with no inhibition of spermatogenesis but with a conspicuously large number of giant cells in the lumen of some tubules. However, a relatively large number of giant cells were also noted in the upper regions of testicular epithelium from two control animals.

Abbreviations: LD: low dose; MD: mid dose; HD: high dose

Study Title: Embryotoxicity Studies of Nifurtimox in Rats and Mice and Study of Fertility and General Reproductive Performance (Lorke 1972)

Reviewer Comment: *This study briefly described in a literature publication included direct measurements of male and female fertility. Limited information was presented and deficiencies included imprecise determination of the possibly variable daily nifurtimox doses based on feed consumption.*

Key Study Findings:

- Male fertility was completely inhibited in rats administered 30-60 mg/kg/day nifurtimox in feed for 10 weeks.
- Female fertility was not affected for female rats undergoing the same dosing regimen.
- In another study, 11 weeks after the end of dosing, fertility was still completely inhibited in 75% of male rats administered nifurtimox for 32 weeks.

Conducting laboratory and location: Institute of Toxicology, BAYER A.G., Wuppertal-Elberfeld, Germany

GLP compliance: No

Table 14. Methods, Male and Female Fertility Study, Lorke (1972)

Method	Details
Dose and frequency of dosing:	Main study: nifurtimox doses of 0, 150 ppm (equivalent to 7-15 mg/kg), 300 ppm (equivalent to 15-30 mg/kg/day), and 600 ppm (equivalent to 30-60 mg/kg/day). Male and female rats received nifurtimox ad libitum in their food for 10 weeks before mating. Recovery study: Male rats were dosed with 600 ppm nifurtimox (equivalent to 30-60 mg/kg/day) for 32 weeks then maintained without treatment for 4 and 11 weeks before mating with untreated females to assess the degree of recovery from nifurtimox-related male infertility.
Route of administration:	Oral in feed
Formulation/vehicle:	No vehicle, the negative control group received no nifurtimox mixed into their food.
Species/strain:	FB 30 (Long Evans) rats
Number/sex/group:	17-20/sex/group
Satellite groups:	None
Study design:	In the main study, male and female rats received nifurtimox for 10 weeks mixed into their food then the animals were mated and female dams were permitted to litter and keep their offspring for 2 weeks. In the recovery study, male rats were dosed for 32 weeks, then left untreated for 4 and 11 weeks before mating with untreated females.
Deviation from study protocol affecting interpretation of results:	Study protocol deviations were not discussed in the literature report. However, it is expected that study deviations did not affect the study results.

Table 15. Observations and Results, Male and Female Fertility Study, Lorke (1972)

Parameters	Major Findings
Mortality	No male or female animals died during the study.
Clinical signs	HD rats receiving 600 ppm (30-60 mg/kg/day) nifurtimox were less active.
Body weights	HD rats receiving 600 ppm nifurtimox had reduced body weights compared to control rats. HD males and females weighed approximately 8 and 10% less than control males and females respectively at the end of dosing. Weight loss continued after dosing with HD males and females weighing approximately 6% and 11% less than control male and females respectively 22 weeks after the end of dosing.
Necropsy findings *[Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc]*	Main study: Only fertility was assessed in males and females. **Males:** Control, LD, and MD males impregnated 95-100% of the females with which they were paired, but HD males had a 0% fertility rate. **Females:** The pregnancy rate for females in the negative control, LD, MD and HD groups was 100%, 100%, 95%, and 90% respectively. Recovery study: Fertility was assessed in HD males dosed for 32 weeks with 600 ppm (30-60 mg/kg/day) nifurtimox. Four weeks after the end of dosing only 4/17 HD males were able to impregnate untreated females. Eleven weeks after the end of dosing, only 4/16 HD males were able to impregnate untreated females. These results are consistent with persistent infertility in the majority of males treated with 600 ppm (30-60 mg/kg/day) nifurtimox after recovery periods of 4 and 11 weeks.

Abbreviations: LD: low dose; MD: mid dose; HD: high dose

Embryo-Fetal Development

Study Title: BAYER 2502: Experiments to Evaluate Embryonic Effects in the Rat (Study No.: 31498; Study Report No.: PH-1457)

Also published in: Embryotoxicity Studies of Nifurtimox in Rats and Mice and Study of Fertility and General Reproductive Performance (Lorke 1972).

Reviewer Comment: *The rat embryo-fetal study was conducted in 1971 and reported in a 14-page study report and a 5-page literature manuscript. Several deficiencies in the methods and reporting for this study limit the acceptability of the study and the degree to which the study can be comprehensively reviewed. In the brief description of methods employed in this study, it is clear that some of the methods are inconsistent with current recommendations. Nifurtimox treatment groups in this study included 10 pregnant females/group which is less than the minimum of 16 pregnant rodents/group recommended in the ICH S5(R3) Guideline. One unusual finding, the absence of any malformations in any control or treatment fetuses in the study may have resulted because too few litters were examined to detect rare malformations. Based on the brief reports provided for this study, it is not clear what methods or criteria were used to evaluate fetal malformations and variations. Finally, a major factor limiting a comprehensive review of the study is the brevity of the study reports. Detailed information regarding methods and results is not available. Also, neither report includes historical control data.*

Key Study Findings:

- Nifurtimox administered to pregnant female rats by oral gavage from GD 6 to GD 15 reduced maternal body weights in a dose-dependent manner with significant reductions of body weight in LD, MD, and HD groups of approximately 11, 18, and 34% respectively compared to the mean body weight for control females.
- Mean values for fetal body weights in the MD and HD groups were significantly reduced by approximately 9% and 16% compared to control values, and the reduced body weights were associated with reduced maternal body weights.
- The NOAEL for maternal and fetal toxicity was considered to be the low dose of 20 mg/kg/day.

Conducting laboratory and location: Farbenfabriken Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany

GLP compliance: No

Table 16. Methods, Rat Embryo-Fetal Study, Lorke (1972)

Method	Details
Dose and frequency of dosing:	0, 20, 50, and 125 mg/kg nifurtimox, administered once per day from Gestation Day (GD) 6 to GD 15
Route of administration:	Oral gavage
Formulation/vehicle:	Nifurtimox was suspended in 1% tragacanth
Species/strain:	Rat Strain FB 30 (Long Evans)
Number/sex/group:	10-11 animals/group
Satellite groups:	None
Study design:	Following mating (insemination detected by vaginal smears), pregnant female rats (10-11 per group) received BAY 2502 (nifurtimox; 0, 20, 50, and 125 mg/kg) daily by oral gavage from the 6th to 15th day of gestation. On GD 20, the animals underwent Cesarean section.
Deviation from study protocol affecting interpretation of results:	Study protocol deviations were not addressed in the brief study report. However, deviations from the study protocol were not considered to have altered the study results.

Table 17. Observations and Results, Rat Embryo-Fetal Study, Lorke (1972)

Parameters	Major Findings
Mortality	All of the dams survived until the end of the study.
Clinical signs	Dams receiving the high dose of nifurtimox (125 mg/kg/day) exhibited shaggy coats, rapid breathing, and less activity than control animals. MD dams receiving 50 mg/kg/day nifurtimox were similarly but less severely affected. Control and low-dose dams reportedly did not exhibit clinical signs.
Body weights	Mean values for maternal body weight at necropsy were significantly reduced in LD, MD, and HD groups by approximately 11, 18, and 34% respectively compared to the mean body weight for control females on GD 20. Nifurtimox was associated with a dose-dependent reduction in maternal body weight gains between GD 6 and 15. Mean values for weight gain were significantly reduced in the MD and HD nifurtimox groups by 34% and 82%, respectively.

Parameters	Major Findings
Necropsy findings Cesarean Section Data	No significant changes in the number of implantations, fetuses, or resorptions were observed. The gestation rates for the control, LD, MD, and HD groups were 100%, 100%, 100% and 90.9%, respectively.
Necropsy findings Offspring	Mean fetal weights were reduced in a nifurtimox dose-dependent manner and significantly reduced in the MD and HD groups by 9% and 16%, respectively, compared to control values. Also the number of fetuses with slight bone changes and the number of small fetuses (runts) tended to increase in a nifurtimox dose-dependent manner but not to a significant degree. Although historical control data was not included with the study report, the authors reported the mean values for slight bone changes and runts to be within control values. No fetal malformations were reported in any group.

Abbreviations: LD: low dose; MD: mid dose; HD: high dose

Study Title: Embryotoxicity Studies of Nifurtimox in Rats and Mice and Study of Fertility and General Reproductive Performance (Lorke 1972)

Reviewer Comment: *The mouse embryo-fetal study was conducted in 1971 and reported only in a 5-page literature manuscript. Several deficiencies in the methods and reporting for this study limit the acceptability of the study and the degree to which the study can be comprehensively reviewed. In the brief description of methods employed in this study, it is clear that some of the methods are inconsistent with current recommendations. Nifurtimox treatment groups in this study included 10 pregnant females/group which is less than the minimum of 16 pregnant rodents/group recommended in the ICH S5(R3) Guideline. One unusual finding, the absence of any malformations in any control or treatment fetuses in the study may have resulted because too few litters were examined to detect rare malformations. Based on the brief report provided for this study, it is not clear what methods or criteria were used to evaluate fetal malformations and variations. Finally, a major factor limiting a comprehensive review of the study is the brevity of the study report. Detailed information regarding methods and results is not available. Also, historical control data is not included.*

Key Study Findings:

- No fetal malformations were reported for any group in the mouse embryo-fetal study.
- The mean fetal weights but not placental weights were significantly reduced by 7.5% in the nifurtimox high-dose group compared to control values.
- The mean number of stunted fetuses tended to increase in the high-dose group, but not to a significant degree.

Conducting laboratory and location: Farbenfabriken Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany

GLP compliance: No

Table 18. Methods, Mouse Embryo Study, Lorke (1972)

Method	Details
Dose and frequency of dosing:	0, 20, 50, and 125 mg/kg nifurtimox, administered once per day from Gestation Day (GD) 6 to GD 15
Route of administration:	Oral gavage
Formulation/vehicle:	Nifurtimox was suspended in 1% tragacanth
Species/strain:	Mice/NMRI strain
Number/sex/group:	10-14 female mice
Satellite groups:	None
Study design:	Following mating (insemination detected by vaginal smears), pregnant female rats (10-14 per group) received BAY 2502 (nifurtimox; 0, 20, 50, and 125 mg/kg) daily by oral gavage from the 6th to 15th day of gestation. On GD 18, the animals underwent Cesarean section.
Deviation from study protocol affecting interpretation of results:	Study protocol deviations were not addressed in the brief study report. However, deviations from the study protocol were not considered to have altered the study results.

Table 19. Observations and Results, Mouse Embryo Study, Lorke (1972)

Parameters	Major Findings
Mortality	No maternal mortality was reported for the study
Clinical signs	No clinical signs were reported. In the manuscript, the mice are reported to be lively and appearing healthy.
Body weights	Mean maternal body weight gain over the course of dosing from GD 6 to GD 15 was decreased in a nifurtimox dose-dependent manner, but not to a significant degree with respective values of 14.4 g, 13.7 g, 12.6 g, and 12.3 g for the control, LD, MD, and HD groups.
Necropsy findings Cesarean section data	In the Cesarean section data for 14, 10, 10, and 10 pregnant mice in the control, LD, MD, and HD groups, mean values for the number of implantations, fetuses and resorptions were not significantly altered by treatment with nifurtimox.
Necropsy findings Offspring	The mean fetal weights but not placental weights were significantly reduced in the nifurtimox HD group by 7.5% compared to control values. The mean number of fetuses with minor bone changes was similar in all groups while the number of stunted forms tended to increase but not to a significant degree in the HD group. No fetal malformations were reported for any group.

Abbreviations: LD: low dose; MD: mid dose; HD: high dose

Study Title: Lampit: Developmental Toxicity Study in Rabbits After Oral Administration (Study No.: T6063160; Study Report No.: PH-33192, Study Conducted in 2003)

Key Study Findings:

- Nifurtimox at a dose of 60 mg/kg/day produced a dose-dependent increase in abortions and maternal toxicity in the form of significantly reduced food consumption and body weights.

- Consistent with the maternal toxicity, the mean number of live fetuses/litter and the percent of live fetuses per total implantations per group were significantly lower in the mid- and high-dose groups compared to the control group.
- A skeletal malformations (fused caudal vertebral bodies) was increased in the nifurtimox low-dose group with fetal and litter incidences that exceeded the historical control range for fetal and litter incidence for the years 1998 to 2008.

Conducting laboratory and location: Bayer HealthCare AG, PH-PD-P-HCT-Experimental Toxicology, D-42096 Wuppertal, Germany

GLP compliance: Yes

Table 20. Methods, Study T6063160

Method	Details
Dose and frequency of dosing:	0, 5, 15, and 60 mg/kg/day nifurtimox administered once per day
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% aqueous Tylopur (methylhydroxyethylcellulose)
Species/Strain:	Himalayan rabbits/CHBB:HM strain
Number/Sex/Group:	20 females/group
Satellite groups:	Toxicokinetic evaluation: three groups (n = 3/group) receiving 5, 15, and 60 mg/kg nifurtimox respectively.
Study design:	Female rabbits were mated with male rabbits then females were administered nifurtimox by oral gavage from GD 6 to GD 20. Cesarean sections were performed on surviving females on GD 29. The drug that was used in the study was: Lampit Mikrofein 20 MCM (nifurtimox), Batch No.: BXA00A7, measured purity of the active ingredient is reported as being 100.2%
Deviation from study protocol affecting interpretation of results:	Deviations from the study protocol were reported. However, none was considered to have altered the results or compromised the integrity of the study.

Table 21. Observations and Results, Study T6063160

Parameters	Major Findings
Mortality	No females died, but those experiencing abortion were euthanized early. Eight HD (60 mg/kg/day) females aborted on GDs 17 (1 female), 19 (1), 20 (1), 21 (1), 22 (3), and 26 (1) and one LD female aborted on GD 21. The abortions in high-dose females were considered to be related to nifurtimox administration, but the abortion in the single low-dose female was not considered to be clearly related to nifurtimox treatment due to a lack of dose dependency and because spontaneous abortions are known to occur in the strain of rabbits used in the study.
Clinical signs	The dams that aborted and were euthanized previously exhibited severely decreased food intake, severe body weight loss (up to -467 grams in one female), cold ears, alopecia, decreased water consumption, reduced, soft or light colored feces. In addition, one LD (5 mg/kg/day) female aborted on GD 21 after exhibiting similar clinical signs. A majority of HD females (18 including those with abortion) exhibited reduced feces and/or soft feces (17 females in total) and/or light colored feces (10 females in total). Also HD females exhibited light yellow urine and or orange discoloration of the urine.

Parameters	Major Findings
Body weights	Nifurtimox treatment resulted in significantly lower weight gain in females with viable fetuses in the HD group during the dosing period (GD 6 – 20) as well as for the entire gestational period in HD females compared to control females. Body weight corrected by subtracting the gravid uterus weight on GD 29 was also reduce in HD dams by 97% compared to control females, but not to a significant degree (see table below).

Body Weight Gain in Females With Viable Fetuses in the Rabbit Embryo-Fetal Study

Category of Weight Gain and Observation Period	Nifurtimox Doses (mg/kg/day) 0	5	15	60
Abs. weight gain (g) GD 6 – GD 20	180.0	170.3	145.7	-106.0** (-159%)
Abs. weight gain (g) GD 0 – GD 29	448.8	437.2	441.1	282.3** (-37%)
Corrected weight gain (g) GD 0 – GD 29[a]	61.1	99.3	119.1	1.8 (-97%)

Source: Study report no.: PH-33192
The percent decrease in weight gain in HD dams compared to control values is shown in parentheses.
[a] Corrected for gravid uterus weight
** p<0.01

Necropsy findings Cesarean section data	Placental findings included a higher incidence of partly necrotic placenta (litter incidence of 16.7%) and coarse grained placenta (litter incidence of 8.3%) in the HD group compared to control values (litter incidences of 5.6% and 0.0%, respectively). Although the HD values were within the historical control range for control groups in studies conducted in the years 1998 to 2008 (placenta partly necrotic: litter incidence of 0 to 22.2%; coarse grained placenta: 0 to 20%), a treatment effect cannot be excluded because a total of 3 HD litters were affected out of a comparably small number of litters (12) due to abortions in the HD group. Placental weights tended to increase with nifurtimox dose, but not to a significant degree. The fertility rate (percentage of mated females with implantations), the mean number of corpora lutea, and the number of implantations in the nifurtimox groups were not significantly different from control values. Trends toward reduced implantations and increased preimplantation loss were evident for the HD group; however, the differences were not significant (see table below titled “Cesarean Section Data for the Rabbit Embryo-Fetal Study”). The percent postimplantation loss per group increased in a dose-dependent manner with significant increases in the MD (11.2%) and HD (12.7%) groups compared to the concurrent control value (1.5%), but the percent postimplantation loss per litter was not significantly increased for the same nifurtimox groups. The percent of postimplantation loss per group and litter in all the nifurtimox treatment groups were within the historical control range (1.9 to 13.0% postimplantation loss/group; 0.1 to 1.4% postimplantation loss per litter). The mean number of live fetuses/litter and the percent of live fetuses per total implantations per group and litter were significantly lower in the MD and HD groups compared to the concurrent control group. Although values were within the historical control range, the significant reductions occurred in a clear dose-dependent manner and are considered related to nifurtimox administration. Fetal sex distribution and fetal weights were similar in all groups (see table below).

Parameters	Major Findings

Cesarean Section Data for the Rabbit Embryo-Fetal Study

Category		Nifurtimox Dose Groups (mg/kg/day) 0	5	15	60	Historical Control Range (1998-2008)[a]
Number of females with viable fetuses (exclusive of females with abortions)		18	17	18	12	----
Mean placental weight (g)		4.41	4.71	4.73	4.93	3.86–5.29
Mean corpora lutea per female		8.1	7.0	6.8	7.1	7.6–9.9
Mean preimplantation loss per female		0.6	0.5	0.4	1.2	0.2–1.6
Mean implantations per female		7.5	6.5	6.4	5.9	6.6–9.0
Mean fetal weight (g)		37.20	39.86	40.42	38.74	32.36–42.67
Mean % males	Per group	45.9	57.3	61.2	43.5	41.7–60.1
	Per litter	46.2	56.1	63.5	41.2	41.2–59.2
Mean live fetuses	Per group	133	103	103	62	----
	Per litter	7.4	6.1	5.7*	5.2**	5.2–8.2
Mean live fetuses as a % of total implantations	Per group	98.5	92.8	88.8**	87.3**	78.4–98.1
	Per litter	98.7 ± 3.8	93.5 ± 10.9	87.2* ± 15.8	85.9* ±19.0	81.6–98.1
Mean postimplantation loss	Total per group	2	8	13**	9**	3–27
	Number of litters affected	2	5	9	6	----
Mean (± SD) postimplantation loss as a % of total implantations	Per group	1.5	7.2	11.2**	12.7**	1.9–21.6
	Per litter	0.1 ± 0.32	0.5 ± 0.80	0.7 ± 1.02	0.8 ± 0.97	0.1–1.4

Source: Study report no. PH-33192

[a] Historical control data was obtained from the same facility where the study was conducted and for the same strain of rabbits. Historical control data for the years 2002 and 2005 was not available.

* Significantly different compared to control values (p<0.05)

** Significantly different compared to control values (p<0.01)

Necropsy findings
Offspring

External, Visceral, and Skeletal Malformations

The total percentages of fetuses and liters with external, visceral, and skeletal malformations was increased in the HD group (8.1% of fetuses with malformations; 33.3% of litters with malformations) compared to the vehicle control group (3.8% of fetuses with malformations; 11.1% of litters with malformations). However, the HD values fell within the historical control range (0.7 to 13.4% control fetuses with malformations; 4.5 to 47.6% control litters with malformations) for both measurements. Also the percent of litters with malformations for the LD group (29.4%) was higher than that for the MD group (11.1%) and similar to the HD value indicating a lack of nifurtimox dose-dependency.

Findings from the rabbit embryo-fetal study include one fetal malformation that occurred with an elevated litter incidence in the LD group relative to the concurrent control group and comparable historical control values provided in the study report for the years 1998-2008 (exclusive of the year 2005 where historical control data were not provided) which encompass 5 years before and after the year the rabbit embryo-fetal study was conducted (2003). One skeletal malformation, fusion of caudal vertebral bodies, occurred in 3 LD fetuses (fetal incidence of 2.9%) in 3 litters (litter incidence of 17.6%) which, respectively, exceed the fetal and litter incidences for the concurrent control group (1 fetus) and the historical control ranges (fetal incidence range of 0 – 1.8%; litter incidence range of 0 – 11.1%). Two other malformations, ventral septal defect and malpositioned forelimbs occurred in nifurtimox treatment groups with fetal and litter incidences above the incidence values in the concurrent control group. However, the fetal and litter incidences of both of these malformations were within the historical control range for the fetal and litter incidences for control groups in the years 1998 to 2008. These results suggest the occurrence of fused caudal vertebral bodies was related to nifurtimox administration in LD fetuses (see the tables below).

Fetal External, Visceral, and Skeletal Malformations in the Rabbit Embryo-Fetal Study

Malformation	Dose (mg/kg b.w./day)			
	0	5	15	60
1st sacral vertebral arch left looks like a lumbar vertebral arch/7th lumbar vertebral arch right looks like the 1st sacral vertebral arch, pelvis left/right shift to caudal/cranial			1	1
12th rib is missing right, one presacral vertebra is missing		1		
4th rib is bifurcated right, 4th-5th ribs right are fused in the cartilaginous part of the ribs				1
supernumerary presacral vertebra	4 (1)			
fusion of caudal vertebral body(ies)	1	3 (3)	1	
eye ball reduced in size and reddish discolored left, orbital cavity reduced in size left		1		
malposition of forelimb(s)			1	3 (2)
ventricular septal defect of the heart			2 (2)	1
number of fetuses per group	133	103	103	62
number of fetuses with malformations	5	5	3	5
malformed fetuses per group (%)	3.8	4.9	2.9	8.1
number of litters per group	18	17	18	12
number of litters with malformations	2	5	2	4
malformed litters per group (%)	11.1	29.4	11.1	33.3

() number of litters affected

Source: Study report no. PH-33192

The Fetal and Litter Incidences for Malformations Occurring With Increased Incidences in Nifurtimox Treatment Groups and Historical Control Ranges

Fetal Malformation	Incidence Category	Highest Incidence in Study No.: T6063160	Historical Control Range (1998–2008)[a]
Fusion of caudal vertebral bodies	Fetus	2.9% (3/103)	0–1.8%
	Litter	17.6% (3/17)	0–11.1%
Ventricular septal defect	Fetus	1.9% (2/103)	0–4.4%
	Litter	11.1% (2/18)	0–21.1%
Malposition of forelimbs	Fetus	4.8% (3/62)	0–5.9%
	Litter	16.7% (2/12)	0–21.1%

[a] No historical control data was available for the year 2005
Source: Study report no. PH-33192

External, Visceral, and Skeletal Variations

Only one visceral deviation was noted, several white punctiform areas in the renal pelvis in one low-dose fetus. Fetal examinations for skeletal variations revealed a significant increase in the incidence of right and left 8th caudal vertebral arches and an increased incidence of incomplete ossification of the 16th caudal vertebral body in LD fetuses when the calculation was performed on a fetal basis but not on a litter basis (see table below). In the absence of dose dependency for any of the 8th vertebral arch or 16th vertebral body variations and a lack of significant changes on a litter basis, the variations was not considered to be related to nifurtimox administration. Also the incidence of the 8th vertebral arches on a fetal basis was within the historical control ranges for fetal (40.3–65.7%) and litter (77–100%) incidences in the year 2000 historical control data. Significantly retarded ossification of the frontal and parietal bones occurred in the HD group when calculation was performed on a fetal basis but not on a litter basis, but both values were within the historical control range (1.5–23.2% for frontal bone incomplete ossification and 0.0–13.1% for parietal bone incomplete ossification)

Fetal Skeletal Variations in the Rabbit Embryo-Fetal Study

		Nifurtimox Dose Group (mg/kg/day)			
Category		**0**	**5**	**15**	**60**
Number of fetuses		133	103	103	62
Number of litters		18	17	18	12
Medial phalanx digits (R and L) - incompletely ossified	Fetuses	2 (1.5)	1 (1.0)	4 (3.9)	3 (4.8)
	Litters	1 (5.6)	1 (5.9)	2 (11.1)	3 (25.0)
8th caudal vertebral aches (L and R) - present	Fetuses	50 (37.6)	59* (57.3)	45 (43.7)	28 (45.2)
	Litters	16 (88.9)	16 (94.1)	18 (100)	9 (75)
16th caudal vertebral aches - present	Fetuses	7 (5.3)	17* (16.5)	14 (13.6)	10 (16.4)
	Litters	5 (27.8)	9 (52.9)	5 (27.8)	4 (33.3)
16th caudal vertebral bodies	Fetuses	3 (2.3)	14** (13.6)	9 (8.7)	6 (9.8)

Parameters	Major Findings					
	– incompletely ossified	Litters	2 (11.1)	8 (47.1)	3 (16.7)	4 (33.3)
	Incompletely ossified frontal bone -bilateral	Fetuses	4 (3.0)	5 (4.9)	1 (1.0)	11** (17.7)
		Litters	3 (16.7)	5 (29.4)	1 (5.6)	4 (33.3)
	Incompletely ossified parietal bone - bilateral	Fetuses	0 (0.0)	2 (1.9)	3 (2.9)	4* (6.5)
		Litters	0 (0.0)	2 (11.8)	2 (11.1)	3 (25)

Source: Study report no. PH-33192
The percent fetal and litter incidences are shown in parentheses.
* = p<0.05; ** = p<0.01
Abbreviations: L, left; R, right

Abbreviations: LD: low dose; MD: mid dose; HD: high dose

Prenatal and Postnatal Development

Study Title: Nifurtimox: Oral (Gavage) Study of Pre- and Postnatal Development in the Rat/Study No.: T102196-9; Study Report No.: R-11265. (Study Initiated on August 4, 2015)

Key Study Findings

- Maternal body weight gains were significantly reduced by 14% and 32% in mid- and high-dose animals by the end of dosing. Also, maternal body weights were significantly reduced by 10% at the end of dosing and daily food consumption was reduced by 15-25% compared to control values during dosing.
- During the lactation period (birth to PND 21) and the postlactation period (PND 21 to PND 65) mean body weights were significantly reduced by 9% to 17% in high-dose males and females. However, the physical development, neurological measurements and reproduction of F1 offspring were not substantially changed in the nifurtimox treatment groups.
- Slightly small testes in conjunction with either small seminiferous tubules or epididymides were observed in 1/20, 4/20, and 3/20 F1 males, respectively, in the low-, mid-, and high-dose groups.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 22. Methods, Study T102196-9

Method	Details
Dose and frequency of dosing:	0 mg/kg/day (vehicle control: Group 1); 15 mg/kg/day (Low dose: Group 2); 30 mg/kg/day (Mid dose: Group 3); and 60 mg/kg/day (High dose: Group 4). Dosing frequency is once daily.
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% Tylose 300 in water
Species/strain:	Rat/Crl:WI (Han)
Number/sex/group:	22 females/group
Satellite groups:	Toxicokinetic animals: 3 females in Group 1, 9 females/group in Groups 2, 3, and 4
Study design:	Four groups of time-mated female rats were administered oral vehicle or nifurtimox in doses of 15, 30, and 60 mg/kg/day from Gestation Day (GD) 6 to Lactation Day (LD) 21.
Deviation from study protocol affecting interpretation of results:	Multiple deviations in the study protocol were recorded. However, none of the deviations are considered to have altered the results or the integrity of the study.

Table 23. Observations and Results, Study T102196-9

Generation	Major Findings
F0 dams	**Survival:** No maternal toxicity was observed. **Clinical signs:** No nifurtimox-related clinical signs were observed. **Body weight** <u>Gestation (GD 6 to GD 21):</u> Maternal body weights were reduced in a dose-dependent manner. MD females tended to have less body weight gain during dosing and overall body weight gain between GD 6 and 21 was significantly reduced by approximately 14% compared to control values. In HD females significantly reduced body weight gain was observed between GD 7 and 9 by 97% and by 32% between GD 6 to 21 compared to control values. Beginning on GD 13 mean body weights for HD dams were significantly reduced by 5% with incrementally greater reductions each day compared to control values until the end of dosing on GD 21 when mean body weights were significantly reduced by 10% compared to control values. <u>Lactation period:</u> Body weight gain was similar in all groups during lactation so that the reduction in maternal weights that HD and MD animals demonstrated during gestation remained reduced during the lactation period. Maternal body weights for the HD group during the lactation period were consistently significantly reduced by approximately 6-9% compared to the control group. Maternal body weights in the MD group tended to be lower than control values by up to 5% throughout the lactation period, but the reductions reached statistical significance on only 3 of 21 days. **Feed consumption** <u>Gestation (GD 6 to GD 21):</u> Daily food consumption was significantly reduced in a dose-dependent manner by approximately 15%-25% for almost all of the daily measurements for HD dams from GD 6 to GD 21 compared to control values. MD dams tended to eat less food on most but not all days during gestion with up to approximately 12% less food consumption compared to control values. Reduced food

Generation	Major Findings
	consumption in MD dams was significantly lower than control values on only 3 days during gestation. Lactation period: During the lactation period, HD females continued to eat significantly less food on a daily basis ranging from approximately 12-27% less food consumption compared to control values. In the MD females, daily food consumption was significantly lower ranging from approximately 7-20% less food consumption than the control group until Lactation Day (LD) 6 then similar to control values in the following lactation days. **Parturition:** A total of 22/22, 19/22, 21/22, and 22/22 females in Groups 1, 2, 3, and 4 respectively became pregnant and all the pregnant females in each group delivered live pups. The live birth index ranged from 97.5 to 100% for all the groups. No still born pups occurred in any of the groups and the mean number of pups born per female was similar in each group. **Uterine content:** All of the uterine content parameters were similar in all groups.
F1 generation	**Survival** All of the F1 pups born alive survived until the planned termination. **Clinical signs** On the day of birth, a greater number of HD animals were small or missing and presumed cannibalized (6 HD litters compared to 2 control litters), but the results could be related to reduced body weights in HD offspring. During subsequent days in the lactation period, none of the observed clinical signs in F1 offspring were considered to be related to maternal nifurtimox administration. **Body weight** Lactation Period: The mean body weights for male and female F1 offspring in the HD group were significantly reduced for each sex and for the combined sexes from PND 1 until the end of lactation on postnatal day (PND) 21 compared to control values. The reduction in body weights for the combined sexes consistently ranged from approximately 11% to 17% on each measurement day compared to control values over this period. Postlactation period: Following the lactation phase, the mean male and female body weights in F1 offspring in the HD group were significantly reduced compared to control values. Mean body weights were reduced by 11% and 9% in HD males and females respectively. Body weights were significantly reduced until PND 65. Mean body weight gain in F1 offspring following the lactation period was similar in all groups, and by PND 78 (the end of the maturation phase), mean male and female body weights in the HD group were nonsignificantly reduced by approximately 5% compared to control values. **Feed consumption** Food consumption was not measured in F1 offspring during or after the lactation period. **Physical development** Lactation period: Nifurtimox had no adverse effect on developmental milestones including surface righting on Day 1, pinna unfolding, incisor eruption, eye opening, and air righting reflex on PND 17.

Generation	Major Findings
	Postlactation period: All animals demonstrated pupillary light reflex on PND 23. Females in the MD and HD groups tended to attain vaginal opening later than control females although the difference was not significant. The mean day of attainment of vaginal opening was PND 34 in MD and HD offspring while it was PND 33 in control and LD females. Males in all the nifurtimox treatment groups demonstrated a 1-2 day delay in attainment of balano-preputial separation compared to control males. Males in the LD and HD groups attained separation on PND 45 which was significantly later than the PND Day 43 separation for control males. Males in the MD group attained separation on PND 44. On the respective days of attainment, mean body weights were similar between groups. **Neurological assessment** Motor activity: No significant differences between groups were observed for motor activity over 30 minutes for males or females. Morris water maze: No nifurtimox-related significant changes in any of the learning and memory parameters were observed. The parameters that were not changed included: latency to the platform, path length and swim speed in the spatial learning test over 4 days; cued learning (mean latency to the platform), reference memory in the probe trial (time spent in the platform quadrant and entries into the platform zone). Auditory startle response: There were no significant effects of maternal nifurtimox administration on male or female mean startle amplitude or prepulse inhibition. **Reproduction** Males: The mating index and fertility index values for F1 males were similar in all groups. Mating index values were 100%, 95%, 90%, and 95%, and fertility index values were 90%, 95%, 85%, and 90% for Groups 1-4, respectively. Females: The mating index and fertility index values for F1 females were similar in all groups. Mating index values were 100% for all groups and fertility index values were 90%, 100%, 95%, and 95% for Groups 1-4, respectively. There were no nifurtimox dose-related effects on the mean number of implantations, pre- or postimplantation losses, early and late resorptions, or the mean number of live F2 fetuses. The mean number of corpora lutea for F1 females in the HD group was slightly lower than the controls (12.8 compared to 14.2 for controls) but the difference was not statistically significant, and the effect may have been related to lower body weights of the F1 females in the HD group through the maturation phase (PND 22 to PND 78). **Other** Gross pathology Males: 1, 4, and 3 F1 males in the LD, MD, and HD groups, respectively, exhibited small testes as well as either small seminal vesicles or small epididymides. These findings did not appear to be related to body weight for the afflicted animals which with the exception of one animal had body weights with the control range. Females: No gross pathology findings in F1 females were considered to be related to maternal nifurtimox administration
F2 generation	The mean number of live F2 generation fetuses was similar in the control and nifurtimox treatment groups.

5.4.6. Other Toxicology Studies

None.

6. Clinical Pharmacology

6.1. Executive Summary

The clinical pharmacology review of this NDA focused on (A) assessment of the proposed age and weight based nifurtimox dosing, (B) assessment of the relative bioavailability (BA) of nifurtimox from different Lampit tablet formulations, and (C) the proposed drug product labeling for Lampit.

6.1.1. Recommendations

The Office of Clinical Pharmacology (OCP) has reviewed the information provided in NDA 213464 for Lampit (Nifurtimox) and recommends approval of this NDA.

Table 24. Review Issues and Recommendations for NDA 213464

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Pivotal evidence of effectiveness is derived from a historically controlled prospective Phase 3 study (Study 16027, also referred to as the CHICO study, Part 1) that evaluated the safety and efficacy of nifurtimox in children aged 0 to <18 years with Chagas disease. The Applicant also conducted four Phase 1 studies: Study 16004: Comparative BA study with tablet formulations with different strengths Study 16005: Food effect study Study 16007: Comparative BA study with 30-mg tablet formulations with different dissolution rates Study 19500: Comparative BA study with different tablet formulations
General dosing instructions	The proposed nifurtimox dosing regimen is based on a patient's body weight and age as given below: **Applicant Proposed Nifurtimox (NFX) Dosing Regimen** **BW Group** — **TDD of NFX (mg/kg BW)** [redacted] (b) (4) (≥40 kg)[a] — 8-10 [redacted] (b) (4) (<40 kg) — 10-20 [a] [redacted] (b) (4) [b] Term newborn with body weight of ≥2.5 kg Abbreviations: BW, body weight; NFX, nifurtimox; TDD, total daily dose Lampit tablets are administered orally three times a day with food for 60 days. The Applicant's proposed nifurtimox dosing regimen based on both weight and age is not acceptable from a clinical pharmacology perspective. The Clinical Pharmacology review team recommends the following weight based nifurtimox dosing regimen for pediatric patients (birth[a] to <18 years of age):

Review Issue	Recommendations and Comments
	Recommended Nifurtimox (NFX) Dosing Regimen **BW Group** / **TDD of NFX [mg/kg BW]** ≥40 kg: 8-10 <40 kg: 10-20 [a] Term newborn with body weight ≥2.5 kg Abbreviations: BW, body weight; NFX, nifurtimox; TDD, total daily dose The proposed dosing frequency, treatment duration, and dosing instructions are acceptable, i.e., Lampit tablets are administered orally three times a day with food for 60 days.
Dosing in patient subgroups (intrinsic and extrinsic factors)	Clinical studies to assess the effect of renal or hepatic impairment on the pharmacokinetics (PK) of nifurtimox have not been conducted. The Applicant proposes that Lampit be administered under close medical supervision in patients with renal and/or hepatic impairment. The Applicant's proposal is acceptable. The Applicant's rationale for the proposed contraindication appears reasonable, however, the proposed language is not acceptable. The Clinical Pharmacology and Clinical reviewers recommend contraindication of Lampit in patients who do not agree to abstain from alcohol use during treatment.
Labeling	The content and format of the proposed labeling language is not aligned with the current labeling guidance and has been revised.
Bridge between the to-be-marketed and clinical trial formulations	Not applicable as the to-be-marketed formulation was used in the Phase 3 study. The CHICO study utilized 30-mg tablet formulations with varying degrees of dissolution rates. The Applicant has conducted a BA study to evaluate the relative BA of nifurtimox 30-mg tablets with different in vitro dissolution rates that encompasses the dissolution rates of clinical trial formulations. Findings from this study show that the 30 mg clinical trial tablet formulations with varying dissolution characteristics have comparable BA.

6.1.2. Postmarketing Requirements and Commitments

None.

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

Lampit tablets are single-scored tablets with two different strengths containing 30 mg or 120 mg nifurtimox. Dosing in pediatric patients who are unable to swallow tablets can be facilitated by dissolving tablets in water to form a slurry as an alternative method of administration.

The Applicant has conducted the following four single-dose Phase 1 studies in adult Chagas patients to characterize the BA of nifurtimox with different formulations and dosing preparations:

- Study 16004: Comparative BA study with four 30-mg tablets, four 30 mg nifurtimox tablets as aqueous slurry, and one 120-mg tablet
- Study 16005: Food effect study
- Study 16007: Comparative BA study with formulations of different dissolution characteristics
- Study 19500: Comparative BA study with different formulations

Findings from the abovementioned studies are summarized below.

Bioavailability Assessment

Strengths and Dosing Preparations

Absolute BA of nifurtimox has not been evaluated. Study 16004 compared nifurtimox BA following a single 120 mg nifurtimox dose administered as a single 120-mg tablet, four 30-mg tablets, and an aqueous slurry prepared from four 30-mg tablets under fed conditions in adult Chagas patients. BA assessment findings from Study 16004 are summarized in Table 25. See Section 20.4.3.1 for additional details on the study design and findings.

Table 25. Bioavailability Assessment, Study 16004

	% Point Estimates (90% CI)	
Parameters	**4x30 mg Aqu. Slurry/4x30-mg Tablets**	**4x30-mg Tablets/1x120-mg Tablet**
AUC_{Last} (mcg·h/L)	93 (84–103)	105 (99–111)
C_{max} (mcg/L)	77 (69–85)	102 (89–116)

Source: Reviewer's analysis
Abbreviations: Aqu, aqueous; AUC_{Last}, area under the concentration versus time curve up to last concentration timepoint; CI, confidence interval, C_{max}, maximum plasma concentration

Study 16004 demonstrated comparable BA of nifurtimox with the 30 mg and 120-mg tablets. The study results also showed that the nifurtimox BA was slightly lower with the slurry preparation (7% lower AUC and 23% lower C_{max}) compared to tablets. The Applicant proposes that for pediatric patients who cannot swallow tablets, nifurtimox tablets can be dissolved in water and administered as a slurry as an alternate method of administration. Information regarding whether subjects received nifurtimox as tablets or as a slurry was not captured in the CHICO study. Given that it is reasonable to assume younger patients (age <6 years) enrolled in the CHICO study may have received nifurtimox treatment as a slurry, PK variability was compared between this subgroup and patients ≥6 years of age. The post hoc mean AUC estimates (CV%) for these two subgroups were 2960 mcg·h/L (42%) and 2270 mcg·h/L (49%), respectively. Therefore, the observed slightly lower BA of nifurtimox with the slurry preparation compared to tablets is considered to be clinically insignificant.

Effect of Dissolution Rate

The CHICO study utilized the 30-mg tablet formulation batches with varying degrees of dissolution rates. The Applicant conducted Study 16007 to evaluate the relative BA of nifurtimox 30-mg tablets with different in vitro dissolution rates (slow, medium, and fast) under fed conditions in adult patients with chronic Chagas disease.

The range of the dissolution rates for the 30-mg tablets used in this study encompasses that of the clinical batches of 30-mg tablets used in the CHICO study. Findings from this study show that the 30-mg tablets with varying dissolution characteristics have comparable BA (Table 26). See Section 20.4.3.3 for additional details on the study design and findings.

Table 26. Bioavailability Assessment, Study 16007

	% Point Estimates (90% CI)	
Parameters	**4 x 30-mg Tablets Fast Dissolution/Slow Dissolution**	**4 x 30-mg Tablets Medium Dissolution/Slow Dissolution**
AUC_{Last} (mcg·h/L)	110 (102 - 118)	103 (97-111)
C_{max} (mcg/L)	105 (93 - 118)	101 (89-114)

Source: Reviewer's analysis
Abbreviations: AUC_{Last}, area under the concentration versus time curve up to last concentration timepoint; CI, confidence interval; C_{max}, maximum plasma concentration.

Effect of Food

Study 16005 evaluated the effect of food on nifurtimox BA and PK parameter estimates in the presence and absence of food (a high-calorie/high-fat meal). Systemic exposure to nifurtimox was increased by 68-72% when administered with a high-calorie/high-fat meal compared to exposures when administered under fasted conditions (Table 27). See Sections 20.4.3.2 for additional details on the study design.

Table 27. Comparison of Mean (CV%) Estimates for Pharmacokinetic Parameters Following Single Dose of 120 mg (4x30 mg) Nifurtimox Tablets Under Fed and Fasted Conditions

Study	Fasted/Fed Condition	N	AUC_{Last} (mcg·h/L)	C_{max} (mcg/L)	T_{max}[a] (h)	$T_{1/2}$ (h)
16005	Fasted conditions	35	1390 (40.6)	277 (36.7)	3 [0.5-6]	3.07 (34.6)
	Fed conditions	35	2390 (21.7)	465 (33.4)	4 [1-8]	3.13 (27.4)

Source: Study 16005 report
[a] Median [Range]
Abbreviations: AUC_{Last}, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum plasma concentration; h, hours; N, number of patients; $T_{1/2}$, elimination half-life.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The nifurtimox dosing regimen proposed by the Applicant is based on a patient's body weight and age as summarized in Table 28.

Table 28. Applicant Proposed Total Daily Nifurtimox Dosages (Given in Three Divided Doses With Meals)

Body Weight Group	TDD of Nifurtimox [mg/kg Body Weight]
(b) (4) (≥40 kg)[a]	8-10
(b) (4) (<40 kg)	10-20

[a] (b) (4)

[b] Term newborn with body weight of ≥2.5 kg
Abbreviations: TDD, total daily dose

The proposed nifurtimox dosing regimen (listed above) was compared to the nifurtimox dosing regimen used in the CHICO study where the pivotal evidence of effectiveness is derived. The comparison showed discrepancies between the proposed nifurtimox dosing regimen and the actual dosing regimen evaluated in the CHICO study. Specifically, the nifurtimox dosing regimen used in the CHICO study was based on only body weight stratification rather than both age and body weight stratifications as proposed by the Applicant. The Clinical Pharmacology review team deems the nifurtimox dosing is acceptable and recommends using the body weight-only cutoff of 40 kg to determine nifurtimox dosing for pediatric patients (Table 29) based on the following considerations:

- Using both age and body weight cutoffs would complicate the dosing instructions for "outlier patients", i.e., pediatric patients (b) (4) years of age weighing ≥40 kg, and pediatric patient (b) (4) years of age weighing <40 kg. (b) (4) no specific dosing instruction for pediatric patients (b) (4) years of age weighing >40 kg was provided.
- Nifurtimox dosing as evaluated in the CHICO study was determined using the body weight stratification of 40 kg regardless of the age of pediatric patients. This weight-based dosing was supported by the efficacy and safety results from the CHICO study. In addition, limited data suggest no discernible differences in efficacy and safety among those "outlier" patients receiving nifurtimox dosing based on the body weight only stratification.
- Age and body weight are highly correlated in pediatric patients. The Applicant's PK simulation analysis showed nifurtimox dosing either based on an age- or a body weight-based stratification would result in comparable nifurtimox exposures for pediatric patients.

- The dose-response (D-R) and exposure-response (E-R) analyses provide supportive evidence for the proposed dosing regimen. The D-R and E-R analyses showed that serological response generally increased with decreasing age and increasing nifurtimox dose/exposure. However, increased nifurtimox dose and exposure were also associated with higher incidence of abdominal pain, particularly in older pediatric patients (>8 years).
- Overall, the available information supports nifurtimox dosing based on a body weight-only stratification of 40 kg.

Table 29. Review Team Recommended Total Daily Nifurtimox Dosages (Given in Three Divided Doses With Meals)

Age	Body Weight Group	Total Daily Dose of Nifurtimox (mg/kg)
Pediatric patients (birth[a] to <18 years)	≥40 kg body weight	8-10
	<40 kg body weight	10-20

[a] Term newborn with body weight of ≥2.5 kg

See Section 6.3.2 for details on the assessment of the proposed nifurtimox dosing.

Therapeutic Individualization

Alcohol Consumption

Based on the chemical class of nifurtimox, i.e., nitrofurans and nitroheterocyclic compounds, use of Lampit is contraindicated in patients who do not agree to abstain from alcohol use during treatment.

Renal and Hepatic Impairment

The effect of renal and hepatic impairment on nifurtimox PK is unknown. Lampit should be administered under close medical supervision in patients with impaired renal and or hepatic function(s).

Outstanding Issues

None.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

The available clinical pharmacology and PK information for nifurtimox is summarized in Table 30. The Applicant has not conducted a human mass balance study using radiolabeled nifurtimox. In addition, the effect of renal or hepatic impairment on nifurtimox PK has not been evaluated. The Applicant has conducted exploratory assessments to characterize nifurtimox's disposition and excretion in humans and the findings from these assessments are summarized in Table 30.

Table 30. Clinical Pharmacology and Pharmacokinetic Summary

Pharmacodynamic Information	
Cardiac Electrophysiology: QT-IRT Review (Date: 05/04/2020) notes that the quality of ECG data submitted from Study 16027 is suboptimal and QT data from a placebo or a positive control arm are not available. Overall, the conclusion from the QT-IRT review is that data are not adequate to exclude a 10-msec mean increase in QTc, i.e., the regulatory threshold in ICH E14 guideline. QT assessment of the available limited data suggest no large QTc prolongation effect (>20 msec) due to nifurtimox exposure from a therapeutically relevant dose.	
Pharmacokinetic Information and Parameter Estimates	
Mean (SD, range) C_{max} (mcg/L) following a single 120 mg nifurtimox oral dose under fasted conditions [a]	295 (109, 145–604)
Mean (SD, range) AUC_{Last} (mcg·hr/L) following a single 120 mg nifurtimox oral dose under fasted conditions [a]	1496 (567, 619–2773)
Absorption	
Median (range) T_{max} (hr) [a]	3 (0.5-6)
Effect of food: With high-fat meal[b] (relative to fasting)	↑68% in C_{max} ↑72% AUC_{Last} ↑T_{max} by 0.5 hr
Distribution	
Protein binding	42%
Elimination	
Mean (SD, range) $T_{1/2}$ (hr)	3.15 (1.1, 1.5–7.1)
Metabolism	
Metabolic pathways	Findings from in vitro studies with human hepatocytes and other test systems suggest the following: • Nifurtimox is not a substrate of CYP or UGT enzymes • Nifurtimox is metabolized primarily by nitroreductases. In human plasma, two major pharmacologically inactive metabolites (M-4, M-6) and several minor metabolites were identified. M-4 is postulated to be a rearranged cysteine conjugate of nifurtimox with a half-life of approximately 28 hours, and M-6 is postulated to be formed by hydrolytic cleavage of the hydrazone moiety with a half-life of approximately 10 hours.

Excretion	
Major route of elimination	Exploratory investigations showed that approximately 44% of the administered dose was recovered in urine mainly as metabolites following administration of a single 120 mg nifurtimox dose to adult Chagas patients under fed conditions. The estimated mean recovery for nifurtimox, M-4, and M-6 in human urine within 24 hours of dosing were 0.07%, 12%, and 21%, respectively.

[a] C_{max}, AUC_{Last}, T_{max}, and $T_{1/2}$ estimates are from the Reviewer's analysis derived from PK data in 35 adult Chagas patients who received a single oral dose of 120 mg nifurtimox (4 x 30-mg tablets) under fasted conditions (Study 16005)
[b] Changes in C_{max}, AUC_{Last}, and T_{max} estimates are from the Reviewer's analysis from a crossover food-effect study in 35 adult Chagas patients who received a single oral dose of 120 mg nifurtimox (4 x 30-mg tablets) with a high-fat, high calorie meal consisting approximately 800 to 1000 kcal in total (approximately 500 to 600 kcal from fat, 150 kcal from protein, and 250 kcal from carbohydrate) (Study 16005)
↑ indicates increase
Abbreviations: AUC_{Last}, area under the concentration versus time curve up to last concentration timepoint, C_{max}, maximum plasma concentration, $T_{1/2}$, elimination half-life, T_{max}, time to reach C_{max},

A cross-study comparison of nifurtimox PK parameter estimates were performed from various BA studies and findings are reported in Table 31. Following a single 120-mg dose with food, mean AUC estimates of nifurtimox ranged from 1842-2450 mcg·h/L with the 120-mg tablet and 1676-2670 mcg·h/L with four 30-mg tablets. Mean C_{max} estimates ranged from 425-509 mcg/L with the 120-mg tablet and 355-568 mcg/L with four 30-mg tablets. Across all the studies, mean estimates for the elimination half-life ranged from 2.42 to 3.61 hours. See Sections 20.4.3.1-20.4.3.4 for additional details on the study designs and findings for these studies.

Table 31. Cross-Study Comparison of Mean (CV%) Estimates for Pharmacokinetic Parameters Following Single Dose of 120 mg Nifurtimox

Study	Dosage Form/Formulation	N	AUC_{Last} (mcg·h/L)	C_{max} (mcg/L)	T_{max}[a] (h)	$T_{1/2}$ (h)
16004	4x30-mg tablets	12	2670 (19.2)	568 (26.4)	4 [2-6]	3.3 (11.6)
	4x30 mg aqueous slurry	12	2490 (23.5)	434 (31.2)	4 [2-8]	3.61 (37.3)
	4x30-mg tablets	24	2560 (26.3)	518 (40.2)	4 [2-6]	2.63 (23.1)
	1x120-mg tablet	24	2450 (25.6)	509 (37.9)	4 [2-6]	2.85 (30.2)
16005	4x30-mg tablets under fasted conditions	35	1390 (40.6)	277 (36.7)	3 [0.5-6]	3.07 (34.6)
	4x30-mg tablets under fed conditions	35	2390 (21.7)	465 (33.4)	4 [1-8]	3.13 (27.4)
16007	4x30-mg tablets with fast in vitro dissolution	36	1844 (26)	372 (31)	3 [2-6]	2.7 (27)
	4x30-mg tablets with medium in vitro dissolution	36	1738 (28)	358.5 (44.7)	4 [0.75-6]	2.61/23.95
	4x30-mg tablets with slow in vitro dissolution	36	1676 (32)	355 (43)	4 [1.5-8]	2.8 (34)
	1x120-mg tablet	12	1842 (32)	425 (50)	3 [2-6]	2.42 (32)
19500[b]	1x120-mg tablet	23	2140 (21)	455 (31)	4 [2-8]	3.09 (31)

Source: Compiled from study reports.
[a] Median [Range]
[b] Study 19500 evaluated and compared nifurtimox BA from the clinical trial formulation of 120 mg nifurtimox tablet and a newly developed 120 mg nifurtimox tablet formulation; however, the PK parameter estimates reported in the table are for the clinical trial formulation only
Abbreviations: AUC_{Last}, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum plasma concentration; h, hours; N, number of patients; $T_{1/2}$, elimination half-life.

6.3.2. Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

The pivotal evidence of effectiveness for this NDA submission is derived from a Phase 3 study, i.e., the CHICO study. The clinical pharmacology program provides supportive evidence of effectiveness in terms of the proposed nifurtimox dosing. See the response to the next question for additional details.

Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

The Applicant's proposed nifurtimox dosing regimen is based on a patient's weight and age for the treatment of Chagas disease (American Trypanosomiasis) caused by *Trypanosoma cruzi (T. cruzi)* in pediatric patients less than 18 years of age (Table 32). The Clinical Pharmacology review team recommends a weight only-based nifurtimox dosing regimen for this patient population (Table 33). The rationale for the review team's recommendation is summarized in the next section.

Table 32. Applicant-Proposed Total Daily Nifurtimox Dosages (To Be Given in Three Divided Doses With Meals)

Age and Body Weight Group	Total Daily Dose of Nifurtimox (mg/kg)
(b) (4) (≥40 kg)	8-10
(b) (4) (<40 kg)	10-20

[a] (b) (4)

[b] Term newborn with body weight of ≥2.5 kg

Table 33. Review Team Recommended Total Daily Nifurtimox Dosages (Given in Three Divided Doses With Meals) by the Clinical Pharmacology Review Team

Age	Body Weight Group	Total Daily Dose of Nifurtimox (mg/kg)
Term newborns[a] to <18 years	≥40 kg body weight	8-10
	<40 kg body weight	10-20

[a] Term newborn with body weight of ≥2.5 kg

Assessment of the Applicant Proposed Age and Body Weight Cutoffs for Nifurtimox Dosing

The proposed nifurtimox dosing regimen was compared to the nifurtimox dosing regimen used in the CHICO study.

There are discrepancies between the proposed nifurtimox dosing and the dosing evaluated in the CHICO study. (b) (4) . The actual nifurtimox doses received

by patients in the CHICO study are presented in Table 34, which show that the main separation in dose level appears to be driven by the weight cutoff rather than the age cutoff, i.e., the majority of patients weighing 40 kg or more received the adolescent dose (orange dots) while the majority of patients weighing less than 40 kg received the children dose (blue dots). Of note, certain deviations from the weight-based dosing is inevitable due to limitations in the formulation strengths (30 and 120-mg tablets).

Table 34. Demographics and Administered Dose of Patients Enrolled in the CHICO Study

Age (yr)	Weight (kg)	Dose (mg/kg/day)	n
<12	<40	<10	12
		≥10	164
	≥40	<10	13
		≥10	0
≥12	<40	<10	5
		≥10	15
	≥40	<10	108
		≥10	13

Source: Reviewer's analysis
The number of patients enrolled in the CHICO study are enumerated by groups of age, weight, and nifurtimox dose with cutoffs for each variable selected based on the Applicant's proposed dosing.

Based on these observations, it appears that the nifurtimox dosing used in the CHICO study was based on body weight stratification only rather than both age and body weight stratifications as proposed by the Applicant.

Exposure/Dose-Response Analysis Supporting Nifurtimox Dosing

A population PK (popPK) model was developed for nifurtimox using PK data in pediatric patients from the CHICO study. The popPK analysis showed that pediatric patients <2 years of age had higher steady state AUC compared to older pediatric patients, due to higher doses (10-20 mg/kg/day) administered and lower weight-normalized clearance in pediatric patients <2 years as shown in Figure 1. Although patients <12 years of age received nifurtimox doses of 10-20 mg/kg/day, patients <2 years of age generally received higher doses relative to patients 2-12 years of age potentially because fewer tablet strengths were available for the youngest patients.

Figure 1. Comparison of Nifurtimox AUC by Age Group

Source: Reviewer's analysis

Exposure-response (E-R) and dose-response (D-R) analyses were conducted for efficacy (i.e., serological response) and safety. In the CHICO study, serological response to nifurtimox treatment was determined as 20% reduction in optical density or seroconversion to negative using the total purified antigen (TPA) ELISA and the recombinant ELISA at 1-year post-treatment follow-up. Both ELISA tests showed superiority in favor of the nifurtimox 60-day arm compared to the nifurtimox 30-day arm. In addition, the seroconversion rate using the F29 ELISA test is numerically higher in the 60-day nifurtimox treatment arm compared to the 30-day nifurtimox treatment arm at 1-year post-treatment follow-up.

The E-R and D-R analyses demonstrate that age and dose/exposure are generally correlated with serological response. The percentage of patients achieving serological response generally decreased as the age of patients increased possibly because of the duration of infection or immunological response to pathogen. In addition, increases in nifurtimox dose and AUC stratified by age were associated with improved serological response assessed using the TPA (only in the D-R analysis) and recombinant assays (in both the D-R and E-R analyses), but not the F29 assay. Note that the calculation method used for the F29 assay endpoint (seroconversion only) relative to the TPA assay and recombinant assay endpoints (≥20% decrease in optical density or seroconversion depending on age) may contribute to the observed difference between these assays (see Section 8 for more details). However, increased nifurtimox dose and AUC were also associated with higher incidence of abdominal pain, particularly in patients 8 years of age and older (see Section 20.4.4 for more details). Based on the D-R analysis, adolescent patients (≥12 years) weighing at least 40 kg may benefit from a nifurtimox dose higher than [redacted] (b) (4) (8-10 mg/kg/day) for efficacy, but only a limited number of patients (13 of 121) in this age and weight group received a nifurtimox dose

≥10 mg/kg/day (Table 34). In addition, increasing the dose for adolescents may cause treatment compliance issues in a 60-day treatment course due to potential increases in the incidence of adverse reactions (e.g., abdominal pain).

Given that: 1) age and body weight are highly correlated in pediatric patients, and 2) nifurtimox dosing using a body weight only stratification was evaluated in the CHICO study and also supported by the efficacy and safety results, the Clinical Pharmacology review team recommends that the nifurtimox dosing regimen be determined using body weight stratification only (Table 33).

Assessment of Additional Nifurtimox Dosing Instructions Proposed by the Applicant

The Applicant has proposed to adjust doses if body weight decreases during treatment due to loss of appetite or nausea/vomiting. The Applicant's proposal is reasonable as body weight has been identified as a significant covariate affecting the PK of nifurtimox. See the PPK Modeling subsection in Section 20.4.4.2.3 for additional details.

The Applicant has also proposed (b) (4) However, the Applicant did not provide any specific analysis or data supporting the proposed dose adjustment criterion. Therefore, the Clinical Pharmacology review team recommends against the inclusion of the criterion (b) (4)

Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

Body weight and age are discussed above.

Gender

During the review of pooled PK data from adults across all studies, it was noted that nifurtimox exposures were higher in female patients (~28%↑) compared to male patients. The Applicant postulated that this observation was due to differences in body weight between females and males when the same dose of nifurtimox was given. The Clinical Pharmacology review team's exploratory analysis suggested that in addition to the extent of nifurtimox absorption, the absorption rate also varied between females and males among various Lampit formulations. The difference in the absorption phase (Study 16004) is illustrated in the figure below.

Figure 2. Mean (±SE) Nifurtimox Concentrations in Plasma (Linear Scale on Y-Axis With log_2 Scale on X-Axis) in Group 2 (N=12), Study 16004

Source: Reviewer's analysis

Similar trends were observed in Study 16005 and Study 19500 with relatively slower/reduced absorption in males compared to females. In Study 16007, a similar trend was observed for patients receiving a 120-mg tablet, but not in other treatment groups. The Applicant postulates that these observations are due to physiological factors impacting rate of absorption leading to the occurrence of "early" and "late" absorbers and/or due to imbalanced distribution in enrolled male versus female patients. The potential reasons for the observed gender differences in nifurtimox absorption provided by the Applicant appear reasonable. Population PK analysis using data from the CHICO study did not identify gender as a covariate that affected nifurtimox PK in pediatric patients. Given nifurtimox dosing for pediatric patients is based on body weight, the observed gender differences in nifurtimox absorption rate from the adult trials are not considered to have a meaningful impact on the overall nifurtimox exposure and the clinical outcome, especially in a multiple dose setting.

Hepatic and Renal Impairment

The elimination pathway of nifurtimox has not been adequately characterized. The effect of renal and hepatic impairment on nifurtimox PK is unknown. The Applicant proposes that nifurtimox be administered under close medical supervision in patients with chronic renal and/or hepatic impairment. The proposed indication is for pediatric patients and the prevalence of renal and hepatic impairment is much lower in pediatrics compared to adults. Therefore, the Applicant's proposal of administering nifurtimox under close medical supervision for patients with renal or hepatic impairment is acceptable.

Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

Yes, there are clinically relevant food-drug interactions with Lampit.

Food Effect

The food-effect study (Study 16005) demonstrated that a high-calorie and high-fat meal increased nifurtimox C_{max} and AUC by 68% and 72%, respectively, compared to fasting conditions. Lampit should be administered with food to mitigate gastrointestinal related side effects. Of note, Lampit was given with food in the CHICO study. See Section 20.4.3.2 for additional details on the design and findings of the food effect study.

Drug-Drug Interactions

Based the nifurtimox's chemical class, i.e., nitrofurans and nitroheterocyclic compound, alcohol consumption may increase the incidence and severity of undesirable effects. Therefore, Lampit's use is contraindicated in patients who do not agree to abstain from alcohol use during treatment. There are no other known drug-drug interaction potentials with nifurtimox treatment. In vitro studies suggest that nifurtimox is not a substrate of P-gp or BCRP, and nifurtimox does not inhibit P-gp, BCRP, and OATP transporters at clinically relevant concentrations. Nifurtimox is not a substrate, inhibitor, or inducer of drug metabolizing enzymes (e.g., CYPs, UGTs). Clinical drug-drug interaction studies with nifurtimox have not been conducted.

7. Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

Table 35. Listing of Clinical Trials Relevant to This NDA

Trial Identity	NCT No.	Trial Design	Regimen/Schedule/Route	Study Endpoints	Treatment Duration/ Total Study Duration	No. of Patients Enrolled	Study Population	No. of Centers and Countries
Controlled studies to support efficacy and safety								
16027	NCT 02625974	Phase 3, MC, R, DB, PG trial	NFX 60-day: Weight-based[1] oral nifurtimox for 60 days NFX 30-day: Weight-based[1] oral nifurtimox for 30 days followed by 30 days of placebo Placebo: Historical placebo treatment for 60 days (de Andrade et al. 1996; Sosa Estani et al. 1998)	≥20% reduction in optical density [OD] measured by two enzyme-linked immunosorbent assays [ELISAs], lysate ELISA and recombinant ELISA at 12 months compared to baseline in subjects ≥8 months to <18 years of age at randomization, or Seroconversion (defined as negative Immunoglobulin G [IgG] concentration) at 12 months in all subjects	60 days of Nifurtimox; 30 days of Nifurtimox and 30 days of placebo / 4 years total, but 420 days available in Part 1 (CHICO)	219 in 60-day; 111 in 30-day; 330 total	Pediatric patients aged 0 to <18 years with Chagas disease	25 centers in Argentina, Bolivia, and Colombia

Trial Identity	NCT No.	Trial Design	Regimen/Schedule/Route	Study Endpoints	Treatment Duration/ Total Study Duration	No. of Patients Enrolled	Study Population	No. of Centers and Countries
Other studies pertinent to the review of efficacy or safety (e.g., clinical pharmacological studies)								
16004	NCT 01927224	Phase 1, open-label, randomized, single-dose, crossover study	Nifurtimox 30 mg and 120-mg tablets (single oral doses)	PK of 30 mg and 120 mg oral tablet formulations	7 to 14 days after the last study drug administration	36	Adult subjects aged 18-45 years with chronic Chagas disease	1 center in Argentina
16005	NCT 02606864	Phase 1, open-label, randomized, single-dose, crossover study	Nifurtimox 30 mg and 120-mg tablets (single oral doses)	Effect of a high calorie/fat diet on PK	7 to 14 days after the last study drug administration	36		1 center in Argentina
16007	NCT 03350295	Phase 1, randomized, noncontrolled, open-label crossover study	Nifurtimox 30 mg and 120 mg tablet (single oral doses)	Relative bioavailability of 3 formulations of NFX	7 to 14 days after the last study drug administration	48		1 center in Argentina
19500	NCT 03708133	Phase 1, randomized, noncontrolled, open-label crossover study	Nifurtimox 120-mg tablet mg (single oral doses; old and new formulation)	Bioequivalence of new NFX oral tablet to old tablet	7 to 14 days after the last study drug administration	24		1 center in Argentina

Source: Reviewer Table

[1] 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.

Abbreviations: DB, double-blinded; MC, multicentered; PG, parallel-group; R, randomized

7.2. Review Strategy

The review of clinical efficacy and safety of NFX for the treatment of Chagas disease was conducted using Study 16027. Supplementary efficacy and safety data were obtained from a summary of literature studies obtained from the Applicant and reviewer literature search.

This NDA was submitted in eCTD format. Data sources include protocols, reporting and analysis plans, study reports, and data sets (in both Study Data Tabulation Model (SDTM) and Analysis Data Model (ADaM) formats). All submitted data sets are available at \\CDSESUB1\evsprod\NDA213464\0001.

Study 16027 provides the primary evidence of efficacy and safety of nifurtimox for this review. The efficacy comes from assessments made within the trial as well as assessments using an external control with limited patient level data provided by the Applicant.

The Applicant's primary efficacy analysis was conducted using combined results of the total purified antigen (lysate) ELISA test and a recombinant ELISA test. Because this analysis was considered difficult to interpret, further analyses were conducted to evaluate the relationship of the two tests and conclusions were based on the results of lysate and recombinant ELISA separately. The F29 ELISA test results were compared to a historical control to supplement the analyses.

Data Quality and Integrity

The datasets for Study 16027 (CHICO) and documentation were generally acceptable. It was possible to reproduce the Applicant's major analyses without complex manipulations.

8. Statistical and Clinical and Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. Study 16027

Trial Design

This study was a randomized, double-blinded, historically controlled, parallel group, Phase 3 trial to assess the safety and efficacy of nifurtimox in the treatment of Chagas disease in pediatric patients from birth to <18 years old. The study was conducted at 25 sites in Argentina, Bolivia and Colombia. A total of 300 patients were planned to be randomized in a ratio of 2:1 to

receive a 60-day regimen or a 30-day regimen of nifurtimox oral tablets, dosed as follows:

- For body weight <40 kg: 10-20 mg/kg/day in 3 divided doses
- For body weight ≥40 kg: 8-10 mg/kg/day in 3 divided doses

For patients <6 years old who were not able to swallow tablets, the tablets were dissolved in water to form a soft slurry and given in a teaspoon. Prior to randomization, patients were stratified according to their age group as follows:

- 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

Within each of the four age groups, patients were to be randomized into one of the two treatment groups. Patients randomized to the 30-day regimen received an additional 30 days of matching placebo to fully blind the trial.

***Reviewer Comment**: The protocol (after amendment 2) states: "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."*

The targeted minimum number of 38 subjects in each age stratum was not met in this trial.

Study Objectives

The primary objective was to assess the superiority of a 60-day regimen of nifurtimox to a historical untreated control at the 12-month follow-up (Visit 11, 360 days from the end of the 60-day treatment period) as measured by ≥20% decrease in optical density or seroconversion.

The secondary objectives were listed as follows:

- To assess the comparability of a 30-day regimen of nifurtimox to a 60-day regimen of nifurtimox in achieving ≥20% decrease in optical density or seroconversion 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), ECG monitoring, vital sign measurements (blood pressure, heart rate, respiratory rate, temperature), AE monitoring, and physical examinations, including neurological examinations
- To evaluate the PK/PD of nifurtimox in children receiving the drug for treatment of Chagas disease

The exploratory objectives included the following:

- To assess the comparability of a 60-day regimen of nifurtimox to historical active control (benznidazole) in achieving ≥20% decrease in optical density 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 qPCR at the 12-month follow-up (360 days from EOT)
- To evaluate the relationship of conventional serology (using ≥20% decrease in optical density or seroconversion) to qPCR using frequencies of matches and mismatches to assess agreement
- To evaluate the relationship of nonconventional serology to conventional serology
- To evaluate the relationship of conventional serology to IHA.

Details of the serological and parasitological methods will be discussed in the Clinical Microbiology section of this review.

Inclusion Criteria

The subject must meet all of the following criteria to be eligible for inclusion:

1) Male and female pediatric subjects aged 0 days to younger than 18 years
2) Chagas disease diagnosed/confirmed by:
    a) Subjects <8 months of age at randomization must demonstrate direct observation of *T. cruzi* by concentration test
    b) 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:
        i) Recombinant ELISA
        ii) Total purified antigen (lysate) ELISA
    c) Additionally, the F29 ELISA test and IHA were obtained; however, a positive result was not required for diagnosis.
    d) Subjects were also eligible if there was acceptable documentation (two ELISA tests) of positive Chagas disease within three months prior to screening and they had not had prior antitrypanocidal or antiparasitic 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).

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 were screened for the study.
4) Females of childbearing potential (i.e., female subjects who had 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.

Exclusion Criteria

Patients were required to have met none of the following exclusion criteria to be eligible for participation in the study:

1) Subjects aged 0 to 27 days who, at birth, were preterm (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 associated with Chagas disease, such as:
    a) Known evidence of Chagas disease-related cardiomyopathy/Chagas heart disease
    b) Known evidence of Chagas disease-related gastrointestinal dysfunction (e.g., megaesophagus, megacolon, or both) or Chagas digestive disease
    c) Serious manifestations of acute Chagas disease, including myocarditis, meningoencephalitis, or pneumonitis
    d) 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)
    e) 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:
    a) Hypersensitivity to nifurtimox or any hydantoin, or to any of the excipients
    b) Suspected or known porphyria
    c) Severe renal impairment defined by the following:
        i) 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)
        ii) For subjects ≥1 year to <18 years of age at randomization, eGFR <80% of LLN appropriate for age

d) Severe hepatic impairment (alanine aminotransferase [ALT] or aspartate aminotransferase [AST] considered clinically significant by the investigator)

e) History of brain injury, predisposition to seizures or epilepsy, psychiatric disease, or serious behavioral alteration

f) 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 were chronic abusers or current users of alcohol or recreational drugs, or the newborn infants of mothers who were 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 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 had treatment with any investigational medicinal product within 30 days before the first dose of study drug or had previously received the study treatment

11) Subjects who were pregnant or breastfeeding

12) Subjects of breastfeeding mothers under treatment with trypanocidal agents

13) Subjects who the investigator considered 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

15) Subjects living in housing conditions where there was no active or effective vector-control to *T. cruzi* reinfection as determined by Ministry of Health guidelines in each country

Study Visits

The visit schedule was designed as follows:

- Visit 1: Screening, Day -14 to -1
- Visit 2: Baseline, Day 1, randomization and drug dispensed
- Visit 3: On therapy, Day 7 (±1 day), lab tests
- Visit 4: Telephone visit, Day 14 (±1 day)
- Visit 5: Telephone visit, Day 21 (±3 days)
- Visit 6: On therapy, Day 30 (±3 days), lab tests
- Visit 7: Telephone visit, Day 42 (±3 days)
- Visit 8: End of Treatment (EOT), Day 60 (±3 days), lab tests
- Visit 9: Follow-up, Day 90 (±7 days), lab tests
- Visit 10: Follow-up, Day 240 (±7 days), lab tests
- Visit 11: Follow-up (12-months following EOT), Day 420 (±7 days), lab tests

Study Endpoints

The primary efficacy variable was a ≥20% reduction in optical density in subjects ≥8 months to <18 years of age at randomization or seroconversion (defined as negative IgG concentration in all subjects) at 12 months post-treatment using two ELISA serologic tests: total purified antigen (lysate) ELISA and recombinant ELISA. Seroconversion or ≥20% decrease in optical density was considered as a "cure" for the primary endpoint which was based on a binary variable (cure, no cure).

For ≥20% decrease in optical density, the average percentage of optical density (OD) reductions was used (e.g., Test #1=15% and Test #2=25%; average =20% and, hence, "cure"). For seroconversion, both test results had to be negative for the subject to be considered as a "cure."

Reviewer Comment: *The endpoint requires subjects <8 months of age to be considered a success only if they seroconverted. The Applicant noted that 12 months of follow-up may not be a long enough duration for a large percent of subjects ≥8 months to convert to negative.*

With a targeted minimum number of 38 subjects in each age stratum, at least 76 subjects in strata 1 and 2 would have contributed to the endpoint with seroconversion results. However, as stated above, this target was not met, resulting in the primary efficacy endpoint relying largely on ≥20% decrease in optical density rather than seroconversion.

The computation method for the ≥20% decrease in optical density rate using the average rate of two different tests is not appropriate. In Section 8.1.2, the statistical reviewer discusses the relationship of lysate ELISA and recombinant ELISA and further analyses using separate test results are provided.

The primary efficacy endpoint was clarified in protocol amendment 1 prior to the start of the trial.

Secondary efficacy endpoints included clinical signs/symptoms of Chagas disease, concentration test for *T. cruzi* (for subjects <8 months of age), nonconventional (F29 ELISA) serologic testing, and disease state determined by qPCR ("cure" defined as Not Detectable / "no cure" defined as Detectable).The indirect hemagglutination assay (IHA) was also considered in the exploratory efficacy analysis.

***M.O. Comment**: Although it is not currently known how much reduction in optical density corresponds to specific decreases in antibody titer, 20% reduction in optical density is a reasonable component of the surrogate endpoint. The rationale for the inclusion of a 20% reduction in optical density in the surrogate endpoint of change in serologic tests is based in part on analysis of data from a prior study of the treatment of Chagas disease patients 6 to 12 years of age (Sosa Estani et al. 1998) that showed a 21% reduction in mean optical density (using a conventional ELISA) in benznidazole-treated subjects versus no change in the mean optical density for the placebo subjects at 12 months post-treatment. In addition, follow-up at 48 months showed continued decline in the optical density values in benznidazole-treated subjects, but not in placebo subjects.*

Statistical Analysis Plan

Analysis Populations

The study had the following analysis populations:

- Full analysis set (FAS): all randomized subjects who received at least one dose of study drug
- Per protocol set (PPS): all randomized subjects treated with study drug who had no major protocol deviations.

Major protocol deviations included the following:

- Subjects who had both conventional serology results missing at the 12-month time point
- Subjects who did not meet inclusion/exclusion criteria but entered the treatment phase
- Subjects who took less than 80% or more than 120% of total assigned doses of study drug
- Subjects whose treatment codes were unblinded during the study
- Subjects who took prohibited medications during the study

The primary efficacy analysis was conducted in FAS. It was repeated in PPS as a sensitivity analysis.

Statistical Methods

The proportion of nifurtimox subjects with ≥20% decrease in optical density or seroconversion (60-day regimen) and the proportion estimated from historical data were compared. The asymptotic 2-sided 95% confidence interval (CI) was calculated with a continuity correction of 1/2n for a single proportion.

The null hypothesis H_0 was $P_{nifurtimox} = P_{placebo}$, where P_t was the cure rate for treatment t. The alternative hypothesis H_1 was $P_{nifurtimox} \neq P_{placebo}$. Superiority would be concluded if the lower limit of the CI for the proportion of nifurtimox subjects (60-day regimen) with ≥20% decrease in optical density or seroconversion is greater than 16%.

Reviewer Comment*: As the study did not contain an active or placebo control, it was planned that the study results of the 60-day nifurtimox treatment arm would be compared to a historical placebo control rate of 16%. Data supporting the 16% placebo control rate were limited. For this reason, the assessment of efficacy focuses on the comparison of the primary endpoint between the randomized treatment arms, rather than relying on a historical placebo control.*

Additionally, seroconversion at 12 months using the F29 ELISA was compared to a historical control. See discussion below.

Protocol Amendments

There were 8 protocol amendments. The first two amendments were made prior to the start of the trial (January 27th, 2016).

Amendment 1 (dated April 2nd, 2015) had the following modifications:

- ECGs would be obtained at the time PK samples were collected when nifurtimox had reached C_{max} or peak steady-state concentrations. Measurements of ECGs would be optional for subjects <5 years of age, and required for subjects 5 years of age and older.
- The efficacy endpoint using reduction in optical density and seroconversion was clarified. Tests required for Chagas disease confirmation were changed to recombinant ELISA and total purified antigen (lysate) ELISA.
- Information about laboratory procedures, assay variability and cutoffs, and facilities to be used for all assays was added.
- Study details were added regarding subjects with change in urine color, safety and compliance questionnaire, terms for adverse event reporting, telephone assessment at Day 14, neurological examinations along with each physical examination, procedures for follow-up on discontinued subjects, treatment compliance monitoring via telephone at Visit 6, phone contact form, and subject diary.

Amendment 2 (dated October 19th, 2015) added a targeted minimum number of 38 subjects in each age stratum, deleted the enrollment procedure based on evaluable subjects, added rescue

therapy at the 30-day visit, and updated the information for the historical control.

Amendment 5 (dated October 12th, 2017) added an additional test (Immunofluorescent antibody, IFA) at Visit 1 and Visit 11. Amendment 6 (dated December 20th, 2017) replaced the IFA test with the indirect hemagglutination assay (IHA). Amendment 7 (dated May 11th, 2018) added a long-term follow-up plan (CHICO SECURE) to extend Study 16027.

8.1.2. Study Results (Study 16027)

Compliance With Good Clinical Practices

On the title page of the CSR, the Applicant stated that the study was conducted in compliance with Good Clinical Practice. In addition, in Section 4, the Applicant stated the study protocol was approved by the IRB at each study site and each subject provided informed consent.

Financial Disclosure

The Applicant certified that none of the investigators for Study 16027 had any disclosable financial interests or arrangements with the Applicant.

Patient Disposition

The study was conducted between January 2016 and July 2018 at 25 sites in Argentina, Bolivia and Colombia. The following table summarizes the disposition of subjects.

A total of 371 subjects were screened in the study, of which 41 were excluded. The 330 eligible subjects were randomized in a 2:1 ratio to the 60-day and 30-day regimen. 318 (96.4%) subjects completed the study, with the most common reason for early discontinuation being withdrawal by subject. 308 (93.3%) subjects completed the treatment, with the most common reason for early treatment termination being due to an adverse event.

Table 36. Subject Disposition, Study 16027

Category	30-Day	60-Day
Subjects randomized (FAS), N	111	219
Subjects treated	111 (100)	219 (100)
PPS	95 (85.6)	187 (85.4)
Completed the treatment	105 (94.6)	203 (92.7)
Did not complete the treatment	6 (5.4)	16 (7.3)
Reason for not completing the treatment:		
Adverse event	2 (1.8)	12 (5.5)
Withdrawal by subject	1 (0.9)	3 (1.4)
Noncompliance with study drug	2 (1.8)	0
Other	1 (0.9)	1 (0.5)
Completed the study	108 (97.3)	210 (95.9)
Did not complete the study	3 (2.7)	9 (4.1)

Category	30-Day	60-Day
Reason for not completing the study:		
Lost to follow-up	1 (0.9)	2 (0.9)
Withdrawal by subject	1 (0.9)	3 (1.4)
Death	0	1 (0.5)
Other	1 (0.9)	3 (1.4)

Source: Clinical Study Report, Figure 8-1, Listing 16.2.1
All values are expressed as n (%) unless stated otherwise. All percentages are calculated from randomized subjects.
Abbreviations: FAS, full analysis set; PPS, per protocol set

Protocol Violations/Deviations

A total of 200 patients (135 [61.6%] 60-day subjects and 65 [58.6%] 30-day subjects) had at least 1 protocol deviation. The two groups were comparable in terms of proportions of total violations. 48 subjects were classified as having major protocol deviations and were excluded from the PPS. Details are summarized in Table 37.

Table 37. Protocol Deviations, Study 16027

Protocol Deviation Category	30-Day (N=111)	60-Day (N=219)
Subjects with any deviation	65 (58.6)	135 (61.6)
Major	16 (14.4)	32 (14.6)
Procedure deviations	3 (2.7)	9 (4.1)
Randomization errors	0	1 (0.5)
Treatment deviations	13 (11.7)	25 (11.4)
Minor	64 (57.7)	132 (60.3)
Other protocol deviations	3 (2.7)	5 (2.3)
Procedure deviations	27 (24.3)	47 (21.5)
Time schedule deviations	47 (42.3)	100 (45.7)
Treatment deviations	22 (19.8)	40 (18.3)

Source: Clinical Study Report, Table 8-2
All values are expressed as n (%).
Subjects may have more than one protocol deviation but were only counted once within each deviation category.

Demographic Characteristics

Demographic characteristics are listed in Table 38. The two groups had similar distributions in these characteristics. All subjects were between 0 and 18 years of age. Approximately 54% of patients were female. The majority of the subjects were white.

Table 38. Demographic Characteristics of the Primary Efficacy Analysis, Study 16027

Demographic Parameters	30-Day (N=111)	60-Day (N=219)
Sex		
Male	52(46.8)	100 (45.7)
Female	59 (53.2)	119 (54.3)
Age		
Mean years (SD)	9.86 (5.07)	9.42 (5.16)
Median (years)	11.00	10.00
Min, max (years)	0.01, 17.00	0.02, 17.00

Demographic Parameters	30-Day (N=111)	60-Day (N=219)
Age group		
0 to 27 days	3 (2.7)	4 (1.8)
28 days to younger than 8 months	4 (3.6)	8 (3.7)
8 months to younger than 2 years	8 (7.2)	17 (7.8)
2 years to younger than 18 years	96 (86.5)	190 (86.8)
Race		
White	81 (73.0)	155 (70.8)
American Indian[1]	30 (27.0)	64 (29.2)
Ethnicity		
Hispanic or Latino	108 (97.3)	217 (99.1)
Not Hispanic or Latino	3 (2.7)	2 (0.9)
Region		
Argentina	59 (53.2)	119 (54.3)
Bolivia	21 (18.9)	41 (18.7)
Colombia	31 (27.9)	59 (26.9)

Source: Clinical Study Report, Table 8-4, Reviewer's analysis
All values are expressed as n (%) unless specified otherwise.
Abbreviations: SD, standard deviation
[1]Defined as indigenous peoples of the Americas

Other Baseline Characteristics

Primary Diagnosis and Indication-Specific Characteristics at Baseline

The primary diagnosis and baseline characteristics related to Chagas disease are presented in the following table (results from central laboratory). Note that eligibility for the study was based on local laboratory results. At baseline, there was one negative result in each "conventional" ELISA test based on central laboratory results. Concentration tests for *T. cruzi* were positive in 100% of subjects <8 months of age at randomization, corresponding to 5.8% of all subjects.

Table 39. Primary Diagnosis and Indication-Specific Characteristics at Baseline, Study 16027

Demographic Parameters	30-Day (N=111)	60-Day (N=219)
Total purified antigen (lysate) ELISA results		
Reactive	110 (99.1)	219 (100)
Nonreactive	1 (0.9)	0
Total purified antigen (lysate) ELISA OD values		
n	111	219
Mean (SD)	1.53 (0.53)	1.47 (0.55)
Median	1.58	1.54
Minimum, maximum	0.20, 2.50	0.25, 2.59
Recombinant ELISA results		
Reactive	110 (99.1)	219 (100)
Nonreactive	1 (0.9)	0
Recombinant ELISA OD values		
n	111	219
Mean (SD)	2.77 (0.61)	2.74 (0.64)
Median	2.98	2.99
Minimum, maximum	0.16, 3.58	0.66, 3.90

Demographic Parameters	30-Day (N=111)	60-Day (N=219)
F29 ELISA test results		
Reactive	72 (64.9)	142 (64.8)
Nonreactive	39 (35.1)	77 (35.2)
Quantitative PCR test results		
Detectable	57 (51.4)	117 (53.4)
Nondetectable	53 (47.7)	99 (45.2)
Nonevaluable	1 (0.9)	1 (0.5)
Missing	0	2 (0.9)
Concentration test for *T. cruzi*[a]		
Positive	7 (6.3)	12 (5.5)
Negative	0	0
Missing	104 (93.7%)	207 (94.5)
Chagas disease clinical signs and symptoms		
No	111 (100.0)	217 (99.1)
Known ECG abnormality	0	2 (0.9)

Source: Clinical Study Report, Table 8-5
All values are expressed as n (%) unless stated otherwise.
[a] Performed in subjects <8 months of age at randomization
Abbreviations: ECG, electrocardiogram; ELISA, enzyme-linked immunosorbent assay; OD, optical density; PCR, polymerase chain reaction; SD, standard deviation

Medical History

According to the clinical study report, 37.6% of all subjects were reported with at least one medical history finding. The two treatment arms were well-balanced with respect to the medical histories reported. The medical history with highest incidences were varicella (7.9% in total population, 8.2% in the 60-Day group and 7.2% in the 30-Day group), anemia (3.3% in total population, 3.2% in the 60-Day group and 3.6% in the 30-Day group), and eosinophilia (2.1% in total population, 1.8% in the 60-Day group and 2.7% in the 30-Day group).

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Treatment Compliance

Treatment compliance is shown in the following table. The compliance was high and comparable between the two groups.

Table 40. Treatment Compliance, Study 16027

Category Analysis/Subcategory Parameter	30-Day (N=111)		60-Day (N=219)
Drug	Nifurtimox	Placebo	Nifurtimox
Actual number of tablets taken			
n	110	106	218
Mean (SD)	148.31 (77.49)	158.29 (82.50)	319.03 (161.12)
Median	124.75	132.25	271.50
Min, max	40.5, 332.5	36.5, 391.0	4.0, 703.0

Category Analysis/Subcategory Parameter	30-Day (N=111)		60-Day (N=219)
% planned number of tablets taken[a]			
n	110	106	218
Mean (SD)	92.89 (14.01)	91.26 (12.89)	92.31 (12.81)
Median	97.62	95.01	96.05
Min, max	34.2, 124.2	32.7, 123.5	3.3, 144.0
Compliance categories, n (%)			
Missing	1 (0.90)	5 (4.50)	1 (0.46)
≤80%	14 (12.61)	10 (9.01)	24 (10.96)
>80%-120%	94 (84.68)	95 (85.59)	193 (88.13)
>120%	2 (1.80)	1 (0.90)	1 (0.46)

Source: Clinical Study Report, Table 9-1
Abbreviations: SD, standard deviation
[a] According to protocol

Prior Medications

According to the clinical study report, 26 (7.9% overall, 9.1% in the 60-Day group and 5.4% in the 30-Day group) subjects in the FAS received at least one prior medication before starting study drug. The most frequently used prior medications were from the following anatomical therapeutic chemical (ATC) classes: anti-infectives for systemic use (n=13, 3.9%), respiratory system (n=8, 2.4%), and genitourinary system and sex hormones (n=8, 2.4%). 5.9% in the 60-Day group and 0.9% in the 30-Day group took at least one medication that started and ended before administration of study drug. Overall use of prior medications was higher among patients in the 60-Day group.

Concomitant Medications

172 (52.1%) subjects received at least one concomitant medication; 121 (55.3%) subjects in the 60-Day group and 51 (45.9%) subjects in the 30-Day group. Of those reported, the most common concomitant medications were shown in the following table. Note that use of concomitant medications were higher in the 60-Day group in most of the listed classes.

Table 41. Concomitant Medications Taken by ≥10% of Subjects in Either Treatment Regimen, Study 16027

ATC Class	30-Day (N=111)	60-Day (N=219)
Stomatological preparations	12 (10.8)	19 (8.7)
Antibacterials for systemic use	20 (18.0)	43 (19.6)
Cardiac therapy	14 (12.6)	43 (19.6)
Other gynecologicals	17 (15.3)	43 (19.6)
Antiinflammatory and antirheumatic products	19 (17.1)	43 (19.6)
Topical products for joint and muscular pain	17 (15.3)	44 (20.1)
Analgesics[a]	11 (9.9)	45 (20.5)
Throat preparations	17 (15.3)	42 (19.2)
Ophthalmologicals	17 (15.3)	40 (18.3)
Otologicals	9 (8.1)	25 (11.4)

Source: Clinical Study Report, Table 8-7
All values are expressed as n (%).
Abbreviations: ATC, anatomical therapeutic chemical

[a] Significantly higher percentage in the 60-Day group

M.O. Comment: *On further review, ibuprofen was the "cardiac therapy" drug. In addition, the "analgesics" were mostly acetaminophen. The imbalance in the arms for these two drug classes likely is a result of treating AEs that occurred more often in subjects in the 60-day arm.*

Efficacy Results—Primary Endpoint

The table below displays the Applicant's results for the primary efficacy endpoint. The Applicant concluded superiority for nifurtimox over historical placebo, as the lower limit of the 95% CI for the 60-Day group (26.4%, 39.3%) was greater than the prespecified cutoff of 16%. There was also a dose response effect noted for the 60-Day regimen versus the 30-Day regimen in the FAS (p-value=0.0043) and in the PPS (p-value=0.0022) with a higher cure rate in the 60-day regimen. The difference in cure rate between the two groups was driven by those with ≥20% decrease in optical density.

Table 42. Primary Efficacy Result, Study 16027—CHICO

Analysis Set	Analysis	30-Day	60-Day
FAS	N	111	219
	Total cure (rate)	21 (18.9)	72 (32.9)
	Seroconversion	5 (4.5)	10 (4.6)
	≥20% decrease in optical density	16 (14.4)	62 (28.3)
	No cure	90 (81.1)	147 (67.1)
	95% CI for cure rate	(11.2, 26.7)	(26.4, 39.3)
PPS	N	95	187
	Total cure (rate)	18 (18.9)	66 (35.3)
	Seroconversion	5 (5.3)	10 (5.3)
	≥20% decrease in optical density	13 (13.7)	56 (29.9)
	No cure	77 (81.1)	121 (64.7)
	95% CI for cure rate	(10.5, 27.4)	(28.2, 42.4)

Source: Clinical Study Report, Table 9-6, Table 9-7, as well as Reviewer's analysis
All values are expressed as n (%) unless stated otherwise.
All 95% CI's in this table adopt a continuity correction of 1/2n following the Applicant's analysis plan.
Abbreviations: CI, confidence interval; FAS, full analysis set; PPS, per protocol set

Reviewer Comment: *The relationship between recombinant ELISA values and antibody titers is not linear. This is discussed in the Clinical Microbiology section and the CDRH comments in Section 4.3 of this review. For this reason, comparison to a historical control rate on change in optical density for the ELISA serologic tests is difficult to interpret. Additionally, as previously discussed, the Applicant's primary analysis was based on the averaged reduction in optical density. The reviewer conducted a further analysis on the relationship of the two "conventional" ELISA tests: TPA (lysate) and recombinant. The following graph shows the reduction in optical density rates for patients using each "conventional" ELISA test (TPA [lysate] ELISA on the x-axis and recombinant ELISA on the y-axis). Note that the dashed line is the defined borderline of*

treatment success (20% average reduction), and the points above it are considered as cure (≥20% decrease in optical density) in the primary analysis. However, we can see that the points are scattered. The correlation of the two separate reduction rates is 0.697. Also note that the two "conventional" ELISA tests detect different kinds of antibodies. Based on this information, we did not consider it appropriate to pool results from two different "conventional" ELISA tests as was done for the Applicant's primary efficacy endpoint. We present the results for each of the individual ELISA tests.

Figure 3. Comparison of the TPA (Lysate) and Recombinant ELISA Reduction Rates After 12 Months, Study 16027

Abbreviations: ELISA, enzyme-linked immunosorbent assay; TPA, total purified antigen

Table 43. Primary Efficacy Result Using Total Purified Antigen (Lysate) ELISA and Recombinant ELISA Tests Separately (FAS), Study 16027

	TPA (Lysate) ELISA		Recombinant ELISA	
Efficacy	**30-Day N=111**	**60-Day N=219**	**30-Day N=111**	**60-Day N=219**
Total cure (rate)	21 (18.9)	70 (32.0)	24 (21.6)	76 (34.7)
Seroconversion	6 (5.4)	11 (5.0)	7 (6.3)	11 (5.0)
≥20% decrease in optical density	15 (13.5)	59 (26.9)	17 (15.3)	65 (29.7)
Cure RD of 60-day vs. 30-day (95% CI)	13.0 (3.5, 22.6)		13.1 (3.2, 23.0)	
p-value to test the diff. betw. groups	0.007		0.010	

Source: Reviewer's analysis

Abbreviations: betw., between; diff., difference; ELISA, enzyme-linked immunosorbent assay; RD, rate difference; TPA, total purified antigen

The above table summarizes the primary efficacy results using the two "conventional" ELISA tests separately. We see that there was a dose response effect for the 60-day regimen compared to the 30-day regimen using both tests.

The following table summarizes mean OD values for both "conventional" ELISA tests by visit. Note that the mean OD values for both "conventional" ELISA tests decreased significantly at Visit 11 compared to Visit 1.

Table 44. Mean OD Values of "Conventional" ELISA Tests by Visit (FAS)

Visit	TPA (Lysate) ELISA		Recombinant ELISA	
Analysis	30-Day	60-Day	30-Day	60-Day
Visit 1 (screening)				
n	111	219	111	219
Mean	1.53	1.47	2.76	2.73
Visit 3 (day 7)				
n	108	218	108	218
Mean	1.55	1.49	2.78	2.71
Visit 6 (day 30)				
n	110	214	110	214
Mean	1.52	1.47	2.72	2.66
Visit 8 (day 60)				
n	107	215	107	215
Mean	1.48	1.41	2.69	2.58
Visit 10 (day 240)				
n	109	211	109	211
Mean	1.33	1.27	2.42	2.34
Visit 11 (day 420)				
n	108	210	108	210
Mean	1.32	1.25	2.38	2.26
Visit 1–visit 11				
n	108	210	108	210
Mean	0.21	0.24	0.38	0.47
p-value*	<0.001	<0.001	<0.001	<0.001

Source: Reviewer's analysis
* Testing if Visit 1-Visit 11>0. The mean change from baseline is difficult to interpret because no data are available for placebo/no treatment. No significant dose response is shown comparing the two treatment groups.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; FAS, full analysis set; OD, optical density; TPA, total purified antigen

The following density plots (Figure 4) compare densities of reduction rates between the 60-day group and the 30-day group in total purified antigen (lysate) ELISA and recombinant ELISA. We can see that the differences between groups are driven by reductions between 10% to 60%. The vertical dashed line represents a 20% reduction. Subjects with data to the right of the line would be considered as having a ≥20% reduction in optical density or seroconversion. The 60-day curve is higher than the 30-day curve in this range, representing that a greater percentage of subjects in the 60-day arm than in the 30-day arm have reduction rates greater than 20%.

Figure 4. Density Plots of Reduction Rate From Baseline in Total Purified Antigen (Lysate) ELISA and Recombinant ELISA

Abbreviations: ELISA, enzyme-linked immunosorbent assay; TPA, total purified antigen

Efficacy Results—Secondary Endpoints

Clinical Signs and Symptoms of Chagas Disease

According to the clinical study report, among the observed signs and symptoms at all visits, none were severe in intensity. While anemia had a maximum intensity of moderate at Visit 3, all other signs and symptoms were mild in intensity. Romana's sign was reported for 1 (0.9%) subject at Visit 6 (end of exposure to nifurtimox) in the 30-day regimen. Anemia, Chagas disease, and hepatomegaly which were observed at earlier visits, were not observed during the follow-up period (Visits 9, 10 and 11). The most frequently reported sign or symptom, "known ECG abnormality", appeared in 2 (0.9%) subjects in the 60-day arm at Visit 1, in 4 (1.8%) subjects in the 60-day arm at Visit 3, in 1 (0.5%) subject in the 60-day arm at Visits 6, 8, 9, 10, and in 3 (1.4%) subjects in the 60-Day arm and 3 (2.8%) subjects in the 30-Day arm at Visit 11.

Concentration Test for *T. cruzi*

The concentration test for *T. cruzi* was performed in subjects <8 months of age at Visits 1, 3, 6, 8, and 9. A summary of these results is shown in the following table. Overall, the incidence of positive results decreased over time. All subjects were positive for *T. cruzi* at Visit 1, and the vast majority of the subjects (94.74%) had a negative test result at Visit 9.

Table 45. Concentration Test for *T. cruzi* by Visit (FAS)

Visit	30-Day (N=7)	60-Day (N=12)
Visit 1 (screening)		
Positive	7 (100.0)	12 (100.0)
Negative	0	0
Missing	0	0
Visit 3 (day 7)		
Positive	1 (14.3)	1 (8.3)
Negative	5 (71.4)	11 (91.7)
Missing	1 (14.3)	0
Visit 6 (day 30)		
Positive	0	0
Negative	7 (100.0)	12 (100.0)
Missing	0	0
Visit 8 (day 60)		
Positive	0	0
Negative	7 (100.0)	12 (100.0)
Missing	0	0
Visit 9 (day 90)		
Positive	0	1 (8.3)
Negative	7 (100.0)	11 (91.7)
Missing	0	0

Source: Clinical Study Report, Table 9-9
All values are expressed as n (%).
Abbreviations: FAS, full analysis set

"Nonconventional" Serologic Testing—F29 ELISA

The F29 ELISA was conducted at Visits 1, 3, 6, 8, 10 and 11. Overall, the proportion of subjects with positive test results decreased with time. Detailed test results by visit are summarized in the following table.

Table 46. F29 ELISA Results by Visit (FAS)

Visit	30-Day (N=111)	60-Day (N=219)
Visit 1 (screening)		
Positive	72 (64.9)	142 (64.8)
Negative	39 (35.1)	77 (35.2)
Missing	0	0
Visit 3 (day 7)		
Positive	64 (57.7)	133 (60.7)
Negative	44 (39.6)	85 (38.8)
Missing	3 (2.7)	1 (0.5)
Visit 6 (day 30)		
Positive	67 (60.4)	131 (59.8)
Negative	43 (38.7)	83 (37.9)
Missing	1 (0.9)	5 (2.3)
Visit 8 (day 60)		
Positive	63 (56.8)	124 (56.6)
Negative	44 (39.6)	91 (41.6)
Missing	4 (3.6)	4 (1.8)

Visit	30-Day (N=111)	60-Day (N=219)
Visit 10 (day 240)		
Positive	61 (55.0)	110 (50.2)
Negative	48 (43.2)	101 (46.1)
Missing	2 (1.8)	8 (3.7)
Visit 11 (day 420)		
Positive	54 (48.7)	96 (43.8)
Negative	54 (48.7)	114 (52.1)
Missing	3 (2.7)	9 (4.1)

Source: Clinical Study Report, Table 9-10
All values are expressed as n (%).
Abbreviations: ELISA, enzyme-linked immunosorbent assay; FAS, full analysis set

Reviewer Comment: *214 patients were seropositive at Visit 1 using the F29 ELISA. 142 were in the 60-day group, and 72 were in the 30-day group. The following table summarizes F29 ELISA seroconversion results at Visit 11 among those positive at baseline. The difference in cure rates between the two treatment groups is not statistically significant.*

Table 47. F29 ELISA Test Results at Visit 11 Among Subjects Seropositive at Baseline

Total Baseline Seropositives	30-Day (N=72)	60-Day (N=142)
Visit 11 seronegative	20 (27.8)	46 (32.4)
Visit 11 seropositive	51 (70.8)	94 (66.2)
Visit 11 missing	1 (1.4)	2 (1.4)
95% CI for cure rate	(16.7, 38.8)	(24.3, 40.4)

Source: Reviewer's analysis
All values are expressed as n (%) except the 95% confidence interval. The 95% CI's in this table adopt a continuity correction of 1/2n.
Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay

Sosa Estani et al. conducted a double-blind placebo-controlled trial in Argentina starting in 1982 (Sosa Estani et al. 1998). Patients from 6 to 12 years of age were randomized to receive benznidazole or placebo for 60 days. The study included information on F29 ELISA seroconversion in the placebo group evaluated at 12 months after the onset of treatment. The Applicant submitted patient-level data from this study. Since age can greatly impact the rate of seroconversion, we evaluated the F29 ELISA results in the patients in Study 16027 who were in the same age group as in the Sosa Estani study and the following table summarizes our findings. Note that only 88 patients 6 to 12 years of age were seropositive at Visit 1 using F29 in Study 16027.

Table 48. F29 ELISA Results at Visit 11 Compared to Sosa Estani Placebo Data

	F29 ELISA Results[a]		Sosa Estani Data[b]
Analysis	**30-Day N=29**	**60-Day N=59**	**Historical Control N=36**
Total			
Seronegative	10 (34.5)	20 (33.9)	1 (2.8)
Seropositive	19 (65.5)	38 (64.4)	30 (83.3)
Missing	0	1 (1.7)	5 (13.9)
95% CI for cure rate	(17.9, 54.3)	(22.1, 47.4)	(0.1, 14.5)
Two-sided p-value comparing to historical control	0.002	<0.001	

Source: Reviewer's analysis
All values are expressed as n (%) except the 95% CIs and p-values. The 95% CI's in this table are Clopper and Pearson exact CI. P-values are from two-sided Fisher's exact tests.
[a] The F29 ELISA results were evaluated at 12 months among subjects between 6 and 12 years of age who were seropositive at baseline.
[b] The Sosa Estani data were provided by the Applicant.
Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay

From the above table we can see that the 60-Day treatment and the 30-Day treatment had a significantly higher seroconversion rate using the F29 ELISA compared to the historical control. The Sosa Estani placebo data from the Applicant is similar to data in the publication, but more conservative since the Kaplan-Meier curve displayed in the paper showed 0 seroconversions at 12 months.

Disease State Determined by qPCR

Quantitative PCR was performed at Visits 1, 3, 6, 8, 10, and 11. Overall, the proportion of subjects with positive test results decreased with time from Visit 1 to Visit 8 in both treatment groups, with a slight increase at Visit 10 and Visit 11. Detailed test results by visit are summarized in the following table.

Table 49. qPCR Testing by Visit (FAS)

Visit	**30-Day (N=111)**	**60-Day (N=219)**
Visit 1 (screening)		
Positive	57 (51.4)	117 (53.4)
Negative	53 (47.8)	99 (45.2)
Nonevaluable	1 (0.9)	1 (0.5)
Missing	0	2 (0.9)
Visit 3 (day 7)		
Positive	21 (18.9)	46 (21.0)
Negative	86 (77.5)	171 (78.1)
Nonevaluable	1 (0.9)	0
Missing	3 (2.7)	2 (0.9)
Visit 6 (day 30)		
Positive	3 (2.7)	4 (1.8)
Negative	105 (94.6)	207 (94.5)
Nonevaluable	2 (1.8)	3 (1.4)
Missing	1 (0.9)	5 (2.3)

Visit	30-Day (N=111)	60-Day (N=219)
Visit 8 (day 60)		
Positive	1 (0.9)	3 (1.4)
Negative	105 (94.6)	210 (95.9)
Nonevaluable	2 (1.8)	2 (0.9)
Missing	3 (2.7)	4 (1.8)
Visit 10 (day 240)		
Positive	2 (1.8)	3 (1.4)
Negative	105 (94.6)	206 (94.1)
Nonevaluable	2 (1.8)	2 (0.9)
Missing	2 (1.8)	8 (3.7)
Visit 11 (day 420)		
Positive	5 (4.5)	3 (1.4)
Negative	102 (91.9)	205 (93.6)
Nonevaluable	1 (0.9)	1 (0.5)
Missing	3 (2.7)	10 (4.6)

Source: Clinical Study Report, Table 9-11
All values are expressed as n (%).
Abbreviations: FAS, full analysis set; qPCR, quantitative polymerase chain reaction

Reviewer Comment: *174 patients tested positive at Visit 1 (117 in the 60-Day group and 57 in the 30-Day group). The table below shows the Visit 11 test results among baseline positive subjects. Note that the overwhelming majority of subjects in both treatment arms became qPCR negative by Visit 11. The results were not significantly different between the two groups.*

Table 50. qPCR Test Result at Visit 11 Among qPCR Baseline Positives

Total Baseline Positives	30-Day (N=57)	60-Day (N=117)
Visit 11 negative	50 (87.7)	106 (90.6)
Visit 11 positive	5 (8.8)	3 (2.6)
Visit 11 nonevaluable	1 (1.8)	1 (0.9)
Visit 11 missing	1 (1.8)	7 (6.0)
95% CI for cure rate	(76.3, 94.9)	(83.8, 95.2)

Source: Reviewer's analysis
All values are expressed as n (%) except the 95% CI. The 95% CI's in this table are Clopper and Pearson exact CI.
Abbreviations: CI, confidence interval; qPCR, quantitative polymerase chain reaction

IHA Testing

IHA was performed at Visit 1 and Visit 11. 327 patients were positive at Visit 1. Among them, 9 in the 60-Day group and 5 in the 30-Day group became negative at Visit 11. There was no significant difference in conversion to negative between the two groups.

Subgroup Analyses for "Conventional" ELISA Tests

A summary of cure rates for the FAS population by baseline demographics is presented in the following table. Note that "cure" is defined as seroconversion or ≥20% decrease in optical density using the individual total purified antigen (lysate) and recombinant ELISA tests.

Table 51. Cure Rates by Demographics

Demographic Parameters	TPA (Lysate) ELISA		Recombinant ELISA	
	30-Day (N=111)	60-Day (N=219)	30-Day (N=111)	60-Day (N=219)
Sex				
Male	12/52 (23.1%)	28/100 (28.0%)	17/52 (32.7%)	30/100 (30.0%)
Female	9/59 (15.3%)	42/119 (35.3%)	7/59 (11.9%)	46/119 (38.7%)
Age group*				
0 to 27 days	3/3 (100.0%)	3/4 (75.0%)	3/3 (100.0%)	3/4 (75.0%)
28 days to <8 months	2/4 (50.0%)	7/8 (87.5%)	2/4 (50.0%)	7/8 (87.5%)
8 months to <2 years	7/8 (87.5%)	15/17 (88.2%)	5/8 (62.5%)	8/17 (47.1%)
2 to 5 years	4/10 (40.0%)	10/22 (45.5%)	1/10 (10.0%)	7/22 (31.8%)
6 to 12 years	4/45 (8.9%)	24/97 (24.7%)	7/45 (15.6%)	29/97 (29.9%)
13 to <18 years	1/41 (2.4%)	11/71 (15.5%)	6/41 (14.6%)	22/71 (31.0%)
Race				
White	18/81 (22.2%)	62/155 (40.0%)	22/81 (27.2%)	67/155 (43.2%)
American Indian[1]	3/30 (10.0%)	8/64 (12.5%)	2/30 (6.7%)	9/64 (14.1%)
Ethnicity				
Hispanic or Latino	21/108 (19.4%)	70/217 (32.3%)	23/108 (21.3%)	76/217 (35.0%)
Not Hispanic or Latino	0/3 (0)	0/2 (0)	1/3 (33.3%)	0/2 (0)
Region				
Argentina	15/59 (25.4%)	51/119 (42.9%)	15/59 (25.4%)	43/119 (36.1%)
Bolivia	3/21 (14.3%)	4/41 (9.8%)	1/21 (4.8%)	6/41 (14.6%)
Colombia	3/31 (9.7%)	15/59 (25.4%)	8/31 (25.8%)	27/59 (45.8%)

Source: Reviewer's analysis
All values are expressed as n (%).
[1]Defined as indigenous peoples of the Americas
* Cure rates for patients less than 8 months of age were all due to seroconversion by definition. Two patients 8 months of age or older were seronegative using Total Purified Antigen (lysate) ELISA at Visit 11, and all other "cured" patients by Total Purified Antigen (lysate) ELISA in this age group were based on ≥20% decrease in optical density. Three patients 8 months of age or older were seronegative using Recombinant ELISA at Visit 11, and all other "cured" patients by Recombinant ELISA in this age group were based on ≥20% decrease in optical density.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; TPA, total purified antigen

From the table we can see that a dose response effect is shown in female subjects, while the "cure" rates in male subjects are similar in both groups. The cure rates also vary largely in different countries, with the lowest observed in subjects from Bolivia.

8.1.3. Literature Data Supportive of Efficacy

The Applicant submitted a summary of literature studies along with the primary publications in support of the efficacy of NFX in the treatment of Chagas disease. These studies were conducted over the past 50+ years and include studies conducted with support from the Applicant. This section will review the 13 studies which provided sufficient data on outcomes.

Table 52 shows three studies from the literature in which patients with acute Chagas disease were treated with NFX. Two of the studies include placebo-treated patients. However, neither of these studies used randomized treatment allocation.

Table 52. Studies of Acute Chagas: Uncontrolled and Partially Controlled Studies

Study Name	Age	Daily Dose and Duration	N	Testing Method	Follow-Up	Efficacy Result
Bocca Tourres (Bocca Tourres 1969)	Included infants, young children, adolescents	4 different regimens: 12 to 30 mg/kg for 30, 60, 90 days	142 (84 completed treatment)	XD, CFT, HAR and IFAT	1 year	58.8% to 78.6% cured with negative XD and serology. Reinfection may have affected results.
Cerisola (Cerisola 1969)	Not reported	Unreported dose for 90 days	246 (NFX); 18 (PBO)	CFT, HAR, IFAT	Up to 18 months	NFX-treated patients had seropositivity rates <10% at 18 months vs. >85% for placebo
Wegner and Rohwedder (Wegner and Rohwedder 1972)	Infants to adults, 93.4% were 0 to 16 years	15 to 20 mg/kg (children), 12.5 to 15 mg/kg (adolescents), each for 90 days; 8 to 10 mg/kg for 120 days (adults)	550 (NFX); 51 (PBO)	Direct examination of parasite for study entry; CFT, HAR, IFAT for efficacy	At least 1.5 years	See Table 53

Source: Reviewer Table
All referenced publications are listed in Section 20.1.
Abbreviations: CFT, complement fixation test, HAR, hemagglutination reaction, IFAT, indirect immunofluorescence, PBO, placebo; XD, xenodiagnosis

Table 53. Serology Test Results From Wegner and Rohwedder (1972)

Test	Result	Months After Diagnosis									
		0	1	3	4	5	6	9	12	18	24
Nifurtimox (N=550)											
CFR	Total cases	407	322	277	272	134	103	293	205	275	169
	Positive	142	136	43	29	16	14	43	21	38	22
	%	34.9	42.2	15.5	10.7	11.9	13.6	14.7	10.2	13.8	13.0
HAR	Total cases	429	350	285	276	135	106	299	211	279	170
	Positive	121	97	35	28	18	16	46	25	44	25
	%	28.2	27.7	12.3	10.1	13.3	15.1	15.4	11.8	15.8	14.7
FAT	Total cases	423	345	266	272	135	106	303	206	278	173
	Positive	264	255	79	66	29	23	67	45	65	38
	%	62.4	74.0	29.7	24.3	21.5	21.7	22.1	21.8	23.4	22.0
Placebo (N=51)											
CFR	Total cases	40	35	32	26	13	15	30	13	27	14
	Positive	14	22	23	24	12	15	27	12	24	13
	%	35.0	62.9	71.9	92.3	92.3	100	90.0	92.3	88.9	92.9
HAR	Total cases	47	35	36	27	14	15	32	19	30	16
	Positive	13	17	18	23	14	15	30	19	29	15
	%	27.7	48.6	50.0	85.2	100	100	93.8	100	96.7	93.8
FAT	Total cases	43	37	34	27	14	15	31	19	30	16
	Positive	31	33	33	27	14	15	30	19	30	16
	%	72.1	89.2	97.1	100	100	100	96.8	100	100	100

CFR= complement fixation reaction; FAT = fluorescent antibody test; HAR = hemaglutination reaction; N = number of patients treated.
Source: Table 13 from (Wegner and Rohwedder 1972).

M.O. Comment: *The studies in acute Chagas disease show high rates of seroconversion to negative with NFX and low rates with placebo. However, there were many cases lost over time. For example, in the Wegner study (Wegner and Rohwedder 1972), there were approximately 400 subjects with a status for each serology result at baseline, but at 24 months this number dropped to around 170. The status of the lost subjects could not be determined. In addition, these studies used a different dose and duration of NFX compared to that in the proposed label which could affect the outcome. These are nonrandomized studies which may make the placebo comparison invalid.*

Table 54 shows two studies from the literature in which patients with chronic Chagas disease were treated with NFX or placebo. These studies were nonrandomized. The method for treatment allocation was not specified in the Ferreira study. The Cichero study stated that patients with abnormal EEGs were preferentially treated with placebo.

Table 54. Studies of Chronic Chagas: Partially Controlled Studies

Study Name	Age	Daily Dose and Duration	N	Testing Method	Follow-Up	Efficacy Result
Cichero et al. (Cichero et al. 1969)	5 to 14 years	25 mg/kg for 15 days then 15 mg/kg for 105 days (0 to 12 years); 15 mg/kg for 120 days (13+ years)	33 (NFX); 7 (PBO)	Serology, XD	240 days post-treatment	10 patients had positive xenodiagnosis at baseline (6 NFX; 4 placebo). All NFX-treated patients had negative xenodiagnosis at last follow-up. Three of 4 (75%) of placebo-treated had positive xenodiagnosis at last follow-up.
Ferreira HO 1990 (Ferreira Hde 1990)	Mostly children and adolescents	15 mg/kg (children); 10 mg/kg (adolescents and adults) for 60 to 120 days	50 (NFX); 50 (BNZ)	XD, CFT, IFAT, and HAR	24 months	NFX- 50% negative by xenodiagnosis, 6% negative by serology; BNZ- 70% negative by xenodiagnosis, 10% negative by serology

All referenced publications are listed in Section 20.1.
Abbreviations: BNZ, benznidazole; CFT, complement fixation test; HAR, hemagglutination reaction; IFAT, indirect immunofluorescence; NFX, nifurtimox; PBO, placebo; XD, xenodiagnosis

M.O. Comment: *The Cichero study (Cichero et al. 1969) was small, but showed that patients who were positive by xenodiagnosis at baseline became negative with NFX treatment. Most of the placebo patients were positive by xenodiagnosis at the last follow-up visit. The Ferreira study (Ferreira Hde 1990) showed that NFX and BNZ had similar rates of parasite clearance by xenodiagnosis and seronegativity. Both studies treated patients for 120 days which is twice as long as the dosing duration in the proposed label.*

Table 55 shows eight studies from the literature in which patients with chronic and congenital Chagas disease were treated with NFX. These studies were uncontrolled and did not have placebo or active comparator cohorts.

Table 55. Studies of Chronic and Congenital Chagas Disease, Uncontrolled Studies

Study Name	Age	Daily Dose/ Duration	N	Testing Method	Follow-Up	Efficacy Result
Altcheh et al. (Altcheh et al. 2005)	15 days to 10 years	10-15 mg/kg for 60 days	168	Conventional serology	3 months to 11 years (median 3 years)	87.2% negative serology
Bianchi et al. (Bianchi et al. 2015)	4 to 19 years	10 to 12 mg/kg (<40 kg) or 8 to 10 mg/kg (>40 kg) for 60 days	62	ELISA, IFAT, qPCR	30 months post-treatment	Negative serology by both ELISA and IFAT was 41.9%; PCR positivity went from 88.4% pretreatment to 12.1%.

Study Name	Age	Daily Dose/ Duration	N	Testing Method	Follow-Up	Efficacy Result
Moya et al. (Moya et al. 1985)	1 day to 16 months (congenital)	15 mg/kg for 2 or 3 months	48	Parasitological methods (XD, blood culture, Strout method) and serology (IFAT, CFT, HAR)	1 to 12 years (mean 5.6 years)	45/48 (94%) had negative serology; 47/48 (98%) had negative parasitological tests
Solari et al. (Solari et al. 1998)	0 to 10 years	7 mg/kg for 60 days	28	XD and PCR	6 months after treatment	100% were negative by xenodiagnosis at 3 and 6 months; 6/28 (21%) negative by PCR at 3 months, and 10/28 (36%) at 6 months
Solari et al. (Solari et al. 2001)	0 to 10 years	7 mg/kg for 60 days	66	Conventional serology, XD, and PCR	36 months post-treatment	At 36 months, all PCR negative, but serology all positive except for 2 youngest patients; positive xenodiagnosis dropped from 79.1% to 1.6% 3 to 6 months after treatment
Rubio and Donoso (Rubio and Donoso 1969)	1 month to 13 years	25 mg/kg for 15 days then 12.5 mg/kg for 90 days	12	CFT, HAR	6 to 24 months after treatment	Of the 9 patients who completed treatment, all 9 seroconverted by CFT; 4/4 congenital cases seroconverted by HAR
Schenone et al. (Schenone et al. 2003)	Children <11 years	7-10 mg/kg for 30 days	99	XD (all positive at baseline)	3 years	All negative by xenodiagnosis
Schmunis et al. (Schmuñis et al. 1978)	1 to 14 years	10-15 mg/kg for 90 days	10	Indirect immunofluorescence	1 to 2 years	All seroconverted

All referenced publications are listed in Section 20.1.
Abbreviations: CFT, complement fixation test; ELISA, enzyme-linked immunosorbent assay; HAR, hemagglutination reaction; IFAT, indirect immunofluorescence; PCR, polymerase chain reaction; qPCR, quantitative PCR; XD, xenodiagnosis

M.O. Comment: *These uncontrolled studies were varied with respect to subject age, efficacy assessments, NFX dose and duration, as well as length of follow up. Using xenodiagnosis, NFX-treated subjects had high rates of conversion to negative across the studies. However, xenodiagnosis may have false negative results in chronic Chagas disease as parasitemia is intermittent and at low levels.*

Seronegativity rates varied from study to study. For example, the Solari 2001 study (Solari et al. 2001) showed low seroconversion at 36 months, but the Schmuñis study (Schmuñis et al. 1978) showed 100% seroconversion. These differences in seronegativity may be a result of differences in the study population, drug dose and duration of therapy, regional differences in the T. cruzi strains, and test characteristics. Overall, these studies provide supportive evidence for the efficacy of NFX in the treatment of Chagas disease in the pediatric population.

We also note that several published observational studies (Viotti et al. 1994; Viotti et al. 2006; Fabbro et al. 2007) provide evidence that seroconversion to negative using conventional serologic tests is associated with a lower risk of development of cardiac abnormalities in adults. Prevention of development of cardiomyopathy would take decades to demonstrate and is clearly a difficult trial endpoint for the pediatric population. The proposed duration Part 2 of Study 16027 (CHICO SECURE) should be sufficient to demonstrate conversion of serologies to negative in a substantial proportion of the study population.

8.2. Statistical Issues

The targeted minimum number of 38 subjects in each age stratum was not met in this trial, resulting in the primary efficacy analysis relying largely on achieving ≥20% decrease in optical density rather than seroconversion. The clinical meaningfulness of ≥20% decrease in optical density is not as clear as seroconversion, since the TPA (lysate) and recombinant ELISAs are primarily designed as qualitative tests rather than quantitative. The relationship between optical density values in the TPA (lysate) and recombinant ELISA and antibody titers is not linear. This makes the interpretation of achieving ≥20% decrease in optical density compared to a historical control difficult, although the study did make the prespecified cut-off point.

The Applicant submitted data from a prior study (Sosa Estani et al. 1998) of Chagas disease in pediatric patients using an ELISA similar to the lysate ELISA. In this study, 90 patients had results at 12, 24 and 48 months after treatment onset. Among them, 26 patients had a >20% decrease in optical density at 12 months and 64 did not. Seroconversion was achieved in 8 patients at 24 months and 6 patients at 48 months. The 24 month seroconversion rate among those who had >20% decrease in optical density at 12 months was 0.23 (6/26) and among those who did not have a >20% decrease was 0.03 (2/64). The 48 month seroconversion rate among those who had >20% decrease in optical density at 12 months was 0.19 (5/26) and among those who did not was 0.02 (1/64). Using Fisher's exact test under the null hypothesis of equal seroconversion rates for 24 months and 48 months, the p-values were both 0.007. Based on these findings, we note that >20% decrease in optical density at 12 months is correlated with seroconversion at 24 months and 48 months.

The Applicant's analysis was based on the averaged reduction in optical density of the TPA (lysate) ELISA and the recombinant ELISA. The reviewer conducted a further analysis on the relationship of the two ELISA tests. The two ELISA tests are based on antibodies for different types of antigens, and reduction in optical density based on the two tests were not consistent for many subjects. Based on that information, it was not considered appropriate to pool results

from the two different ELISA tests for the primary efficacy endpoint. The reviewer analyzed the results of the TPA (lysate) ELISA and recombinant ELISA separately. Both tests show a dose response for the 60-day treatment arm compared to the 30-day treatment arm using the seroconversion/≥20% decrease in optical density endpoint.

The reviewer also conducted analyses using the F29 ELISA. Comparing the seroconversion results from this trial to the Sosa Estani study placebo group in the same age category (6 to 12 years of age), the F29 seroconversion rates for both nifurtimox treatments are significantly higher than the historical placebo. The Division requested that the Applicant provide data on the untreated/spontaneous seroconversion rate at 12 months, using the F29 ELISA, in the following pediatric age groups: 0 to <6 years and 13 to <18 years of age. The Applicant was not able to identify any literature to support a placebo seroconversion rate on F29 ELISA for the aforementioned additional pediatric age groups.

Further evidence with long-term follow-up is needed. This information will be provided in the CHICO SECURE study, which comprises an additional 3-year follow-up of Study 16027 (CHICO) which started in July 2018.

8.3. Conclusions and Recommendations

Study 16027, a randomized, double-blind, controlled trial provides evidence that there is a treatment effect for nifurtimox on the endpoint of seroconversion or ≥20% decrease in optical density using the lysate and recombinant ELISAs in pediatric patients with Chagas disease. Our conclusions are based on the dose response effect observed in the 60-day nifurtimox treatment group as compared to the 30-day treatment group in both the lysate and recombinant ELISA tests using the seroconversion/≥20% decrease in optical density endpoint, in combination with the superiority of the two nifurtimox treatments compared to historical control using the F29 ELISA seroconversion endpoint. Benefit should be confirmed in the long-term follow-up study, CHICO SECURE, based on seroconversion using the recombinant ELISA and IHA tests.

9. Clinical Microbiology Review

9.1. Nonclinical Microbiology

9.1.1. Mechanism of Action

The mechanism of action of nifurtimox is not fully understood. Studies suggest that nifurtimox is activated by nitroreduction leading to production of toxic intermediate metabolites that induce DNA damage and cell death. Two *T. cruzi* nitroreductase (TcNTR) enzymes, Type I and Type II, play an important role in the activation of nifurtimox. Type I nitroreductase (NTR), present in many prokaryotes including some of the protozoan parasites but absent from humans, is oxygen independent (i.e., oxygen insensitive) and leads to production of nitrile but

not to the production of reactive oxygen species. Type II NTR, an ubiquitous enzyme present in both prokaryotes and eukaryotes, is oxygen sensitive and important in maintaining respiratory rate as well as release of hydrogen peroxide and/or other reactive oxygen species (free radicals) resulting in oxidative stress and reducing the formation of thiols, such as glutathione, important in conferring defense to the parasites.

9.1.1.1. Effect on Oxidative Stress, DNA and Protein Synthesis

The studies suggest that activation of nifurtimox, by Type I and Type II NTRs leads to production of toxic intermediate metabolites and/or reactive oxygen species, a decrease in thiols, DNA damage, inhibition of protein synthesis, and cell death of the intracellular (amastigotes) and extracellular (epimastigotes and trypomastigotes) forms of *T. cruzi*.

9.1.1.2. Effect on Ultrastructure

Nifurtimox induces damage of the intracellular and extracellular forms of *T. cruzi* leading to loss of infectivity and death of the parasites. For example, Gönnert and Bock (1972) reported that **trypomastigotes** incubated with nifurtimox for an hour were damaged and had reduced infectivity of the HeLa cells.

Nifurtimox induced damage to the intracellular **amastigotes** within 8 to 10 hours; some of the changes reported include the presence of an increased number of cytoplasmic vacuoles, membrane bound lipoid droplets, and dense particles in the kinetoplast. The cytoplasmatic damage and swelling of the mitochondria gradually increased over time and by 72 hours, most of the parasite cells were damaged. The kinetoplast was reported to be morphologically least influenced and considered most resistant (Voigt et al. 1972a).

After 24 hours, the nifurtimox induced damage to the parasites was irreversible (Gönnert and Bock 1972).

For details see Sections 20.6.1.1 and 20.6.1.2.

9.1.2. In Vitro Activity

Several studies reported the activity of nifurtimox against different stages of *T. cruzi.* The methods for measurement of in vitro sensitivity of *T. cruzi* are not standardized and limited to testing in research laboratories.

The experimental design used in different studies varied. Some of the variations include different stages and strains of the parasite, inoculum concentration, duration of incubation and the methods used to measure activity such as microscopic examination and ^{3}H-thymidine uptake. The activity against the epimastigotes was measured in axenic cultures whereas the activity against the amastigote stage was measured in mammalian cell cultures. There is a possibility that different stages of the parasite may be present in culture (asynchronous cultures) and the proportions of different developmental forms may vary with the *T. cruzi* strain.

The results show that the 50% inhibitory concentration (IC_{50}) values based on inhibition of growth, against different *T. cruzi* strains (~50) irrespective of the stage of the parasite, were ≤21.1 mcg/mL. The activity against epimastigote, trypomastigote or amastigote forms of the parasite appears to be similar and concentration- and time-dependent. Faster growing strains may require higher concentrations of the drug for inhibition. Based on a small number of strains tested, there does not appear to be any difference in the nifurtimox IC_{50} against the different distinct typing units (DTUs) and different strains of *T. cruzi* from different geographic regions.

There is a trend towards higher IC_{50} values against the strains that are considered to be nonresponsive compared to the responsive strains (nonresponsiveness/responsiveness of strains was based on studies in murine models of *T. cruzi*). The nonresponsive strains are more infective and proliferate faster in vitro compared to the responsive strains.

One study (Martínez et al. 2013) reported the effect of nifurtimox on both growth and motility based on testing of 11 *T. cruzi* strains; the motility, compared to growth, was inhibited at 2- to 52-fold higher nifurtimox concentrations; the IC_{50} values ranged between 1.8 and 110.5 mcg/mL.

There was no effect of nifurtimox on the uninfected HeLa cells in culture.

The IC_{50} values against some of the strains are higher than the peak concentrations in serum from subjects with Chagas disease. It is unclear if nifurtimox can accumulate in phagocytic cells, where the amastigotes replicate and release trypomastigotes into circulation. The activity of nifurtimox against the intracellular stage (amastigotes) of *T. cruzi* is important as the amastigote stage is the predominant form in humans. Also, the killing of the parasite requires that drug cross the host cell membrane.

For details see Section 20.6.1.2.

9.1.3. In Vivo Activity (Animal Studies)

Several studies reported the activity of nifurtimox in acute and/or chronic infection models of *T. cruzi*. Approximately 62 strains/isolates were tested in several animal species that include mice, rats, hamsters, guinea pigs, rabbits and dogs. Several aspects of *T. cruzi* infection in these animal models mimic human disease. Like humans, parasitemia develops after incubation or a prepatent phase; the patent phase is followed by a subpatent or latent phase. However, the duration of different phases of infection vary with the experimental conditions that include strain of the parasite, animal species and strains; immune status of the host plays a role in conferring protection. In general, the *T. cruzi* infection of animals is not fatal; however, acute infection of mice with *T. cruzi* can be fatal and untreated animals may die within a month postinfection. Mice with chronic infection may survive up to 2 years postinfection. Animals may develop cardiac abnormalities that include myocardial fibrosis and persistence of parasites in tissues. The lesions and characteristic chronic pathology, in general, are similar to those observed in human disease, and include diffuse myocarditis and signs of heart failure.

The experimental design for different studies varied (for details see Section 20.6.1.3). A majority of the studies were conducted in mice and reported the effect of nifurtimox treatment on parasite burden or survival. Few studies reported the effect of nifurtimox treatment on serological response and compared with parasite burden.

9.1.3.1. Effect on Parasite Burden

The activity of nifurtimox varies with the strain of *T. cruzi*, inoculum concentration, time of initiation of treatment, and other experimental conditions. Overall, the studies suggest that nifurtimox is effective in reducing parasitological burden and/or duration of parasitemia in animals with acute and chronic infection. The survival of mice with acute infection was also improved. The activity of nifurtimox decreases if there is a delay in initiation of treatment, e.g., in the chronic phase of *T. cruzi* infection.

Reactivation of infection was observed after immunosuppression.

9.1.3.2. Effect on Anti-*T. cruzi* Antibody Response

Some studies, in murine and dog infection models, reported the effect of nifurtimox treatment on both serological response and parasite burden.

9.1.3.2.1. Mice

Acute Infection Models

Andrade et al. (Andrade et al. 1985) reported that cure rates in mice, infected with 30 different strains, varied between 5.8% and 100%; the cure rates were based on microscopic evidence of parasite(s) in blood, xenodiagnosis, inoculation in newborn mice, and hemoculture. All animals that remained infected after treatment were seropositive by the IFA test, a conventional serological test; antibody titer ranged between 1:10 to 1:80. The antibody titer was ≤1:10 in parasitologically cured mice; however, 82.0% of the mice were seropositive.

Similar observations were reported by de Oliveira et al. (de Oliveira et al. 2017) in mice infected with 4 clinical isolates (3 of DTU TCII and 1 of TCVI) obtained from Brazil. Briefly, treatment with nifurtimox (100 mg/kg for 20 days) was initiated on the day mice became parasitemic and followed for survival and parasitemia until Day 90 postinfection. Cure was based on hemoculture, PCR, as well as anti-*T. cruzi* antibodies by conventional ELISA (anti-*T. cruzi* IgG antibodies by the method of Voller et al. (Voller et al. 1975) and nonconventional test (flow cytometry for detecting anti-live trypomastigotes IgG antibodies) at Days 90, 180 and 360 after treatment. Nifurtimox was effective in reducing parasitemia to undetectable levels in a majority of mice by Day 90 and all mice by Day 360 (Figure 5). By conventional ELISA, a majority of the mice remained seropositive until the end of the study; only 25% of the mice infected with the strain # 501 became seronegative. However, by the nonconventional flow cytometry assay that detect anti-*T. cruzi* antibodies against the live parasites, ≥63% of nifurtimox-treated mice became seronegative irrespective of the strain (Table 56). Cardiac inflammation was decreased

in treated mice (Figure 5); there was no effect on fibrosis. All mice survived the period of observation.

Table 56. Effectiveness of Nifurtimox Treatment During Acute and Chronic Murine Infection With Distinct *T. cruzi* Isolates[a]

T. cruzi Genotype (Isolate Code)		Cure Assessement										
		Parasitological Methods (% of negative results)						Serological Methods (% of negative results)				Kretlli & Brener Cure Criteria
								Conventional			Non-conventional	
		HC			PCR			ELISA			FC-ALTA	
		90dat	180dat	360dat	90dat	180dat	360dat	90dat	180dat	360dat	360dat	360dat
Acute Phase	TcII (501)	63 (5/8)	13 (1/8)	100 (8/8)	13 (1/8)	100 (8/8)	100 (8/8)	0 (0/8)	25 (2/8)	25 (2/8)	75 (6/8)	75[d] (6/8)
	TcII (452)	100 (8/8)	100 (8/8)	100 (8/8)	25 (2/8)	100 (8/8)	100 (8/8)	0 (0/8)	0 (0/8)	0 (0/8)	88 (7/8)	88[d] (7/8)
	TcII (728)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)	63 (5/8)	100 (8/8)	0 (0/8)	0 (0/8)	0 (0/8)	25 (2/8)	25[a,b,c] (2/8)
	TcVI (1337)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)	75 (6/8)	100 (8/8)	0 (0/8)	0 (0/8)	0 (0/8)	63 (5/8)	63[d] (5/8)
Chronic Phase	TcII (501)	25 (2/8)	100 (8/8)	100 (8/8)	50 (4/8)	50 (4/8)	100 (8/8)	0 (0/8)	0 (0/8)	0 (0/8)	0 (0/8)	0 (0/32)
	TcII (452)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)	0 (0/8)	0 (0/8)	0 (0/8)	0 (0/8)	0 (0/32)
	TcII (728)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)	50 (4/8)	100 (8/8)	0 (0/8)	0 (0/8)	0 (0/8)	0 (0/8)	0 (0/32)
	TcVI (1337)	50 (4/8)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)	0 (0/8)	0 (0/8)	0 (0/8)	0 (0/8)	0 (0/32)

[a] Data are presented as percentage of negative results in parasitological e aserological methods (number of negative aninals/total number of animals); dat: day after treatment HC: hemoculture; PCR: polymerase reaction chain; ELISA: IgG anti-*T. cruzi* epimastigote enzine-linked immunosorbent assay; FC-ALTA: Flow cytometric anti-live trypomastigote IgG antibody. Krettli & Brener cure criteria as proposed by Krettli & Brener (1982).

Source: NDA

Figure 5. Response to Nifurtimox Treatment During Acute and Chronic Murine Infection With TcII and TcVI Strains of *T. cruzi*

Parasitemia curves and cardiac inflammatory infiltrate analysis in Swiss mice infected with TcI, TcII and TcVI *Trypanosoma cruzi* samples. Parasitemia data are reported as number of trypomastigotes per 0.01 mL of blood animals infected with TcI (Pr 150 –◻), TcVI (1337 –), TcVI (748 –), TcII (501 – ●), TcII (452 – ▲) and TcII (728 – ■). Comparative analysis of parasitemia curves observed for distinct *T. cruzi* genotypes was performed considering the area under the curve *via* ANOVA followed by Newman-Keuls post-test. AUC values are provided in the figures. The cardiac inflammatory infiltrate was evaluated in animals infected with TcVI (▬) and TcII (▬) *Trypanosoma cruzi* samples. Data are reported as the mean number ± standard deviation of nucleated cells in a total area of 0.8×10^6 µm². The comparison of cardiac inflammatory infiltrated observed for distinct *T. cruzi* genotypes was carried out by Kruskall-Wallis followed by Dunn's post-test. In all cases, significant differences at $p \leq 0.05$ were identified by letters "a", "b", "c" and "d" as compared to TcVI (1337), TcII (501), TcII (452) and TcII (728), respectively.

Source: NDA

Chronic Infection Model

Meckert et al. reported parasitological cure of BALB/c mice infected intraperitoneally with the Tulahuen strain of *T. cruzi* and treated with nifurtimox (Meckert et al. 1988). However, the effect on serological response varied depending on the test. Anti-*T. cruzi* antibodies were detected by conventional serological (IFA and hemagglutination) tests in the sera of all treated and untreated mice; however, complement-dependent lysis of the parasite or antibody-dependent cell-mediated cytotoxicity (ADCC) activity was not detected after treatment. Two months after the end of the 30-day treatment, no parasites were recovered by subinoculation into suckling mice. No mortality was observed in either the untreated or the treated mice.

Another study (de Oliveira et al. 2017) reported that Swiss mice infected with 4 clinical isolates and treated with nifurtimox during the chronic phase (90 days postinfection) were aparasitemic by Day 360. However, none of the mice became seronegative by either the conventional ELISA or nonconventional flow cytometry assay (that detects antibodies against the live parasites) up to Day 360. There was no effect on cardiac inflammation or fibrosis (Figure 5; Table 56). All mice survived the period of observation.

9.1.3.2.2.Dogs

The effect on parasitemia and serological response in Beagle dogs infected with the Ecuador strain was reported in one study ((Haberkorn and Gönnert 1970) and (Haberkorn and Gonnert 1972)). Treatment was initiated during the subacute and chronic phase of *T. cruzi* infection.

Subacute Infection

Treatment was initiated in 7 dogs, 46 days after inoculation when the last positive microscopical demonstration of the parasites was made at least a week previously in 8 of the 14 infected animals; one dog was parasitemic on the day of treatment and the body temperature was normal in all animals. Nifurtimox was administered daily (30 mg/kg/day for 15 days followed by 20 mg/kg/day for 75 days). No evidence of parasitemia was reported at Months 6 and 18 postinfection whereas all 7 untreated dogs tested positive; parasitemia was measured by subinoculation of blood from treated dogs into naïve mice. All treated dogs became seronegative for anti-*T. cruzi* antibodies by three serological (IFA, ADCC, and hemagglutination) tests by Month 7 postinfection; the untreated dogs remained seropositive by all three tests (Figure 6).

There was no change in liver and kidney function.

Chronic Infection

Of the 7 untreated dogs from the above subacute infection part of the study, four were treated with nifurtimox for 120 days at 1½ years postinfection. No parasites were detected by subinoculation of mice from the blood of dogs for the duration of the study. All the treated dogs remained seropositive during the 4-month postobservation period (Figure 6).

Figure 6. Effect of Nifurtimox on Parasitemia and Serological Response (Anti-*T. cruzi* Antibodies) in Dogs Experimentally Infected With the Ecuador Strain of *T. cruzi*

Effect on Parasitemia | **Effect on Anti-*T. cruzi* Antibody Response**

Source: NDA

Overall, the studies suggest that nifurtimox is active against the intracellular and extracellular stages of *T. cruzi* and effective in improving survival, decreasing parasitic burden, decreasing inflammation of the heart as well as decreasing antibody response to *T. cruzi* antigens especially by nonconventional assays against the live trypanosomes. Cure rates varied with the parasite strain, animal species, as well as the time of initiation of treatment postinfection. Treatment with nifurtimox initiated early after infection, especially during the acute or subacute phase, appears to be most effective; parasitological cure appears to be associated with seroconversion when testing is performed using assays that detect antibodies against the live parasites by nonconventional assays. Early treatment may reduce organ damage; however, organ damage cannot be reversed by nifurtimox treatment.

9.1.4. Drug Resistance

The studies in vitro and in murine animal models suggest a potential for development of resistance to nifurtimox by the *T. cruzi* parasites. The mechanism of resistance appears to be due to down-regulation of Type I NTR (oxygen insensitive enzyme), increased efflux pump, and higher glutathione concentration. The clinical relevance of these findings is not known.

For details see Section 20.6.1.4.

9.1.5. Cross-Resistance

Nonclinical studies suggest cross-resistance between nifurtimox and benznidazole. This appears to be due to down regulation of Type I NTR. The clinical relevance of these findings is not known.

For details see Section 20.6.1.5.

9.2. Clinical Microbiology—Study 16027

The parasitological assessments in the Phase 3 randomized, double-blind, 2-arm (60-Day and 30-Day dosing regimen) parallel-group historically controlled 2-part trial (Study 16027) in 330 children, <18 years of age, include detection of anti-*T. cruzi* antibodies by serological tests, parasites in blood by microscopy, and/or parasite DNA in blood by the real time quantitative PCR (RT-qPCR). These assessments were conducted prior to initiation of therapy, i.e., at baseline for confirming diagnosis/patient enrollment as well as at follow-up visits to evaluate treatment response. The trial was conducted at 25 sites; 18 in Argentina, 3 in Bolivia, and 4 in Colombia. However, the parasitological testing was conducted at a central laboratory (b) (4)), unless specified otherwise (for details of the Study protocol see Section 8).

The majority of the assays used for parasitological assessments are not cleared by the FDA. The Center for Devices and Radiological Health (CDRH) was consulted to determine the adequacy of the performance of the assays for the intended context of use.

A description of the parasitological tests used as well as information supporting the performance of the assays for the intended context of use, i.e., patient enrollment and for assessment of efficacy including a comparison of the assays with the historical control studies, and the clinical trial results based on patients who completed the study, i.e., the per protocol (PP) population, are summarized.

9.2.1. Description of the Parasitological Tests

9.2.1.1. Detection of Anti-*T. cruzi* Antibodies by Serological Tests

The diagnosis of subjects, for enrollment, was based on testing by recombinant-ELISA and lysate-ELISA performed at the site laboratories. However, the specimens, collected at baseline, during treatment and follow-up visits were shipped to the central laboratory for testing by four different serological tests.

The serological tests used in Study 16027 as well as the historical control published studies are as follows.

9.2.1.1.1. Serological Tests Used by the Applicant for Study 16027

The Applicant used four serological tests for detecting anti-*T. cruzi* antibodies that include Recombinant-ELISA, Lysate-ELISA (referred to as total purified antigen ELISA by the Applicant), IHA, and F29-ELISA. The antigens and the probes used to detect anti-*T. cruzi* antibodies vary for the four different serological tests (for details see Table 57).

Three of the four tests (Recombinant-ELISA, Lysate-ELISA and IHA) are commercially available in Argentina. Of the 3 commercial tests, one assay (recombinant-ELISA), appears to be the same as that cleared by the FDA and the manufacturer is the same (Wiener Lab). All three commercially available tests are for qualitative use (reactive/nonreactive); the Applicant used the test cut-off values specified by the test manufacturers to characterize specimens as seropositive (reactive) or seronegative (nonreactive). The antibodies measured by the three tests are against the parasite extracts or a cocktail of *T. cruzi* antigens (Table 57; for details see Section 20.6.2).

The F29-ELISA is not commercially available in any country. Studies (Engman et al. 1989; Porcel et al. 1996) show that the recombinant F29 (29 kDa) antigen used in the assay, is a flagellar calcium-binding protein encoded by several genes, highly conserved among different *T. cruzi* strains. As F29 contributes to the rapid motility of the trypanosomes, anti-*T. cruzi* antibodies against the F29 antigen may reflect antibody response to live parasites. Some of the published studies suggest that antibodies, such as the lytic antibodies measured by a complement mediated lysis (CoML) assay, are directed against the live trypomastigotes; these lytic antibodies did not persist in patients that were negative by xenodiagnosis (Krettli et al. 1982). Also, the lytic antibodies were detected in mice with live infection but not in those vaccinated with the dead parasites.

Table 57. Summary of Antigens and Anti-*T. cruzi* Antibodies Detected as Well as Concordance Among Different Serological Tests

	Assay Characteristics		Overall Concordance Among Serological Tests**			
Test (Cut-Off^)	***T. cruzi* Antigen(s)**	**Anti-*T. cruzi* Antibody Class Detected**	**Recombinant-ELISA (n=70)**	**Lysate-ELISA (n=70)**	**IHA**	**F29-ELISA (n=67)**
Recombinant-ELISA*# (0.313 based on an average negative control readings plus 0.300 OD)^^	Six recombinant antigens (1, 2, 13, 30, 36 and SAPA) obtained from epimastigote and trypomastigote forms.	Immunoglobulins (IgM and IgG)	NA	94.3%	ND	86.6%
Lysate-ELISA* (0.221 based on an average of the negative control OD readings plus 0.200)^^	Cytoplasmic and membrane antigens.	IgG	94.3%	NA	ND	85.1%
IHA* (titers ≥1:16 is considered seropositive)	Cytoplasmic and membrane antigens.	Agglutinating antibodies (IgM and IgG)	ND	ND	NA	ND
F29-ELISA (0.170 based on an average OD ±3 SD)	Recombinant F29 antigen (a flagellar calcium-binding protein from epimastigotes)	Immunoglobulins (IgM and IgG)	86.6%	85.1%	ND	NA

* Commercially available from Wiener Lab, Rosario, Argentina.
** Comparison based on testing in the central laboratory.
# Recombinant-ELISA used by the Applicant appears to be the same as the FDA cleared assay by the same manufacturer.
^ Cut-off used to define the results as reactive (seropositive) or nonreactive (seronegative).
^^ For the clinical trial specimens: the OD cut-offs, based on information in the datasets are as follows:

- Recombinant-ELISA: ≥0.262 seropositive; ≤0.25 listed as seronegative.
- Lysate-ELISA: ≥0.247 seropositive; ≤0.23 listed as seronegative.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; NA, not applicable; ND, not done; OD, optical density; SAPA, shed acute phase protein; SD, standard deviation

The Applicant proposed to assess efficacy based on seroconversion OR a ≥20% decrease in optical density (OD) by Recombinant-ELISA and Lysate-ELISA for patients that do not seroconvert within the study observation period.

Seroconversion

The serological tests used by the Applicant are adequate to assess seroconversion, i.e., the presence or absence of anti-*T. cruzi* antibodies. Generally, at least two different serological tests that detect anti-*T. cruzi* antibodies against different antigens used in combination are considered as evidence of exposure to *T. cruzi*.

≥20% Decrease in Optical Density

Recombinant ELISA

The package insert of the FDA cleared test for the Recombinant-ELISA described the results from testing serial dilutions of two reactive specimens. The relationship between the dilution factor and the Recombinant-ELISA OD was nonlinear (Figure 7; for details see Section 4.3 and Section 20.6.2.1).

Figure 7. Recombinant-ELISA OD Based on Serial Dilution of 2 Reactive Specimens

Source: Dr. Noel Gerald, CDRH
Abbreviations: ELISA, enzyme-linked immunosorbent assay; OD, optical density

Lysate-ELISA

No information was available to support an efficacy assessment based on a ≥20% OD decrease by the Lysate-ELISA used in Study 16027.

9.2.1.1.2.Serological Tests in the Historical Control Studies

The Applicant compared the serological test results, at Month 12, of nifurtimox treated patients in Study 16027, with the results of the placebo group in 2 published studies in children 6 to 12 (Sosa Estani et al. 1998) and 7-12 (de Andrade et al. 1996) years of age. In response to the Division's request, the Applicant provided the SOP and data for the F29-ELISA for the published study by Sosa-Estani et al. (Sosa Estani et al. 1998). The available information for the serological tests used, in the 2 published studies that are relevant to the tests used by the Applicant, is summarized in Table 58 (for details see Section 20.6.2.5). Briefly, none of the assays used in the published studies are commercially available assays. The conventional ELISA in the published studies appears to be similar to the Lysate-ELISA used by the Applicant; the IHA test and F29-ELISA in the published studies and those used by Applicant appear to be similar.

Table 58. Comparison of Serological Tests Used in the Published Studies With Those Used by the Applicant, Study 16027

	Sosa-Estani et al. (1998)		de Andrade et al. (1996)		
Test	**Method (Cross-Referenced Publication)**	**Results**	**Method (Cross-Referenced Publication)**	**Results**	**Comments**
ELISA	A soluble extract derived from the Peru strain of *T. cruzi.* Cut-off: >0.4* (Voller et al. 1975)	Of the 44 patients in the placebo-group, 2 (4.5%) became seronegative at month 48. Cure rates at month 12 not specified.	A soluble extract derived from the Peru strain of *T. cruzi.* Cut-off: Index ≥1.2 (Voller et al. 1975)	Cure rates not reported at any of the time points.	The assay appears to be similar to the lysate-ELISA used by the Applicant; however, the cut-offs used are different.
IHA	Cytoplasmic and membrane antigens. Cut-off: Not specified. (Cerisola et al. 1971)	No significant change in IHA titer up to month 48 in the placebo group. Cure rates not specified.	Parasite extract Cut-off: ≥1:16 (Camargo et al. 1973)	An initial decrease in IHA titer between months 2 and 5 followed by a gradual increase up to Month 36. Cure rates not specified.	The antigens appear to be similar to the test used by the Applicant; however, the cut-offs used are different.
F29-ELISA	Testing performed at Instituto Nacional de Parasitologıa (INP) Dr. Mario Fatala Chaben. The cut-off used was not specified in the publication. Cut-off: 0.170**	**Based on publication:** None of the children (age 6 -12 years) in the placebo-group seroconverted to a negative result at Month 12 and up to the end of follow-up (Month 48). **Based on dataset**: ***Month 12*:** 1 of 31 (3.2%)! subjects in the placebo group became seronegative; 5 subjects were not tested at Month 12. ***Month 48:*** 4 of 36 (11.1%) subjects, became seronegative.	Not applicable		The method is same as that used by the Applicant.

All referenced publications are listed in Section 20.1.
* 0.4 based on cross reference by Voller et al., 1975. It is stated that a reading of 0.4 was measured at 400 nm in a spectrophotometer with a 1 cm light path. A reading of 0·4 or less was considered negative.
** It is stated in the SOP that testing was performed on 50 sera from healthy individuals and the cut-off point determined based on the following formula: Average of the optical densities ±3 standard deviations.
! 5 subjects were not tested at Month 12; these were tested at other time points such as baseline, Months 3, 6, 18, 24 and/or 48.

Reviewer Comments: *All the serological tests used by the Applicant are qualitative tests and adequate for determining cure rates based on seroconversion. Seroreduction based on a reduction in antibody titer, by the IHA test, is also an appropriate measure of efficacy.*

Based on the information in the FDA cleared package insert for the recombinant ELISA, the change in OD values in serially diluted specimens was non-linear; this is a limitation of the enzyme immunoassays. The correlation between ≥20% decrease in OD value and a decrease in antibody titer or seroconversion is not known.

The results of the placebo group of patients in the published studies, for the conventional ELISA and IHA test, were based on tests that were not commercially available; whereas the Applicant used commercially available tests. The antigens used for ELISA and IHA tests in the two historical control studies appear to be similar to the commercially available Lysate-ELISA and IHA test, respectively, used by the Applicant. One study (Sosa Estani et al. 1998) reported Month 48 cure rate of 4.5% in the placebo group of patients by the ELISA; however, the Month 12 cure rates in the placebo group of patients were not reported in either of the studies.

The F29-ELISA used by the Applicant appears to be same as that used in the published study by Sosa-Estani et al. (Sosa Estani et al. 1998); 3% of the placebo group of patients became seronegative at Month 12.

The comparison of cure rates based on seroconversion for Study 16027 with the placebo group in historical control studies based on Lysate-ELISA, IHA test and F29-ELISA is appropriate.

9.2.1.2. Detection of Parasites in Blood by Microscopy

The detection of *T. cruzi* parasites in blood, collected at different visits, was performed by the concentration test, in children <8 months old. Briefly, the parasites in whole blood were concentrated by centrifugation to separate different components of the blood; the parasites present between the WBCs and the plasma layers were detected based on characteristic movement and not morphology.

Testing was performed at the site laboratories and samples were processed immediately after collection (for details see Section 20.6.2.6).

9.2.1.3. Detection of Parasite DNA by Real Time-Quantitative PCR

The detection of parasite DNA by real time-qPCR assay was performed in the central laboratory. The assay was based on the amplification of a 166 bp segment in the satellite (Sat) DNA of *T. cruzi* by the method of Ramirez et al. (Ramírez et al. 2015); a multiplex RT-qPCR assay and TaqMan probes were used for quantification of *T. cruzi* SatDNA in blood samples (Duffy et al. 2013). Based on the available information in the publications and some of the testing performed in the central laboratory, it appears that the different *T. cruzi* lineages can be detected but the analytical sensitivity varies by up to 16-fold in spiked samples of different strains. A patient was considered RT-qPCR positive when the cycle threshold (Ct) value was <40 (for details see Section 20.6.2.7).

A comparison of the results of the RT-qPCR assay for the Study 16027 with historical control studies was not performed.

9.2.2. Overall Concordance Among the Different Parasitological Tests—Verification in the Central Laboratory

9.2.2.1. Overall Concordance Among Different Serological Tests

A comparison of the performance of the three ELISA tests (Recombinant-ELISA, Lysate-ELISA, and F29-ELISA) was performed in the central laboratory. Serum samples from 70 patients were tested by the Recombinant-ELISA and Lysate-ELISA; 67 of the 70 samples were tested by the F29-ELISA. No details of patient history were provided (for details see Section 20.6.2). The results show 94% overall agreement between the Recombinant-ELISA and Lysate-ELISA; overall agreement of the F29-ELISA with the Recombinant-ELISA and Lysate-ELISA was 87% and 85%, respectively (Table 57); 25 samples were seronegative by all three ELISAs.

A comparison of the IHA test with any of the three ELISAs was not performed.

9.2.2.2. Overall Concordance of Serological Tests With Microscopic Findings

A comparison of any of the serological tests with the detection of parasites by microscopy was not performed in the central laboratory.

9.2.2.3. Overall Concordance of Serological Tests With RT-qPCR

The clinical sensitivity and specificity of the RT-qPCR assay was assessed in the central laboratory based on testing of 20 samples from patients with serological and molecular diagnosis and 20 samples from healthy individuals with negative serology and without the risk of getting Chagas in acute form. The details of the serological or molecular tests used previously for the testing of these samples were not specified. There was 100% agreement in sensitivity and specificity (for details see Section 20.6.2.7).

9.2.3. Results—Parasitological Assessments

Diagnosis of the 311 children enrolled, between 8 months to 17 years of age, was based on testing by the Recombinant-ELISA and Lysate-ELISA performed at the site laboratories. The enrollment of 19 infants, 0 to <8 months of age, was based on evidence of the *T. cruzi* parasites in blood, by microscopy. ECG abnormalities were reported in 2 patients in the 60-Day treatment group.

Of the 330 patients enrolled, 282 were in the PP set (95 in the 30-Day treatment group and 187 in the 60-Day treatment group), i.e., nifurtimox treated patients who had no major protocol deviations and completed the trial. Parasitological findings in patients in the PP set, at the time of enrollment and follow-up visits, are summarized below in Sections 9.2.3.1 and 9.2.3.2, respectively.

9.2.3.1. Parasitological Findings at the Time of Enrollment

9.2.3.1.1. Serology

The following is the anti-*T. cruzi* antibody status by the different serological tests, prior to treatment, based on testing in the central laboratory:

- **By Recombinant-ELISA and Lysate-ELISA:** Of the 282 patients in the PP set that were enrolled based on testing at the site laboratories, 280 (99.3%) were seropositive, based on testing in the central laboratory, by both Recombinant-ELISA and Lysate-ELISA (Table 59); the following two patients were seronegative by one of the tests:
  - ID (b) (6) seronegative by Recombinant-ELISA: 30-Day treatment group.
  - ID (b) (6) seronegative by Lysate-ELISA: 30-Day treatment group.
- **By IHA:** Of the 282 patients in the PP set that were enrolled based on testing by Recombinant-ELISA and Lysate-ELISA at the site laboratories, 279 (99.3%) were seropositive by the IHA test; one patient was not tested (Table 59). The following 2 patients were seronegative by the IHA test:
  - ID (b) (6): 60-Day treatment group.
  - ID (b) (6): 60-Day treatment group.

Reviewer Comments: *The CDC recommends that the diagnosis of Chagas disease be based on testing by two or more tests that use different techniques and detect antibodies to different antigens for diagnosis of Chagas disease. The diagnosis of the patients enrolled based on serological findings is consistent with the CDC guidelines as all subjects were seropositive by at least two tests; 98.6% tested positive by Recombinant-ELISA + Lysate-ELISA + IHA; 99.3% tested positive by Recombinant-ELISA + Lysate-ELISA; all subjects were seropositive by either Recombinant-ELISA + IHA or Lysate-ELISA + IHA.*

Table 59. Patient Characteristics at the Time of Enrollment (Visit 1)—Number Positive or Negative by One or More of the Serological Tests and/or RT-qPCR

Test - Parameter	Positive	Negative	Discordance*
1 Test			
Recombinant-ELISA (N=282)	281 (99.6)	1 (0.4)	NA
Lysate-ELISA (N=282)	281 (99.6)	1 (0.4)	NA
IHA (N=281)	279 (99.3)	2 (0.7)	NA
F29-ELISA (N=282)	185 (65.6)	97 (34.4)	NA
RT-qPCR (N=279)	151 (54.1)	128 (45.9)	NA
2 Serological Tests			
Recombinant-ELISA + lysate-ELISA (N=282)	280 (99.3)	0 (0.0)	2 (0.7)
IHA and recombinant-ELISA (N=281)	278 (98.9)	0 (0.0)	3 (1.1)
IHA and lysate-ELISA (N=281)	278 (98.9)	0 (0.0)	3 (1.1)
IHA + recombinant-ELISA or lysate-ELISA (N=281)	279 (99.3)	0 (0.0)	2 (0.7)
IHA + F29-ELISA (N=281)	184 (65.5)	1 (0.4)	96 (34.1)
F29-ELISA + recombinant-ELISA (N=282)	185 (65.6)	1 (0.4)	96 (34.0)
F29-ELISA + lysate-ELISA (N=282)	185 (65.6)	1 (0.4)	96 (34.0)
3 Serological Tests			
IHA + recombinant-ELISA + lysate-ELISA (N=281)	277 (98.6)	0 (0.0)	4 (1.4)
4 Serological Tests			
IHA, recombinant-ELISA, lysate-ELISA, and F29-ELISA (N=281)	184 (65.4)	0 (0.0)	97 (34.5)
RT-qPCR + Serological Tests			
RT-qPCR + F29-ELISA (N=279)	120 (43.0)	65 (23.3)	94 (33.7)
RT-qPCR + recombinant-ELISA (N=279)	151 (54.1)	1 (0.4)	127 (45.5)
RT-qPCR + lysate-ELISA (N=279)	151 (54.1)	1 (0.4)	127 (45.5)
RT-qPCR + IHA (N=278)	150 (54.0)	2 (0.7)	126 (45.3)

All values are expressed as n (%).
*Represents discordance based on testing by 2 or more tests
Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; RT-qPCR, real-time quantitative polymerase chain reaction; NA, not applicable.

- **F29-ELISA:** The results of the F29-ELISA were not used for enrollment; however, 185 of 282 (65.6%) patients were seropositive at the time of enrollment (Table 59). The concordance between the F29-ELISA and Recombinant-ELISA or Lysate-ELISA was 65.6%; this is lower than that reported based on testing of 67 specimens for verification of the assay performance in the central laboratory (Table 57); reasons for this are unclear as no information regarding patient history or the serological tests used for verification was provided.

9.2.3.1.2.RT-qPCR

Of the 282 patients in the PP set, 279 were tested by the RT-qPCR assay; 151 of 279 (54.1%) patients tested positive by RT-qPCR were seropositive by Recombinant-ELISA or Lysate-ELISA (Table 59).

Reviewer Comments: *Overall concordance between the RT-qPCR and serological findings in the patients enrolled is low compared to 100% reported for verification of the assay performance in the central laboratory (for details see Section 9.2.2.3); reasons for this are unclear. No information regarding patient history or the serological tests used for verification was provided.*

9.2.3.1.3.Detection of Parasites in Blood

All 19 infants, <8 months of age, were parasitemic based on evidence of the parasites by the concentration test, at the time of enrollment; 7 were in the 30-Day treatment group and 12 in the 60-Day treatment group. Of the 19 infants, 15 (78.9%) were RT-qPCR positive.

Reviewer Comments: *Although the lower limit of detection by RT-qPCR is 0.1 parasite equivalent/mL, 4 (21%) of the infants positive by the concentration test were negative by RT-qPCR; the reasons for negative results by RT-qPCR with microscopic evidence of parasitemia are unclear.*

9.2.3.2. Parasitological Assessments at Follow-Up Visits

The follow-up of patients includes detection of anti-*T. cruzi* antibodies by the 4 serological tests to determine seroconversion and/or seroreduction, parasitemia based on detection of parasite DNA by RT-qPCR and microscopic findings.

9.2.3.2.1.Effect on Seroconversion

Effect on Proportion of Patients Who Became Seronegative

The proportion of patients that became seronegative at Month 12 (Visit 11), for anti-*T. cruzi* antibodies, by either of the four serological tests as well as based on two or more serological tests were analyzed in the PP set (n=282) and a subset of PP set patients that were seropositive by the F29-ELISA at the time of enrollment (n=185).

Seroconversion at Month 12 Based on Either of the Serological Tests

<u>PP Subset</u>

The proportion of patients, in Study 16027, that became seronegative at Month 12 (Visit 11) was between 5.3% and 7.4%, in the 60-day and 30-Day treatment groups, by either Recombinant-ELISA, Lysate-ELISA or IHA (Table 60). In previously published studies (de Andrade et al. 1996; Sosa Estani et al. 1998), Month 12 cure rates were not reported for the conventional ELISA (the assay appears to be similar to Lysate-ELISA used by the Applicant); however, at Month 48, 4.5% of the subjects in the placebo group became seronegative by conventional ELISA (Sosa Estani et al. 1998). The cure rates based on Recombinant-ELISA or IHA test were not reported in the published studies.

By F29-ELISA, over 7-fold higher proportion of patients in Study 16027, became seronegative compared to other serological tests. There is a trend towards a higher proportion (56.1%) of seronegative patients, by F29-ELISA, in the 60-Day treatment group compared to the 30-Day treatment group (50.5%) (Table 60). About 3% of the placebo group of patients in the historical control study (Sosa Estani et al. 1998), became seronegative by the F29-ELISA at Month 12.

Subset of PP Set Based on F29-ELISA Seropositive at Baseline

In the subset of patients (n=185) that were seropositive by the F29-ELISA at the time of enrollment, the proportion of patients that became seronegative at Month 12, compared to baseline, was between 1.6% and 5.7% by either IHA, Recombinant-ELISA or Lysate-ELISA in the two treatment groups. By F29-ELISA, 28.6% and 34.4% of the patients became seronegative in the 30-Day and 60-Day treatment groups, respectively (Table 136). There appears to be a trend towards a higher proportion of seronegative patients in the 60-Day treatment group compared to the 30-Day treatment group by F29-ELISA as well as other serological tests.

Seroconversion at Month 12 Based on Two or More Serological Tests

IHA + Recombinant-ELISA and/or Lysate-ELISA

Of the 282 patients in the PP set, 12 (4.3%) became seronegative at Month 12 (4 in the 30-Day treatment group and 8 in the 60-Day treatment group) by either IHA + Recombinant-ELISA or IHA + Lysate-ELISA or all three serological tests (Table 61). All the 12 patients that became seronegative by all 3 serological tests, were <8 months old and were aparasitemic based on microscopic evidence of *T. cruzi*.

Recombinant-ELISA + Lysate-ELISA + IHA + F29-ELISA

The proportion of subjects that became seronegative at Month 12, by all four serological tests, was similar in the 60-Day and 30-Day treatment groups (Table 61). All the patients that became seronegative at Month 12 were <8 months old.

9.2.3.2.2.Effect on Seroreduction

IHA

Serial dilutions of serum samples collected prior to treatment (baseline) and at Month 12 after discontinuation of treatment (Visit 11) were tested by the IHA test. The results show a decrease in antibody titer by IHA at Month 12 compared to baseline; however, there was no difference between the two treatment groups (Table 62). In the placebo group of patients in the study by Sosa-Estani et al. (Sosa Estani et al. 1998) no change in antibody titer by IHA was reported up to Month 48; whereas de Andrade et al. (de Andrade et al. 1996) reported an initial decrease in IHA titer between Months 2 and 5 followed by a gradual increase up to Month 36.

ELISAs

A decrease in antibody titer, based on serial dilutions of the serum specimens, by any of the ELISAs (Recombinant-ELISA, Lysate-ELISA, or F29-ELISA) was not measured.

Table 60. Seronegative or Parasitologically Negative Patients, by Different Tests at Baseline, During Treatment, and Follow-Up Visits, PP Set, Study 16027

			Treatment Period – Day 30 or 60 (Visit)			Follow-Up Month (Visit)	
Test (Cut-Off)	**Treatment Group**	**Baseline: Visit 1**	**Day 7: Visit 3**	**Day 30: Visit 6**	**Day 60: Visit 8**	**Month 6: Visit 10****	**Month 12: Visit 11**
IHA*	30-day (n=95)	0 (0.0)	ND	ND	ND	ND	5 (5.3%)
	60-day (n=187)	2 (1.1)	ND	ND	ND	ND	10 (5.3%)
Recombinant-ELISA	30-day (n=95)	1 (1.1)	1 (1.1)	1 (1.1)	1 (1.1)	7 (7.4)	7 (7.4)
	60-day (n=187)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (5.3)	11 (5.9)
Lysate-ELISA	30-day (n=95)	1 (1.1)	1 (1.1)	1 (1.1)	1 (1.1)	6 (6.3)	6 (6.3)
	60-day (n=187)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (5.3)	10 (5.3)
F29-ELISA	30-day (n=95)	32 (33.7)	39 (41.1)	37 (38.9)	38 (40.0)	41(43.2)	48 (50.5)
	60-day (n=187)	65 (34.8)	75 (40.1)	76 (40.6)	81 (43.1)	92 (49.2)	105 (56.1)
RT-qPCR	30-day (n=95)	44 (46.3)	75 (78.9)	91 (97.9)	93 (97.9)	92 (96.8)	90 (94.7)
	60-day (n=187)	84 (44.9)	146 (78.1)	179 (95.7)	182 (97.3)	182 (97.3)	183 (97.9)
Concentration test**	30-day (n=7)	0 (0.0)	5 (71.4)#	7 (100.0)	7 (100.0)	ND	ND
	60-day (n=12)	0 (0.0)	11 (91.7)	12 (100.0)	12 (100.0)	Only 2 pts tested, 1 was positive**	ND

Patients with a missing value were imputed as seropositive.
All values are expressed as n (%).
! Nifurtimox tablets administered three times daily for 30 or 60 days. Treatment initiated on Day 1 (Visit 2).
* IHA testing performed only at baseline (Visit 1) and Visit 11.
** For the concentration test, represent results at Visit 9 i.e., 30 days after discontinuation of treatment; Not tested at Visits 10 and 11. For other tests represents Day 40 i.e., Visit 10 results.
#1 patient was not tested and was imputed as positive.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; ND, not done; PP, per protocol; pts, patients; RT-qPCR, real-time quantitative polymerase chain reaction

Table 61. Seroconversion at Month 12 (Visit 11) Based On ≥2 Serological Tests, PP Set, Study 16027

Test	30-Day Trt Group (N=95)	60-Day Trt Group (N=187)
2 serological tests:		
IHA and recombinant ELISA	4 (4.2)	8 (4.3)
IHA and lysate-ELISA	4 (4.2)	8 (4.3)
3 serological tests:		
IHA, recombinant ELISA, and lysate ELISA	4 (4.2)	8 (4.3)
4 serological tests:		
IHA, recombinant ELISA, lysate ELISA, and F29-ELISA	4 (4.2)	5 (2.7)

Source: Reviewer's analysis
All values are expressed as n (%).
Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; N, total number of patients in treatment group; n, number of seronegative patients; PP, per protocol; trt, treatment

Table 62. Antibody Titer by IHA at Baseline and Month 12, PP Set, Study 16027

Visit	30-Day Treatment Group	60-Day Treatment Group
Visit 1 (baseline)** (N=281)	7.54±1.67 / 1876.31 (n=95)	7.32±1.72 / 1514.39 (n=186)
Visit 11 (month 12) (N=282)	6.62±2.08 / 748.25* (n=95)	6.47±1.99 / 646.68* (n=187)

Source: Table constructed by Dr Jie Cong, PhD (Statistics reviewer, CDER)
Results are based on Log transferred reciprocal titer by IHA based on serial dilutions of specimens. All values are expressed as mean ± standard deviation / geometric mean of reciprocal antibody titer.
* Significant difference (p<0.001) between IHA titer at Visit 11 compared to Visit 1
** At baseline, the antibody titer by IHA was ≥1:32 in 279 patients in the PP set; antibody titer was <1:16 for 2 patients and one patient was not tested.
Abbreviations: IHA, indirect hemagglutination assay; N, total number of patients tested at visit; n, number of patients in treatment group; PP, per protocol

Reviewer Comments: *Most of the serological assays are based on detection of IgG antibodies against T. cruzi that can persist for a long time after treatment. The results of Study 16027 show >50% of the subjects who completed the study became seronegative, by the F29-ELISA after treatment with nifurtimox compared to baseline, i.e., prior to initiation of treatment; there is a trend towards a higher proportion of seronegative subjects treated for 60 days (56.1%) compared to 30 days (50.5%). About 3% of the placebo group of subjects in the historical control study became seronegative. Another study (Fabbro et al. 2013) reported seronegative findings in 2 of 37 (5.4%) untreated adult subjects with chronic Chagas disease monitored for about 20 years.*

By Recombinant-ELISA + Lysate-ELISA + IHA, 12 patients became seronegative at Month 12 and were considered cured. All 12 patients were <8 months of age with acute infection based on evidence of parasitemia prior to initiation of therapy.

A decrease in antibody titer was observed by the IHA test, based on serial dilutions of the serum samples, at Month 12 compared to baseline. A decrease in antibody titer, based on serial dilutions of the serum specimens, by other serological tests (Recombinant-ELISA, Lysate ELISA or F29-ELISA) was not measured.

It is often challenging to use serology for diagnosis to reflect active infection or as a marker for cure of the disease after the successful treatment of T. cruzi infection. The long-term follow-up and clinical relevance was reported in one study in adults (Viotti et al. 2006) treated with benznidazole; the proportion of patients that changed clinical group to a more severe Kuschnir

group as well as those that remained seropositive were lower in the benznidazole treated group compared to placebo.

Seroconversion, by F29-ELISA or by two or more conventional serological tests, is an appropriate measure of efficacy; published studies suggest no significant change in seropositivity if left untreated.

9.2.3.2.3.Effect on Parasite DNA by RT-qPCR

Over 75% of the patients became RT-qPCR negative by Day 7 (Visit 3) and ≥95% by Day 30 (Visit 6) of treatment in both the PP set and a subset of patients that were seropositive by the F29-ELISA at the time of enrollment (Table 60 and Table 136).

In a subset of patients that were RT-qPCR positive prior to treatment, 60% became PCR negative by Day 7 and >90% by Day 30 (Table 137).

The majority of the patients that became RT-qPCR negative after start of treatment remained negative for the duration of the trial. There were 6 patients (3 in each of the treatment arm) that became RT-qPCR positive at follow-up Visits 10 and/or 11, after being PCR negative. It is unclear whether the recurrence of RT-qPCR positivity is due to re-infection, relapse, or limitation of the assay. There is no placebo group of patients for comparison.

A majority (97.6%) of the patients that were RT-qPCR negative at baseline, remained negative for the duration of the trial.

Reviewer Comments: *A decrease in the proportion of PCR positive patients occurred by Day 7 of treatment with nifurtimox; a majority (>90%) of the subjects that were PCR positive prior to treatment, became PCR negative by Day 30 of treatment; there were no control group data available for comparison. The results of PCR should be interpreted with caution as a positive PCR finding may be a marker for treatment failure, however, a negative PCR result may be indicative only of the absence of circulating DNA at the moment when blood is drawn for testing and a PCR negative result may not reflect that the patient is cured.*

9.2.3.2.4.Effect on Parasitemia by the Concentration Test

Follow-up by detection of *T. cruzi* parasites in blood, by the concentration test, was performed in all 19 infants, <8 months of age. A majority of the patients [16/19 (84.2%)] were negative by the concentration test within 7 days of start of treatment (Table 60). One patient in the 60-Day treatment group was positive by the concentration test on Day 7 (Visit 3), negative on Days 30 and 60 but became positive at Month 3 after the start of treatment (Visit 9). Another patient was positive on Day 7 and then had negative results for the rest of the follow-up period (Table 63).

Table 63. Parasitological Findings (Microscopic, RT-qPCR and Serological) in Two Patients Who Were Positive by the Concentration Test After the Start of Treatment

		Treatment Days 30 or 60			Follow-Up	
Patient ID (Trt Arm) Test	**Baseline: Visit 1**	**Day 7: Visit 3**	**Day 30: Visit 6**	**Day 60: Visit 8**	**Month 6: Visit 10**	**Month 12: Visit 11**
(b) (6) (60 day)						
Concentration test	+ve	+ve	-ve	-ve	+ve*	ND
RT-qPCR	+ve	+ve	+ve	+ve	+ve*	ND
F29-ELISA	-ve	-ve	-ve	-ve	+ve*	ND
Recombinant-ELISA	+ve	+ve	+ve	+ve	+ve*	ND
Lysate-ELISA	+ve	+ve	+ve	+ve	+ve*	ND
IHA titer	1:2048	ND	ND	ND	1:2048*	ND
(b) (6) (30 day)						
Concentration test	+ve	+ve	-ve	-ve	-ve*	ND
RT-qPCR	+ve	+ve	-ve	-ve	-ve	-ve
F29-ELISA	-ve	-ve	-ve	-ve	-ve	-ve
Recombinant-ELISA	+ve	+ve	+ve	+ve	-ve	-ve
Lysate-ELISA	+ve	+ve	+ve	+ve	-ve	-ve
IHA titer	1:512	ND	ND	Nd	ND	1:32

Source: Reviewer's analysis
*Represent Visit 9 (90 days after treatment start) results.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; ND, not done; RT-qPCR, real-time quantitative polymerase chain reaction; trt, treatment; +ve, positive; -ve, negative; Trt, treatment

Of the 2 patients that were parasitemic by the concentration test at Day 7 of treatment, one was RT-qPCR positive at Month 3 (Visit 9) after the last dose. The second patient was RT-qPCR positive on Day 7, negative on Day 30 and remained negative until Month 3 (Visit 9) after the last dose (Table 63). One patient became seropositive by F29-ELISA at Visit 10 whereas the second patient remained seronegative. Both patients were seropositive by Recombinant-ELISA, Lysate-ELISA and IHA at all visits (Table 63).

There appears to be a correlation between the concentration test and RT-qPCR; however, a higher proportion of patients in the 30-Day treatment group became RT-qPCR negative compared to the 60-Day treatment group (Table 64).

A high proportion of infants became seronegative at Month 12 by all the serological tests (Table 61Table 64) compared to older children.

Table 64. Seronegative or RT-qPCR Negative Patients by Different Tests at Baseline, During Treatment, and Follow-Up Visits, Parasitologically Positive Patients by the Concentration Test at Baseline, Study 16027

			Treatment Days 30 or 60 (Visit)			Follow-Up	
Test	**Treatment Arm**	**Baseline: Visit 1**	**Day 7: Visit 3**	**Day 30: Visit 6**	**Day 60: Visit 8**	**Month 6: Visit 10**	**Month 12: Visit 11**
IHA*	30-day (N=7)	0 (0.0) [1:256-1:4096]	ND	ND	ND	ND	5 (71.4) [1:16-1:32]
	60-day (N=12)	0 (0.0) [1:128-1:4096]	ND	ND	ND	ND	8 (66.7) [1:16-1:32]
Recombinant-ELISA	30-day (N=7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (71.4)	5 (71.4)
	60-day (N=12)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (83.3)	10 (83.3)
Lysate-ELISA	30-day (N=7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (71.4)	5 (71.4)
	60-day (N=12)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (83.3)	10 (83.3)
F29-ELISA	30-day (N=7)	5 (71.4)	4 (57.1)	5 (71.4)	4 (57.1)	5 (71.4)	5 (71.4)
	60-day (N=12)	5 (41.7)	7 (58.3)	8 (66.7)	9 (75.0)	9 (75.0)	7 (58.3)
RT-qPCR	30-day (N=7)	0 (0.0)	0 (0.0)	6 (85.7)	6 (85.7)	7 (100.0)	7 (100.0)
	60-day (N=12)	4 (33.3)	3 (25.0)	11 (91.7)	11 (91.7)	10 (83.3)	10 (83.3)

Source: Reviewer's analysis
All values are expressed as n (%). IHA values are expressed as n (%) [range of antibody titer].
Patients with a missing value were imputed as seropositive or PCR positive
! Nifurtimox tablets administered three times daily for 30 or 60 days. Treatment initiated on Day 1 (Visit 2).
*IHA testing performed at baseline and Visit 11 for all children, except 1, <8 months of age.
Serological and RT-qPCR testing performed at all visits except for one patient performed at Visit 9 instead of Visits 10 and 11
Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; ND, not done; RT-qPCR, real-time quantitative polymerase chain reaction

Reviewer Comments: *In infants <8 months of age with acute phase infection with documented parasitemia based on microscopic evidence prior to initiation of therapy, 84.2% became aparasitemic at Day 7 and 94.7% at Month 3 after the last dose; 89% were negative by RT-qPCR as well.*

The seroconversion rate at Month 12, by Recombinant-ELISA, Lysate-ELISA, IHA, or F29-ELISA, was higher in infants with acute infection, compared to older children. This may be due to earlier treatment of infants with acute infection compared to older children with subacute or indeterminate infection; the circulating parasites are intermittent, scarce or absent in the indeterminate and chronic phases of the disease.

9.2.4. Interpretive Criteria

The Applicant has not requested any interpretive susceptibility testing criteria in the labeling. This is appropriate as the tests to measure in vitro sensitivity of the *T. cruzi* parasites are not standardized and their use is limited to research laboratories.

9.3. Conclusions

The studies suggest that nifurtimox is active against the intracellular and extracellular stages of *T. cruzi*. The cure rates in *T. cruzi* infected animals vary with the parasite strain, animal species, as well as the time of initiation of treatment postinfection. Treatment with nifurtimox initiated early after infection, especially during the acute or subacute phase, appears to be most effective; parasitological cure is associated with seroconversion when testing is performed using assays that detect antibodies against the live parasites. Early treatment may reduce organ damage; however, organ damage cannot be reversed by nifurtimox treatment. Also, lytic antibodies were reported in mice with live infection but not in those vaccinated with dead parasites.

The clinical diagnosis of Chagas disease and its pathology are related to the host immune response. Seroconversion, by serological tests that detect antibodies against parasite extract or a cocktail of antigens, usually occurs several years after treatment requiring long-term follow-up and may take over a decade. However, by F29-ELISA, seroconversion occurs earlier as it reflects antibody response to viable parasites. This is based on published studies that suggest lytic antibodies are directed against the live trypomastigotes; the lytic antibodies did not persist in patients that were negative by xenodiagnosis.

The trial is currently ongoing and long term follow-up of patients will be useful. It is recommended that antibody titers by serial dilution of all available serum samples be measured by the F29-ELISA, Recombinant-ELISA and Lysate-ELISA in addition to IHA.

10. Review of Safety

10.1. Safety Review Approach

The safety review focused on Study 16027 which studied pediatric patients less than 18 years of age with Chagas disease. It should be noted that Study 16027 evaluated two dosing regimens of NFX, but did not have an active control or placebo arm. As a result, there was no comparative group for the safety analysis. The Applicant provided a 4-month safety update of subjects enrolled in the ongoing Part 2 of Study 16027 (CHICO SECURE). This update did not provide any additional relevant safety findings. Supportive safety information was obtained from the literature review submitted by the Applicant. In addition, the M.O. independently reviewed the literature for additional safety data. The literature data are discussed in Section 10.10.

The nifurtimox product labeling from outside the United States notes neurological and psychiatric-type symptoms as “secondary events.” As a result, these symptoms were noted as submission-specific safety issues and will be discussed in Section 10.5.

10.2. Review of the Safety Database

Overall Exposure

The safety data for nifurtimox is derived primarily from Study 16027 which studied two dosing regimens of the drug in pediatric subjects and used a historical placebo control for efficacy purposes. Minimal additional safety information was obtained from four phase 1 PK studies conducted in adults. These data are summarized in Table 65.

Table 65. Safety Population for Nifurtimox, Size, and Denominators

Clinical Trial Groups	Population (N=474)
Controlled trials conducted for this indication (study 16027)	330
All other than controlled trials conducted for this indication	0
Controlled trials conducted for other indications (4 PK studies in adults)	144

Source: Reviewer Table
The safety data for nifurtimox were derived from individuals exposed to the drug for the treatment of Chagas disease.
Abbreviations: N, sum of all available numbers; PK, pharmacokinetics

The demographics of the study population are shown in Table 38. Most of the subjects were in the 2 years to younger than 18 years age group and all subjects were from South America.

In Study 16027, 219 subjects were in the 60-day arm and 111 subjects were in the 30-day arm. The mean duration of treatment for nifurtimox in the 60-day arm was 58.9 days, while it was 29.4 days in the 30-day arm. Subjects in both arms received a weight-based dose of nifurtimox three times daily. The mean number of administered doses was 164.4 in the 60-day arm and 81.2 in the 30-day arm.

Of note, the Applicant provided a summary of published studies from the literature which provide additional safety information. However, the primary data are not available from these studies and so these studies only provide supportive safety data.

Adequacy of the Safety Database

The number of subjects and duration of exposure of subjects enrolled in Study 16027 provides an adequate safety database to evaluate NFX.

10.3. Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

Review of the data fitness by OCS and the clinical reviewer did not find major issues regarding data integrity and submission quality.

Categorization of Adverse Events

The Applicant defined treatment-emergent AEs as, "events which first occurred or worsened at or after first application of study drug during the course of the study up to and including 7 days after last application of study drug." As a result, AEs occurring more than 7 days after the last administration of study drug were not included as TEAEs. This review uses the Applicant's definition of TEAE for the primary analysis. However, a secondary analysis which includes these later AEs is included in Section 20.5.

MedDRA version 21.0 was used to code AEs in Study 16027. The Applicant's coding of verbatim terms to MedDRA terms was acceptable.

Routine Clinical Tests

Routine coagulation, hematology, blood chemistry, and urinalysis tests were performed at screening, Visit 3 (Day 7), Visit 6 (Day 30), Visit 8 (Day 60), and Visit 9 (Day 90).

10.4. Safety Results

10.4.1. Deaths

There was one death in the Applicant-conducted clinical development program. A 12-year-old boy in the 60-day arm of Study 16027 committed suicide by hanging approximately 11 months after completing study drug.

M.O. Comment: *It is unlikely that this death was related to the study drug as it occurred many months after the last dose.*

10.4.2. Serious Adverse Events

In Study 16027, there were 20 SAEs reported from 17 subjects (5.1%). This included 12 in the 60-day arm (5.5%) and 5 subjects in the 30-day arm (4.5%). Nine subjects experienced at least one SAE while taking the study drug and are summarized in Table 66. All SAEs from these 9 subjects were reported as recovered or resolved. A discussion of the 8 subjects that experienced at least one SAE after completing the study drug follows this section.

M.O. Comment: *There does not appear to be a differential effect on the incidence of SAEs related to duration of NFX use (60 days versus 30 days).*

Table 66. SAEs Occurring During Study Drug Treatment, Study 16027

Treatment Arm Subject ID	Start Day of AE	Preferred Term of AE	Severity of AE	Action Taken With Study Drug
6				
(b) (6)	37	Cellulitis	Moderate	Dose not changed
	24	Urticaria	Severe	Drug withdrawn
	38	Apnea	Severe	Dose not changed
	10	Pelvic inflammatory disease	Moderate	Dose not changed
	9	Seizure	Moderate	Dose not changed
	31	Seizure	Moderate	Drug withdrawn
	50	Syncope	Mild	Dose not changed
30 days				
(b) (6)	11	Febrile convulsion	Moderate	Drug interrupted
	11	Pneumonia	Moderate	Drug interrupted
	13	Ear infection	Severe	Dose not changed
	10	Abdominal pain	Moderate	Drug withdrawn

Source: Reviewer Table

M.O. Comment: *The M.O. reviewed the narratives for these subjects. The notable SAEs are the two subjects with seizures or convulsions, one with syncope, one with urticaria, and one with abdominal pain as they appear possibly related to the study drug. Narrative summaries of these cases are found below.*

Subject (b) (6) was a 17-year-old boy randomized to the 60-day treatment arm with no significant medical history who developed a rash on Day 22 and hives on Day 24. Treatment with the study drug was interrupted for one day and then discontinued. The hives were treated and were reported as resolved on Day 31.

M.O. Comment: *This case of skin rash and hives appears to be related to the study drug as it occurred after initiation of study drug and studies from the literature suggest skin rashes are associated with nifurtimox.*

Subject (b) (6) was a 10-year-old girl randomized to the 60-day treatment arm with no medical history, but a family history of seizures, who experienced a seizure on Day 9 after taking dimenhydrinate. She was treated in the hospital and sent home the next day without anticonvulsant medication. She experienced another seizure on Day 31 also in conjunction with dimenhydrinate. Treatment of the seizure required hospitalization. A CT scan of the head and

laboratory work did not reveal any abnormalities. She was discharged home two days later with anticonvulsant medication. After the second seizure, the study drug was stopped.

M.O. Comment: *The dose of dimenhydrinate in each case was noted as 50 mg given by the subject's father, but the Applicant's narrative describes it as an overdose. Dimenhydrinate overdose can lead to seizures, but this would not be expected if only 50 mg was given in each case. The seizures in this case appear to be at least partially related to the study drug as they occurred twice after starting study drug in a subject without a prior history of seizure. In addition, the nifurtimox label used outside the United States lists seizure as a possible adverse reaction.*

Subject (b) (6) was a 11-year-old boy randomized to the 60-day treatment arm with no medical history who was reported to have accidently tangled himself in a rope leading to syncope on Day 50. His parents immediately removed the rope and he regained consciousness. He was monitored in the hospital and sent home the next day with a consult to see a psychologist. The study drug was not discontinued.

M.O. Comment: *The mental status of the subject is unclear. The consult to see a psychologist suggests he may have intended self-harm. In that case, the study drug may be related to this behavior as it occurred after starting study drug and the nifurtimox label used outside the United States lists "excitatory states" and "psychotic behavior" as possible side effects.*

Subject (b) (6) was a 12-year-old girl randomized to the 30-day treatment arm with a medical history of chronic constipation treated with enemas. On Day 11, she experienced two generalized tonic clonic seizures lasting 2 to 4 minutes each (reported as complex febrile seizure). She was hospitalized and found to have a pneumonia caused by *M. pneumoniae*. A CT scan of the head was normal. No body temperature measurement was provided on Day 11. She was treated for the pneumonia which was considered resolved on Day 25. Study drug was interrupted for two days, but restarted at hospital discharge on Day 13. No outpatient anticonvulsant medication was prescribed.

M.O. Comment: *Febrile seizures are more common in children aged between 6 months and 5 years. Therefore, this seizure being reported as a febrile seizure in a 12-year-old is unusual. As nifurtimox is known to be associated with seizures, it is likely that the study drug also played a role in this case.*

Subject (b) (6) was a 17-year-old female randomized to the 30-day treatment arm with no medical history who experienced moderate abdominal pain and fever on Day 10 which led to hospitalization. The fever resolved the next day. Laboratory studies showed a peripheral leukocytosis and 1+ leukocytes in the urine. She was treated with antibacterial drugs and the abdominal pain and laboratory abnormalities were resolved by Day 15. Because of these AEs, the study drug was stopped on Day 12.

M.O. Comment: *The investigator and Applicant assessed the abdominal pain as related to the study drug. However, it appears that the subject had a UTI which could also have led to this symptom.*

Eight subjects experienced at least one SAE after completing the study drug and are summarized in Table 67. All SAEs from these subjects were reported as recovered or resolved except for the case of suicide which is summarized above.

Table 67. SAEs Occurring After Completion of Study Drug, Study 16027

Treatment Arm	Subject ID	Start Day of AE	Preferred Term of AE	Severity of AE
60 days	(b) (6)	356	Upper limb fracture	Moderate
		208	Chronic tonsillitis	Moderate
		418	Completed suicide	Severe
		342	Burns second degree	Moderate
		349	Abdominal pain	Mild
		223	Seizure	Moderate
		206	Snake bite	Moderate
30 days		257	Gastroenteritis	Moderate
		101	Abortion induced	Moderate

Source: Reviewer Table
Abbreviations: AE, adverse event; SAE, serious adverse event

M.O. Comment: *Review of the narratives for these subjects did not reveal any cases where the study drug was likely to have been related to the SAE. Of note, the seizure in Subject* (b) (6) *was attributed to neurocysticercosis. Also, the abortion in Subject* (b) (6) *was elective. All cases of pregnancy in the study will be discussed Section 10.9 below.*

10.4.3. Dropouts and/or Discontinuations Due to Adverse Effects

A total of 14 subjects discontinued the study drug due to at least one AE (4.2%). This includes 12 subjects in the 60-day arm (5.5%) and 2 in the 30-day arm (1.8%). Table 68 below summarizes these subjects. All AEs from these subjects were reported as recovered or resolved. Of note, 3 of these subjects were reviewed in the SAE section above (b) (6)).

M.O. Comment: *There appears to be a differential effect on the number of discontinuations related to the duration of NFX use (60 days versus 30 days). That is, the rate of discontinuations increased with a longer duration of NFX use.*

Table 68. Discontinuations Due to Adverse Events, Study 16027

Treatment Arm	Subject ID	Day of D/C	Preferred Term of AE(s)	Severity of AE(s)
60 days	(b) (6)	21	Rash	Moderate
		13	Asthenia Abdominal pain upper Decreased appetite Rash macular Nausea Headache	Mild to moderate
		49	Urticaria	Mild
		57	Urticaria	Severe
		46	Gastroenteritis viral	Mild
		46	Gastroenteritis viral	Mild
		48	Rash	Moderate
		34	Seizure	Moderate
		13	Pyrexia	Mild
		13	Abdominal pain Cough Odynophagia Headache Leukocytosis	Mild to moderate
		32	Leukopenia Neutropenia	Mild to moderate
		30	Headache	Moderate
30 days		12	Pyrexia Abdominal pain	Moderate
		10	Pyrexia Cough Headache	Mild

Source: Reviewer Table

M.O. Comment: *Review of the narratives for these subjects showed that the AEs appeared to be at least possibly related to the study drug. Common AEs observed that led to study drug discontinuation include skin reactions, headache, and gastrointestinal complaints.*

10.4.4. Significant Adverse Events

Three subjects experienced a TEAE with a severity of “severe” and are discussed in Table 66 above.

10.4.5. Treatment Emergent Adverse Events and Adverse Reactions

In Study 16027, 586 TEAEs were reported from 213 subjects (64.5%). This included 147 subjects (67.1%) in the 60-day arm and 66 subjects (59.5%) in the 30-day arm. Table 69 shows these subjects by SOC and Table 70 shows them by preferred term.

Table 69. TEAEs by System Organ Class and Treatment Arm, Study 16027

System Organ Class*	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Blood and lymphatic system disorders	6 (5.4)	15 (6.8)	21 (6.4)
Ear and labyrinth disorders	0	1 (0.5)	1 (0.3)
Eye disorders	1 (0.9)	1 (0.5)	2 (0.6)
Gastrointestinal disorders	33 (29.7)	78 (35.6)	111 (33.6)
General disorders and administration site conditions	6 (5.4)	24 (11.0)	30 (9.1)
Hepatobiliary disorders	0	3 (1.4)	3 (0.9)
Immune system disorders	1 (0.9)	0	1 (0.3)
Infections and infestations	22 (19.8)	59 (26.9)	81 (24.5)
Injury, poisoning and procedural complications	2 (1.8)	4 (1.8)	6 (1.8)
Investigations	1 (0.9)	9 (4.1)	10 (3.0)
Metabolism and nutrition disorders	8 (7.2)	25 (11.4)	33 (10.0)
Musculoskeletal and connective tissue disorders	4 (3.6)	3 (1.4)	7 (2.1)
Nervous system disorders	19 (17.1)	34 (15.5)	53 (16.1)
Psychiatric disorders	2 (1.8)	1 (0.5)	3 (0.9)
Renal and urinary disorders	1 (0.9)	2 (0.9)	3 (0.9)
Reproductive system and breast disorders	0	1 (0.5)	1 (0.3)
Respiratory, thoracic and mediastinal disorders	7 (6.3)	19 (8.7)	26 (7.9)
Skin and subcutaneous tissue disorders	6 (5.4)	22 (10.0)	28 (8.5)

Source: Reviewer Table
All values are expressed as n (%).
* Subjects with more than one AE in a given SOC were counted only once per SOC.

M.O. Comment: *SOCs with high rates of AEs include gastrointestinal disorders and infections and infestations. The SOCs of general disorders and administration site conditions, investigations, and skin and subcutaneous tissue disorders have notable higher rates of AEs in the 60-day arm compared to the 30-day arm. This suggests that there may be a differential effect related to the duration of NFX exposure. Review of the AEs in the general disorders SOC reveals that the AEs of chest pain and pyrexia account for the difference between the arms. Review of the AEs in the investigations SOC reveal that the AE of weight decreased accounts for the difference between the arms. Review of the AEs in the skin disorders SOC reveals that the AEs of rash and urticaria account for the difference between the arms. These AEs are discussed below.*

Table 70. TEAEs by Preferred Term and Treatment Arm Occurring in >2% of Subjects, Study 16027

Preferred Term	30-Day Arm (N=111)	60-Day Arm (N=219)	Total (N=330)
Upper respiratory tract infection[1]	11 (9.9)	36 (16.4)	47 (14.2)
Headache	16 (14.4)	28 (12.8)	44 (13.3)
Vomiting	9 (8.1)	32 (14.6)	41 (12.4)
Abdominal pain[2]	12 (10.8)	29 (13.2)	41 (12.4)
Nausea	14 (12.6)	18 (8.2)	32 (9.7)
Decreased appetite	8 (7.2)	23 (10.5)	31 (9.4)
Pyrexia	3 (2.7)	16 (7.3)	19 (5.8)
Diarrhea	6 (5.4)	10 (4.6)	16 (4.8)
Rash[3]	1 (0.9)	12 (5.5)	13 (3.9)
Bronchitis[4]	2 (1.8)	8 (3.7)	10 (3.0)
Cough	2 (1.8)	7 (3.2)	9 (2.7)
Dizziness	2 (1.8)	6 (2.7)	8 (2.4)
Eosinophilia	3 (2.7)	5 (2.3)	8 (2.4)
Anemia	1 (0.9)	6 (2.7)	7 (2.1)
Weight decreased	1 (0.9)	6 (2.7)	7 (2.1)
Urticaria	1 (0.9)	5 (2.3)	6 (1.8)
Chest pain	0	5 (2.3)	5 (1.5)
Urinary tract infection	0	5 (2.3)	5 (1.5)
Asthenia	3 (2.7)	2 (0.9)	5 (1.5)
Back pain	3 (2.7)	0	3 (0.9)

Source: Reviewer Table
All values are expressed as n (%).
[1] Upper respiratory tract infection includes the PTs of upper respiratory tract infection, catarrh, laryngitis, nasopharyngitis, pharyngitis, pharyngotonsillitis, rhinitis, rhinorrhoea, sinusitis, tonsillitis, and viral tonsillitis
[2] Abdominal pain includes the PTs of abdominal pain and abdominal pain upper
[3] Rash includes the PTs of rash, rash macular, rash maculo-papular, rash morbilliform, and rash papular
[4] Bronchitis includes the PTs of bronchitis and bronchitis viral
Abbreviations: PT, preferred term; TEAE, treatment-emergent adverse event

M.O. Comment: *The AEs of nausea, vomiting, headache, abdominal pain, decreased appetite, pyrexia, diarrhea, rash, dizziness, urticaria, weight decreased, and asthenia are all possibly related to the study drug and are listed in the Applicant's proposed label. The AEs of eosinophilia and anemia could be related to the study drug and are not included in the proposed label. The AEs of pyrexia, rash, anemia, urticaria, and weight decreased are notably higher in the 60-day arm compared to the 30-day arm suggesting that there is a differential effect related to the duration of NFX exposure. Upper respiratory tract infection and bronchitis (with associated cough and chest pain) are common in children and are unlikely to be related to the study drug. It is not clear why there is an imbalance in the number of subjects with the AEs of chest pain and urinary tract infection between the treatment arms. This finding could have occurred by chance.*

10.4.6. Laboratory Findings

Review of the laboratory data revealed decreases in hemoglobin, leukocytes, and platelets and increases in eosinophil count which will be discussed first. Next, the subjects with transaminase elevations will be discussed. In addition, there were a few subjects with notable laboratory outlier values.

Figure 8. Mean Hemoglobin Level (g/dL) by Visit and Treatment Arm

Source: Reviewer Figure
Abbreviations: HGB, hemoglobin

Figure 9. Maximum Decrease in Hemoglobin (g/dL) From Baseline

Source: Reviewer Figure

M.O. Comment: *The mean hemoglobin values in both treatment arms decreased a small amount from baseline (Visit 1) to Visit 8 (Day 60) and then increased afterwards. This suggests the study drug was related to the decreased hemoglobin values. However, it should be noted that the mean hemoglobin values at all timepoints were in the normal range.*

The median maximum hemoglobin decrease was 0.6 g/dL. However, 8 subjects had hemoglobin decreases from baseline of greater than 4 g/dL which is a significant decrease. Review of these subjects revealed 5 were in the 60-day arm and 3 in the 30-day arm. Importantly, 5 of 8 subjects were 0 to 27 days old when enrolled in the study. The remaining 3 subjects ranged in age from

11 months to 16 years. Of note, there were only 7 subjects total in the 0 to 27 day age group. The normal hemoglobin of a neonate is 17 to 22 g/dL and decreases in the first weeks and months of life such that the normal hemoglobin in a child is 11 to 13 g/dL. This physiological decrease likely accounts for some of the greater decreases in hemoglobin that were observed. Still, anemia was noted as an AE in 2.7% of subjects in the 60-day arm suggesting it was identified as an abnormality in those subjects. Of note, this reviewer recommends adding anemia as an adverse reaction to the Applicant's proposed product label.

Figure 10. Mean Leukocyte Count (10^3/µL) by Visit and Treatment Arm

Source: Reviewer Figure
Abbreviations: WBC, white blood cell

M.O. Comment: *The mean WBC count decreases from baseline in both treatment arms through Visit 6 (Day 30) before increasing through Visit 9 (Day 90). The mean decrease is modest at about 500/µL. The fact that the mean WBC count increases to a greater extent in the 30-day arm at Visit 8 (Day 60) suggests the decrease was related to NFX exposure. Leukopenia will be added to the Applicant's proposed product label as an adverse reaction.*

Figure 11. Mean Platelet Count (10^3/mcL) by Visit and Treatment Arm

Source: Reviewer Figure

M.O. Comment: *The mean platelet count decreases from baseline in both treatment arms at Visit 6 (Day 30) before increasing at the later visits. The mean decrease is mild at about 10 to 15 x 10^3/mcL. The fact that the mean platelet count increases at the later timepoints suggest that the decrease was related to NFX exposure. Thrombocytopenia was noted as an adverse reaction in postmarketing data and will be listed in the product label.*

Figure 12. Mean Absolute Eosinophil Count (10^3/mcL) by Visit and Treatment Arm

Source: Reviewer Figure
Abbreviation: EOS, eosinophil

Figure 13. Mean Eosinophil/Leukocyte (%) by Visit and Treatment Arm

Source: Reviewer Figure
Abbreviation: EOS, eosinophil; LE, leukocyte

Figure 14. Maximum Increase in Absolute Eosinophil Count (103/mcL) From Baseline

Source: Reviewer Figure

M.O. Comment: *The mean absolute eosinophil and eosinophil percentage levels increased from baseline in both arms, but the increase was more sustained in the 60-day arm compared to the 30-day arm. These findings suggest the study drug was related to the increase. Some subjects had eosinophil count increases from baseline of greater than 3000 x 10^3/mcL, but the median maximum increase was approximately 180 x 10^3/mcL. One possible explanation is that the study drug causes the trypanosome to release antigens and elicits the eosinophila. Alternatively, the drug itself could cause a hypersensitivity reaction. Of note, the baseline mean absolute and*

eosinophil count percentage was already above the normal range. This elevation could be from the Chagas disease or from other parasitic infections present at baseline. Eosinophilia was reported as an TEAE in 2% to 3% of subjects, but is not listed as an adverse reaction in the Applicant's proposed product label.

Using an absolute eosinophil count increase from baseline of greater than 1000 x 10^3/mcL as a cutoff resulted in 32 subjects. Review of the AEs from these subjects found parasitic gastroenteritis in 3 subjects which may explain the eosinophilia is these subjects.

M.O. Comment: *The remaining subjects did not have a clearly identified alternative explanation for the eosinophilia. This finding suggests the study drug could be causing the eosinophilia. Eosinophilia will be added to the product label as an adverse reaction.*

Review of the transaminase values showed 7 subjects with at least one AST or ALT value greater than or equal to 3 times the upper limit of normal. Of these subjects, 2 had the elevated values only at baseline. Additionally, 2 subjects had the first elevation at Visit 9 (Day 90). One of these subjects (b) (6) had an ALT of 284 U/L and AST of 198 U/L, but returned to normal at an unscheduled follow up visit. Notably, 3 subjects had the elevation during the study drug treatment, but returned to normal values at the next visit. For these 3 subjects, the peak AST/ULN ranged from 2.2 to 3.4 and the peak ALT/ULN ranged from 0.9 to 3.3. There were no possible Hy's Law cases.

M.O. Comment: *The study drug is unlikely to be related to the elevated transaminases at baseline or at Day 90. However, for the 3 subjects with elevations during the study drug treatment, it is possible NFX may have been related to the increase. However, the elevations were modest and there were no cases that met Hy's Law criteria. Overall, there was not a concerning signal for hepatotoxicity for NFX in Study 16027.*

Otherwise, review of the coagulation, chemistry, and hematology laboratory values did not show any clinically significant changes over time or by treatment arm.

Individual Outliers

Subject (b) (6) in the 60-day arm had an elevated serum creatinine value of 2.8 mg/dL at Day 90, but had normal values before and afterwards.

Subject (b) (6) in the 60-day arm had an elevated alkaline phosphatase level of 3649 U/L (normal range 320 to 872 U/L) at the Day 90 visit. No additional alkaline phosphatase levels were reported after Day 90. Prior alkaline phosphatase levels were not elevated. Bilirubin, ALT, and AST values at all timepoints were also not elevated.

M.O. Comment: *As all the elevated laboratory values occurred at Day 90 and were normal at Day 60, it suggests the study drug was likely not related to these increased values. Review of these subjects did not reveal an alternate cause for these one-time elevations.*

10.4.7. Vital Signs

Decrease in weight was noted as an adverse event. As a result, the weight and BMI vital signs data were reviewed.

Figure 15. Change From Baseline Weight (kg) by Visit and Treatment Arm

Source: Reviewer Figure

Figure 16. Change From Baseline Body Mass Index (BMI) by Visit and Treatment Arm

Source: Reviewer Figure

Figure 17. Maximum Decrease in Weight (kg) From Baseline

Source: Reviewer Figure

M.O. Comment: *The mean reduction in weight and BMI from baseline increased over time with the largest decrease noted at Visit 8 (Day 60) which was the end of treatment. The decrease in weight and BMI was more prominent in the 60-day arm which suggests that the study drug was related to the weight decrease. Both weight and BMI increased after Day 60. Some subjects lost up to 6 and 8 kg compared to baseline, but the median maximum decrease in weight was 1.1 kg in the 30-day arm and 1.3 kg in the 60-day arm. "Weight decreased" was noted as a TEAE. Decreased appetite and weight loss are listed as a Warning in the product label.*

Figure 18. Change From Baseline Height (cm) by Visit and Treatment Arm

Source: Reviewer Figure

M.O. Comment: *The mean change from baseline height increased over time with greater increases at Visit 10 (Day 240) and Visit 11 (Day 420) showing that the subjects continued to grow in height.*

Review of the blood pressure, heart rate, body temperature, and respiratory rate data did not reveal any significant findings.

10.4.8. Electrocardiograms (ECGs)

ECGs were obtained at Visits 1, 2, 3, 6, 8, 9, 10, and 11, but were optional for subjects <5 years of age. A total of 301 subjects had at least one ECG collected (199 in the 60-day arm; 102 in the 30-day arm). Two subjects in the 60-day arm had left anterior fascicular block at baseline and continued to have this finding at multiple follow-up visits. Thirteen subjects (7 in the 60-day arm; 6 in the 30-day arm) had a finding of right bundle branch block (RBBB) noted during the study. Seven subjects were noted to have the RBBB at only one visit (Day 7, 60, and 90). The other 6 subjects had RBBB noted at more than one visit. Of note, all 13 subjects were enrolled in Argentina. An additional subject in Colombia who was in the 60-day arm had right ventricular hypertrophy noted as a miscellaneous finding at Visits 8 and 9 (Days 60 and 90).

M.O. Comment: *Left anterior fascicular block, right bundle branch block, and right ventricular hypertrophy are findings of cardiac Chagas disease and are unlikely to be related to NFX treatment. The preponderance of subjects in Argentina with these ECG findings may be related to regional differences in trypanosomes or host factors. Alternatively, as the ECGs were evaluated manually by the investigator at each site, this finding could be investigator-dependent.*

10.4.9. QT Assessment

Markers of QTcF elevations at the different timepoints in the study are shown in the tables below.

Table 71. QTcF Elevations at Visit 1 (Screening)

QTc Measurement	30-Day Arm N=94	60-Day Arm N=194
QTcF >450 msec	1 (1.1)	5 (2.6)
QTcF >480 msec	0	1 (0.5)
QTcF >500 msec	0	0

Source: Reviewer Table
All values are expressed as n (%).

M.O. Comment: *At the screening visit, the proportion of subjects with elevations in QTcF was low in both treatment arms.*

Table 72. QTcF Elevations at Visits 2, 3, and 6 (on Active Study Drug)

QTc Measurement	30-Day Arm N=100	60-Day Arm N=195
QTcF >450 msec	2 (2.0)	6 (3.1)
QTcF >480 msec	0	1 (0.5)
QTcF >500 msec	0	0
Increase >30 msec from baseline	16 (16.0)	26 (13.3)
Increase >60 msec from baseline	4 (4.0)	6 (3.1)

Source: Reviewer Table
All values are expressed as n (%).

M.O. Comment: *Visits 2, 3, and 6 occurred between Day 1 and Day 30 when subjects in both treatment arms were on active drug. The proportion of subjects with QTcF >450 msec did not meaningfully change from the screening visit. Regarding the subjects with an increase of >60 msec from baseline, one of them* (b) (6) *; 60-day arm) experienced an AE of somnolence starting on Day 8 and ending on Day 23. The QTcF on Day 8 was 381 msec which was increased from 320 msec at baseline. It does not appear that this subject's somnolence was related to QT prolongation as the absolute QTcF was not prolonged and the somnolence resolved prior to the completion of treatment.*

Table 73. QTcF Elevations at Visit 8 (Day 60)

QTc Measurement	30-Day Arm N=96	60-Day Arm N=190
QTcF >450 msec	1 (1.0)	3 (1.6)
QTcF >480 msec	0	0
QTcF >500 msec	0	0
Increase >30 msec from baseline	10 (10.4)	18 (9.5)
Increase >60 msec from baseline	4 (4.2)	2 (1.1)

Source: Reviewer Table
All values are expressed as n (%).

M.O. Comment: *At Visit 8, subjects in the 60-day arm were on active study drug, but the 30-day arm subjects had been on placebo for the preceding 30 days. As the proportion of subjects with increases of >30 msec and >60 msec from baseline were similar in each arm, it suggests the increases were not related to the study drug. In addition, the proportion of subjects with QTcF >450 msec remained low.*

Table 74. Visits 9, 10, and 11 (Poststudy Drug)

QTc Measurement	30-Day Arm N=100	60-Day Arm N=189
QTcF >450 msec	4 (4.0)	4 (2.1)
QTcF >480 msec	0	1 (0.5)
QTcF >500 msec	0	1 (0.5)
Increase >30 msec from baseline	22 (22.0)	35 (18.5)
Increase >60 msec from baseline	5 (5.0)	2 (1.1)

Source: Reviewer Table
All values are expressed as n (%).

M.O. Comment: *At Visits 9, 10, and 11, all subjects were off study drug. Similar to Visit 8, the proportion of subjects with QTcF >450 msec remained low. Overall, the results from Study 16027 do not appear to show a significant QT prolongation effect for NFX.*

Per the QT-IRT consult team, these ECG data can rule out a QT prolongation >20ms. In addition, the animal data only shows QT prolongation with very high exposures. Therefore, based on these data and experience from use of NFX outside the United States, the risk of QT prolongation due to NFX is low and a thorough QT study does not appear to be warranted.

10.4.10. Immunogenicity

Not applicable for this NDA.

10.5. Analysis of Submission-Specific Safety Issues

10.5.1. Neurological Adverse Events

In Study 16027, 53 subjects (16.1%) experienced a TEAE in the nervous system disorders SOC. These TEAEs are shown in Table 75.

Table 75. TEAEs in the Nervous System Disorders SOC

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Headache	16 (14.4)	28 (12.8)	44 (13.3)
Dizziness	2 (1.8)	6 (2.7)	8 (2.4)
Somnolence	0	2 (0.9)	2 (0.6)
Syncope	1 (0.9)	2 (0.9)	3 (0.9)
Paresthesia	0	1 (0.5)	1 (0.3)
Seizure	0	1 (0.5)	1 (0.3)
Tremor	0	1 (0.5)	1 (0.3)
Autonomic nervous system imbalance	1 (0.9)	0	1 (0.3)
Febrile convulsion	1 (0.9)	0	1 (0.3)

Source: Reviewer Table
All values are expressed as n (%).
Abbreviations: SOC, system organ class; TEAE, treatment-emergent adverse event

M.O. Comment: *These AEs include central nervous system-related symptoms such as dizziness, somnolence, and seizure, and also possible peripheral nervous system symptoms such as paresthesia and tremor. The M.O. recommends that seizure, somnolence, and syncope be included as adverse reactions in the NFX label.*

10.5.2. Psychiatric Adverse Events

The ex-U.S. label for NFX lists psychiatric symptoms as “undesirable effects.” To investigate these types of reactions, psychiatric AEs were analyzed. In Study 16027, 3 subjects (0.9%) experienced a TEAE in the psychiatric disorders SOC. Two subjects experienced irritability and one subject experienced anxiety. All these TEAEs were nonserious and mild in severity. Both irritability and anxiety were also reported in the literature reviewed in Section 10.10.

10.5.3. Hypersensitivity Reactions

Studies from the literature and postmarketing data note hypersensitivity and skin reactions as adverse reactions associated with NFX. To investigate these types of reactions, a standardized MedDRA query (SMQ) for hypersensitivity was performed. The findings are shown in Table 76.

Table 76. Subjects With Hypersensitivity SMQ by Treatment Arm

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Broad SMQ	12 (10.8)	30 (13.7)	42 (12.7)
Narrow SMQ	3 (2.7)	19 (8.7)	22 (6.7)

Source: Reviewer Table
All values are expressed as n (%).
Abbreviations: SMQ, standardized MedDRA query

M.O. Comment: *There appears to be a dose effect with more subjects in the 60-day arm having PTs associated with hypersensitivity compared to the 30-day arm. The difference is more pronounced for the narrow SMQ. The narrow SMQ includes PTs related to skin reactions, such as rash and urticaria, as well as bronchospasm. The broad SMQ includes the narrow SMQ PTs plus other PTs such as eosinophilia, erythema, and pneumonitis. It appears hypersensitivity reactions were fairly common in Study 16027. The proposed label includes hypersensitivity as a warning.*

10.6. Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

There are no COA data that are applicable to the safety analysis.

10.7. Safety Analyses by Demographic Subgroups

The proportion of subjects with at least one TEAE in various demographic subgroups is shown in Table 77.

Table 77. TEAEs by Demographic Subgroups and Treatment Arm

Subgroup	30-Day Arm	60-Day Arm	Total
All subjects	66/111 (59.5)	147/219 (67.1)	213/330 (64.5)
Sex			
Female	36/59 (61.0)	77/119 (64.7)	113/178 (63.5)
Male	30/52 (57.7)	70/100 (70.0)	100/152 (65.8)
Race			
American Indian[1]	18/30 (60.0)	42/64 (65.6)	60/94 (63.8)
White	48/81 (59.3)	105/155 (67.7)	153/236 (64.8)
Age			
0 to 27 days	2/3 (66.7)	3/4 (75.0)	5/7 (71.4)
28 days to younger than 8 months	1/4 (25.0)	4/8 (50.0)	5/12 (41.7)
8 months to younger than 2 years	5/8 (62.5)	15/17 (88.2)	20/25 (80.0)
2 years to younger than 18 years	58/96 (60.4)	125/190 (65.8)	183/286 (64.0)

Subgroup	30-Day Arm	60-Day Arm	Total
Country			
Argentina	34/59 (57.6)	78/119 (65.5)	112/178 (62.9)
Bolivia	11/21 (52.4)	26/41 (63.4)	37/62 (59.7)
Colombia	21/31 (67.7)	43/59 (72.9)	64/90 (71.1)

Source: Reviewer Table
[1]Defined as indigenous peoples of the Americas
All values are expressed as n/N (%).
Abbreviations: TEAE, treatment-emergent adverse event

As weight loss is concerning in a pediatric population, this adverse reaction was analyzed by age group. All 7 subjects with the preferred term "weight decreased" were aged 2 years or older. Analysis of the vital sign data using a cutoff of ≥5% weight loss from baseline is shown in Table 78.

Table 78. Subjects With >5% Decrease in Baseline Weight by Age Group and Treatment Arm

Age Group	30-Day Arm	60-Day Arm	Total
0 to 27 days	0/3 (0.0)	3/4 (75.0)	3/7 (42.9)
28 days to younger than 8 months	0/4 (0.0)	0/8 (0.0)	0/12 (0.0)
8 months to younger than 2 years	3/8 (37.5)	10/17 (58.8)	13/25 (52.0)
2 years to younger than 18 years	33/96 (34.4)	89/190 (46.8)	122/286 (42.7)

Source: Reviewer Table
All values are expressed as n/N (%).

M.O. Comment: *The proportion of subjects with at least one TEAE did not meaningfully vary between the listed demographic subgroups. With the exception of the 28 day to 8 month age group, the proportion of subjects in each age group with ≥5% weight loss from baseline was similar. Of note, there were limited numbers of subjects in the younger age subgroups such that a change in the status of a few subjects could have resulted in large fluctuations in the percentages.*

10.8. Specific Safety Studies/Clinical Trials

There were no specific safety studies conducted.

10.9. Additional Safety Explorations

Human Carcinogenicity or Tumor Development

In Study 16027, there were no AEs in the Neoplasms SOC. However, there are nonclinical data that nitrofurans, which have similar chemical structures to nifurtimox, are carcinogenic in mice and rats. Refer to Section 5.5.4.

Human Reproduction and Pregnancy

In Study 16027, there were two subjects in the 30-day arm with a positive pregnancy test (Subjects (b) (6)). For Subject (b) (6), a serum pregnancy test was positive on Day 91. Prior urine pregnancy tests at screening and through Day 60 were negative. The subject had an elective abortion. For Subject (b) (6), a urine pregnancy test was

positive on Day 95. Prior urine pregnancy tests at screening and through Day 63 were negative. The subject gave birth to a healthy infant at gestational age 41 weeks. No fetal/infant abnormalities were noted.

M.O. Comment: *As both of these subjects completed active study drug on Day 30 and had negative pregnancy tests at about Day 60, it is unlikely that either fetus was exposed to NFX.*

There were an additional 3 subjects in the 60-day arm with reported pregnancies. In all three cases, conception occurred several months after the last dose of study drug as all pregnancy tests conducted during the study were negative. In two cases, the subjects gave birth to healthy term infants without any noted abnormalities. In the other case, the subject gave birth at gestational age 40 weeks without any prenatal care. At birth, the treating physician (not the investigator) diagnosed intrauterine growth restriction. However, when the infant was 9 months old, the subject had a visit with the investigator who assessed the infant as healthy and with normal development.

M.O. Comment: *As all of these subjects completed active study drug several months before conception, it is unlikely that any of the fetuses were exposed to NFX. As a result, there are insufficient data from Study 16027 to evaluate the effect of NFX on pregnancy and fetal outcomes.*

Pediatrics and Assessment of Effects on Growth

NFX was studied in a pediatric population in Study 16027. In this study, loss of appetite and weight loss were reported as TEAEs. In addition, measurement of subject weights confirmed weight loss. For subjects who received the proposed duration of 60 days, the peak loss of weight occurred at Day 60. In addition, the median maximum weight loss for these subjects was 1.3 kg. However, at the last follow up visit (Day 420), subjects had surpassed their baseline weight (Figure 15).

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

NFX does not have any known potential for drug abuse or dependence.

10.10. Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

As NFX has been available outside the United States for several decades there are safety data available from the literature and from reports to the Applicant. Both of these types of data will be discussed in this section.

Literature Data

The Applicant reports on two nonrandomized, placebo-controlled studies of NFX in children and adolescents with chronic Chagas disease published in 1969 (Bustos et al. 1969; Cichero et al. 1969). The dose of NFX in these studies ranged from 15 to 25 mg/kg/day for 120 days. This daily dose is slightly higher than the Applicant's proposed dose, but the duration is twice as long. A total of 85 patients were treated with NFX and 22 with placebo. In these two studies, NFX was discontinued due to skin allergy/rash, psychiatric symptoms, dizziness, and convulsions. Frequently reported adverse events included decrease in appetite/anorexia, weight loss, nausea, vomiting, vertigo, dizziness, disorientation, asthenia, excitation, aggressiveness, and depression.

M.O. Comment: *The safety signals from these two studies generally fall into the SOCs of gastrointestinal disorders, neurological disorders, and psychiatric disorders in addition to rash.*

The Applicant also reports on several uncontrolled studies and case series of NFX in children and adolescents with chronic Chagas disease including congenital Chagas. Frequent adverse events reported from these studies are shown in Table 79.

Table 79. Frequently Reported Adverse Events in Literature Studies of NFX in Children and Adolescents

Category	Reported Adverse Events
Skin disorders	Rash, skin allergy
Gastrointestinal symptoms	Anorexia, weight loss, nausea, vomiting, abdominal pain
Neurological symptoms	Insomnia, amnesia, irritability, anxiety, dizziness, headaches, asthenia, polyneuritis[1], convulsions
Hematological findings	Leukopenia, thrombocytopenia
Musculoskeletal symptoms	Myalgia

Source: Reviewer Table
[1]Severe ascending polyneuritis was reported in a single patient, but resolved after stopping NFX treatment.
Abbreviations: NFX, nifurtimox

M.O. Comment: *The listed frequent AEs from the literature are included in the Applicant's proposed label except for seizures/convulsions, leukopenia, and thrombocytopenia. All these AEs should be included as adverse reactions in the label.*

Studies in adults confirmed that NFX was associated with similar adverse events in adults as seen in children, but symptoms appeared to be worse with older age. For example, an observational study conducted in Switzerland among 81 adult Latin American immigrants treated with NFX found that 98% of patients developed at least one AE (Jackson et al. 2010). Six patients (7.4%) developed suspected unexpected serious adverse reactions (SUSARs) during or shortly after completing NFX treatment. These SUSARs included DRESS, myocarditis, angioedema, and anaphylaxis. Of note, these 6 patients were reported to the Applicant and are included in the postmarking data discussed below. The types of reported AEs were similar to those noted in published pediatric studies and included gastrointestinal, neurological, constitutional, musculoskeletal, and dermatological events. Importantly, 32 patients (39.5%) did not complete treatment because of an AE. An additional 8 subjects were lost to follow up. Crespillo-Andújar et al (Crespillo-Andújar et al. 2018) summarized the safety profile of NFX in

adults from five studies which included the Jackson study. The rate of treatment discontinuation ranged from 18.36% to 49.1%. Treatment discontinuation data for NFX in children were available for 3 of the studies provided by the Applicant and ranged from 0 to 18.2%. Similarly, the rate of discontinuation in the 60-day arm in Study 16027 was 5.5%.

M.O. Comment: *It appears the types of AEs related to NFX use are similar between adults and children. However, the incidence of AEs and AEs resulting in treatment discontinuation may be higher in adults as compared to children.*

Reported Postmarket Data

The Applicant reports 1371 single case postmarketing reports for NFX. However, the vast majority of these cases were from patients who were treated with NFX and eflornithine which is recommended therapy for African Trypanosomiasis. As it is difficult to separate the contribution of NFX, African Trypanosomiasis, and eflornithine to the development of any adverse reactions, these cases were not included in this analysis. Similarly, the 5 reports from patients being treated for neuroblastoma with combination chemotherapy and NFX were not included in this analysis.

The Applicant reports 22 spontaneous serious solicited postmarketing reports containing 125 AEs for nifurtimox used for the treatment of Chagas disease. Of note, each report is for a single patient each with one or more AEs. These reports included AEs from adults and children. In response to an information request, the Applicant supplied a list of preferred terms from these patients. Review of these reports revealed several safety signals which are listed in Table 80. In addition, there were two notable cases which are discussed below.

Table 80. Safety Signals Identified From Postmarket Data

Category	Reported Adverse Events
Hypersensitivity reactions	Anaphylaxis, rash, DRESS
Gastrointestinal symptoms	Nausea, vomiting, abdominal pain, diarrhea
Neurological symptoms	Insomnia, amnesia, paresthesia, seizure, tremor
Hematological findings	Neutropenia, thrombocytopenia

Source: Reviewer Table
Abbreviations: DRESS, drug reaction with eosinophilia and systemic symptoms

M.O. Comment: *The safety signals identified from the postmarket data are similar to what were found from the literature data. The notable addition was five cases of DRESS which should be added to the label as an adverse reaction.*

There was a report of a 4-year-old boy in Bolivia with acute Chagas disease with heart failure who was treated with 12 mg/kg NFX daily. Ten days later he developed ascending polyneuropathy and NFX was stopped. The next day he developed quadriplegia. However, he slowly recovered and was discharged with normal mobility, but later died from unclear causes.

M.O. Comment: *The polyneuropathy and quadriplegia could be related to NFX. However, the patient's heart failure could have led to thromboembolic disease and stroke which could also explain the neurological adverse events.*

In addition, there was a 47-year-old patient in Venezuela with depression and completed suicide. However, there was insufficient information in this report to make an assessment. For example, it is unknown how long the patient took NFX.

M.O. Comment: *With limited information from a single case, it is difficult to make an association between NFX and suicide.*

Expectations on Safety in the Postmarket Setting

NFX will likely be used off-label in adult patients for whom AEs occur more frequently compared to children (see *Literature Data* above).

10.11. Integrated Assessment of Safety

The safety data for nifurtimox (NFX) in the treatment of Chagas disease in children evaluated in this review were mainly derived from Study 16027 which evaluated NFX in two dosing regimens, but without an active control or placebo. The subjects in Study 16027 were children aged 0 to <18 years living in endemic countries. Their ethnicities were almost all Hispanic/Latino which is reflective of the population with Chagas disease in the United States. The M.O. reviewed the literature and postmarketing data to develop a better understanding of NFX's safety profile in the treatment of Chagas disease. The collective safety issues identified from these sources will be summarized in this section.

Hypersensitivity reactions were noted in Study 16027, in the literature, and in the postmarketing data. These reactions include rash, urticaria, DRESS, eosinophilia, and anaphylaxis. In Study 16027, rash and urticaria led several subjects to discontinue the study drug. Rash was noted in 5.5% of subjects in the 60-day arm. DRESS and anaphylaxis were not observed in Study 16027, but were reported in the literature and postmarketing data.

Neurological and psychiatric adverse reactions were noted in Study 16027, in the literature, and in the postmarketing data. Neurological reactions include peripheral nervous system symptoms such as polyneuritis, paresthesia, and tremor, but also central nervous system symptoms, such as headache, somnolence, asthenia (weakness), dizziness, syncope, and memory loss. Headache was reported in 12.8% of 60-day arm subjects, while the other neurological reactions were uncommon. There was also a case of quadriplegia reported in the postmarketing data in a patient with acute Chagas disease and heart failure. The polyneuropathy and quadriplegia could have been related to NFX. However, the patient's heart failure could have led to thromboembolic disease and stroke which could also explain the neurological adverse events. In addition, seizure/convulsion was reported in 2 subjects in Study 16027 and in the literature and postmarketing data. Psychiatric adverse reactions include insomnia, irritability, and anxiety. In addition, the Applicant's ex-U.S. label lists psychotic behavior as an adverse reaction.

Similar gastrointestinal and nutritional adverse reactions were noted from several data sources. These reactions include poor appetite and weight loss which were confirmed by analysis of the

vital sign data. In addition, symptoms of nausea, vomiting, diarrhea, and abdominal pain were reported. These symptoms were commonly reported, but were mild to moderate in severity.

Hematological adverse reactions were noted from several data sources. These reactions include anemia, leukopenia, neutropenia, and thrombocytopenia. Anemia was observed in 2.7% of subjects in the 60-day arm in Study 16027. Leukopenia, neutropenia, and thrombocytopenia were not commonly reported in Study 16027 as adverse events, but there were decreases in hemoglobin, WBC count, and platelets during NFX exposure. In addition, these adverse reactions were reported in the literature and from the postmarketing data.

10.12. Conclusions and Recommendations

Nifurtimox is associated with several adverse reactions including hypersensitivity reactions, neuropsychiatric reactions, gastrointestinal reactions, and hematological reactions. Some of these types of reactions can be serious including anaphylaxis, DRESS, seizure, and psychiatric symptoms. However, these risks can be managed by appropriate labeling. See Section 13 of this review for labeling recommendation.

11. Advisory Committee Meeting and Other External Consultations

No advisory committee meeting was held, and no external consultations were obtained as there were no issues that needed input from external experts.

12. Pediatrics

Nifurtimox was studied in a pediatric population exclusively. The Applicant is exempt from submitting a pediatric study plan as NFX was designated an orphan drug for Chagas disease.

13. Labeling Recommendations

13.1. Prescription Drug Labeling

Table 81. Significant High-Level Labeling Changes (Not Direct Quotations)

Section	Proposed Labeling	Tentative Labeling
HIGHLIGHTS	Established Pharmacological Class (EPC) was "antiprotozoal"	• New EPC is "nitrofuran antiprotozoal."
1 INDICATIONS AND USAGE	• LAMPIT tablets are indicated in term newborns, infants, children and adolescents less than 18 years of age • For the accelerated approval statement, described the surrogate endpoint as an average 20% reduction in IgG antibody or seroconversion	• Revised age and added weight to read as follows: LAMPIT is indicated in pediatric patients (birth to less than 18 years of age and weighing at least 2.5 kg) • Revised to describe endpoint as seroconversion or 20% reduction in optical density in two different IgG antibody tests
2 DOSAGE AND ADMINISTRATION	• Recommended dose based on age and body weight • Reference to dissolving tablets	• Dose based on body weight alone • Tablets can be prepared as a slurry for patients who cannot swallow tablets • Added instructions on how to split tablets and prepare slurry as an alternate method of administration • Pregnancy testing recommended prior to treatment • Removed risk mitigation information as it is included in the Warnings and Precautions section

M.O. Comment: *The Applicant sent revised labeling on 27 Feb 2020 in response to a CMC IR about dissolving tablets in which they replaced dissolving with slurry.*

Associate Director for Labeling Comment: OND Policy Labeling Development Team (LDT) Recommendation (7/13/20)*: The rationale for describing the approved age groups in the Indications and Usage section as pediatric patients (birth to less than 18 years of age) includes the following:*

- *Although 21 CFR 201.57(c)(9)(iv)(A) defines pediatric patients as birth to 16 years (and CDER has generally considered the upper age group to encompass the entire 16th year), 21 CFR 201.57(c)(9)(iv)(G) permits use of an appropriate, alternative statement if statements described in paragraphs (A) through (F) of 21 CFR 201.57(c)(9)(iv) are not appropriate or relevant to the drug's labeling.*
- *The information in the INDICATIONS AND USAGE section should be concise and use terminology that is clinically relevant and understandable to healthcare providers (HCP). Accordingly, "pediatric patients birth to less than18 years of age" is a more concise and understandable phrase for communicating the approved age groups to the HCP than "pediatric patients (birth to less than 17 years old) and adult patients aged 17 to less than 18 years old."*

Section	Proposed Labeling	Tentative Labeling
4 CONTRAINDICATIONS	• (b) (6) • •	• Pregnancy and porphyria contraindications were replaced with warnings • Modified contraindication related to alcohol use to a contraindication in patients who consume alcohol during NFX treatment
5 WARNINGS AND PRECAUTIONS	•	• Added that patients with neurological and psychiatric conditions may experience worsening of their conditions • Warning on alcohol consumption removed as it is listed as a contraindication • Added warning for patients with porphyria, potential for genotoxicity and carcinogenicity, and embryo-fetal toxicity • Removed warnings for (b) (4) as they did not describe adverse reactions related with the drug
M.O. Comment: *There is a theoretical risk that NFX could precipitate acute attacks of porphyria based on data from other nitrofuran derivatives.* (b) (6) *removed and replaced with a warning about embryo-fetal toxicity and a recommendation for pregnancy testing and contraception.*		
6 ADVERSE REACTIONS	• (b) (6)	• Incidence of adverse reactions reported only for subjects in the 60-day treatment arm • Preferred terms for rash combined together • Added somnolence, seizure, syncope, leukopenia, and neutropenia to list of adverse reactions occurring <1% • Added anaphylaxis, DRESS, and thrombocytopenia as postmarketing adverse reactions • Removed postmarking adverse reactions already listed in Section 6.1
M.O. Comment: *As the recommended duration of treatment is 60 days, adverse reaction rates occurring in the 60-day arm are provided in the label. The adverse reactions of anaphylaxis, DRESS, and thrombocytopenia were noted by the Applicant in response to an IR.*		
7 DRUG INTERACTIONS	• Advised to discontinue consumption of alcohol during treatment	• NFX is contraindicated for patients who consume alcohol during treatment

Section	Proposed Labeling	Tentative Labeling
8 USE IN SPECIFIC POPULATIONS	• [redacted] (b) (6)	• NFX is not recommended during pregnancy for treatment of chronic Chagas. The decision to treat acute Chagas disease in a pregnant woman should be made on a case-by-case basis • Recommendation for contraception and pregnancy testing of females of reproductive potential • Recommendation for condom use by males of reproductive potential with female partners • Observation that based on findings in rodents, LAMPIT may impair fertility in males of reproductive potential
M.O. Comment: *The revised language regarding use of NFX in pregnancy highlights the need for contraception in women of childbearing potential and pregnancy testing, but also consideration of treatment of acute Chagas disease in pregnant women. As chronic Chagas disease is not imminently life-threatening, the recommendation is not to treat pregnant women with chronic Chagas disease.*		
10 OVERDOSAGE	• [redacted] (b) (4)	• Removed Section 10 as it did not include any data that would be useful to healthcare providers
12 CLINICAL PHARMACOLOGY	• The Applicant provided data on pharmacokinetics and microbiology	• This section was rewritten to align the content and format with the current labeling guidance. • Added *Cardiac Electrophysiology* section to note nifurtimox does not result in increases of >20 ms in the QTc interval
M.O. Comment: *ECG data from Study 16027 was limited but was sufficient to rule out large increases in the QTc interval. Despite use of NFX outside the United States for several decades, there are no reports of prolonged QTc intervals associated with NFX use.*		
13 NONCLINICAL TOXICOLOGY	• The Applicant provided data on embryo-fetal toxicity, fertility, genotoxicity, and carcinogenesis	• These data were rewritten more concisely and the data on embryo-fetal toxicity were removed from this section
14 CLINICAL STUDIES	• Efficacy measured using 20% decrease in optical density based on an average of two ELISAs and seroconversion and compared to historical control • Additional efficacy data from F29 ELISA and PCR • Listed adverse reaction data	• Described efficacy as based on 20% decrease in optical density and seroconversion using two ELISAs analyzed separately and comparison of 60-day and 30-day arms • Included historical placebo control data for F29 ELISA • Removed PCR results • Moved adverse reaction data to Section 6

Section	Proposed Labeling	Tentative Labeling
M.O. Comment: *As noted in Section 1 of this review, the efficacy of NFX was determined using seroconversion and ≥20% decrease in optical density on the lysate and recombinant ELISAs that showed a dose-response effect in the 60-day vs. 30-day NFX treatment arms in Study 16027 with additional data from F29 ELISA results compared to a historical placebo control.*		

Source: Reviewer Table

M.O. Comment: *The Patient Package Insert and the Instructions for Use have been revised to reflect the nifurtimox product labeling.*

14. Risk Evaluation and Mitigation Strategies (REMS)

No risk evaluation and mitigation strategies are needed at this time. The risks of nifurtimox may be adequately managed in the postmarketing setting through labeling and routine pharmacovigilance.

15. Postmarketing Requirements and Commitment

Postmarketing Requirements (PMRs)

Clinical

PMR-3868-1: Complete the CHICO SECURE part of Study 16027 to determine the seroconversion rate in subjects treated with nifurtimox at the 4-year timepoint. Obtain antibody titers by serial dilution for all available serum samples measured by the F29-ELISA, Recombinant-ELISA and Lysate-ELISA in addition to IHA.

Final protocol submission: June 2018 (submitted)

Study completion: August 2021

Final study report submission: February 2022

PMR-3868-2: Conduct a worldwide descriptive study that collects prospective and retrospective data in women exposed to LAMPIT (nifurtimox) during pregnancy to assess risk of pregnancy and maternal complications, adverse effects on the developing fetus and neonate, and adverse

effects on the infant. Infant outcomes will be assessed through at least the first year of life. The study will collect information for a minimum of 10 years.

Draft protocol submission: May 2021

Final protocol submission: January 2022

Interim study report: January 2023

Interim study report: January 2024

Interim study report: January 2025

Interim study report: January 2026

Interim study report: January 2027

Interim study report: January 2028

Interim study report: January 2029

Interim study report: January 2030

Interim study report: January 2031

Study completion: January 2032

Final study report submission: June 2032

Nonclinical

PMR-3868-3: Conduct a GLP-compliant embryo-fetal study in rats administered oral nifurtimox during organogenesis to assess the risk to pregnancy.

Draft protocol submission: December 2020

Final protocol submission: April 2021

Study completion: October 2021

Final study report submission: October 2022

Postmarketing Commitment (PMC)

Chemistry, Manufacturing, and Controls (CMC)

PMC-3868-4: Nifurtimox 30 mg tablets did not meet the updated dissolution criteria on (NLT (b) (4) % (Q) in 60 minutes) at the 43-day and 67-day time points for the in-use stability studies. Conduct an identical in-use stability study for nifurtimox 30 mg tablets with an appropriately aged batch. All the critical quality attributes (such as appearance, assay, impurities, dissolution, disintegration, % water, microbial purity, resistance to crushing, etc.) should be tested to demonstrate in-use stability of the split tablets. The data and final study report should be submitted as a prior approval (PAS) supplement.

Draft protocol submission: September 2020

Final protocol submission: October 2020

Study completion: January 2021

Final study report submission: February 2021

16. Division Director (DAI) Comments

I agree with the review team's assessment and recommendations.

17. Office Director (OID) Comments

I agree with the review team's assessment and recommendations.

18. Appendices

18.1. References

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18.2. Financial Disclosure

Table 82. Covered Clinical Study (Name and/or Number):

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 88		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: _____ Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in S Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) 0		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

18.3. Nonclinical Pharmacology/Toxicology

Table 83. Identities and Proposed Structures of Nifurtimox Metabolites Identified in In Vitro Incubations and In Vivo Studies

Assignment	^{12}C-mass $[M+H]^+$ [m/z]	Mass shift to drug [Da]	Structure proposal
Drug BAYA002502	288	--	
M-1	258	-30	
M-2	292	+4	
M-3	277	-11	
M-4	362	+74	
M-5	404	+116	
M-6	165	-123	

(Continued on next page.)

Assignment	^{12}C-mass $[M+H]^+$ [m/z]	Mass shift to drug [Da]	Structure proposal
M-7	352	+64	
M-8	394	+106	
M-9	293	+5	
M-10a/b	419	+131	
M-11	304	+16	
M-12	320	+32	

(Continued on next page.)

Assignment	^{12}C-mass $[M+H]^+$ [m/z]	Mass shift to drug [Da]	Structure proposal
M-13a/b	336 $[M-H]^-$	+50	
M-14	355 $[M-H]^-$	+69	
M-15	277	-11	
M-16	306	+18	
M-17a/b	256	-32	
M-18a/b	548	+260	

Source; Study Report No.: 40552

18.4. OCP Appendices (Technical Documents Supporting OCP Recommendations)

18.4.1. Summary of Bioanalytical Method Validation and Performance

Review of the bioanalytical methods utilized to quantify nifurtimox in the clinical pharmacology studies are summarized in Table 84.

Table 84. Bioanalytical Method Validation and Performance Findings

Method/Report	Findings	
Study No. 16027		
Analyte/assessment	Nifurtimox	
Method	HPLC-MS/MS	
Matrix	Plasma using lithium heparinized micro hematocrit sampling glass capillaries	
Validation report	Validation report provided: MW1716v01 and 02 *Notes: Validation reports for studies MW1716 v01 and 02 have not been submitted, instead, the findings from these reports are provided as a part of Report PH-40730.*	☐ Yes ☒ No
	Validation report acceptable Range: 5 ng/mL to 5000 ng/mL *Notes: Report PH-40730 does not contain information on the intrarun and inter-run accuracy and precision information for calibration samples, however, this information is available for quality control samples. Extraction recovery was reported to be ~60% compared to ~80% reported in Report ANI10740.01; however, the recovery estimates were reproducible at 25 ng/mL and 5000 ng/mL (n=6).*	☒ Yes ☐ No
Performance report	Samples analyzed within the established stability period *Notes: All samples were stored at or below -15°C and analyzed within 477 days after sampling. The reported stability is up to 544 days in lithium heparinized human plasma in glass capillaries and 371 days in lithium heparinized human plasma in glass capillaries in the presence of centrifuged blood cells.*	☒ Yes ☐ No
	Quality control samples range acceptable QC: 15, 250, and 4000 ng/mL	☒ Yes ☐ No
	Chromatograms provided	☐ Yes ☒ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable: *Notes: ISR findings showed 44/75 (58.7%) samples had difference within 20%. It is typically recommended that at least 66% of the investigated ISR sample results should be within 20% based on the respective mean of the first and second result.*	☒ Yes ☐ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No

Method/Report	Findings	
Study No. 16004		
Analyte/assessment	Nifurtimox	
Method	HPLC-MS/MS	
Matrix	EDTA K_2 Plasma	
Validation report	Validation reports provided: SOP: ANI10678_02/ANI10740.01 Report: KINE 120188	☒ Yes ☐ No
	Validation report acceptable Range: Project 135112AIZN: 10 to 10000 ng/mL Project 135112AJTI: 10 to 2000 ng/mL	☒ Yes ☐ No
Performance report	Samples analyzed within the established stability period	☒ Yes ☐ No
	Quality control samples range acceptable QC: 30, 150, 1000, and 1500 ng/mL	☒ Yes ☐ No
	Chromatograms provided *Notes: Only example chromatograms provided.*	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable	☒ Yes ☐ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No
Study No. 16005		
Analyte/assessment	Nifurtimox	
Method	HPLC-MS/MS	
Matrix	EDTA K_2 Plasma	
Validation report	*Same as Study 16004*	
Performance report	Samples analyzed within the established stability period	☒ Yes ☐ No
	Quality control samples range acceptable QC: 30, 150, 1000, and 1500 ng/mL	☒ Yes ☐ No
	Chromatograms provided *Notes: Only example chromatograms provided.*	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable *Notes: ISR was repeated after high failure rate in initial samples. An investigation was ensued for the high failure rate; however, reasons were not identified.*	☒ Yes ☐ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No

Method/Report	Findings	
Study No. 16007		
Analyte/assessment	Nifurtimox	
Method	HPLC-MS/MS	
Matrix	EDTA K_2 Plasma	
Validation report	*Same as Study 16004*	
Performance report	Samples analyzed within the established stability period	☒ Yes ☐ No
	Quality control samples range acceptable QC: 30, 150, 1000, and 1500 ng/mL	☒ Yes ☐ No
	Chromatograms provided	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable	☒ Yes ☐ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No
Study No. 19500		
Analyte/assessment	Nifurtimox	
Method	HPLC-MS/MS	
Matrix	EDTA K_2 Plasma	
Validation report	Same as Study 16004	
Performance report	Samples analyzed within the established stability period	☒ Yes ☐ No
	Quality control samples range acceptable QC: 0.6, 5, 8, 20, and 30 mcg/mL	☒ Yes ☐ No
	Chromatograms provided	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable	☒ Yes ☐ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No

18.4.2. In Vitro Studies

18.4.2.1. Metabolism and Transporters Characterization

Table 85. Metabolism and Transporters Characterization, Study PH-40552

Title	Nifurtimox: Metabolite Identification and Structure Elucidation from In Vitro and In Vivo Metabolism Studies
Objectives	In vitro biotransformation of nifurtimox (BAYA002502) was investigated in different in vitro test systems including hepatocytes and different subcellular fraction of rat and man, and recombinant bacterial nitroreductase.[1]

[1] Data from only human derived hepatocytes and different subcellular fractions were reviewed.

Methods	
Systems	Human derived hepatocytes: Cryopreserved human hepatocytes Subcellular fractions: Human kidney S9 Human feces Nitroreductase: Recombinant nitroreductase from *Escherichia coli*
Methodology	**Test conditions for subcellular fractions:** • Test concentration: 1 - 20 mcM [^{14}C]BAYA002502 as a 1:100 dilution prepared from respective stock solutions dissolved in acetonitrile • Incubation buffer: 50mM potassium phosphate pH 7.4+1mM EDTA • Temperature: 37°C • NADPH-generating system: 1mM NADP or NADH 5mM glucose 6-phosphate • 1.5 U/mL glucose 6-phosphate dehydrogenase • Protein concentration: up to 2 mg/mL • Incubation time: up to 90 min **Test conditions for hepatocyte incubation conditions:** • Test concentration: 1 and 10 mcM [^{14}C]BAYA002502 added from a 0.1 and 1mM stock solution dissolved in acetonitrile, respectively • Incubation buffer: William's E Medium • Temperature: 37°C • Incubation time: 0, 4 h

Control	**Activities Expressed as CL_{int} in [mcL/min/10^6 Cells]**
Granisetron	2.7
Phenacetin	3.2
Repaglinide	37.3
Diclofenac	40.9
Dextromethorphan	77.9
Midazolam	95.6

Test conditions for human feces:
- Test concentration: 50 mcL of a solution of [^{14}C]BAYA002502 (0.252 mg/mL in acetonitrile:water (1:1, V/V)
- Matrix: Fresh human feces (about 13 g) were added to 50 mL degassed phosphate buffer pH 7:water (1:5, V/V).
- Temperature: 37°C
- Incubation time: 20 h
- Control: [^{14}C]BAYA002502 was incubated without feces under the same conditions

Results & Applicant's conclusion

- After 10 min incubation with nitroreductase, complete turnover of nifurtimox was observed
- Incubations with human kidney S9 with 1mM cysteine or acetylcysteine resulted in formation of M-4 or M-5, respectively. However, formation was also present without kidney S9, indicating nonenzymatic direct formation of M-4 and M-5.
- Incubation with hepatocytes resulted in traces of M-4, which postulated to be from a nonenzymatic direct reaction with cysteine, present in the incubation medium. In addition, a very small amounts of a very polar not identified product was found. The hepatocytes were incubated in parallel with prototypic substrates (positive controls) and exhibited good activity. When human hepatocytes were incubated under reductive conditions in nitrogen (N2) atmosphere, no formation of any product was detected.
- Incubation with human blood resulted in traces of a very polar unidentified component in addition to small amounts of two glutathione conjugates M-18a and M-18b, and M-4.
- Nifurtimox was found to be susceptible to reducing agents.
- Incubation with human feces indicated nifurtimox was not stable and several products including significant amounts of saturated nitrile M-1 were formed after incubation with feces.

The parent compound was readily degraded and more than 35 nifurtimox related metabolites/products could be identified. All products seem to derive from chemical processes like reduction or direct nucleophilic conjugation and not enzymatic reactions confirming the metabolic stability of nifurtimox in the in vitro investigation. It was also found that nifurtimox seems to be unstable in human feces. Nifurtimox is therefore considered to be not a substrate for prototypical drug metabolizing enzymes (e.g., CYPs, UGTs)

Reviewer comments

The study evaluated hepatocytes' activity by incubating prototypic substrates (positive controls). However, from the provided information on the incubation conditions with hepatocytes for positive controls and nifurtimox, it is not clear if the concentrations of acetonitrile in incubation mediums were similar. Since acetonitrile can affect the viability of hepatocytes, information on acetonitrile concentrations in the incubation medium for nifurtimox and positive controls appear to be valuable.

Table 86. Metabolism and Transporters Characterization, Study PH-40156

Title	Nifurtimox: In Vitro Studies in L-MDR1 Cells to Evaluate the Substrate Characteristics and the Inhibitory Potential of Nifurtimox Towards P-gp
Methods	
System	P-gp-transfected LLC-PK1 (L-MDR1 cells) Nontransfected LLC-PK1 cells (Control cells)
Methods	The mass transfer of Lucifer Yellow from the donor to the receiver compartment was evaluated to assess the cell line's permeability.

Transporter/Objective	**Substrate: Concentration**	**Inhibitor: Concentration**
P-gp/substrate characterization	Nifurtimox: 0.2 mcM, 2 mcM, and 10 mcM	Ivermectin: 1 mcM and 5 mcM
	Dipyridamole (positive control): 1 mcM and 2 mcM	
P-gp/inhibitory potential	Digoxin: 25 mcM Dipyridamole: 1 mcM	Nifurtimox: 0-10 mcM

Results & Applicant's conclusion

Nifurtimox efflux ratios with transfected cells (transfected LLC-PK1) were comparable to ratios with control cells (nontransfected LLC-PK1 cells), i.e., ratios of 0.6±0.02, 0.5±0.04, and 0.4±0.04 vs. 0.55±0.04, 0.51, and 0.53±0.04 at concentrations of 0.2, 2, and 10mcM, respectively.

Nifurtimox showed no concentration dependent inhibition of the efflux of dipyridamole or digoxin in the investigated concentration range from 0.1 to 100 mcM (Table below)

Inhibitory Effect of Nifurtimox on the Efflux Ratio of the P-gp Substrate

A: Digoxin (25 µM) in L-MDR1 cells (n=3)

Test item	Conc.	P_{app} A-B		P_{app} B-A		Efflux ratio		% of control
	[µM]	Mean [nm/s]	SD [nm/s]	Mean [nm/s]	SD [nm/s]	Mean	SD	[%] SD
control	0	8.1		92	3.6	11.3	0.4	100 +- SD
BAYA002502	0.1	6.7	0.7	93	1.3	13.9	1.4	123.5
BAYA002502	0.3	10.0	1.3	105	7.0	10.5	1.5	93.2
BAYA002502	1	6.5	0.5	78	4.5	12.0	1.2	106.5
BAYA002502	3	10.1	1.5	83	4.2	8.2	1.3	72.9
BAYA002502	10	6.4	0.1	81	6.5	12.6	1.0	112.1
BAYA002502	30	10.1	1.5	85	4.4	8.4	1.3	74.2
BAYA002502	100	7.0	1.0	93	4.5	13.3	2.0	118.2

B: Dipyridamole (1 µM) in L-MDR1 cells (n=3)

Test item	Conc.	P_{app} A-B		P_{app} B-A		Efflux ratio		% of control
	[µM]	Mean [nm/s]	SD [nm/s]	Mean [nm/s]	SD [nm/s]	Mean	SD	[%] SD
control	0	10	0.3	685	20	66	2.7	100 +- SD
BAYA002502	0.1	12	2.4	706	31	58	12	87.4
BAYA002502	0.3	16	5.5	715	26	44	15	66.8
BAYA002502	1	13	1.7	686	13	53	7.0	80.2
BAYA002502	3	11	0.9	712	7.6	65	5.3	97.3
BAYA002502	10	11	0.5	736	4.0	69	3.0	103.1
BAYA002502	30	11	0.4	763	46	69	4.7	103.1
BAYA002502	100	13	0.4	709	22	53	2.3	79.1

Nifurtimox is not a P-gp substrate or inhibitor. Therefore, the risk of drug-drug interactions due to interaction of nifurtimox with P-gp can be ruled out.

Table 87. Metabolism and Transporters Characterization, Study PH-40200

Title	Nifurtimox: In Vitro Studies in MDCKII-BCRP Cells to Evaluate the Substrate Characteristics and the Inhibitory Potential of Nifurtimox Towards BCRP
Methods	
System	BCRP-transfected MDCKII cells (MDCKII-BCRP) MDCKII wild-type cells (control cells)
Methods	The mass transfer of Lucifer Yellow from the donor to the receiver compartment was evaluated to assess the permeability.

Transporter/Objective	Substrate: Concentration	Inhibitor: Concentration
BCRP/substrate characterization	Nifurtimox: 0.1-50 mcM Topotecan (positive control): 2 mcM	KO143: 1 mcM
BCRP/inhibitory potential	Topotecan: 2 mcM	Nifurtimox: 0-100 mcM

Results & Applicant's conclusion
• Nifurtimox efflux ratios with transfected cells (MDCKII-BCRP) were comparable to ratios with control cells (MDCKII-WT), i.e., ratios of 0.5±0.05 and 0.4±0.03, and 0.5±0.04 and 0.7±0.06 (for 10 mcM) at concentrations of 0.1-50 mcM, respectively. • Nifurtimox showed in the tested concentration range (1-100 mcM) just a slight inhibitory effect towards the efflux of the BCRP probe substrate topotecan with the IC_{50} value of >100 mcM. Nifurtimox is not a BCRP substrate or inhibitor. Therefore, the risk of a drug-drug interactions due to interaction of nifurtimox with BCRP can be ruled out.
Reviewer comments
The Applicant's conclusions appear reasonable. The observed maximum inhibition of nifurtimox on the efflux ratio of topotecan was approximately 34% at the concentration of 30 mcM compared to the positive control. However, at the other nifurtimox concentrations (1, 3, 10, and 100 mcM) the observed inhibition of nifurtimox on the efflux ratio of topotecan compared to the positive control ranged between approximately -11 to 17%. These findings suggest that the observed nifurtimox's inhibition potential towards BCRP in this study was not concentration dependent at the concentrations ranging from 1 to 100 mcM.

18.4.2.2. In Vitro Drug Interaction Studies—Cytochrome P450 Enzyme

Table 88. In Vitro Drug Interaction With CYP450 Enzyme, Study No. 37686

Title	BAY A 2502 *[Nifurtimox]*: Determination of the Inhibitory Potency Towards Human CYP Isoforms In Vitro
Methods	
System	Human liver microsomes pool
Methods	• Activity of various CYPs in pooled human liver microsomes was determined by measuring the rate of formation of metabolites following incubation with enzyme specific substrates. • Incubation mixture included human liver microsomes at protein concentrations defined in the table below, NADPH-regenerating system (1mM NADP, 5mM glucose 6-phosphate, glucose 6-phosphate dehydrogenase (1.5 U/mL), 1mM EDTA, nifurtimox at six different concentrations (50, 25, 12.5, 6.3, 3.1, 1.6 mcM), probe substrate at concentrations close to reaction Km values, and phosphate buffer (50mM, pH 7.4) in a total volume of 200 mcL. • Known direct-acting and metabolism-dependent inhibitors of the respective enzymes ("standard inhibitors") were included as positive controls. • For time-dependent inhibition evaluation, nifurtimox at six different concentrations (50, 25, 12.5, 6.3, 3.1, 1.6 mcM) was preincubated with microsomes and NADPH-regenerating system in the absence of the probe substrate (midazolam, testosterone) for 30 min at 37°C. Following preincubation, probe substrates dissolved in water (midazolam) or water/methanol (testosterone) were added at the concentrations specified for the coincubations, and the reactions were allowed to proceed. The known metabolism-dependent inhibitor mibefradil served as positive control.

Incubation Conditions, Study No. 37686

CYP	Substrate (concentration)	Protein [µg/mL][A]	Incubation time [min]	ISTD	Standard inhibitor (IC_{50})
1A2	Phenacetin (45 µM)	600	15	[$^2H_3$]Acetaminophen	Furafylline (9.4 µM)
2A6	Coumarin (2.0 µM)	20	10	7-Hydroxy[$^2H_5$]coumarin	Tranylcypromine (0.41 µM)
2B6	Bupropion (100 µM)	250	30	[$^2H_6$]Hydroxybupropion	Ticlopidine (1.3 µM)
2C8	Amodiaquine (2.0 µM)	14	15	[$^2H_5$]Desethylamodiaquine	Montelukast (0.056 µM)
2C9	Diclofenac (4 µM)	14	15	4'-Hydroxy[$^{13}C_6$]diclofenac	Sulfaphenazole (0.021 µM)
2C19	Mephenytoin (50 µM)	500	30	4'-Hydroxy[$^2H_3$]-mephenytoin	(-)-*N*-3-Benzylphenobarbital (1.3 µM)
2D6	Dextromethorphan (5 µM)	600	15	[$^2H_3$]Dextrorphan	Fluoxetine (0.17 µM)
2E1	Chlorzoaxazone (50 µM)	127	15	6-Hydroxy[$^{13}C_6$]-chlorzoxazone	4-Methylpyrazole (10 µM)
3A4	Midazolam (2.5 µM)	60	10	[$^2H_3$]Omeprazole	Ketoconazole (0.0023 µM)
	with preincubation (2.5 µM)	60	30/10	Omeprazole	Mibefradil (0.014 µM)
3A4	Testosterone (50 µM)	100	15	6β-Hydroxy[$^2H_3$]-testosterone	Ketoconazole (0.092 µM)
	with preincubation (50 µM)	100	30/15	6β-Hydroxy[$^2H_3$]-testosterone	Mibefradil (0.068 µM)

A = human liver microsomes

The CYP-mediated activities in the presence of inhibitors were evaluated as percentages of the corresponding control values and IC_{50} values were calculated using a nonlinear least-squares regression.

Results & Applicant's conclusion

- Estimated IC_{50} values for positive controls were in the range expected from literature.
- Coincubation of nifurtimox did not affect CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 activities as indicated by IC_{50} values >50 mcM.
- Preincubation of 30 mins of nifurtimox NADPH-supplemented human liver microsomes did not indicate time-dependent inhibition of CYP3A4.

Based on these in vitro inhibition results and data on the exposure of nifurtimox, clinical drug-drug interactions through inhibition of CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 in the presence of nifurtimox are unlikely.

Reviewer comments

This study evaluated potential for time-dependent inhibition with nifurtimox against CYP3A4 only. Information on the time-dependent inhibition potential against other CYP450 enzymes is available in Study 40946.

Table 89. In Vitro Drug Interaction With CYP450 Enzyme, Study No. 40946

Title	Nifurtimox: Determination of the Time-Dependent Inhibitory Potency of Nifurtimox Towards Human CYP Isoforms In Vitro
Methods	
System	Human liver microsomes
Methods	• Activity of various CYPs in pooled human liver microsomes was determined by the Human liver microsomes' supplier based on measuring the rate of formation of metabolites following incubation with enzyme specific substrates.

- Pre-incubation (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6) in the absence and presence of NADPH: For experiments with pre-incubation in the absence of NADPH, incubation mixture included human liver microsomes at protein concentrations defined in table (see below), NADPH-regenerating system (1mM NADP, 5mM glucose 6-phosphate, glucose 6-phosphate dehydrogenase (1.5 U/mL), 1mM EDTA, nifurtimox at six different concentrations (50, 25, 12.5, 6.3, 3.1, 1.6 mcM), probe substrate at concentrations defined in table (see below) close to reaction Km values, and phosphate buffer. For experiments with pre-incubation in the presence of NADPH, the incubation mixture was prepared similarly except that the solution was pre-incubated with NADPH-regenerating system for 30 min at 37°C prior to addition of the probe substrates.
- Known direct-acting and metabolism-dependent inhibitors of the respective enzymes were included as positive controls.

Incubation Conditions, Study No. 40946

CYP	Substrate (concentration)	Protein[a] [µg/mL]	Incubation time [min]	Positive control Standard inhibitor	IC_{50} [µM]	Literature value for standard inhibitor IC_{50} [µM]
1A2	Phenacetin (45 µM)	100	30/15	Furafylline		
				- NADPH	>2.5	>10
				+ NADPH	0.59	0.021 (0.020, 0.022)
2B6	Bupropion (100 µM)	250	30/30	Ticlopidine		
				- NADPH	0.79	0.84 (0.69, 0.99)
				+ NADPH	0.63	0.048 (0.042, 0.053)
2C8	Amodiaquine (2 µM)	10	30/15	Gemfibrozil glucuronide		
				- NADPH	5.5	26 (25, 26)
				+ NADPH	1.8	0.46 (0.55, 0.37)
2C9	Diclofenac (4 µM)	10	30/15	Tienilic acid		
				- NADPH	>0.1	1.7 (1.4, 1.9)
				+ NADPH	0.019	0.049 (0.054, 0.044)
2C19	Mephenytoin (50 µM)	500	30/30	Clopidogrel		
				- NADPH	>74	57.3
				+ NADPH	38	23.2
2D6	Dextromethorphan (5 µM)	100	30/15	Paroxetine		
				- NADPH	0.78	1.1 (1.05, 0.98, 0.96)
				+ NADPH	0.072	0.066 (0.076, 0.059, 0.063)

a = human liver microsomes

The CYP-mediated activities in the presence of inhibitors were evaluated as percentages of the corresponding control values and IC_{50} values were calculated using a nonlinear least-squares regression.

Results & Applicant's conclusion

- No change in the inhibitory potency of nifurtimox on biotransformation reactions catalyzed by CYPs 1A2, 2B6, 2C8, 2C9, 2C19, and 2D6 after preincubation with NADPH was observed up to the highest test concentration (IC_{50}>50 mcM), indicating no metabolism-dependent (time-dependent) inhibition of nifurtimox on these CYPs.

There is no risk to observe clinical PK drug-drug interactions with substrates of the investigated CYP isoform under treatment with nifurtimox.

Table 90. In Vitro Drug Interaction With CYP450 Enzyme, Study# 40954

Title	Nifurtimox: Determination of the Direct and Time-Dependent Inhibitory Potency of Metabolites M-4 (BAY 3062888) and M-6 (BAYA004013) of Nifurtimox towards Human CYP Isoforms In Vitro
Methods	
System	Human liver microsomes
Methods	• Activity of various CYPs in pooled human liver microsomes was determined by the Human liver microsomes' supplier based on measuring the rate of formation of metabolites following incubation with enzyme specific substrates. • Co-incubation (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4): Incubation mixture included human liver microsomes, NADPH-regenerating system (1mM NADP, 5mM glucose 6-phosphate, glucose 6-phosphate dehydrogenase (1.5 U/mL), 1mM EDTA, M-4 at six different concentrations (20, 10, 5, 2.5, 1.3, 0.6 mcM) or M-6 at six different concentrations (100, 50, 25, 12.5, 6.3, 3.1 mcM), probe substrate at concentrations close to reaction Km values, and phosphate buffer. For experiments with pre-incubation in the presence of NADPH, the incubation mixture was prepared similarly except, the solution was pre-incubated with NADPH-regenerating system for 30 min at 37°C prior to addition of the probe substrates. • Pre-incubation (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) in absence and presence of NADPH: For experiments with pre-incubation in the absence of NADPH, incubation mixture included human liver microsomes, NADPH-regenerating system (1mM NADP, 5mM glucose 6-phosphate, glucose 6-phosphate dehydrogenase (1.5 U/mL), 1mM EDTA, BAY3062888 at six different concentrations (20, 10, 5, 2.5, 1.3, 0.6 mcM) or BAYA004013 at six different concentrations (100, 50, 25, 12.5, 6.3, 3.1 mcM), probe substrate at concentrations close to reaction Km values, and phosphate buffer. For experiments with pre-incubation in the presence of NADPH, the incubation mixture was prepared similarly except, the solution was pre-incubated with NADPH-regenerating system for 30 min at 37°C prior to addition of the probe substrates. • Known direct-acting and metabolism-dependent inhibitors of the respective enzymes were included as positive controls. • The CYP450-mediated activities in the presence of inhibitors were evaluated as percentages of the corresponding control values and IC_{50} values were calculated using a nonlinear least-squares regression.
Results & Applicant's conclusion	
• No change in the inhibitory potency of metabolites M-4 and M-6 on biotransformation reactions catalyzed by CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 after co-incubation and after preincubation with NADPH was observed up to the highest test concentration (M-4: IC_{50}>20 mcM; M-6: IC_{50}>100 mcM), indicating no metabolism-dependent (time-dependent) inhibition of M-4 and M-6 on these CYPs. There is no risk to observe clinical PK drug-drug interactions with substrates of the investigated CYP isoform due to inhibition caused by metabolites M-4 and M-6 of nifurtimox.	

Table 91. In Vitro Drug Interaction With CYP450 Enzyme, Study No. 41133

Title	Nifurtimox: In Vitro Evaluation of Nifurtimox and its Metabolites M-4 and M-6 as Inducers of Cytochrome P450 Expression
Methods	
System	Human hepatocytes
Methods	• Three preparations of different cryopreserved human hepatocytes from three donors were utilized • Hepatocyte viability was determined by dividing the absorbance found in the test compound treated cells by the mean absorbance found in the solvent vehicle treated control cells. • Enzymes' activities were assessed using the substrates listed in table below

Enzyme Activity Assays, Study No. 41133

Enzyme	Reagent use	Name
CYP1A2	Substrate	Phenacetin
	Substrate metabolite	Acetaminophen
	Internal standard	Acetaminophen-d_3
CYP2B6	Substrate	*S*-Mephenytoin
	Substrate metabolite	Nirvanol
	Internal standard	Nirvanol-d_5
CYP2C19	Substrate	*S*-Mephenytoin
	Substrate metabolite	4'-Hydroxymephenytoin
	Internal standard	Nirvanol-d_5
CYP3A4	Substrate	Testosterone
	Substrate metabolite	6β-Hydroxytestosterone
	Internal standard	6β-Hydroxytestosterone-d_3

- Omeprazole, rifampicin, and phenobarbital were used as the positive controls
- Enzyme activity was measured based on mRNA analysis using quantitative RT-PCR and by relative quantification measures in the change in mRNA expression.
- Fold change in enzymes' activity was assessed by comparing enzymatic rates between the test compound and the vehicle control.
- Fold change in enzymes' activity was also compared against positive controls using the following equation:

$$\text{Percent positive control (\%)} = \frac{(\text{Activity of test article treated cells} - \text{Activity of vehicle control})}{(\text{Activity of positive control} - \text{Activity of vehicle control})} x\ 100$$

Results & Applicant's conclusion

- In the in vitro hepatocyte cultures, nifurtimox and its metabolites M-4 and M-6 did not induce CYP3A4, CYP2B6, CYP2C19, and CYP1A2 in any of the three donors.

Nifurtimox and its metabolites M-4 and M-6 bear no risk for clinically relevant drug-drug interactions due to CYP enzyme induction.

18.4.2.3. In Vitro Drug Interaction Studies—Transporter-Mediated Drug Interactions

Table 92. In Vitro Transporter-Mediated Drug Interactions, Study No. PH-41046

Title	Nifurtimox: In Vitro Studies in L-MDR1 Cells to Evaluate the Inhibitory Potential of the Metabolites M-4 (BAY 3062888) and M-6 (BAYA004013) towards P-gp
Methods	
System	P-gp-transfected LLC-PK1(L-MDR1 cells) Nontransfected LLC-PK1 cells (control cells)
Methods	The mass transfer of Lucifer Yellow from the donor to the receiver compartment was evaluated to assess the cell line permeability.

Transporter/Objective	Substrate: Concentration	Inhibitor: Concentration
P-gp/inhibitory potential	Digoxin: 20 mcM Dipyridamole: 2 mcM	BAY 3062888 (M-4): 0.05 to 5 mcM with Digoxin 0.05 to 20 mcM with Dipyridamole
	Digoxin: 20 mcM Dipyridamole: 2 mcM	BAYA004013 (M-6): 0.2-200 mcM
		Ivermectin (5 mcM) as a reference inhibitor

Results & Applicant's conclusion
• Neither M-4 (BAY 3062888) or M-6 (BAYA004013) showed any concentration dependent inhibition of the efflux of dipyridamole or digoxin. Nifurtimox metabolites M-4 (BAY 3062888) and M-6 (BAYA004013) are not inhibitors of P-gp.
Reviewer comments
Inhibition potential of nifurtimox towards P-gp was evaluated in Study PH-40156.

Table 93. In Vitro Transporter-Mediated Drug Interactions, Study PH-41047

Title	Nifurtimox: In Vitro Studies in MDCKII-BCRP Cells to Evaluate the Inhibitory Potential of the Metabolites M-4 (BAY 3062888) and M-6 (BAYA004013) towards BCRP
Methods	
System	BCRP-transfected MDCKII cells (MDCKII-BCRP cells) MDCKII wild-type cells (control cells)
Methods	The mass transfer of Lucifer Yellow from the donor to the receiver compartment was evaluated to assess the cell line permeability.

Transporter/Objective	Substrate: Concentration	Inhibitor: Concentration
BCRP/Inhibitory potential	Topotecan: 2 mcM Fluvastatin: 2 mcM	BAY 3062888 (M-4): 0.05 to 20 mcM
		BAYA004013 (M-6): 0.2- 200 mcM
		KO143 (1 mcM) as a reference inhibitor

Results & Applicant's conclusion

- Neither M-4 (BAY 3062888) or M-6 (BAYA004013) showed any concentration dependent inhibition of the efflux of topotecan or fluvastatin.

Nifurtimox metabolites M-4 (BAY 3062888) and M-6 (BAYA004013) are not inhibitors of BCRP

Table 94. In Vitro Transporter-Mediated Drug Interactions, Study PH-41132

Title	Nifurtimox: In Vitro Studies in HEK-OATP1B1 and HEKOATP1B3 Cells to Evaluate the Inhibitory Potential of Nifurtimox and Metabolites M-4 (BAY 3062888) and M-6 (BAYA004013) Following Pre-incubation		
Methods			
System	OATP1B1-transfected HEK cells (HEK-OATP1B1) OATP1B3-transfected HEK cells (HEK-OATP1B3)		
Methods	The mass transfer of Lucifer Yellow from the donor to the receiver compartment was evaluated to assess the permeability.		
	Transporters/Objective	**Substrate: Concentration**	**Inhibitor: Concentration**
	OATP1B1 and OATP1B3/inhibitory potential	Pravastatin: 5 mcM	Nifurtimox: 0.2 to 50 mcM
			BAY 3062888 (M-4): 0.05 to 20 mcM
			BAYA004013 (M-6): 0.2-50 mcM
			Rifamycin: 30 mcM as a reference inhibitor

Results & Applicant's conclusion

- Nifurtimox, M-4 (BAY 3062888) and M-6 (BAYA004013) are not inhibitors for OATP1B1
- Nifurtimox and M-6 (BAYA004013) are not inhibitors for OATP1B3, whereas M-4 (BAY 3062888) showed inhibitory potential towards OATP1B3 with an IC_{50} value of 10 mcM.

M-4 (BAY 3062888) showed inhibitory potential towards OATP1B3.

Reviewer comments

In an extrapolatory analysis, i.e., Study PH-40959, the reported C_{max} of M-4 and M-6 in plasma are 0.207 mcmol/L and 2.25 mcmol/L. This study reports IC_{50} values for OATP1B3 as 10 mcM and >50 mcM for M-4 and M-6, respectively. In a separate report, the Applicant concludes that, "Based on the results of the in vitro *investigations and considering the clinical exposure of metabolites M-4 and M-6 following 120 mg QD doses of nifurtimox, clinically relevant pharmacokinetic DDIs with the investigated CYP enzymes and transporters are unlikely." The Applicant's conclusion appears reasonable.*

Table 95. In Vitro Transporter-Mediated Drug Interactions, Study PH-13501

Title	Nifurtimox: In vitro studies to determine the inhibitory potential of Nifurtimox, BAYA004013 (M-6), and BAY 3062888 (M-4) towards human efflux transporters hMATE1 and hMATE2K
Methods	
System	HEK293 cell lines transfected with the SLC47A1 and SLC47A2 cDNAs expressing the human uptake transporters hMATE1 and hMATE2K. HEK293 cells transfected with the empty vector pcDNA5/FRT serving as control cell lines.
Methods	Information on the cell line permeability assessment is not provided

Transporter/Objective	Substrate: Concentration	Inhibitor: Concentration
hMATE1/Inhibitory potential	Metformin: 20 mcM	Nifurtimox: 2 mcM, 20 mcM M−6: 3 mcM, 30 mcM M−4: 0.5 mcM, 5 mcM Cimetidine (positive control): 50 mcM
hMATE2K/Inhibitory potential	Metformin: 90 mcM	

Results & Applicant's conclusion

- Nifurtimox, M-4, or M-6 did not show notable inhibition of hMATE1-mediated metformin-uptake with mean maximum inhibition of 24% compared to 95% inhibition of metformin uptake by positive control cimetidine
- Nifurtimox, M-4, or M-6 did not show notable inhibition of hMATE2-mediated metformin-uptake with mean maximum inhibition of 5.6% compared to 90% inhibition of metformin uptake by positive control cimetidine

Nifurtimox, M−6, and M−4 are not inhibitors of human uptake transporters hMATE1 or hMATE2K, in vitro.

Reviewer comments

Overall, for any of the three analytes, the observed inhibition in metformin uptake was less than 25% and 6% for MATE1 and MATE2K transporters, respectively, and IC50 values could not be determined. In a clinical study observed Cmax values following a single dose 120 mg nifurtimox are 2 mcM, 2.25 mcM, and 0.207 mcM for nifurtimox, M-6, and M-4, respectively. These concentrations are covered by the concentrations used in this in vitro study, i.e., up to 20 mcM, 30 mcM, and 5 mcM for nifurtimox, M−6, and M−4, respectively. And, the findings from this invitro study show nifurtimox, M-6, or M-4 did not inhibit MATE1 or MATE2K transporters up to aforementioned concentrations.

Table 96. In Vitro Transporter-Mediated Drug Interactions, Study PH-13502

Title	Nifurtimox: In vitro study to determine the inhibitory potential of Nifurtimox, BAYA004013 (M-6), and BAY 3062888 (M-4) towards human uptake transporters hOAT1, hOAT3 and hOCT2
Methods	
System	HEK293 cell lines transfected with the SLC22A6, SLC22A8, and SLC22A2 cDNAs expressing the human uptake transporter hOAT1, hOAT3, and hOCT2, respectively. HEK293 cells transfected with the empty vector pcDNA5 serving as control cell line for the hOAT1- and hOAT3-HEK cell lines HEK293 cells transfected with the empty vector pRcCMV and serving as control cell line for the hOCT2-HEK cell line
Methods	Information on the cell line permeability assessment is not provided.

Transporter/ Objective	Probe substrates: Concentration	Inhibitor: Concentration
OAT1/Inhibitory potential	p-aminohippuric acid (PAH): 10 mcM	Nifurtimox: 2 mcM, 20 mcM M−6: 3 mcM, 30 mcM M−4: 0.5 mcM, 5 mcM Probenecid (positive control for OAT1 and OAT3): 100 mcM Decynium22 (positive control for OCT2): 50 mcM
OAT3/Inhibitory potential	Estrone 3-sulfate (ES): 1 mcM	
OCT2/Inhibitory potential	1-methyl-4-phenylpyridinium (MPP): 10 mcM	

- Inhibition of hOAT1-mediated PAH uptake was measured after 5 min of incubation
- Inhibition of hOAT3-mediated ES uptake was measured after 1 min of incubation
- Inhibition of hOCT2-mediated MPP uptake was measured after 1 min of incubation
- All experiments were performed in triplicates on two separate days

Results & Applicant's conclusion

- Nifurtimox, M-4, or M-6 did not show notable inhibition of hOAT1-mediated PAH uptake with mean maximum inhibition of 4.1% compared to 93% inhibition of PAH uptake by positive control probenecid
- Nifurtimox, M-4, or M-6 did not show notable inhibition of hOAT3-mediated ES uptake with mean maximum inhibition of 27% compared to 92% inhibition of ES uptake by positive control probenecid
- Nifurtimox, M-4, or M-6 did not show notable inhibition of hOCT2-mediated MPP net-uptake with mean maximum inhibition of 10% compared to 81% inhibition of MPP uptake for positive control Decynium22

Nifurtimox, M−6, and M−4 are not inhibitors of human uptake transporters hOAT1, hOAT3, and hOCT2, in vitro.

Reviewer comments

The concentrations of nifurtimox, M-6, and M-4 analyzed this study were same as the concentrations in Study PH-13501 and covers the expected Cmax values in humans. The findings from this in vitro

study show nifurtimox, M-6, or M-4 did not inhibit OAT1, OAT3, or OCT2 transporters up to concentrations 20 mcM, 30 mcM, or 5 mcM, respectively.

18.4.2.4. Other In Vitro Study

Table 97. Study PH-39990

Title	Nifurtimox: Investigations on Binding to Plasma Proteins in Different Species and Determination of the Blood/Plasma Partitioning of Nifurtimox In Vitro
Methods	
Systems	• Protein binding: Equilibrium dialysis with Williams E Medium +10% Fetal calf serum (FCS) • Partitioning between Blood Cells and Plasma: Heparinized fresh blood
Methods	• Protein binding of nifurtimox was investigated in plasma of mouse, rat, dog, monkey, and man over the concentration range of 90.8 - 9528 mcg/L (100 - 10000 mcg/L) for [^{3}H]Nifurtimox (Radiolabeled Test Item). • The blood to plasma concentration ratios (Cb/Cp) were investigated for rat (male), dog (female), and man (male) at one concentration in the range between 981 and 1026 mcg/L (nominal concentration was 1000 mcg/L) [^{3}H]Nifurtimox. • Incubations were performed at 37°C for 4 h. • The radioactivity was determined by liquid scintillation counting.
Results	

Study results are summarized in the table below:

Overview of plasma protein binding and blood to plasma concentration ratio in different species

Species	Concentration (range) for protein binding assay [µg-eq/L]	f_u [%]	C_b/C_p
Human (m)	93.0 - 8648	57.6	0.857
Wistar rat (m)	100 - 6910	62.1	1.10
Beagle dog (f)	92.1 - 8856	57.1	0.810
NMRI mouse (m)	90.8 - 6330	71.7	
Cynomolgus monkey (f)	95.9 - 9528	62.3	
Williams Medium + 10% FCS	106 - 10143	89.7	

Abbreviations: Cb/Cp, blood to plasma concentration ratio; f, female; Fu, fraction unbound; m, male

18.4.2.5. Pharmacokinetics in Healthy Adults

All Phase 1 PK studies enrolled adult Chagas patients instead of healthy adult subjects given the known risk profile of nifurtimox. All single dose clinical pharmacology studies were conducted in adult male and female Chagas patients, to whom further treatment was offered after the study, if medically indicated.

18.4.3. Pharmacokinetics Assessments in Chagas Patients

18.4.3.1. Study 16004

Overview

This was an open-label, randomized, single-dose cross-over study that assessed and compared bioavailability (BA) of nifurtimox with a single 120 mg nifurtimox dose administered as four 30-mg tablets and in a form of aqueous slurry prepared from four 30-mg tablets (Group 1). The study also assessed and compared BA of nifurtimox between the 120-mg tablet and four 30-mg tablets (Group 2). Both groups enrolled adult male and female patients with chronic Chagas disease and the assigned treatments were administered following high calorie-high fat meal. In total, 37 patients were randomized with 13 in Group 1 and 24 in Group 2. One patient who did not receive any study drug withdrew from the study and remaining 36 patients completed the study.

<u>Group 1</u>

This group enrolled 12 white female patients. The mean age of enrolled patients was 34 years (Range: 22 to 42 years). The enrolled patients received the following two treatments as a single dose under fed conditions (high fat, high calorie meal) in a cross-over manner:

- Treatment A: 4 x 30 mg nifurtimox tablets
- Treatment B: 4 x 30 mg nifurtimox tablets as aqueous slurry

The patients remained at the study center until 24 hours after receiving the assigned treatment. There was a washout period of ≥5 days and up to 10 days between dosing in both groups. For PK assessments, 16 blood samples were collected between predose to 24 hours postdose and nifurtimox concentrations in plasma were measured using validated LC-MS/MS methods. Urine was also collected over the 24-hour period postdose to estimate renal excretion. The reported mean plasma concentration-time profiles for both the treatment groups are presented in Figure 19. Nifurtimox plasma concentrations were subjected to noncompartmental analysis to derive PK parameter estimates, which are reported and compared (for BA assessments) in Table 98 and Table 99, respectively.

Figure 19. Geometric Mean (SD) Concentrations of Nifurtimox in Plasma, Group 1, Study 16004

Source: Study Report
A: linear scale, B: semilogarithmic scale in Group 1 (PK analysis set, N=12)
Abbreviations: SD, standard deviation

Table 98. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates, Group 1, Study 16004

Parameters	Treatment A	Treatment B
$AUC_{0\text{-t.last}}$ (mcg·h/L)	2670 (19.2)	2490 (23.5)
C_{max} (mcg/L)	568 (26.4)	434 (31.2)
T_{max}^{a}(h)	4 [2-6]	4 [2-8]
$T_{1/2}$ (h)	3.3 (11.6)	3.61 (37.3)

Source: Study Report
Treatment A: 4x30-mg tablets (n=12)
Treatment B: 4x30 mg aqueous slurry (n=12)
[a] Median [Range]
Abbreviations: Aq, aqueous; CV, coefficient of variation; h, hours

Table 99. Summary of the Bioavailability Assessment, Group 1, 4x30 mg Aqueous Slurry/4x30-mg Tablets, Study 16004

Parameters	% Point Estimates (90% CI)
$AUC_{0\text{-t.last}}$ (mcg·h/L)	93 (84–103)
C_{max} (mcg/L)	77 (69–85)

Source: Study Report
Abbreviations: CI, confidence interval

Group 2

This group enrolled 24 white patients including 16 female patients (67%). The mean age of enrolled subjects was 32 years (Range: 18 to 45 years). The enrolled subjects received the following two treatments as a single dose under fed conditions (high fat, high calorie) in a cross-over manner:

- Treatment A: 4x30 mg nifurtimox tablets
- Treatment B: 1x120 mg nifurtimox tablet

The patients remained at the study center until 24 hours after receiving the assigned treatment. There was a washout period of ≥5 days and up to 10 days between dosing in both

groups. For PK assessments, 16 blood samples were collected between predose to 24 hours postdose and nifurtimox concentrations in plasma were measured using validated LC-MS/MS methods. Urine was also collected over the 24-hour period postdose to estimate renal excretion. The reported mean plasma concentration-time profiles for both the treatment groups are presented in Figure 20. Nifurtimox plasma concentrations were subjected to noncompartmental analysis to derive PK parameter estimates, which are reported and compared (for BA assessments) in Table 100 and Table 101, respectively.

Figure 20. Geometric Mean (SD) Concentrations of Nifurtimox in Plasma, Group 2, Study 16004

Source: Study Report
A: linear scale, B: semilogarithmic scale in Group 2 (PK analysis set, N=24)
Abbreviations: PK, pharmacokinetic; SD, standard deviation

Table 100. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates, Group 2, Study 16004

Parameters	Treatment A	Treatment B
$AUC_{0\text{-}t.last}$ (mcg·h/L)	2560 (26.3)	2450 (25.6)
C_{max} (mcg/L)	518 (40.2)	509 (37.9)
T_{max}^{a} (h)	4 [2-6]	4 [2-6]
$T_{1/2}$ (h)	2.63 (23.1)	2.85 (30.2)

Source: Study Report
Treatment A: 4x30-mg tablets (n=24)
Treatment B: 1x120-mg tablet (n=24)
[a] Median [Range]
Abbreviations: CV, coefficient of variation; h, hours

Table 101. Summary of the Bioavailability Assessment, Group 2, 4x30-mg Tablets/1x120-mg Tablet, Study 16004

Parameters	% Point Estimates (90% CI)
$AUC_{0\text{-}t.last}$ (mcg·h/L)	105 (99–111)
C_{max} (mcg/L)	102 (89–116)

Source: Study Report
Abbreviations: CI, confidence interval

Conclusions

- The 30-mg tablet formulation was bioequivalent to the 120-mg tablet formulation under fed conditions in adult male and female patients with chronic Chagas disease.
- Systemic exposure to nifurtimox following administration of a single 120-mg dose in a form of aqueous slurry (4 x 30-mg tablets disintegrated in tap water) was comparable to systemic exposure following administration of four whole 30-mg tablets.
- PK data were not available at the time of this report to describe the renal excretion of nifurtimox. These will be provided in an amended report after completion of the assay validation.

Reviewer Comments: *The reported point estimates (90% CI range) following administration as slurry versus tablet for $AUC_{0\text{-}t.last}$ and C_{max} are 0.93 (0.84-1.03) and 0.77 (0.69-0.85), respectively. To evaluate the potential impact of the observed relatively lower BA of nifurtimox from slurry preparation on efficacy, an information request was sent to the Applicant for patients' listings who received nifurtimox treatment with tablets and who received treatment with slurry in Study 16027 (CHICO), which is the pivotal efficacy study for this application. The Applicant's response (Date: February 7, 2020) noted that Study 16027 (CHICO) did not record the information on which patients received nifurtimox treatment with tablets and which patients received treatment with slurry.*

It is reasonable to assume that younger patients (<6 years of age) enrolled in Study 16027 may have received nifurtimox treatment as a slurry. The estimated variability of post hoc PK parameters for the younger (<6 years of age) and older patient (6-18 years of age) subgroups ranged between 42-49%. Considering these findings, the observed differences in BA of nifurtimox, i.e., approximately 7% lower AUC and 23% lower C_{max}, from slurry preparation compared to tablets, would be clinically insignificant in multiple dosing settings.

18.4.3.2. Study 16005

Overview

This was a nonblinded, randomized, single center, single dose, cross-over study that assessed the effect of a high calorie/high fat meal on the nifurtimox PK and BA from four 30-mg tablets in adult male and female patients with chronic Chagas disease. In total, 36 patients were enrolled in this study and 35 patients completed study. One patient who discontinued received the first dose of study drug under fed conditions and subsequently withdrew consent. All the enrolled patients were white including 32 (88.9%) female patients. The mean age of enrolled subjects was 34 years (Range: 26 to 45 years). The enrolled patients were randomized to one of two treatment sequences:

- Treatment A: 4x30 mg nifurtimox tablets under fasted conditions

- Treatment B: 4x30 mg nifurtimox tablets under fed conditions (high calorie-high fat breakfast)

The high calorie, high fat meal contained 800 to 1000 calories (approximately 500 to 600 kcal from fat, 150 kcal from protein, and 250 kcal from carbohydrate). The subjects remained at the study center until 24 hours after receiving the assigned treatment. There was a washout period of ≥5 days between treatments. For PK assessments, 16 blood samples were collected between predose to 24 hours postdose and nifurtimox concentrations in plasma were measured using validated LC-MS/MS methods. Urine was also collected over the 24-hour period following dosing to estimate renal excretion. The reported mean plasma concentration-time profiles on linear and semilogarithmic scales are presented for both the treatment groups in Figure 21. Nifurtimox plasma concentrations were subjected to noncompartmental analysis to derive PK parameter estimates. The resultant estimates are reported and compared (for BA assessments) in Table and Table 103, respectively.

Figure 21. Geometric Mean (SD) Concentrations of Nifurtimox in Plasma, Study 16005

Source: Study Report
A: linear scale, B: semilogarithmic scale (N=35)
Abbreviations: SD, standard deviation

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Table 102. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates, Study 16005

Parameter	Treatment A	Treatment B
$AUC_{0\text{-}t.last}$ (mcg·h/L)	1390 (40.6)	2390 (21.7)
C_{max} (mcg/L)	277 (36.7)	465 (33.4)
T_{max}^{a}(h)	3 [0.5-6]	4 [1-8]
$T_{1/2}$ (h)	3.07 (34.6)	3.13 (27.4)

Source: Study Report
Treatment A: 4x30-mg tablets under fasted conditions (n=35).
Treatment B: 4x30-mg tablets under fed conditions (n=35).
[a] Median [Range]
Abbreviations: CV, coefficient of variation; h, hour(s)

Table 103. Summary of the Bioavailability Assessment, 4x30-mg Tablets Under Fed Conditions/Fasted Conditions, Study 16005

Parameters	% Point Estimates (90% CI)
$AUC_{0\text{-}t.last}$ (mcg·h/L)	172 (154–192)
C_{max} (mcg/L)	168 (150–187)

Source: Study Report

Conclusions

- Systemic exposure to nifurtimox increased when a single oral dose of 120 mg nifurtimox (4x30-mg tablets) was administered under fed conditions (high calorie, high fat meal) compared with fasted conditions: AUC, $AUC_{0\text{-}tlast}$, and C_{max} were increased by 71%, 72%, and 68%, respectively, under fed conditions.
- Food slightly increased the time to reach peak nifurtimox plasma concentrations, with a median t_{max} of 4 hours (fed) versus 3 hours (fasted), suggesting a mildly reduced rate of absorption under fed conditions.
- A single oral dose of 120 mg nifurtimox was well tolerated and there were no new safety or tolerability findings. Two cases of QTc increase >60 ms that occurred 1.5-2.5 hours after dosing, which were judged to be without clinical relevance by the investigator.

18.4.3.3. Study 16007

Overview

This was an open-label, randomized, single-dose, cross-over study that evaluated the relative BA, safety, and tolerability of single doses of three nifurtimox 30-mg tablet formulations with different in vitro dissolution rates (Group 1). The study also evaluated the relative BA of nifurtimox 30 mg and 120-mg tablets (Group 2).

Both groups enrolled 48 adult male and female white patients with chronic Chagas disease (36 in Group 1 and 12 in Group 2) and all patients completed the study. Assigned treatments were administered under fed conditions.

Group 1: The 36 patients enrolled in this group included 29 (80.6%) female patients. The mean age of enrolled patients was 31 years (Range: 19 to 43 years). The enrolled patients received the following three treatments as a single dose under fed conditions (after a high calorie, high fat breakfast) in a crossover manner:

- Treatment A: 4 x 30-mg tablets with fast in vitro dissolution rate
- Treatment B: 4 x 30-mg tablets with medium in vitro dissolution rate
- Treatment C: 4 x 30-mg tablets with slow in vitro dissolution rate

The patients remained at the study center until approximately 24 hours after receiving the assigned treatments. There was a washout period of ≥5 days. For PK assessments, 14 blood

samples were collected between predose to 15 hours postdose and nifurtimox concentrations in plasma were measured using validated LC-MS/MS methods. The reported mean plasma concentration-time profiles on linear and semilogarithmic scales are presented for all treatment groups in Figure 22. Nifurtimox plasma concentrations were subjected to noncompartmental analysis to derive PK parameter estimates, which are reported and compared (for BA assessments) in Table 104 and Table 105, respectively.

Figure 22. Geometric Mean (SD) Concentrations of Nifurtimox in Plasma, Study 16007

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Source: Study Report
A: linear scale, B: semilogarithmic scale in Group 1 (PK analysis set, N=36)
Abbreviations: PK, pharmacokinetic; SD, standard deviation

Table 104. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates, Study 16007

Parameter	Treatment A	Treatment B	Treatment C
$AUC_{0\text{-}t.last}$ (mcg·h/L)	1844 (26)	1738 (28)	1676 (32)
C_{max} (mcg/L)	372 (31)	358.5/44.7	355 (43)
T_{max}^{a} (h)	3 (2-6)	4 (0.75-6)	4 (1.5-8)
$T_{1/2}$ (h)	2.7 (27)	2.61/23.95	2.8 (34)

Source: Study Report
Treatment A: 4x30-mg tablets with fast in vitro dissolution (n=36).
Treatment B: 4x30-mg tablets with medium in vitro dissolution (n=36).
Treatment C: 4x30-mg tablets with slow in vitro dissolution (n=36).
[a] Median [Range]
Abbreviations: CV, coefficient of variation; h, hours

Table 105. Summary of the Bioavailability Assessment, Study 16007

Parameters	Treatment A	Treatment B
$AUC_{0\text{-}t.last}$ (mcg·h/L)	110 (102–118)	103 (97–111)
C_{max} (mcg/L)	105 (93–118)	101 (89–114)

Source: Reviewer's analysis
All values are expressed as % point estimate (90% confidence interval).
Treatment A: 4x30-mg tablets, fast dissolution/slow dissolution
Treatment B: 4x30-mg tablets, medium dissolution/slow dissolution

Group 2

This group enrolled 12 white patients including 8 female patients (67%). The mean age of enrolled patients was 35 years (Range: 28 to 45 years). The enrolled patients received the

following two treatments as a single dose under fed conditions (after a high calorie, high fat breakfast) in a crossover manner:

- Treatment A: 1x30-mg tablet (medium in vitro dissolution)
- Treatment B: 1x120-mg tablet

The patients remained at the study center until 24 hours after receiving the assigned treatment. There was a washout period of ≥5 days between dosing in both groups. For PK assessments, 14 blood samples were collected between predose to 24 hours postdose and nifurtimox concentrations in plasma were measured using validated LC-MS/MS methods. The reported mean plasma concentration-time profiles on linear and semilogarithmic scales are presented for both the treatment groups in Figure 23. Nifurtimox plasma concentrations were subjected to noncompartmental analysis to derive PK parameter estimates. The resultant estimates are reported and compared (for BA assessments) in Table 106 and Table 107, respectively.

Figure 23. Geometric Mean (SD) Concentrations of Nifurtimox in Plasma, Group 2, PK Analysis Set, Study 16007

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Source: Study Report
A: linear scale, B: semilogarithmic scale (N=12)
Abbreviations: PK, pharmacokinetic; SD, standard deviation

Table 106. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates, Group 2, Study 16007

Parameter	Treatment A	Treatment B
$AUC_{0\text{-}t.last}$ (mcg·h/L)	351 (40)	1842 (32)
C_{max} (mcg/L)	93 (49)	425 (50)
T_{max}^{a} (h)	4 [2-6]	3 [2-6]
$T_{1/2}$ (h)	2.38 (25)	2.42 (32)

Source: Study Report
Treatment A: 1x30 mg tablets (n=12)
Treatment B: 1x120-mg tablet (n=12)
[a] Median [Range]
Abbreviations: CV, coefficient of variation; h, hours

Table 107. Summary of the Bioavailability Assessment, Group 2, 1x120-mg Tablet/4x30-mg Tablet, Study 16007

Dose Normalized Parameters	% Point Estimates (90% CI)
$AUC_{0\text{-}t.last}$_D (mcg·h/L)	131 (112–154)
C_{max}_D (mcg/L)	114 (92–141)

Source: Study Report
Abbreviations: CI, confidence interval

Conclusions

- In Group 1, across all the formulations, the geometric mean values of AUC, $AUC_{(0\text{-tlast})}$ and C_{max} were similar or at the most marginally increased. Therefore, BA of nifurtimox can be considered comparable when comparing single oral doses of 120 mg nifurtimox administered as 4 x 30-mg tablets with fast (Treatment A), medium (Treatment B), and slow (Treatment C) in vitro dissolution rates.
- In Group 2, the upper limit of point estimate's 90% CI for $AUC_{(0\text{-tlast})_D}$ exceeded 1.25 and the range of 90% CI excluded 1. The Applicant attributes this observation to the greater number of concentration time points measurable during absorption and terminal phase for the 120-mg dose. For C_{max_D}, the range of 90% CI for point estimate included 1 and the upper limit of point estimate's 90% CI range exceeded 1.25. The latter is attributed to the high variability of absorption which is leading to a very high geometric SD of the plasma concentration time courses during the first 4 to 6 h after drug administration and the highly variable time to reach peak concentrations of 2 to 6 h in combination with the paucity of quantifiable drug concentrations up to 2 h after dosing for the 30 mg single dose treatment. Thus, although the extent of absorption expressed as C_{max_D} appeared slightly increased for the 120-mg tablet compared to the 30-mg tablet, this result does not conflict with the assumption of dose proportionality.

Reviewer Comments: *The conclusions drawn by the Applicant based on the PK findings from Group 1 are reasonable, including that the systemic exposure to nifurtimox was comparable from all the formulations with varying degrees of dissolution rates evaluated in this study. However, the data showed a trend of faster dissolution rates associated with slightly higher C_{max} and AUC estimates (Figure 22 and Table 104).*

Study 16027, the pivotal efficacy study, used the 30-mg tablet batches with varying degrees of dissolution rates. The dissolution profile range of the fast and slow formulation used in Group 1 of this study encompasses the dissolution profile range of batches used in Study 16027 (Figure below) and findings from this study show that the three batches have comparable concentration-time profiles and comparable BA.

Figure 24. Dissolution Profiles of Nifurtimox 30-mg Tablet Batches Used in Study 16027 (Mean of n=6) and Dissolution Profiles of the Fast (KM601B0) and Slow (KM60192) 30 mg Batches Used in Study 16007 (Mean of n=12)

The Applicant's conclusion that PK results from Group 2 do not conflict with the assumption of dose proportionality is not reasonable. This study evaluated only two dose levels and for these two dose levels, dose normalized C_{max} and AUC estimates for 120-mg tablet were 14% and 31% higher, respectively, compared to 30-mg tablet. Also, the lower bound of 90% CI for point estimate for AUC was 1.12, i.e., 90% CI range excludes 1. However, it is noteworthy that Study 16004 has demonstrated that the 4×30 mg nifurtimox oral tablets were bioequivalent to the marketed 120 mg nifurtimox oral tablet, under fed conditions in 24 adult patients with Chagas disease.

18.4.3.4. Study 19500

Overview

This was an open-label, randomized, single dose, cross-over study that assessed bioequivalence between current formulation of 120 mg nifurtimox tablet and a newly developed 120 mg nifurtimox tablet formulation. Both the treatments were administered under fed conditions to adult male and female patients with chronic Chagas disease. In total, 24 patients were randomized and 23 patients completed study. One patient who discontinued received the first treatment with new formulation under fed conditions and subsequently withdrew consent. All enrolled patients were white and 21 (87.5%) patients were female. The mean age of enrolled subjects was 35 years (Range: 23 to 44 years). The enrolled patients received following treatments in a crossover manner:

- Treatment A: 1 x 120 mg new formulation tablet
- Treatment B: 1 x 120 mg current formulation tablet

Patients received treatments following a high-calorie, high-fat breakfast. The patients remained at the study center approximately for 24 hours post-treatment. There was a washout period of ≥5 days between treatments. For PK assessments, 14 blood samples were collected between predose to 15 hours postdose and nifurtimox concentrations in plasma were measured using validated LC-MS/MS methods. The reported mean plasma concentration-time profiles on linear and semilogarithmic scales are presented for both the treatment groups in Figure 25. Nifurtimox plasma concentrations were subjected to noncompartmental analysis to derive PK parameter estimates. The resultant estimates are reported and compared (for BA assessments) in Table 108 and Table 109, respectively.

Figure 25. Geometric Mean (SD) Concentrations of Nifurtimox in Plasma, Study 19500

A: linear scale, B: semilogarithmic scale

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Table 108. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates, Study 19500

Parameter	Treatment A	Treatment B
$AUC_{0\text{-}t.last}$ (mcg·h/L)	2080 (24)	2140 (21)
C_{max} (mcg/L)	402 (53)	455 (31)
T_{max}^{a} (h)	3 (1.5-8)	4 (2-8)
$T_{1/2}$ (h)	3.38 (39)	3.09 (31)

Source: Study Report
Treatment A: 120 mg nifurtimox, new formulation (n=24)
Treatment B: 120 mg nifurtimox, current formulation (n=23)
[a] Median [Range]
Abbreviations: CV, coefficient of variation; h, hours

Table 109. Summary of the Bioavailability Assessment, 120-mg Tablet Formulations (New/Current), Study 19500

Parameters	% Point Estimates (90% CI)
$AUC_{0\text{-}t.last}$ (mcg·h/L)	98 (91–105)
C_{max} (mcg/L)	87 (73–103)

Source: Study Report

Conclusions

- Nifurtimox BA following a single 120-mg dose with the new nifurtimox formulation was found similar to the current formulation with respect to $AUC_{0\text{-}tlast}$ estimates.
- Bioequivalence between new and current formulation could not be established for C_{max} which was 13.16% lower for the new formulation. The lower limit of the 90% CI (0.73-1.03) fell below the lower border of the acceptance interval for bioequivalence of 0.8 to 1.25. High intra-individual variability of C_{max} (CV =35%) has to be taken into account which clearly exceeded the anticipated CV of 18% in the sample size and power calculation.

Reviewer Comments: *The new 120 mg nifurtimox tablet formulation that was used in this study is deemed to be outside of the scope of this NDA submission by the Applicant. However, the PK findings for the current formulation were used from this study in the cross-study comparison discussed in Section 6.3.1 of this review.*

18.4.3.5. Study 40959

Overview

This study evaluated nifurtimox's (BAYA002502) metabolites in human plasma samples pooled from six Chagas disease patients receiving a single dose of 120 mg nifurtimox in Study 16007. This study used a nonvalidated bioanalytical method using liquid chromatography-tandem high resolution mass spectrometry. Therefore, the assessments conducted in this study are considered exploratory assessments.

The concentrations of nifurtimox and metabolites M-1, M-2, M-3, M-4, M-5, M-6 in plasma samples were calculated based on calibration curves in blank plasma spiked with the respective synthetic reference standards. The calibration is based on the relative peak area as a function of

concentration, which is obtained by linear regression with 1/y weighting for nifurtimox, M-2, M-3, M-4, M-5, and M-6 and a 1/x2 weighting for M-1. The quantification was performed on a mass basis [ng/mL] or [mcg/L], but the final calculations for the exposure in plasma were done on a molar basis. Using analytes' concentrations, PK parameters were determined for each analyte. Concentrations and PK parameter estimates are presented in Figure 26 and Table 110 below, respectively.

Figure 26. Plasma Concentrations Vs. Time Profiles for Nifurtimox and Metabolites in Pooled Plasma Samples of Six Patients Following 120 mg Nifurtimox Dose, Study No. 16007

Source: Study Report

Table 110. Pharmacokinetic Parameters Estimates for Nifurtimox and Metabolites in Pooled Plasma Samples of Six Patients Following 120 mg Nifurtimox Dose, Study 16007

Parameter	Unit	Nifurtimox [nmol/L]	M-1 [nmol/L]	M-2 [nmol/L]	M-3 [nmol/L]	M-4 [nmol/L]	M-5 [nmol/L]	M-6 [nmol/L]
AUC	nmol·h/L	8680	913	691	616	4550	256	35600
AUC	**%**	**16.9**	**1.78**	**1.35**	**1.20**	**8.87**	**0.499**	**69.4**
AUC(0-t_{last})	nmol·h/L	8630	844	637	313	2350	226	27800
t_{last}	h	24.0	15.0	15.0	24.0	24.0	15.0	24.0
AUC(0-24h)	nmol·h/L	8630	n.c.	n.c.	313	2350	n.c.	27800
%AUC(0-t_1)	%	0.1	1.9	0.6	1.1	0.5	2.8	0.0
%AUC(t_{last}-inf)	%	0.6	7.6	7.8	49.2	48.4	11.7	21.8
C_{max}	nmol/L	1730	113	85.4	28.4	207	30.0	2250
t_{max}	h	3.00	4.00	6.00	4.00	4.00	4.00	4.00
$t_{½}$	h	3.14	3.16	3.30	28.9	27.5	3.80	10.1
RPoints		6	4	3	3	3	4	4
RRange	h	4.00-24.0	6.00-15.0	8.00-15.0	12.0-24.0	12.0-24.0	6.00-15.0	8.00-24.0

Source: Adapted from Study Report

Conclusions

Besides nifurtimox there were two metabolites M-6 and M-4 with significant exposure.

Reviewer Comments: *As the Applicant concluded, this exploratory assessment showed that two metabolites of nifurtimox: M-6 and M-4 have significant systemic exposure. In addition, the elimination half-lives for M-6 and M-4 are longer than that for nifurtimox (Table 110). Both of these metabolites are inactive from an efficacy perspective; however, it is not clear to this Reviewer how much safety information is available for M-4 and M-6. Therefore, it will be recommended to the Applicant that the elimination half-lives for both M-6 and M-4 be included in the labeling under section 12.3.*

18.4.3.6. Study 40569

Overview

This study evaluated nifurtimox's (BAYA002502) metabolites in human urine samples from 6 Chagas disease patients receiving a single peroral dose of 120 mg nifurtimox in Study 16005. This study used a nonvalidated bioanalytical method using liquid chromatography-tandem high resolution mass spectrometry. Therefore, the assessments conducted in this study are considered exploratory assessments.

In this study, the concentrations of BAYA002502 and metabolites M-1, M-2, M-3, M-4, M-5, M-6 in urine samples were calculated based on calibration curves in blank urine spiked with the respective standards. The calibration curves were based on the relative peak area as a function of concentration, which is obtained by linear regression with 1/y2 weighting for BAYA002502, M-2, and M-5, a 1/x weighting for M-1, M-3 and M-6, and equal weighting for M-4. The quantification was performed on a mass basis [mcg/mL], but the final calculations for the percentage of dose excreted in urine were done on a molar basis using the administered dose of 120 mg nifurtimox as 418 mcmol. The findings are summarized in Table 111 below.

Table 111. Relative Excreted Amounts of Nifurtimox (BAYA002502) and Its Metabolites in Human Urine Collected Over 24 Hours Following Single Dose of 120 mg[a] Nifurtimox in Six Patients in Fasted (Treatment A) and Fed (Treatment B) State

Patient	BAYA002502 [%] of dose	M-1 [%] of dose	M-2 [%] of dose	M-3 [%] of dose	M-4 [%] of dose	M-5 [%] of dose	M-6 [%] of dose	Total amount[b)] $\%A_{E,ur}$(0-24h) [%] of dose
Pat 25, treatment A	0.0499	5.84	0.633	n.c.	3.90	2.37	15.2	**28.0**
Pat 25, treatment B	0.117	2.48	3.42	0.313	16.0	8.57	25.0	**55.9**
Pat 26, treatment A	n.c.	1.49	0.754	n.c.	4.47	2.97	9.94	**19.6**
Pat 26, treatment B	0.0993	1.78	2.29	n.c.	13.1	8.20	21.4	**46.9**
Pat 27, treatment A	0.0535	1.32	1.62	0.187	7.82	4.34	8.54	**23.9**
Pat 27, treatment B	n.c.	1.42	1.87	n.c.	12.2	5.27	22.8	**43.6**
Pat 28, treatment A	n.c.	2.56	0.745	n.c.	4.53	2.31	10.1	**20.3**
Pat 28, treatment B	0.0834	2.13	2.35	0.305	11.4	4.78	17.3	**38.3**
Pat 29, treatment A	0.0467	3.97	1.26	0.173	6.47	4.45	15.6	**31.9**
Pat 29, treatment B	0.0533	2.23	2.39	0.147	9.06	6.47	16.6	**37.0**
Pat 30, treatment A	n.c.	4.18	1.24	n.c.	8.25	4.03	23.0	**40.7**
Pat 30, treatment B	0.0947	2.08	1.85	0.127	11.9	6.24	21.4	**43.7**
Mean, treatment A	0.0250	3.23	1.04	0.0600	5.91	3.41	13.7	**27.4**
Mean, treatment B	0.0746	2.02	2.36	0.149	12.3	6.59	20.8	**44.2**

a) = 120 mg is equivalent to 418 µmol
b) = Total amount = sum of nifurtimox, M-1, M-2, M-3, M-4, M-5, and M-6

Source: Study Report

Conclusions

- In a few samples, degradation of internal standard (stable labeled nifurtimox) was observed compared to other samples. However, this process was slow and had no influence in the time frame the individual urine fractions were analyzed. Therefore, the results of quantification were not biased through this instability.
- To further understand this instability, in a predose (0 h) urine it was elucidated, that nifurtimox was degraded to M-1, identified by the detection and structure elucidation of the corresponding stable labeled M-1.
- This was further confirmed by incubation of radiolabeled nifurtimox at 37°C. These results are reported elsewhere (Report No. PH-40569 Version 2).
- This could explain, at least in part, the low excretion (<0.5%) of unchanged nifurtimox. Based on glomerular filtration rate, fraction unbound, and AUC, an unchanged excretion of close to 10% of dose would be expected.
- Overall, on average about 44% of the dose in human urine could be attributed to nifurtimox and six metabolites when administered with food.

Reviewer Comments: *Report No. PH-40569 Version 2 could not be located within the NDA submission. However, based on the reported findings in Version 1, the Applicant's conclusion that on average about 44% of the dose in human urine could be attributed to nifurtimox and six metabolites when administered with food is reasonable.*

18.4.4. Pharmacometrics Review

18.4.4.1. Results of Applicant's Analysis

Population Pharmacokinetic Modeling Review

The Applicant submitted Population Pharmacokinetic (PPK) Report 16018 to support the approval of nifurtimox in patients with Chagas disease. The studies assessed in this PPK report are shown in Table 112.

Table 112. Studies Included in PPK Report 16018

Study ID	Study Design	Planned Number of Subjects	Planned Number of Samples / Subject	Dose/Treatment Schedule	Application
BP-16004	Open-label, randomized, single-dose cross-over study to assess bioequivalence between single 120 mg nifurtimox tablet and four 30 mg nifurtimox tablets	36	15	120 mg or 4x30 mg administered as single dose	Referenced preliminary model and present model development
BP-16005	Non-blinded, randomized, single center, single dose, cross-over study to assess the effect of a high calorie/high fat meal on the pharmacokinetics of four 30 mg tablets	36	15	4x30 mg administered as single dose	Referenced preliminary model and present model development
BP-16007	Open-label, randomized, single-dose, cross-over phase I study to evaluate the relative bioavailability, safety, and tolerability of single doses of nifurtimox 30 mg tablets exhibiting different in vitro dissolution characteristics, and to evaluate the relative bioavailability of nifurtimox 30 mg and 120 mg tablets	48	15	30-120 mg	Present model development
BP-16027	Prospective, historically controlled, randomized (to dosing regimen), age-stratified, double-blind, parallel-group study to evaluate the efficacy and safety of a new pediatric formulation of nifurtimox in children aged 0 to 17 years with Chagas disease	390	5 (sparse)	Dosing based on age-bodyweight combination 15-300 mg TID	Pediatric PK assessment

Studies BP-16004, BP-16005, and BP-16007 were healthy volunteer studies conducted in adults while Study BP-16027 was conducted in the target pediatric patient population. Initially, PK

data from the three adult studies were used to generate PPK Model 446, with the intent that it would be extended to the pediatric PK data after finalization. However, Model 446 was not able to adequately describe the pediatric PK data; notably, the trough concentrations were overpredicted and the maximum concentration (C_{max}) values were improperly fit. Thus, the Applicant used only pediatric data (Study BP-16027) to generate PPK Model 620. The structures of both models are shown in Figure 27.

Figure 27. Structures for Applicant PPK Models 446 and 620

Model 446

Model 620

Abbreviations: CL, clearance; FR, fraction absorbed through indicated transit compartment; k_a, absorption rate constant, k_{xy}, rate constant of distribution from compartment x to compartment y; V, volume of distr bution in indicated compartment

Reviewer Comments: *The pharmacometrics review team focused their assessment on only the pediatric PK data and model. The pediatric PK data are the most relevant because nifurtimox is only being proposed for pediatric patients in this application. There is no need for comparison of PK exposure to adults because efficacy studies were completed in pediatric but not adult patients. Additionally, there is the possibility that clearance mechanisms may be different between pediatric and adult patients. Nifurtimox is in part cleared by microbiota in the gastrointestinal (GI) tract. Changes in the microbiome, length of the GI tract, and GI transit time could all account for differences in adult and pediatric PK.*

Parameter estimates for the Applicant's final pediatric Model 620 are shown in Table 113. The associated estimates of shrinkage were 45.1% and 33.2% for ETA1 (CL/F) and ETA2 (V1/F), respectively.

Table 113. Parameter Estimates of Applicant's Final Pediatric PPK Model 620

Parameter	Alias	Estimate	Relative SE (%)	95% CI
θ_1	Apparent systemic clearance ($L{\cdot}h^{-1}$)	112.0	6.3	(98 - 126)
θ_2	Apparent central volume of distribution (L)	191.0	13.7	(139 - 242)
θ_3	Absorption rate constant (h^{-1})	0.252	7.3	(0.216 - 0.288)
θ_5	Allometric scaling for weight on (CL/F ($L{\cdot}h^{-1}$))	0.750	.....	NC
θ_6	Allometric scaling for weight on (V1/F (L))	1.00	.....	NC
θ_7	Age effect on clearance	-0.439	21.1	(-0.621 - -0.257)
$\omega_{1.1}$	$\omega^2_{CL/F}$	0.101	42.3	(0.0173 - 0.184)
$\omega_{2.2}$	$\omega^2_{V1/F}$	0.564	31.0	(0.221 - 0.906)
θ_4	σ_{prop}	0.828	5.6	(0.737 - 0.92)

The age effect on clearance was defined as a proportional multiplier on patients younger than 2 yr of age relative to patients greater than or equal to 2 yr.
Abbreviations: CL/F: apparent systemic clearance; V_1/F: apparent central volume of distr bution, ω_X: variance of the interindividual variability of parameter X. σ_{prop}: proportional residual error.

Goodness-of-fit (GOF) plots and a prediction-corrected visual predictive check (VPC) are shown in Figure 28 and Figure 29, respectfully.

Figure 28. Goodness-of-Fit Plots for PPK Model 620

CWRES: Conditional Weighted Residuals. The red line represents the trend of the data relative to the line of unity (black line).

Figure 29. Prediction-Corrected Visual Predictive Check for Model 620

Source: Study R-12597 Report
Circles: Observations, Solid Blue Line: Median of the observed nifurtimox concentrations, Dashed Lines: 2:5th and 97:5th percentiles of the observed nifurtimox concentrations, Shaded Area: The shaded areas indicate the 95% CI around the prediction-corrected median (green area), and 2:5th and 97:5th percentiles of the simulated concentrations (grey areas).

Reviewer Comments: *The GOF and the VPC plots both show potential misspecification of the model. There appears to be some underprediction of the observed estimates. This is evident in the individual prediction versus observed concentrations panel in the GOF plot and around the* t_{max} *in the VPC plot. Additionally, the GOF plots may not be informative due to high shrinkage (30-50%) on clearance and volume.*

18.4.4.2. Reviewer's Analysis

18.4.4.2.1. Introduction

The pharmacometrics team was concerned about the high shrinkage in Model 620. Thus, they attempted to modify the model to produce lower estimates of shrinkage. In doing so, they also evaluated the covariate relationships in the model, particularly those involving weight.

18.4.4.2.2. Methods

Data Sets

Data sets used are summarized in Table 114.

Table 114. Analysis Data Sets

Study Number	Name	Link to EDR
16015	Analysis of the Population Pharmacokinetics of Nifurtimox in Adult and Pediatric Patients with Chagas' Disease	\\CDSESUB1\evsprod\NDA213464\0001\m5\datasets\16018\analysis\legacy\datasets \\CDSESUB1\evsprod\NDA213464\0011\m5\datasets\16018\analysis\legacy

Software

NONMEM v7.4 was used for population pharmacokinetics modeling. Rv3.6.1 was used for data visualization and statistical analyses.

PPK Modeling

The pharmacometrics review team assessed different methods to improve the Applicant's PPK model. The data was considered both in the normal and log-transformed domain. Covariate relationships were assessed for significance in a backwards selection scheme. The contribution of each covariate was considered in terms of change of objective function value, change in associated ETA, and the graphical relationship between the covariate and the associated ETA.

Exposure-Response Analysis

Exposure-response (E-R) analyses for both serological response (as a surrogate marker of efficacy) and safety was conducted using the Applicant's model predictions. AUC was preferred over C_{max} due to misspecification identified in the PK profiles. E-R analyses were performed in the subset of pediatric patients in Study BP-16027 in whom PK samples were collected, 111 patients. The relationship between AUC and efficacy or safety was assessed using logistic regression. Age and weight were considered as potential covariates in the analysis. Once an E-R relationship was identified, the same relationship (with average daily dose instead of AUC) was assessed in the full dose-response (D-R) dataset.

18.4.4.2.3. Results

PPK Modeling

The pharmacometrics review team revised the Applicant's PPK Model 620 to generate Model 625d. A comparison of the estimated parameters for Models 620 and 625d is shown in Table 115. Model 625d improves upon Model 620 by reducing ETA shrinkage by analyzing the

concentration data in the untransformed domain instead of the log-transformed domain. Additionally, the updated model reassessed the relationships among the PK parameters, weight, and age.

Table 115. Comparison of Parameters in Models 620 and 625d

	Model 620 (Applicant Model)			625d (Revised Model)		
Parameter	**Estimate**	**RSE**	**Shrinkage**	**Estimate**	**RSE**	**Shrinkage**
Fixed effects						
CL (L/h)	112	6.3%		94.5	9.5%	
V (L)	191	13.6%		132	16.6%	
k_a (1/h)	0.252	7.3%		0.299	6.9%	
WT_CL	0.75*			0.723	17.0%	
WT_V	1*			0.66	16.4%	
AGE_CL	-0.439	21.1%		-0.456	35.7%	
Intersubject variability						
CL	0.101	42.2%	45.1%	0.196	26.3%	23.1%
V	0.564	31.0%	33.2%	0.742	23.7%	15.1%
Residual error						
Proportional	0.828	5.7%	8.2%	0.341	9.3%	10.5%
Additive	0*			128	40.9%	10.5%

This table contains parameter estimates for both the applicant's model and the reviewer's revised model as run by the reviewer.
* Fixed to indicated value.
Abbreviations: CL, clearance, k_a: absorption rate constant, RSE, residual standard error; V, volume of distribution; WT, weight, X_Y, effect of covariate X on PK parameter Y

Goodness of Fit

Goodness-of-fit plots for the reviewer's final PPK model for nifurtimox, 625d, are shown in Figure 30. The model generally appears to describe the PK data reasonably well. In the individual predictions versus observed concentrations plot, there is a trend towards underestimation of the true value at the higher extreme of observed concentrations. Otherwise, the conditionally weighted residuals appear to be centered at 0 and homoscedastically dispersed.

Figure 30. NFX Model 625d GOF Plots

The red line represents the trend of the data relative to the line of unity (black line).
Abbreviations: CWRES, conditional weighted residuals, GOF, goodness-of-fit; NFX, nifurtimox

The covariates from the final model were all evaluated by backwards selection. Each covariate was removed to assess whether they significantly affected the PPK model by comparing the change in objective function value (OFV), corresponding intersubject variability (ETA, η) term, and graphical fit of the ETA term and the corresponding covariate. As shown in Table 116, each of the three covariates had a significant impact on the final model. Removal of each increased the OFV by at least 3.84, corresponding to a p-value of 0.05, and increased the corresponding ETA value. Graphically, removal of the weight covariates resulted in a linear trend in the residuals. Based on these results, the covariate relationships were kept in the final model.

Table 116. Assessment of Impact of PPK Model Covariates

Covariate Relationship	Model 625d (Final)	Backwards Selection
Effect of weight on clearance		
	OFV: 5040.379; ηCL: 0.196; Shrinkage: 23.10%	OFV: 5069.333; ΔOFV: 28.954; ηCL: 0.3; ΔηCL: 53%; Shrinkage: 19%
Effect of age (age <2 years) on clearance		
	OFV: 5040.379; ηCL: 0.196; Shrinkage: 23.10%	OFV: 5044.466; ΔOFV: 4.087; ηCL: 0.207; ΔηCL: 6%; Shrinkage: 23%
Effect of weight on volume		
	OFV: 5040.379; ηV: 0.742; Shrinkage: 15.10%	OFV: 5069.827; ΔOFV: 29.448; ηV: 1.04; ΔηV: 40%; Shrinkage: 12%

The first column shows covariate relationship in the final model with the associated graph and parameters. The second column shows the covariate relationship in the final after having the covariate removed (backwards selection) with the associated graph and parameters. OFV: Objective Function Value, ETA1: ETA on CL, ETA2: ETA on V

Visual Predictive Check

A visual predictive check (VPC) of Model 625d with and without stratification by age is shown in Figure 31. The VPC appears to capture the general trends in the PK profile. However, C_{max} and t_{max} do not appear to be well characterized. This is explained partially by the administration of multiple formulations in the pediatric study without a record of which patients received which formulation. These formulations had different absorption profiles, specifically in terms of C_{max},

as discussed further in Section 20.4.3.1 describing the results of Study 16004. Thus, C_{max} estimated by the model may be biased, but AUC estimates are likely acceptable.

Figure 31. VPC for Nifurtimox Model 625d Without (Top) and With (Bottom) Stratification by Age

The black lines represent the median (solid) and 5th and 95th percentiles (dashed) of observed concentrations. Median concentration. The shaded areas represent the 90% prediction interval of the median (blue) and 5th and 95th percentiles (light blue). Abbreviations: AGEg, age group; VPC, visual predictive check

Model Assessment

Overall, the PPK appears to reasonably describe nifurtimox PK in pediatric patients, with the caveat that values of C_{max} may be biased.

18.4.4.2.4. Exposure-Response Analyses

Exposure-Response Analysis for Efficacy

The review team assessed the E-R relationship for serological response based on three assays:

- Recombinant ELISA: Seroconversion in patients <8 months or ≥20% reduction in optical density in patients ≥8 months at 1-year post treatment follow-up
- Total Purified Antigen (TPA) ELISA: Seroconversion in patients <8 months or ≥20% reduction in optical density in patients ≥8 months at 1-year post treatment follow-up
- F29 ELISA: Seroconversion at 1-year post treatment follow-up

Refer to Section 9 for further discussion on the significance of each assay related to Chagas disease.

Steady-state nifurtimox AUC and age were identified as significant predictors of efficacy determined using the recombinant assay by logistic regression ($p<0.05$). With the TPA assay, nifurtimox AUC was not a statistically significant predictor of efficacy after age (which was found to be statistically significant, $p<0.05$) was included in the analysis. For the F29 assay, neither nifurtimox AUC nor age were statistically significant predictors of efficacy. Figure 32 depicts the relationship between nifurtimox AUC and efficacy as determined using all three assays.

Figure 32. Relationship Between Nifurtimox AUC and Serological Response Using the Recombinant (Left), Total Purified Antigen (Middle), and F29 Assay (Right)

The relationship between nifurtimox AUC, age, and serological response using the recombinant assay was further assessed in Figure 33. The slope of the relationship between AUC and response appears to be similar in patients between 8 months (0.656 yr) and 12 yr. Patients younger than 8 months tend to have a higher overall probability of response while patients 12

yr and older tend to have a lower probability of response. Additionally, in patients 12 yr and older nifurtimox AUC appears to have lower impact on response as shown visually by the slope.

Figure 33. Relationship Between Nifurtimox AUC and Serological Response Determined Using the Recombinant Assay Stratified by Age

To further assess trends in the surrogate markers of efficacy, the relationship between nifurtimox dose and response was assessed. In contrast to the E-R relationship, nifurtimox dose and age were significant predictors of serological response determined with both the TPA and recombinant assays. The trend toward increasing response appeared to be generally consistent within each age group. The D-R relationships are shown in Figure 34 and Figure 35.

Figure 34. Relationship Between Nifurtimox Dose and Surrogate Markers for Efficacy Using the Recombinant (Left), Total Purified Antigen (Middle), and F29 Assay (Right)

Figure 35. Relationship Between Nifurtimox Dose and Surrogate Markers for Efficacy Using the Recombinant (Left) and Total Purified Antigen Assay (Right) Stratified by Age

Exposure-Response Analysis for Safety

E-R relationships were assessed for the most common adverse events in the E-R dataset. Based on an initial analysis, further evaluation was performed for the adverse events of abdominal pain.

Nifurtimox AUC was found to be a statistically significant predictor of abdominal pain. Patients younger than 12 yr appear to have no relationship between nifurtimox AUC and abdominal pain in contrast to patients greater than or equal to 12 yr. However, this relationship should be interpreted with caution because only six instances of abdominal pain were present within the E-R dataset.

Figure 36. Relationship Between Nifurtimox AUC and Abdominal Pain Without (Left) and With (Right) Stratification by Age

To further assess the relationship between nifurtimox AUC and abdominal pain identified in the E-R dataset, the review team analyzed the full D-R dataset. The D-R dataset had 41 instances of abdominal pain in 330 patients in contrast with the E-R dataset, which had 6 instances of abdominal pain in 111 patients.

In the D-R dataset, age but not nifurtimox weight-normalized dose was a statistically significant predictor of abdominal pain by logistic regression. A CART analysis was performed to assess the trends between the three variables as shown in Figure 37. Note that age was a statistically significant cutoff but dose again was not. Figure 38 shows the D-R relationship with the age cutoff suggested by the CART analysis.

Figure 37. CART Analysis of Age, Dose, and Abdominal Pain

Abbreviations: AGE1, age (years), CART, classification and regression tree; DOSE_WT, weight-based nifurtimox dose (mg/kg/day)

Figure 38. Relationship Between Nifurtimox Dose and Abdominal Pain Stratified by Age

Considering the results of these analyses, nifurtimox AUC but not dose is a statistically significant predictor of abdominal pain. However, there is a visual trend between nifurtimox dose and abdominal pain in pediatric patients greater than or equal to 8 years. If the Applicant had collected PK data in all 330 patients, it is likely both age and nifurtimox AUC *would* be statistically significant predictors of abdominal pain. While AUC is correlated with weight-normalized dose, intersubject variability is likely masking the full relationship. There is a trend that suggests higher exposures of nifurtimox in patients 8 years or older are more likely to lead to abdominal pain. It is not clear if this is due to the mechanism of nifurtimox or if younger patients are less likely to report abdominal pain.

Assessment

Overall, the E-R analysis for serological response demonstrates that higher nifurtimox exposure is associated with improved response when assessed using the recombinant assay. The D-R analysis demonstrates similar relationship for both the recombinant and TPA assays. Given that the D-R dataset is significantly larger than the E-R dataset (330 versus 111 patients), the assessment from the D-R analysis appears to be more robust. The analysis for safety suggests that there is a higher probability of abdominal pain with increasing nifurtimox exposure/dose in patients older than 8 years.

18.5. Clinical Appendices

Table 117. TEAEs Occurring Up to 7 Days After Last Dose of NFX by PT and Treatment Arm Occurring in <2% of Subjects (Applicant Definition of TEAE)

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Gastritis	1 (0.9)	4 (1.8)	5 (1.5)
Stomatitis	0	4 (1.8)	4 (1.2)
Gastroenteritis	2 (1.8)	3 (1.4)	5 (1.5)
Bronchospasm	0	3 (1.4)	3 (0.9)
Cellulitis	0	3 (1.4)	3 (0.9)
Toothache	0	3 (1.4)	3 (0.9)
Conjunctivitis	2 (1.8)	2 (0.9)	4 (1.2)
Leukocytosis	2 (1.8)	2 (0.9)	4 (1.2)
Dyspnoea	1 (0.9)	2 (0.9)	3 (0.9)
Impetigo	1 (0.9)	2 (0.9)	3 (0.9)
Parasitic gastroenteritis	1 (0.9)	2 (0.9)	3 (0.9)
Respiratory disorder	1 (0.9)	2 (0.9)	3 (0.9)
Syncope	1 (0.9)	2 (0.9)	3 (0.9)
Blood bilirubin increased	0	2 (0.9)	2 (0.6)
Dyspepsia	0	2 (0.9)	2 (0.6)
Gastroenteritis viral	0	2 (0.9)	2 (0.6)
Myalgia	0	2 (0.9)	2 (0.6)
Oral candidiasis	0	2 (0.9)	2 (0.6)
Pneumonia influenzal	0	2 (0.9)	2 (0.6)
Pruritus	0	2 (0.9)	2 (0.6)
Somnolence	0	2 (0.9)	2 (0.6)
Xeroderma	0	2 (0.9)	2 (0.6)

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Tooth abscess	2 (1.8)	1 (0.5)	3 (0.9)
Chalazion	1 (0.9)	1 (0.5)	2 (0.6)
Dermatitis	1 (0.9)	1 (0.5)	2 (0.6)
Enterobiasis	1 (0.9)	1 (0.5)	2 (0.6)
Food poisoning	1 (0.9)	1 (0.5)	2 (0.6)
Generalised erythema	1 (0.9)	1 (0.5)	2 (0.6)
Influenza	1 (0.9)	1 (0.5)	2 (0.6)
Irritability	1 (0.9)	1 (0.5)	2 (0.6)
Animal bite	0	1 (0.5)	1 (0.3)
Apnoea	0	1 (0.5)	1 (0.3)
Burns first degree	0	1 (0.5)	1 (0.3)
Choluria	0	1 (0.5)	1 (0.3)
Dehydration	0	1 (0.5)	1 (0.3)
Dermatitis allergic	0	1 (0.5)	1 (0.3)
Dermatitis contact	0	1 (0.5)	1 (0.3)
Dyslipidemia	0	1 (0.5)	1 (0.3)
Eye injury	0	1 (0.5)	1 (0.3)
Fatigue	0	1 (0.5)	1 (0.3)
Gingival pain	0	1 (0.5)	1 (0.3)
Glossitis	0	1 (0.5)	1 (0.3)
Hepatic calcification	0	1 (0.5)	1 (0.3)
Hepatic enzyme increased	0	1 (0.5)	1 (0.3)
Hepatic steatosis	0	1 (0.5)	1 (0.3)
Hepatomegaly	0	1 (0.5)	1 (0.3)
Leukopenia	0	1 (0.5)	1 (0.3)
Lymphadenopathy	0	1 (0.5)	1 (0.3)
Neck pain	0	1 (0.5)	1 (0.3)
Neutropenia	0	1 (0.5)	1 (0.3)
Odynophagia	0	1 (0.5)	1 (0.3)
Oropharyngeal pain	0	1 (0.5)	1 (0.3)
Paresthesia	0	1 (0.5)	1 (0.3)
Pelvic inflammatory disease	0	1 (0.5)	1 (0.3)
Proteinuria	0	1 (0.5)	1 (0.3)
Respiratory tract infection viral	0	1 (0.5)	1 (0.3)
Seizure	0	1 (0.5)	1 (0.3)
Skin abrasion	0	1 (0.5)	1 (0.3)
Subcutaneous abscess	0	1 (0.5)	1 (0.3)
Tremor	0	1 (0.5)	1 (0.3)
Varicella	0	1 (0.5)	1 (0.3)
Vertigo	0	1 (0.5)	1 (0.3)
Vulvovaginal pruritus	0	1 (0.5)	1 (0.3)
Ear infection	2 (1.8)	0	2 (0.6)
Allergy to arthropod sting	1 (0.9)	0	1 (0.3)
Anxiety	1 (0.9)	0	1 (0.3)
Arthralgia	1 (0.9)	0	1 (0.3)
Autonomic nervous system imbalance	1 (0.9)	0	1 (0.3)
Dysuria	1 (0.9)	0	1 (0.3)
Erythema	1 (0.9)	0	1 (0.3)
Febrile convulsion	1 (0.9)	0	1 (0.3)
Flatulence	1 (0.9)	0	1 (0.3)

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Gastrooesophageal reflux disease	1 (0.9)	0	1 (0.3)
Intestinal dilatation	1 (0.9)	0	1 (0.3)
Omphalitis	1 (0.9)	0	1 (0.3)
Photosensitivity reaction	1 (0.9)	0	1 (0.3)
Pneumonia	1 (0.9)	0	1 (0.3)
Pneumonitis	1 (0.9)	0	1 (0.3)
Productive cough	1 (0.9)	0	1 (0.3)
Sunburn	1 (0.9)	0	1 (0.3)
Thermal burn	1 (0.9)	0	1 (0.3)
Umbilical hernia	1 (0.9)	0	1 (0.3)

Source: Reviewer Table
All values are expressed as n (%).

Regarding Table 118, Table 119, and Table 120, the alternate definition of TEAEs includes all AEs that occurred after treatment was initiated. In contrast, the Applicant definition of TEAEs excluded AEs which occurred more than 7 days after the last dose of study drug.

M.O. Comment: *Using the alternate definition of TEAEs did not reveal any new safety signals compared to the Applicant's definition.*

Table 118. Subjects With Alternative Definition of TEAEs by SOC and Treatment Arm

System Organ Class*	30-Day Arm N=111	60-Day Arm N=219	Total N=330
All subjects	73 (65.8)	163 (74.4)	236 (71.5)
Blood and lymphatic system disorders	7 (6.3)	17 (7.8)	24 (7.3)
Ear and labyrinth disorders	0	1 (0.5)	1 (0.3)
Eye disorders	1 (0.9)	2 (0.9)	3 (0.9)
Gastrointestinal disorders	35 (31.5)	85 (38.8)	120 (36.4)
General disorders and administration site conditions	7 (6.3)	30 (13.7)	37 (11.2)
Hepatobiliary disorders	0	3 (1.4)	3 (0.9)
Immune system disorders	1 (0.9)	0	1 (0.3)
Infections and infestations	34 (30.6)	83 (37.9)	117 (35.5)
Injury, poisoning and procedural complications	4 (3.6)	10 (4.6)	14 (4.2)
Investigations	1 (0.9)	11 (5.0)	12 (3.6)
Metabolism and nutrition disorders	9 (8.1)	28 (12.8)	37 (11.2)
Musculoskeletal and connective tissue disorders	4 (3.6)	5 (2.3)	9 (2.7)
Nervous system disorders	19 (17.1)	37 (16.9)	56 (17.0)
Psychiatric disorders	2 (1.8)	2 (0.9)	4 (1.2)
Renal and urinary disorders	1 (0.9)	2 (0.9)	3 (0.9)
Reproductive system and breast disorders	0	2 (0.9)	2 (0.6)
Respiratory, thoracic and mediastinal disorders	9 (8.1)	25 (11.4)	34 (10.3)
Skin and subcutaneous tissue disorders	10 (9.0)	27 (12.3)	37 (11.2)
Surgical and medical procedures	1 (0.9)	0	1 (0.3)
Vascular disorders	0	2 (0.9)	2 (0.6)

Source: Reviewer Table
All values are expressed as n (%).
* Subjects with more than one AE in a given SOC were counted only once per SOC.

Table 119. Alternative Definition of TEAEs by Preferred Term and Treatment Arm Occurring in ≥2% of Subjects

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Upper respiratory tract infection[1]	19 (17.1)	55 (25.1)	74 (22.4)
Abdominal pain[2]	14 (12.6)	33 (15.1)	47 (14.2)
Headache	16 (14.4)	30 (13.7)	46 (13.9)
Vomiting	10 (9.0)	33 (15.1)	43 (13.0)
Decreased appetite	8 (7.2)	25 (11.4)	33 (10.0)
Nausea	14 (12.6)	18 (8.2)	32 (9.7)
Pyrexia	4 (3.6)	19 (8.7)	23 (7.0)
Diarrhea	6 (5.4)	16 (7.3)	22 (6.7)
Bronchitis[3]	6 (5.4)	9 (4.1)	15 (4.5)
Rash[4]	1 (0.9)	12 (5.5)	13 (3.9)
Cough	3 (2.7)	9 (4.1)	12 (3.6)
Dizziness	3 (2.7)	7 (3.2)	10 (3.0)
Eosinophilia	4 (3.6)	6 (2.7)	10 (3.0)
Gastroenteritis	3 (2.7)	6 (2.7)	9 (2.7)
Anemia	1 (0.9)	7 (3.2)	8 (2.4)
Urinary tract infection	2 (1.8)	6 (2.7)	8 (2.4)
Chest pain	0	7 (3.2)	7 (2.1)
Gastritis	1 (0.9)	6 (2.7)	7 (2.1)
Urticaria	1 (0.9)	6 (2.7)	7 (2.1)
Weight decreased	1 (0.9)	6 (2.7)	7 (2.1)
Conjunctivitis	2 (1.8)	5 (2.3)	7 (2.1)
Asthenia	3 (2.7)	3 (1.4)	6 (1.8)

Source: Reviewer Table
All values are expressed as n (%).
[1] Upper respiratory tract infection includes the PTs of upper respiratory tract infection, acute sinusitis, adenoiditis, catarrh, chronic tonsillitis, laryngitis, nasopharyngitis, pharyngitis, pharyngotonsillitis, rhinitis, rhinorrhoea, sinusitis, tonsillitis, viral tonsillitis
[2] Abdominal pain includes the PTs of abdominal pain and abdominal pain upper
[3] Bronchitis includes the PTs of bronchitis and bronchitis viral
[4] Rash includes the PTs of rash, rash macular, rash maculo-papular, rash morbilliform, and rash papular

Table 120. Alternative Definition of TEAEs by PT and Treatment Arm Occurring in <2% of Subjects

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Stomatitis	0	4 (1.8)	4 (1.2)
Varicella	0	4 (1.8)	4 (1.2)
Enterobiasis	1 (0.9)	3 (1.4)	4 (1.2)
Respiratory disorder	1 (0.9)	3 (1.4)	4 (1.2)
Syncope	1 (0.9)	3 (1.4)	4 (1.2)
Leukocytosis	2 (1.8)	2 (0.9)	4 (1.2)
Back pain	3 (2.7)	1 (0.5)	4 (1.2)
Bronchospasm	0	3 (1.4)	3 (0.9)
Cellulitis	0	3 (1.4)	3 (0.9)
Toothache	0	3 (1.4)	3 (0.9)
Bronchiolitis	1 (0.9)	2 (0.9)	3 (0.9)
Chalazion	1 (0.9)	2 (0.9)	3 (0.9)
Dermatitis	1 (0.9)	2 (0.9)	3 (0.9)
Dyspepsia	1 (0.9)	2 (0.9)	3 (0.9)
Dyspnoea	1 (0.9)	2 (0.9)	3 (0.9)
Impetigo	1 (0.9)	2 (0.9)	3 (0.9)

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Parasitic gastroenteritis	1 (0.9)	2 (0.9)	3 (0.9)
Skin lesion	1 (0.9)	2 (0.9)	3 (0.9)
Ear infection	2 (1.8)	1 (0.5)	3 (0.9)
Generalised erythema	2 (1.8)	1 (0.5)	3 (0.9)
Tooth abscess	2 (1.8)	1 (0.5)	3 (0.9)
Animal bite	0	2 (0.9)	2 (0.6)
Blood bilirubin increased	0	2 (0.9)	2 (0.6)
Gastroenteritis viral	0	2 (0.9)	2 (0.6)
Myalgia	0	2 (0.9)	2 (0.6)
Oral candidiasis	0	2 (0.9)	2 (0.6)
Pallor	0	2 (0.9)	2 (0.6)
Pneumonia influenzal	0	2 (0.9)	2 (0.6)
Pruritus	0	2 (0.9)	2 (0.6)
Seizure	0	2 (0.9)	2 (0.6)
Somnolence	0	2 (0.9)	2 (0.6)
Xeroderma	0	2 (0.9)	2 (0.6)
Dental caries	1 (0.9)	1 (0.5)	2 (0.6)
Food poisoning	1 (0.9)	1 (0.5)	2 (0.6)
Influenza	1 (0.9)	1 (0.5)	2 (0.6)
Irritability	1 (0.9)	1 (0.5)	2 (0.6)
Obesity	1 (0.9)	1 (0.5)	2 (0.6)
Oral herpes	1 (0.9)	1 (0.5)	2 (0.6)
Pyoderma	1 (0.9)	1 (0.5)	2 (0.6)
Abscess limb	0	1 (0.5)	1 (0.3)
Acarodermatitis	0	1 (0.5)	1 (0.3)
Acid peptic disease	0	1 (0.5)	1 (0.3)
Apnoea	0	1 (0.5)	1 (0.3)
Burns first degree	0	1 (0.5)	1 (0.3)
Burns second degree	0	1 (0.5)	1 (0.3)
Cardiac murmur	0	1 (0.5)	1 (0.3)
Choluria	0	1 (0.5)	1 (0.3)
Completed suicide	0	1 (0.5)	1 (0.3)
Costochondritis	0	1 (0.5)	1 (0.3)
Dehydration	0	1 (0.5)	1 (0.3)
Dermatitis allergic	0	1 (0.5)	1 (0.3)
Dermatitis contact	0	1 (0.5)	1 (0.3)
Dermatitis diaper	0	1 (0.5)	1 (0.3)
Dyslipidemia	0	1 (0.5)	1 (0.3)
Eye injury	0	1 (0.5)	1 (0.3)
Fatigue	0	1 (0.5)	1 (0.3)
Fungal skin infection	0	1 (0.5)	1 (0.3)
Gingival pain	0	1 (0.5)	1 (0.3)
Glossitis	0	1 (0.5)	1 (0.3)
Helminthic infection	0	1 (0.5)	1 (0.3)
Hepatic calcification	0	1 (0.5)	1 (0.3)
Hepatic enzyme increased	0	1 (0.5)	1 (0.3)
Hepatic steatosis	0	1 (0.5)	1 (0.3)
Hepatomegaly	0	1 (0.5)	1 (0.3)
Hyperuricemia	0	1 (0.5)	1 (0.3)
Leukopenia	0	1 (0.5)	1 (0.3)

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Ligament sprain	0	1 (0.5)	1 (0.3)
Lymphadenopathy	0	1 (0.5)	1 (0.3)
Metrorrhagia	0	1 (0.5)	1 (0.3)
Myiasis	0	1 (0.5)	1 (0.3)
Nail avulsion	0	1 (0.5)	1 (0.3)
Neck pain	0	1 (0.5)	1 (0.3)
Neutropenia	0	1 (0.5)	1 (0.3)
Odynophagia	0	1 (0.5)	1 (0.3)
Oropharyngeal candidiasis	0	1 (0.5)	1 (0.3)
Oropharyngeal pain	0	1 (0.5)	1 (0.3)
Paresthesia	0	1 (0.5)	1 (0.3)
Pelvic inflammatory disease	0	1 (0.5)	1 (0.3)
Proteinuria	0	1 (0.5)	1 (0.3)
Respiratory tract infection viral	0	1 (0.5)	1 (0.3)
Rhinitis allergic	0	1 (0.5)	1 (0.3)
Scoliosis	0	1 (0.5)	1 (0.3)
Skin abrasion	0	1 (0.5)	1 (0.3)
Skin ulcer	0	1 (0.5)	1 (0.3)
Snake bite	0	1 (0.5)	1 (0.3)
Subcutaneous abscess	0	1 (0.5)	1 (0.3)
Tooth infection	0	1 (0.5)	1 (0.3)
Transaminases increased	0	1 (0.5)	1 (0.3)
Tremor	0	1 (0.5)	1 (0.3)
Upper limb fracture	0	1 (0.5)	1 (0.3)
Vertigo	0	1 (0.5)	1 (0.3)
Vulvovaginal pruritus	0	1 (0.5)	1 (0.3)
Abortion induced	1 (0.9)	0	1 (0.3)
Allergy to arthropod sting	1 (0.9)	0	1 (0.3)
Anxiety	1 (0.9)	0	1 (0.3)
Arthralgia	1 (0.9)	0	1 (0.3)
Arthropod bite	1 (0.9)	0	1 (0.3)
Autonomic nervous system imbalance	1 (0.9)	0	1 (0.3)
Bronchial obstruction	1 (0.9)	0	1 (0.3)
Dysuria	1 (0.9)	0	1 (0.3)
Eczema	1 (0.9)	0	1 (0.3)
Erythema	1 (0.9)	0	1 (0.3)
Febrile convulsion	1 (0.9)	0	1 (0.3)
Flatulence	1 (0.9)	0	1 (0.3)
Gastrooesophageal reflux disease	1 (0.9)	0	1 (0.3)
Intestinal dilatation	1 (0.9)	0	1 (0.3)
Limb injury	1 (0.9)	0	1 (0.3)
Omphalitis	1 (0.9)	0	1 (0.3)
Otitis media	1 (0.9)	0	1 (0.3)
Otitis media acute	1 (0.9)	0	1 (0.3)
Photosensitivity reaction	1 (0.9)	0	1 (0.3)
Pneumonia	1 (0.9)	0	1 (0.3)
Pneumonitis	1 (0.9)	0	1 (0.3)
Productive cough	1 (0.9)	0	1 (0.3)
Prurigo	1 (0.9)	0	1 (0.3)
Retained deciduous tooth	1 (0.9)	0	1 (0.3)

Preferred Term	30-Day Arm N=111	60-Day Arm N=219	Total N=330
Sunburn	1 (0.9)	0	1 (0.3)
Thermal burn	1 (0.9)	0	1 (0.3)
Umbilical hernia	1 (0.9)	0	1 (0.3)

Source: Reviewer Table

18.6. Clinical Microbiology Appendix

18.6.1. Nonclinical Studies

The nonclinical studies supporting the mechanism of action, activity in vitro as well as in animal models, drug resistance and cross-resistance are summarized.

18.6.1.1. Mechanism of Action

The mechanism of action of nifurtimox, a nitroheterocyclic molecule, is not fully understood. Studies (Maya et al. 2007; Boiani et al. 2010; Cerecetto and González 2011; Hall et al. 2011) suggest that nifurtimox is activated by nitroreduction leading to the production of toxic intermediate metabolites that induce DNA damage and cell death. Two *T. cruzi* nitroreductase (TcNTR) enzymes, Type I and Type II, play an important role in the activation of nifurtimox.

Type I nitroreductase (NTR), present in many prokaryotes including some of the protozoan parasites but absent from humans, is oxygen independent (i.e., oxygen insensitive) and leads to production of nitrile but not to the production of reactive oxygen species. Two enzymes with Type I activity have been identified in *T. cruzi*; prostaglandin F2α synthase (also known as the Old Yellow Enzyme) and nicotinamide adenine dinucleotide, reduced (NADH)–dependent mitochondrial Type I NTR. Prostaglandin F2α synthase can mediate 2-electron reduction under anaerobic conditions (Kubata et al. 2002). NADH–dependent mitochondrial Type I NTR is an O_2-insensitive flavin mononucleotide-dependent enzyme that catalyzes the 2-electron reduction of nitroheterocyclic compounds within the parasite, producing toxic metabolites without significant generation of superoxide (Wilkinson et al. 2008; Wilkinson et al. 2011; Hall and Wilkinson 2012).

Type II NTR, an ubiquitous enzyme present in both prokaryotes and eukaryotes, is oxygen sensitive and important in maintaining respiratory rate with concomitant hydrogen peroxide release and/or other reactive oxygen species (free radicals) leading to oxidative stress and reducing the formation of thiols such as glutathione.

The studies supporting the effect of nifurtimox on low molecular weight thiols, important in conferring defense to the parasite as well as DNA and protein synthesis are summarized below.

18.6.1.1.1. Effect on Thiols

Maya et al. (Maya et al. 1997) reported that the low molecular weight thiols (glutathione-trypanothione) status influenced the sensitivity to nifurtimox, of the trypomastigotes and amastigotes forms of the LQ, Dm28c, MF, and Brener strains/clones of *T. cruzi*. Nifurtimox

(20 mcM i.e., 5.7 mcg/mL) reduced the free thiols by 65 – 80% in LQ, Dm28c, and Brener strains/clones of *T. cruzi*; at a higher nifurtimox concentration, the free thiol levels were decreased to nearly zero and parasites were lysed. The amastigotes of the MF strain contained the lowest amount of total thiols (291nM of glutathione-equivalent per g of parasite fresh weight) compared to trypomastigotes (428nM) and epimastigotes (890nM).

Similar observations were reported by Moncada et al. (Moncada et al. 1989) and Repetto et al. (Repetto et al. 1996) using Tulahuen, DM28c and LQ strains/clones of *T. cruzi*. Moncada et al. (Moncada et al. 1989) reported that decreased sensitivity of epimastigotes of the LQ and Tulahuen strains of *T. cruzi* may be due to higher glutathione concentration; addition of buthionine sulfoximine decreased the free or conjugated glutathione concentration by 50% and increased the in vitro sensitivity of the parasites to nifurtimox and benznidazole.

Boiani et al. (Boiani et al. 2010) reported a significant reduction in the level of parasitic thiols in the presence of nifurtimox was not linked to the production of reactive oxidant species.

Overall, the studies suggest that the concentrations of free thiols vary among different strains as well as among the different stages (epimastigote, trypomastigote and amastigote forms) of *T. cruzi* and influence the sensitivity of *T. cruzi* parasites to nifurtimox.

18.6.1.1.2. Effect on DNA

Goijman et al. (Goijman et al. 1985) reported that pretreatment of the epimastigotes of the Tulahuen strain of *T. cruzi* with nifurtimox (10 and 100 mcM i.e., 2.9 and 28.7 mcg/mL, respectively) stimulated nuclear and kinetoplast (k) DNA cleavage. DNA damage was reversible since re-incubation in drug-free fresh medium for 24 hours restored electrophoretic and sedimentation patterns to normal.

Some of the in vitro studies conducted to measure the antiproliferative activity of nifurtimox were based on incorporation of ^{3}H-thymidine, essential for DNA synthesis (see Section 20.6.1.2). The results showed a reduction in the incorporation of ^{3}H-thymidine supporting that nifurtimox inhibited DNA synthesis.

18.6.1.1.3. Effect on Protein Synthesis

Maya et al. (Maya et al. 2004) reported that nifurtimox (2.6mM i.e., 747.0 mcg/mL) decreased the metallothionein (MT) content, by 74%, of the epimastigotes of the Tulahuen strain of *T. cruzi* in vitro. MT is a protein that is rich in sulfhydryl groups known to be heat stable and binds cadmium. As the MT content varies in different strains, the sensitivity of the parasites to nifurtimox may vary.

18.6.1.1.4. Effect on Immune System

Lelchuk et al. (Lelchuk et al. 1977) reported improvement in leukocyte migration inhibition (LMI) of peripheral blood mononuclear cells from adult patients with chronic Chagas disease treated with nifurtimox for 2 months compared to untreated patients; the LMI was similar to that of the healthy controls; the antigen used for testing was derived from epimastigotes of the

Tulahuen strain of *T. cruzi*. However, there was no difference in the number of T-cells and B-cells as measured by rosette formation, the number of lymphocytes bearing surface membrane Ig (SMIg), nonspecific stimulation with phytohemagglutinin, and ADCC. A comparison of different parameters prior to treatment was not assessed.

18.6.1.2. In Vitro Activity

The in vitro activity of nifurtimox against extracellular epimastigotes and trypomastigotes as well as intracellular amastigotes of the laboratory strains and clinical isolates of *T. cruzi* was based on inhibition of growth and/or motility or ultrastructure changes.

18.6.1.2.1. Effect on Growth or Motility

The in vitro activity of nifurtimox was reported against several strains of *T. cruzi* that belong to different distinct typing units (DTUs)/lineages (Gönnert and Bock 1972; Neal and van Bueren 1988; Revollo et al. 1998; Luna et al. 2009; Boiani et al. 2010; Mejía-Jaramillo et al. 2011; Martínez et al. 2013; Fonseca-Berzal et al. 2014; Moraes et al. 2014; Moraes et al. 2015; Contreras-Ortiz et al. 2017; Quebrada Palacio et al. 2018), study reports PH-345 (Bock and Haberkorn 1967), PH-2119 (Bock 1970), PH-2416 (Voigt et al. 1970), PH-3806 (Voigt et al. 1972b), R-257 (Bock et al. 1959), and NRR_R-13157 (Kaiser 2019). The experimental design used in different studies varied; variations include different stage of the parasite and the methods used to measure activity (for details see Table 121). Briefly, the activity against the epimastigotes was measured in axenic cultures whereas the activity against the amastigote stage was measured in mammalian cell cultures such as the Vero cells (a lineage of cells originally isolated from kidney epithelial cells of the African green monkey), HeLa cells (derived from cervical cancer cells), and murine peritoneal macrophages or fibroblasts. It should be noted that there is a possibility that different stages of the parasite may be present in culture. For example, Polak and Richle (Polak and Richle 1978) reported about 80% epimastigotes and 20% trypomastigotes in culture whereas Neal and van Bueren (Neal and van Bueren 1988) reported the proportion of different stages of the parasite present in culture varied with the strain [Colombiana-100% amastigotes; Sonya clone-50% mixture of epimastigotes and amastigotes; other 3 strains (Tulahuen, Y, and Peru)-100% epimastigotes]. Gönnert and Bock (Gönnert and Bock 1972) reported that in untreated HeLa cell cultures infected with the WBH (Wellcome-Brazil-Hamburg) strain, the number of infected cells/1000 counted cells increased around Days 6 – 7 and an increase in the number of pseudocysts with numerous mastigotes (transitional forms) shortly before release of the organism. This suggests a completion of the development cycle occurring on the 5th-6th day followed by infection of other host cells. Under normal conditions, the amastigotes divide about every 12 hours; however, the cell infection may be delayed and multiplication of the parasites in the cells may not be synchronous. On the 7th day postinfection, an increase in cells of early stage (≤10) amastigotes was reported suggesting the beginning of a second development cycle.

The activity of nifurtimox at the end of the incubation period was determined by direct microscopic examination or by ^{3}H-thymindine incorporation after incubation for 4 to 5 days in a

majority of the studies; in one study, incubation was performed for up to 12 days depending on the strain as the growth of different strains varied. The results showed that the 50% inhibitory concentrations (IC_{50}s) against different strains (~50), irrespective of the stage of the parasite, were ≤21.1 mcg/mL (for details see Table 121).

Table 121. Summary of Studies Supporting In Vitro Activity of Nifurtimox Against Extracellular and Intracellular Stages of the Laboratory Strains and Clinical Isolates of *T. cruzi*

Parasite Stage (Reference) and Experimental Design	Strain (DTU)	IC_{50} mcM (mcg/mL)
Amastigotes (Gönnert and Bock 1972) HeLa-cells (1-2 Day old) were infected with 2-4 x 10^5 trypanosomes/mL culture medium, washed to remove the parasites in the supernatant; NFX concentrations of 1 ppm (1 mcg/mL) and 0.1 ppm (0.1 mcg/mL), which were known to be active based on previous experience) were added, cultures incubated for 24 h, washed and fresh medium added. At the end of the test, cultures were washed, and processed for microscopic examination by fixing in Susa solution and stained while wet with Giemsa solution. The number of infected cells/1000 HeLa-cells, the number of organisms/infected cell as well as the stage of development of the organisms was determined based on the following five groups: I. 1-5 amastigotes/cell II. 6-10 amastigotes/cell III. 11-50 amastigotes/cell IV. 50 amastigotes/cell V. Last stage of development, pseudocysts with numerous mastigotes shortly before release of the organism. The minimally active concentrations for trypanocidal activity after 24 h was 0.1 mcg/mL and after several days action was 0.01 mcg/mL suggesting a concentration- and dose-dependent activity; 10 mcg/mL NFX did not result in damage to the HeLa-cells based on microscopic examination.	WBH	Not determined
Epimastigotes (Neal and van Bueren 1988) The epimastigotes were cultured in Schneider's insect culture medium and subcultured >10-times. The 10^6 epimastigotes from the log phase were incubated with and without drug, at 26°C, for 4 to 12 days depending on the strain. The growth was measured by ^{3}H-thymidine incorporation; 1 generation time was added before harvesting as the growth of different strains varied (mean generation time for Sonya clone-66 h; Colombiana and Y strains-22 h). The parasite stages present in culture varied (Colombiana-100% amastigotes; Sonya clone-50% mixture of epimastigotes and amastigotes; for other strains-100% epimastigotes). No flagellum or dividing amastigotes were observed by electronmicrography in Colombiana and	Sonya clone* (not specified)	0.2 (0.06)
	Colombiana* (I)	2.9 (0.83)
	Tulahuen** (VI)	14.0 (4.02)
	Y** (II)	2.4 (0.69)

Parasite Stage (Reference) and Experimental Design	Strain (DTU)	IC_{50} mcM (mcg/mL)
Sonya clone cultures and uptake of ^{3}H-thymidine was lowest. The uptake of ^{3}H-thymidine was highest for the Y strain; Colombiana and Sonya clones had the lowest uptake, 13% and 21%, respectively of the Y strain.	Peru** (not specified)	2.0 (0.57)
Amastigotes (Neal and van Bueren 1988) Mouse peritoneal macrophages (1.75x10^5) were infected with trypomastigotes (1.8-2.5x10^5); after 24 h the medium was replaced and incubated with or without drug for 3 to 5 days. The number of infected and uninfected macrophages were counted after Giemsa staining. The responsive strains (Tulahuen, Y and Peru) lysed macrophages in 3 days and infectivity was 40-90%, whereas the nonresponsive strains (Sonya clone and Colombiana) required 4 to 5 days and showed lower infectivity of macrophages (□20-26%).	Sonya clone* (not specified)	1.0 (0.3)
	Colombiana* (I)	0.6 (0.2)
	Tulahuen** (VI)	0.1 (0.03)
	Y** (II)	0.5 (0.1)
	Peru** (Not specified)	0.1 (0.03)
Epimastigotes/Trypomastigotes/Amastigotes (Revollo et al. 1998) The activity of NFX was measured against the different stages of the 21 different stocks, that represent 4 major genetic groups (19, 20, 32, and 39), of *T. cruzi*. Briefly, epimastigotes (10^6/mL), trypomastigotes (10^7/mL) and amastigotes (10 parasites:1 Vero cell) were incubated with NFX and the activity determined by incorporation of ^{3}H-thymidine (epimastigotes and trypomastigotes) or by Giemsa staining of the cells (amastigotes). The NFX IC_{50}s were similar against all 3 stages of the clones 19 and 20; however, for clones 32 and 39, the IC_{50}s were slightly lower against the epimastigotes compared to trypomastigotes and amastigotes. Overall, the mean IC_{50}s were ≤4.4 mcM i.e., 1.3 mcg/mL	**Epimastigotes**	
	19 (5 stocks)/ 20 (5 stocks)	4.4 (1.3)
	32 (5 stocks)	0.5 (0.1)
	39 (6 stocks)	0.9 (0.3)
	Trypomastigotes	
	19 (5 stocks)/ 20 (5 stocks)	3.6 (1.0)
	32 (5 stocks)	2.8 (0.8)
	39 (6 stocks)	4.3 (1.2)
	Amastigotes	
	19 (5 stocks)/ 20 (5 stocks)	3.4 (1.0)
	32 (5 stocks)	2.0 (0.6)
	39 (6 stocks)	1.8 (0.5)
Epimastigotes (Luna et al. 2009) Epimastigotes (5x10^5) of 8 Colombian and 3 other strains were incubated in LIT medium with different dilutions of NFX for 72 h at 28ºC. Each drug concentration was tested in triplicate and IC_{50} and IC_{90} values determined. The IC_{50} and IC_{90} values varied between 1.6-5.1 mcM (0.5-1.5 mcg/mL) and 10.6-35.3 mcM (3.0-10.1 mcg/mL), respectively. *Amastigotes* Vero cells (3x10^5 cells/mL) were allowed to adhere to wells in 16 well plates for 24 h; cells were infected with trypanosomes of 7 Colombian and Y strains in the ratio of 10:1 (parasites:cells), incubated for 24 h and free parasites removed by washing. Infected cells were incubated without and with different concentration (each concentration tested in quadruplicate) of NFX for 72 h; cultures were fixed and processed for Giemsa staining and IC_{50} and IC_{90} values determined. Represent results of 2 experiments.	**Epimastigotes**	
	Colombia 215 (Z3)	<3.2 (0.9)
	Colombia 209 (I)	5.1 (1.5)
	Colombia 204 (I)	4.2 (1.2)
	Colombia 132 (I)	3.8 (1.1)
	Colombia 070I06 (I)	2.3 (0.7)
	Colombia 320I01 (I)	2.5 (0.7)
	Colombia 421I02 (I)	3.8 (1.1)
	Colombia 1874-01 (I)	2.3 (0.7)
	Esmeraldo (II)	<3.2 (0.9)
	Silvio X10 (I)	1.6 (0.5)
	Y (II)	3.5 (1.0)
	Amastigotes	
	Colombia 209 (I)	>1.1–1.3 (0.3-0.4)
	Colombia 204 (I)	1.8-1.4 (0.5-0.4)
	Colombia 132 (I)	1.8–1.5 (0.5-0.4)
	Colombia 070I06 (I)	2.6-1.5 (0.8-0.4)
	Colombia 320I01 (I)	3.4 (1.0)
	Colombia 421I02 (I)	1.6-3.2 (0.5-0.9)
	Colombia 1874-01 (I)	0.5-3.6 (0.1-1.0)

Parasite Stage (Reference) and Experimental Design	Strain (DTU)	IC_{50} mcM (mcg/mL)
The IC_{50} and IC_{90} values varied between 0.5-3.4 mcM (0.1-1.0 mcg/mL) and 5.0-44.8 mcM (1.4-12.9 mcg/mL), respectively. NFX was more active than BZN against both epimastigotes (p<0.001) and intracellular amastigotes (p=0.0004).	Y (II)	2.7 (0.8)
Epimastigotesl (Boiani et al. 2010) The effect of the drugs on parasite proliferation was analyzed by determining two parameters (A) cell growth and (B) cell viability. (A) For the cell growth assay, parasites were grown in the presence of the drug, whereas in the viability assay, parasites were exposed to the drug for 4 h and then resuspended in drug-free medium to follow growth. Briefly, axenic cultures of the mid-log phase epimastigotes ($4x10^6$/mL) were incubated with different concentrations of NFX for 5 days and followed for growth by nephelometry at 610 nm; absorbance readings were directly proportional to cell density. The IC_{50} value producing a 50% reduction in parasite growth as compared to control (no drug added) was determined. (B) For the viability assay, parasites (4×10^6 cells/mL) were exposed to different concentrations of NFX for 4 h, cells were harvested and washed 2-times to eliminate nonincorporated drugs. Parasites were then resuspended in drug-free culture medium and growth was followed as described above for 5 days.	Tulahuen 2 strain (VI)	Cell growth assay: 7.0 (2.0) Viability assay: 25.0 (7.2)
Epimastigotes (Mejía-Jaramillo et al. 2011) The epimastigotes (10^7/mL) of a BZN sensitive strain were incubated with different concentrations of NFX for 96 h at 28ºC; MTT was added and cultures incubated for 90 minutes. The cell density was monitored by the reduction of MTT to formazan crystals at 595 nm and IC_{50} determined.	Gal61 (DTU I);	2.3 (0.7)
Epimastigotes (Martínez et al. 2013) Axenic cultures of 10^6 epimastigotes of different strains/isolates of Mexican origin in LIT medium were incubated with and without NFM for 24 and 48 h and number of parasites counted. The activity of the drug on motility (M) and growth (G) was reported at 48 h.	Silvio (I)	M: >384.6 (110.5) G: 73.4 (21.1)
	MHOM/MX/0000/ EA (I)	M: 6.5 (1.9) G: 7.0 (2.0)
	MHOM/MX/0000/H1 (I)	M: >384.6 (110.5) G: 59.2 (17.1)
	MHOM/MX/2001/ INC9 (I)	M: 10.2 (2.9) G: 4.5 (1.3)
	MHOM/MX/2000/ INC10 (I)	M: 9.0 (2.6) G: 5.0 (1.4)
	MHOM/MX/1994/ Ninoa (I)	M: >384.6 (110.5) G: 0.2 (0.1)
	TBAR/MX/0000/ Queretaro (I)	M: 0.9 (0.3) G: 0.4 (0.01)
	MHOM/MX/1998/ JJO (I)	M: >384.6 (110.5) G: 7.7 (2.2)
	MHOM/MX/2001/ Mor3 (I)	M: >384.6 (110.5) G: 7.2 (2.1)
	MDID/MX/1991/Ver6	M: 6.0 (1.7) G: 0.7 (0.2)

Parasite Stage (Reference) and Experimental Design	Strain (DTU)	IC_{50} mcM (mcg/mL)
	CL Brener (VI)	M: 6.4 (1.8) G: 0.5 (0.1)
Trypanosomes/Amastigotes (Moraes et al. 2014) U2OS cells (osteosarcoma-derived human cell line) were incubated with tissue-derived trypomastigotes for 24 h and different concentrations of NFX added; cultures were incubated for 4 days for all strains except for Dm28c strain (incubation was for 3 days). Activity was measured by high content imaging. The NFX IC_{50}s were 4- to 8-fold higher for the two strains (Dm28c and Y) that were more virulent and proliferated faster in vitro compared to the other 5 strains. Exposure of any of the strains to 50 mcM (14.4 mcg/mL) NFX for 72 h reduced the infection to uninfected control levels.	Dm28c (I)	1.5 (0.4)
	Y (II)	1.4 (0.4)
	ARMA13 cl1 (III)	0.2 (0.1)
	ERA cl2 (IV)	0.2 (0.1)
	92.80 cl2 (V)	0.2 (0.1)
	Tulahuen (VI)	0.2 (0.1)
	CL Brenner (VI)	0.4 (0.1)
Epimastigotes (Fonseca-Berzal et al. 2014) Parasites were grown axenically at 28ºC in LIT medium; tissue culture-derived trypomastigotes were obtained by the infection of nonconfluent monolayers of murine fibroblasts (L929) with epimastigotes in stationary phase of growth. The cultures were washed and incubated for 7 days and trypomastigotes stained.	CL, clone B5 (VI) transfected with E. coli β-galactosidase gene	3.6 (1.0)
Amastigotes (Fonseca-Berzal et al. 2014) Murine fibroblasts (L929) cells (adhered to the cell surface) were infected with 10^6 trypomastigotes in the ratio of 1:6, cultures incubated for 24 h, washed, and incubated with different concentrations of the drug for 7 days and activity measured by colorimetry after adding chromogenic substrate chlorophenol red β- D-galactopyranoside and cultures incubated for 3 h. *Results showed 100% inhibition at a concentration of 8 mcM.	CL, clone B5 transfected with E. coli β-galactosidase gene	<8* (2.3)
Stage of parasite used not specified (Moraes et al. 2015) Details of the method not included in the publication. The activity of NFX and 2 enantiomers (R and S) was measured and shown to be similar. Cytotoxicity against mammalian cells was measured using noninfected rat skeletal myoblast L6 cell line and EC_{50} determined. The NFX racemate EC_{50} values against the rat cells were >200-fold higher (range 64 and 161 mcM i.e., 18.4 and 46.3 mcg/mL) compared to the parasites; similarly, the R and S enantiomers were >180-fold less sensitive against the rat cells compared to the parasites.	Dm28c (I)	3.4 (1.0)
	Y (II)	1.0 (0.3)
	ARMA13 cl1 (III)	0.9 (0.3)
	ERA cl2 (IV)	0.6 (0.2)
	92.80 cl2 (V)	0.3 (0.1)
	Tulahuen (VI)	0.3 (0.1)
	CL Brenner (VI)	0.8 (0.2)

Parasite Stage (Reference) and Experimental Design	Strain (DTU)	IC_{50} mcM (mcg/mL)
Trypomastigotes (Contreras-Ortiz et al. 2017)	Ninoa (I)	ND
$5x10^5$ trypomastigotes or $2x10^4$ Vero cells were incubated with or without NFX (400 mcg/100 mcL) for 24 h and metabolic activity, representing viability, was measured by colorimetry at 490 nm wavelength after adding MTS (3-[4,5,dimethylthiazol-2-yl]-5-[3-carboxymethoxy-phenyl]-2-[4-sulfophenyl]-2H-tetrazolium, inner salt). The results show NFX (400 mcg/mL) was effective in reducing viability of the parasites by □50% in culture. However, there was no effect on the viability of Vero cells.		
Amastigotes		
The activity of NFX was measured against intracellular parasites by adding NFX (400 mcg/mL) to Vero cells cultured with trypomastigotes for 96 h. Viability of the parasites was measured after 24 h as summarized above. The results showed absence of any viable parasites in the presence of NFX.		
Epimastigotes (Quebrada Palacio et al. 2018)	Sylvio-X10/4 (I)	0.5 (0.1)
Axenic cultures of epimastigote (10^6 /mL), for 8 isolates from Argentina, were initiated in LIT media and incubated with NFX at 28°C for 72 h. The motile parasites were counted in a Neubauer's chamber using a phase microscope and the IC_{50} values determined.	BOL-FC5C (I)	8.1 (2.3)
	BOL-FC10A (V)	5.3 (1.5)
	AR-FC19A (V)	3.3 (0.9)
	AR-SEC23C (TV)	5.1 (1.5)
	AR-FC553 (V)	5.5 (1.6)
	AR-FC202113 (V)	1.2 (0.3)
	AE-FC195205 (V)	14.1 (4.1)
Amastigotes (Quebrada Palacio et al. 2018)	AR-SE23C (V)	1.3 (0.4)
Peritoneal macrophages of C3H/HeN mice were seeded onto coverslips ($5x10^5$ cells/cover slip) in 24 well-plates and cultured for 24 h; nonadherent cells were removed by washing and the cultures infected with trypomastigotes (of 2 isolates from Argentina) at a ratio of 1:1 for 16 h. Noninternalized parasites were removed and infected cultures exposed to serial dilutions (0.01±100 mcg/mL) of NFX in triplicate for 72 h; after fixation with methanol and staining with Giemsa, the number of infected cells and intracellular amastigotes/cell was quantitated in microphotographs of randomly selected 200x microscopic fields of the stained smears. A total of 600 cells (200 cells per cover slip) were analyzed for each experimental condition in 2 independent experiments and IC_{50} values determined.	BOL-FC10A (TV)	0.2 (0.1)
Cytotoxicity assays performed on uninfected peritoneal macrophages showed that the NFX CC_{50} was 1000>-fold higher (4.84mM i.e., 1.39 mg/mL) the IC_{50} values.		

Parasite Stage (Reference) and Experimental Design	Strain (DTU)	IC_{50} mcM (mcg/mL)
Trypomastigotes (Quebrada Palacio et al. 2018) Vero cell-derived trypomastigotes (1x10^6/mL) were incubated without (control) or with serial dilutions (0.01±100 mcg/mL) of NFX for 24 h at 4°C. The number of motile parasites was determined using phase microscope and IC_{50} values determined.	AR-SE23C (V)	0.10 (0.03)
	BOL-FC10A (V)	40.2 (11.6)
Amastigotes (Kaiser 2019) L-cells (rat skeletal myoblasts) were infected with the trypomastigotes and different concentrations of NFX added at 48h PI. Cells were incubated for 96 h and activity determined photometrically. The IC_{50} values for the two metabolites (M-4 and M-6 IC_{50} values 71 and 70 mcg/mL, respectively) were higher than that of NFX. The cytotoxicity IC_{50} against the L-cells was 19.5 mcg/mL.	Tulahuen (VI) C2C4 containing the β-galactosidase	0.7 (0.2)

±Nonresponsive strains and **Responsive strains based on long-term treatment of mouse (CD1 mice) infections with nifurtimox (NFX) and benznidazole (BZN).
Vero cells = African green monkey kidney cells
Abbreviation: BE, Berenice; DTU, discrete typing units; h, hours; LIT = liver infusion tryptose; PI = post infection;

One study (Martínez et al. 2013) reported the effect of nifurtimox on both growth and motility of epimastigotes; the motility of the 11 strains, compared to growth, was inhibited at 2- to 52-fold higher nifurtimox concentrations; IC_{50} values ranged between 1.8 and 110.5 mcg/mL (for details see Table 121.

The trypomastigotes, obtained from HeLa-cultures, incubated with nifurtimox concentrations of 10 ppm, i.e., 10 mcg/mL for 1 hour at 22° C, were damaged and had reduced infectivity of the HeLa cells (Gönnert and Bock 1972).

There is a trend towards higher IC_{50} values against the strains that are considered to be nonresponsive compared to the responsive strains based on studies in animal models. The activity of nifurtimox appears to be concentration- and time-dependent; after 24 hours the nifurtimox induced damage to the parasites was irreversible (Gönnert and Bock 1972).

Activity of Enantiomers and Metabolites of Nifurtimox

The activity of the two enantiomers of nifurtimox was reported to be similar (Moraes et al. 2015). However, the two metabolites (M-4 and-M6) of nifurtimox, found in humans, appear to be not active against Tulahuen strain of *T. cruzi* as the IC_{50} values were 350-fold higher than that of nifurtimox (Kaiser 2019).

18.6.1.2.2. Effect on Ultrastructure

Gönnert and Bock (1972) reported that nifurtimox induced damage to the amastigote stage of the WBH strain of *T. cruzi* in vitro; the changes observed by light- and electron-microscopy include alteration in the protoplasm (appeared lighter and bloated compared to the untreated controls) and damage to the nuclei that became small and pyknotic and lost their round form.

In another study by the same group of investigators (Voigt et al. 1972a), the effect of 1 mcg/mL nifurtimox on HeLa cells infected with the WBH strain of *T. cruzi* up to 10 days after exposure to nifurtimox was reported. Briefly, HeLa cells infected with trypanosomes were incubated with nifurtimox at 37°C, and drug containing medium renewed every 24 hours. Within 8 to 10 hours of incubation, first visible changes in the parasite cell observed included the presence of a larger number of cytoplasmic vacuoles, increased numbers of membrane bound lipoid droplets, as well as strongly osmiophilic bodies and dense particles in the kinetoplast in nifurtimox containing cultures compared to the controls.

At 24 hours, marked damage was observed in most of the parasites, including pyknotic shrunken cells with dense cytoplasm and a preponderance of more or less swollen cells characterized by fewer organelles. Other changes include a decrease in ribosomes (which at this time are present in relatively intact parasite cells predominantly as polysomes), an increase in apparently empty vacuoles, irregular broadening of the perinuclear space, disappearance of the peripherally arranged chromatin in the cell nuclei but the nucleolus remained intact. The kinetoplast appeared to be morphologically the least affected; it was surrounded by an increased number of irregular point-like densifications within the mitochondrion.

After 48 hours of incubation with nifurtimox, morphological and intracellular changes increased that include reduction in the ribosomes in the cytoplasm, an enlarged perinuclear space, swollen mitochondria in the kinetoplast, and the Golgi apparatus represented largely by vesicular components.

After 72 hours, most of the parasite cells were damaged and hardly any undamaged cell organelles were present; however, a few parasites were only slightly changed morphologically. The kinetoplast was stated to be the most resistant and the pellicular fibrils were very stable organelles.

There was no effect of nifurtimox on the uninfected HeLa cells in culture.

18.6.1.3. In Vivo Activity (Animal Models)

Several studies reported the activity of nifurtimox in acute and/or chronic infection models of *T. cruzi*. Approximately 62 strains/isolates were tested in several animal species that include the following:

- Mice
  - Study Reports
    - PH-305 (Haberkorn 1967)
    - PH-1777 (Haberkorn 1970a)
    - PH-1918 (Haberkorn 1970b)
    - PH-2098 (Bock et al. 1970)
    - PH-2451 (Haberkorn and Gönnert 1970)
    - R-258 (Study Report R-258: Preclinical study report n.d.)
  - Haberkorn and Gönnert (Haberkorn and Gonnert 1972)
  - Andrade et al. (Andrade et al. 1985; Andrade et al. 1991)

  - Meckert et al. (Meckert et al. 1988)
  - Neal and van Bueren (Neal and van Bueren 1988)
  - Marretto and Andrade (Marretto and Andrade 1994)
  - Urbina et al. (Urbina et al. 2003)
  - Bustamante et al. (Bustamante et al. 2014)
  - Moraes et al. (Moraes et al. 2015)
  - Contreras-Ortiz et al. (Contreras-Ortiz et al. 2017)
  - de Oliveira et al. (de Oliveira et al. 2017)
  - Mazzeti et al. (Mazzeti et al. 2018)

- Rats
  - Study Report PH-2451 (Haberkorn and Gönnert 1970)
  - Haberkorn and Gönnert (Haberkorn and Gonnert 1972)

- Hamsters
  - Study Report PH-2451 (Haberkorn and Gönnert 1970)
  - Haberkorn and Gönnert (Haberkorn and Gonnert 1972)

- Guinea pigs
  - Study Report PH-2451 (Haberkorn and Gönnert 1970)
  - Haberkorn and Gönnert (Haberkorn and Gonnert 1972)

- Rabbits
  - Teixeira et al. (Teixeira et al. 1990)

- Cats
  - Study Report PH-2451 (Haberkorn and Gönnert 1970)
  - Haberkorn and Gönnert (Haberkorn and Gonnert 1972)

- Dogs
  - Study Report PH-2451 (Haberkorn and Gönnert 1970)
  - Haberkorn and Gönnert (Haberkorn and Gonnert 1972)

The experimental design for different studies varied. Some of the variations include the strain of *T. cruzi* used for infecting animals, the trypomastigote inoculum concentration and route of infection, time of initiation of treatment postinfection, dose(s), duration and route of nifurtimox treatment, test(s) used for measuring parasitological response (microscopic examination for the presence of parasites in blood, histological evaluations of tissues, hemoculture, xenodiagnosis, PCR and/or survival) as well as the duration of follow-up. The activity of nifurtimox was based on measurement of parasitemia and/or survival in a majority of the studies; few studies reported the effect of treatment on serological response (for details see Table 122 and Table 123).

The studies suggest that nifurtimox is effective in reducing parasite burden and improving survival; however, a delay in initiation of treatment decreases the activity of nifurtimox. In some of the studies, reactivation of infection was reported in mice that were immunosuppressed, after becoming aparasitemic post-treatment with nifurtimox.

Overall, the study suggests that treatment with nifurtimox leads to a loss of resistance to re-infection with a large number of trypanosomes; however, it is maintained when challenged with a few parasites.

Table 122. Acute Infection Models—Summary of Nonclinical Studies Supporting Activity of Nifurtimox in Animals Infected With *T. cruzi.*

***T. cruzi* Strains/Clones (Reference)**	**Study Summary**
	Mice
8 strains: WBH, Y and Belo Horizonte (Brazil), Cardoba (Argentine), WBH_{1F}, Tulahuen (Chile DTU VI), Ecuador, and one strain from USA) [Study Reports PH-1918 (Haberkorn 1970b), -2098 (Bock et al. 1970), -2451 (Haberkorn and Gönnert 1970), R-258 (Study Report R-258: Preclinical study report n.d.); and Haberkorn and Gönnert (Haberkorn and Gonnert 1972)]	Several experiments were performed. Briefly, CF_1 and/or NMRI mice were infected SC with 10^6 trypanosomes of either of the 8 strains of *T. cruzi*. The mice were treated SC with different doses of NFX to measure survival (4x25 mg/kg and 4x50 mg/kg) or cure rates (4x500 mg/kg and 10x10 mg/kg). The time of initiation of treatment was not specified. The authors state that there was marked myotropy of the Ecuador strain and particularly severe course of the disease after infection with the Tulahuen strain, which reproduces very readily in nearly all organs. Parasitological evaluations were based on xenodiagnosis as well as the subinoculation of blood to naïve mice for the indirect demonstration of parasites, and histological observations. The survival time of untreated mice (NMRI and CF_1) infected with the 8 different strains varied between mean of 9.8 and 15.1 days; similarly, the mean survival time for treated mice infected with the different strains varied between 25 and 62 days and cure rates varied between 0 and 100%. Tulahuen strain was least sensitive to treatment; all mice died by Day 25 and none of the mice were cured. In another experiment, mice were infected SC with 9000-14000 trypanosome forms of the Ecuador strain and treatment with different doses (5x500, 10x250, or 10x100 mg/kg) of NFX initiated either 1- or 14-days PI. No microscopically demonstrable parasitemia was observed after the end of treatment; a negative xenodiagnosis and no demonstration of parasites in histological sections as well as in cell smears from the liver, spleen, lungs, brain, kidneys, heart, skeletal muscles (upper thigh), bladder, epithelium. NFX was more effective in improving survival time when administered 2 days PI compared to 14 days PI. The NFX doses of 10x250 and 10x100 mg/kg, initiated 2 days PI, were most effective and increased the mean survival time to 175 and 205 days, respectively. In another experiment, the effect of NFX was measured in mice infected with different inoculum concentrations (10^2, 10^4, or 10^6) of trypanosomes; CF_1 mice were infected with the Tulahuen strain and mice followed for 49 days whereas NMRI mice were infected with the WBH strain and followed up to 65 days. Mice were treated with either 0, 25, 50 or 100 mg/kg of NFX for 4 days and followed up to Day 49. The results show an increase in the survival time with a decrease in the inoculum concentration; 100 mg/kg NFX dose was most effective. WBH strain was more sensitive to treatment compared to the Tulahuen strain. Parasitological cure was observed in 6 of 6 and 2 of the 6 CF1 mice infected with 10^2 trypanosomes of the WBH strain and treated with NFX doses of 50 or 100 mg/kg doses, respectively; in other groups, the activity of NFX was suppressive as parasitological cure was not reported. Time of initiation of treatment was not specified. In another experiment, the effect of a single dose (25, 50 and 100 mg/kg) of NFX, administered by different routes was compared in mice infected with the

***T. cruzi* Strains/Clones (Reference)**	**Study Summary**
	WBH, Y, and Tulahuen strains of *T. cruzi*. Treatment was started on Day 1 PI. The results showed oral and SC routes of drug administration were most effective in improving survival of mice; whereas the IV route was least effective in improving survival. In another experiment, the effect of 4 doses of NFX administered SC or orally was compared. The SC route of NFX administration was most effective in improving survival. In mice infected with the Ecuador strain and treated with 50 - 100 mg/kg of NFX for 5-10 days, there was a reduction in the number of pseudocysts in the skeletal muscles compared to that of untreated mice. Similarly, in mice infected with the Tulahuen strain and treated with one oral dose of NFX (1000 mg/kg), a week PI, there was 97-98% reduction in the number of nuclei in the liver and spleen 24 h post-treatment. Overall, the study supports the activity of NFX against the **amastigotes** of *T. cruzi*. In mice treated with NFX either 1 h before, at the same time or 1, 2, and 4 h PI, NFX (oral dose of 500 mg/kg for 6 days) delayed the development of parasitemia (Days 11-15) compared to untreated mice (Day 4) suggesting activity against the **trypomastigotes**. All the untreated mice died between Days 15-17 (Mean 16 days) whereas the mean survival time in treated mice was 40 (range 13->63) days. Addition of NFX up to 1000 ppm to mouse blood for at least 8 h at 22° decreased the number of trypanosomes from 10^7 to 10^3 and at 2 h to 10^2; the infectivity of the blood to naïve mice was also reduced. In another experiment, CF_1 mice infected with the Tulahuen strain were treated with low SC dose of NFX (50 mg/kg) for 4 days between Days 5 and 8 PI and the parasite burden measured in different organs. In the brain, heart, lungs, kidneys and bladder parasite burden decreased rapidly after three doses; the few residual parasites found on Days 9, 10 and 14 PI in the spleen, due to inadequate treatment, could lead to a relapse. In CF1 mice infected with the Tulahuen strain and treated with a single dose of NFX (1000 mg/kg on Day 7 PI), trypanosomes were not detected within 26 h post-treatment; however, this was followed by a slight increase on Day 15 and a gradual decline. Histological assessment showed morphological changes in the trypanosomes ~10-12 h after NFX administration. The nucleus appeared larger and the cytoplasm contained vacuoles and the parasites distended. The authors state that at first these forms cannot be distinguished from the "stout" trypanosomes, as they occur in every blood smear from animals infected with *T. cruzi*. These stout forms when compared with the slender forms of trypanosomes apparently represent a degenerate form and are not able to penetrate into cells. In mice infected with the WBH or Tulahuen strains, repeat dosing and longer treatment duration that include a high loading dose followed by small doses of NFX were more effective in improving survival compared to single dose treatment. In one experiment, the effect of delay in treatment with NFX was evaluated in mice infected with the WBH or Tulahuen strains. The results show that oral or SC administered NFX is least effective in improving survival when administered on Day 1 PI. NFX doses of 100 and 250 mg/kg were most effective when treatment was initiated on ≥ Day 5 PI (when there is maximal metabolic activity by the parasites).

***T. cruzi* Strains/Clones (Reference)**	**Study Summary**
	There does not appear to be any prophylactic activity of NFX.
Tulahuen [Study Report PH-1777 (Haberkorn 1970a)]	NMRI infected mice were treated with 250 / 200, 100 or 50 mg/kg NFX in the form of 1 to 6 pulses, each lasting 5 days. Treatment pulses were separated by a 9-day period without treatment. In one group, the intervals between treatments lasted 3 weeks. The results show that continuous treatment (20x50 mg/kg) was better than intermittent treatment (5x250 mg/kg followed by 5 x 5 doses of 200 mg/kg).
30 strains: 3 Type I (Y-Brazil; Peruvian-Peru; Buenos Aires- Argentina), 14 Type II (from Mambai, Sao Felipe, and San Luis) 13 Type III (from Montalvania, La Pampa, Santa Cruz la Sierra, and Columbia [Colombiana]) (Andrade et al. 1985)	Mice were infected IP with $2x10^5$ blood stage (trypomastigotes) of different *T. cruzi* strains maintained by serial passage in mice; mice were treated orally with 200 mg/kg/day NFX for 4 days followed by 50 mg/kg/day up to 90 days; BZN (comparator) was administered at a daily dose of 100 mg/kg for 90 days. Time of initiation of treatment was not specified. The authors state that Type I strain is characterized by a rapid course of infection in mice, high levels of parasitemia and mortality around the 9th and 10th day PI; there is predominance of slender forms and macrophage-tropism during the acute phase of the infection. Type II strains are characterized by increasing parasitemia from the 12th to the 20th day of infection, a low rate of mortality, a predominance of broad forms of the parasites, and myocardial tropism. Type III strains show a slow development of parasitemia that reaches a high level 20-30 days after inoculation, a low mortality, and predominance of parasitism in skeletal muscles. The activity was measured by direct microscopic examination, xenodiagnosis, inoculation of blood in new-born mice, hemoculture and a serological test (IFA). Cure rates were based on parasite clearance in surviving mice between Days 30-90 post-treatment. The cure rates of mice infected with the Type I stains were between 35% and 87%; Type II strains between 0% and 100%; and Type III strains 0% against all strains except 1 (Strain 1-MONT). Type I strains displayed high susceptibility, Type II strains medium to high susceptibility, and Type III strains were highly resistant. The authors state that all animals that remained infected after treatment were seropositive and antibody titer ranged between 1:10 to 1:80 by the IFA test. The antibody titer was ≤1:10 in cured mice; however, 82.0% were seropositive.
5 strains: Sonya clone*, Colombiana*, Tulahuen**, Y** and Peru**. (Neal and van Bueren 1988)	CD-1 mice were infected SC with 10^5 trypomastigotes of the 5 strains of *T. cruzi*. Oral treatment with NFX (30-120 mg/kg for 28-30 days) was initiated about 7 days PI, when parasitemia occurred. Mice were followed for parasitemia by microscopic examination of blood collected at the end of dosing and 28 days later. If all samples were negative, blood cultures were performed in triplicate. Mice were necropsied and tissues (skeletal muscles from the back of the thigh, heart, bladder, kidney, liver, esophagus, ileum, colon and spleen) collected were processed for histological examination of pseudocysts. In all untreated mice infected with either of the strain, high parasitemia was reported leading to death. The mean time to death was 13.8 days for Tulahuen and Y strains; 14.8 days for Peru strain; 17.9 days for Sonya clone; and 21.1 days for Colombiana strain. Semiquantitative analysis of the pseudocysts in different tissue showed that Tulahuen, Y and Peru strains infected all tissues including muscles; the greatest density of pseudocysts was found in tissues of the reticulo-endothelial system. However, for the Colombiana and Sonya strain/clone, the heaviest infection, was in the muscle tissue and liver. Pseudocysts were observed in the muscle layer of the bladder with all strains of *T. cruzi*, but only Colombiana showed scanty pseudocysts in the esophagus and colon. Apart from sporadic occurrence of pseudocysts of Y and Peru

***T. cruzi* Strains/Clones (Reference)**	**Study Summary**
	strains, kidney sections were uniformly negative. No pseudocyst was observed in any of the other tissues examined. Treatment with NFX (100 or 120 mg/kg/day) was effective in curing a majority of the mice infected with the responsive strains [Tulahuen (91%), Y (100%) and Peru (64%) strains]. However, all mice infected with the Colombiana strain or Sonya clone remained hemoculture positive. Survival of treated mice at different time points was not shown.
12 SF or Montalvania strain: Type II (Andrade et al. 1991)	Swiss mice were infected IP with ~10^5 blood stage parasites. NFX dose and duration was same as summarized above for the study by Andrade et al. (Andrade et al. 1985). BZN was administered 5 days a week for 90 days. Parasitological response was measured by xenodiagnosis and hemoculture at 3-6 months after the end of treatment. During the acute phase of the infection, the untreated positive control mice developed high levels of parasitemia 7-10 days PI and were euthanized when parasitemia was maximum. Parasitological cure was reported in 82.7% of the mice, treated for 90 days, with either NFX or BZN. Cured mice were persistently serum antibody positive by the IFA test, using culture forms of *T. cruzi* parasites, for 3-6 months after therapy; the titers varied between 1:10 to 1:160. The uncured mice as well as the chronically infected, untreated controls mice were parasitemic and had higher antibody titers between 1:160 to 1:640. Spleens from treated and untreated mice were processed for immunoelectron microscopy; briefly, sections were incubated with polyclonal rabbit anti-*T. cruzi* antibody followed by anti-rabbit immunoglobulin conjugated with peroxidase and reaction developed with diaminobenzidine and hydrogen peroxide. The sections were then treated with osmium tetroxide and processed for electron microscopy. Immunolabeling on the external membrane of dendritic cell processes in the germinal centers of lymphoid follicles was reported in the spleen sections of all treated mice (parasitologically cured or uncured). These processes were broad, without phagosomes, and sometimes contained granular material. The labeling on the membrane appeared as fine granules and there were no parasite forms in the sections. In the spleen sections from mice that were pretreated with anti-*T. cruzi* antibody, the intracellular amastigote forms of *T. cruzi* were immunolabelled with peroxidase on the parasite surface membrane, intracellular vacuole membrane, and disintegrated fragments. However, in untreated mice no immunolabeling was observed. Overall, the study suggests that immunological memory can be maintained by certain cells such as dendritic cells in the spleen.
Peruvian (I), 21 SF (II), and Colombian (III) strains (Marretto and Andrade 1994)	Swiss mice were infected IP with blood form trypanosomes (0.5-1x10^5) and treated with NFX 100 mg/kg/day for 90 days. The cure rates (based on microscopic findings of parasites in blood, hemoculture and xenodiagnosis) and mortality varied with the strain: Peruvian – 15% died and 37.7% cured. 21 SF – 12% died and 75% cured. Colombian – 65% died and none were cured.

***T. cruzi* Strains/Clones (Reference)**	**Study Summary**
Y (TCII) (Urbina et al. 2003)	NMRIIVIC albino mice were infected with 10^5 trypomastigotes, IP, of the Y strain. Treatment with NFX (50 mg/kg/day for 28 days by gavage followed by a 7-day rest and 15 days of treatment) was initiated 4 days PI and mice followed until Day 60. A majority (75 – 82%) of the untreated mice died during the period of observation; all the surviving animals were parasitemic. All the treated mice survived the period of observation of >100 days and were aparasitemic; however, parasitemia relapsed in most animals, after 40 days PI.
Brazil (Bustamante et al. 2014)	C57BL/6 (Ly5.2$^+$) immunosuppressed mice were infected IP with 10^3 trypomastigotes of a Brazilian strain (BZN sensitive) of *T. cruzi*. Oral treatment with 100 mg/kg NFX was administered continuously for 40 days (Days 15 to 55 PI) or intermittently (once every 5 days for a total of 13 doses). The mice were immunosuppressed with cyclophosphamide (200 mg/kg/day) IP at 2 to 3-day intervals for a total of 5 doses between Days 100 and 112 PI. Hemoculture was performed for immunosuppressed mice every week for 2 months. On Day 120, no parasites were detected by PCR and there was no histological evidence of infection in the skeletal muscles of all treated mice compared to untreated mice. The frequency of *T. cruzi*–specific CD8$^+$ T cells on Day 90 PI (i.e., prior to immunosuppression) in treated mice was similar to untreated mice; however, the frequency of CD8-cell memory phenotype (TCM memory phenotype (CD127^{h}i) for CD8$^+$ TSKB20$^+$ T cells expressing CD127) in mice treated continuously or intermittently was higher compared to untreated mice.
Tulahuen (Moraes et al. 2015)	Swiss mice were infected with $5x10^5$ trypomastigotes IP and treated with NFX 10 mg/kg daily for 20 days, by gavage, initiated on Day 8 PI. No parasitemia was detected at the end of treatment. Relapse was observed after parasitemia negative animals were immunosuppressed IP with cyclophosphamide (3 cycles; each cycle consisted of 4 days of cyclophosphamide administration and 3-day rest period).
Ninoa (Contreras-Ortiz et al. 2017)	Balb/c mice were infected, IP, with 10 trypomastigotes and treated orally with 100 mg/kg/day NFX starting on Day 12 PI until parasites were not detected in the blood. Parasitemia was analyzed by fresh blood smear test, twice a week starting on Day 5, until Day 60 PI, or when parasitemia was undetectable microscopically in fresh blood preparations. Heart and spleen tissues were collected for histopathological as well as parasitological assessments, after mice died from infection or when euthanized. Control, untreated mice remained parasitemic and died by 22-28 days PI. NFX was effective in reducing parasitemia, compared to untreated mice; no parasites were detected ≥22 days PI. All treated mice survived up to 60 days of observation. NFX treatment had no effect on the size of heart although spleen size was reduced compared to untreated control infected mice. In the left ventricle of the NFX treated infected mice, no light focal lymphoplasmacytic infiltrate, necrotic cardiomyocytes, or amastigote nests, were observed; in untreated mice there were large number of amastigote nests and the myocardium displayed diffuse inflammatory infiltrates, represented mainly by lymphoplasmacytes. In the spleen of untreated infected mice, morphological changes observed include hyperplasia of lymphoid follicles and loss of characteristic shape; in NFX treated mice, lymphoid follicles were normal. Serum levels of malondialdehyde (MDA; used as a marker of oxidative stress induced by *T. cruzi* infection in the myocardium) were increased in mice treated with NFX compared to untreated mice suggesting induction of oxidative stress.

***T. cruzi* Strains/Clones (Reference)**	**Study Summary**
4 clinical isolates from Brazil (Berilo and Januária, Minas Gerais State) 501, 452, 728 (TcII) and 1337 (TcVI) N=4 (de Oliveira et al. 2017)	Swiss mice were infected with 10^4 blood trypomastigotes (previously maintained in mice) and oral treatment with 100 mg/kg NFX for 20 days was started on the 1st day parasitemia developed i.e., mice became patent. Mice were followed for survival and parasitemia until Day 90 PI. Cure was based on hemoculture, PCR, and conventional serological test (IgG anti-*T. cruzi*) and nonconventional serology by flow cytometry for detecting anti-live trypomastigotes IgG antibodies (FC- ALTA), at Days 90, 180 and 360 after treatment. Based on the cure criteria used, the therapeutic response was classified as resistant (cure rates ≤33%), partially susceptible (cure rates >33% <67%) and susceptible (cure rates ≥67%). Cardiac histopathological assessment was performed during acute and chronic phases, before and 360 days after NFX treatment. The development of parasitemia infected with different isolates varied; parasitemia in 1337 infected mice was low. The duration of prepatent and patent period also varied. The results show that parasitemia was reduced in NFX treated mice by Day 90; relapse occurred in mice infected with the isolate #728. Cure rates were higher and cardiac inflammatory infiltration decreased in mice treated in the acute phase. Collagen deposition was similar in the acute and chronic phase. None of the mice died. All mice, except for those infected with the isolate # 501, remained seropositive by the conventional ELISA; however, by the nonconventional assay, ≥63% of the mice became seronegative on Day 360 PI; 25% and 75% of the mice infected with the isolate # 501 became seronegative by the conventional and nonconventional assays, respectively.
Y (TcII) (Mazzeti et al. 2018)	Swiss mice were infected with 5 x 10^3 trypanosomes and treated with 50 mg/kg NFX for 7 days starting Day 5 PI. Untreated and treated mice were followed for parasitemia, survival and clinical signs that include appearance (hunched, piloerection, sunken eyes), changed behavior (withdrawal, vocalization, scratching, reluctance to move, irritability, anorexia, abnormal posture), and pain signs (writhing, twitching). Cure was based on parasitemia during and up to 60 days after treatment, cyclophosphamide immunosuppression 30 days after treatment and negative results in a real-time PCR assay on Days 30 and 180 post-treatment. The results show that all treated mice (infected with the Y strain) survived until Day 30 post-treatment; parasitemia was cleared by Day 7 but relapse occurred by 7 days after the end of treatment. All the untreated mice died. In another experiment, mice were treated with either 25 or 50 mg/kg for 20 days or 50 mg/kg for 40 days. All mice treated with 50 mg/kg for 20 or 40 days survived; 6 of 7 (85.7%) mice treated with 20 mg/kg dose for 20 days survived. No parasites were detected in the blood. However, cure (based on parasitemia during and up to 60 days after treatment, cyclophosphamide immunosuppression 30 days after treatment and negative results in a real-time PCR assay on Days 30 and 180 post-treatment) was reported in 14.3% and 42.9% mice treated with 50 mg/kg NFX for 20 or 40 days, respectively; none of the mice treated with 20 mg/kg dose for 20 days were parasitologically cured.

***T. cruzi* Strains/Clones (Reference)**	**Study Summary**
	Rats
Tulahuen and Ecuador [Study report PH-2451 (Haberkorn and Gönnert 1970) and Haberkorn and Gönnert (Haberkorn and Gonnert 1972)]	Wistar rats were infected SC with 10^4 trypomastigotes; oral treatment was initiated on Day 1 PI with 0 or 100 mg/kg of NFX for 12 days. All rats, treated and untreated, survived until Day 365 PI. No parasitemia was reported in treated rats whereas untreated rats had moderate parasitemia.
	Hamsters
Tulahuen [Study report PH-2451 (Haberkorn and Gönnert 1970) and Haberkorn and Gönnert (Haberkorn and Gonnert 1972)]	Golden hamsters were infected SC with 10^4 trypomastigotes; SC treatment was initiated on Day 1 PI with 0, 100 or 200 mg/kg of NFX for 2 days; animals were followed to 120 days PI. The results show that 250 mg/kg dose was effective in suppressing parasitemia and all animals survived up to Day 120. At a lower dose of 100 mg/kg, parasitemia was suppressed; however, all hamsters died between Days 17 and 28. Untreated animals died between Days 9 and 18 and had mild to moderate parasitemia.
	Guinea pigs
Tulahuen [Study report PH-2451 (Haberkorn and Gönnert 1970) and Haberkorn and Gönnert (Haberkorn and Gonnert 1972)]	Guinea pigs were infected SC with 2.7 x 10^4 trypomastigotes; SC treatment was initiated on Day 1 PI with 0 or 200 mg/kg of NFX for 2 days. NFX was not effective in improving survival; untreated mice died on Day 23 and 26 whereas the 2 treated animals died on Day 26.
	Cats
Tulahuen [Study report PH-2451 (Haberkorn and Gönnert 1970) and Haberkorn and Gönnert (Haberkorn and Gonnert 1972)]	Young (3 weeks old) and older (8 to 10 weeks old) cats were infected SC with either 6.5 x 10^5 or 10^4 trypomastigotes, respectively and treated with 0 or 250 mg/kg of NFX for 7 days; for older cats, treatment was initiated either on Day 7 or 13 PI; for younger cats, treatment was initiated on Day 1 PI. NFX was not effective in improving survival; older treated cats died between Days 36 and 50 and untreated cats on Day 50. All the younger cats died on Day 47 PI. However, parasitemia was reduced in both older cats treated with NFX; 1 of the 2 younger cats remained parasitemic.
	Dogs
WBH or Tulahuen [Study report PH-2451 (Haberkorn and Gönnert 1970) and Haberkorn and Gönnert (Haberkorn and Gonnert 1972)]	Mongrel and beagle dogs were infected SC with 10^4 trypomastigotes; oral treatment was initiated between 1- and 13-days PI. Dogs infected with the **WBH** strain were treated with either 0, 100, 250 or 350 mg/kg of NFX for 1, 3, 4 or 6 days; the treatment start day varied between Days 6 and 29; the number of doses and the treatment start day varied for each dog. One untreated dog survived until Day 12 whereas the 2nd dog survived until Day 223. The survival time for treated dogs varied between 11 and 223 days. Parasitemia was decreased in treated dogs compared to untreated animals. **Tulahuen** infected dogs were treated with 50, 75, or 100 mg/kg dose of NFX for 2 to 15 days; treatment start date was Day 12 for 3 dogs and Day 2 for one dog. The 3 dogs treated with either 75, 100 mg/kg for 10 -12 days or one dog treated with 50 mg/kg NFX for 13 days followed by 100 mg/kg for 2 days survived up to Day 152. One dog treated with 50 mg/kg NFX for 10 days developed pneumonia and died on Day 21. The untreated dogs died on Days 15, 21 and 33 and 152. Parasitemia was reduced in treated dogs compared to untreated dogs.

*represent non responsive; **represent responsive; #partially responsive strains

Abbreviations: BZN, benznidazole; IFA, indirect fluorescent antibody test; IP, intraperitoneal; NFX, nifurtimox; PBMC, peripheral blood mononuclear cells; PI, postinfection; SC, subcutaneous; WT, wild type

Table 123. Subacute and Chronic Infection Models—Summary of Nonclinical Studies Supporting Activity of Nifurtimox in Animals Infected With *T. cruzi.*

***T. cruzi* Strains/Clones (Reference)**	**Study Summary**
	Mice
Tulahuen [Study report PH-2451 (Haberkorn and Gönnert 1970) and Haberkorn and Gönnert (Haberkorn and Gonnert 1972)]	Mice were treated with NFX (100 or 250 mg/kg for 10 days) 2 months PI with 10^2-10^3 trypanosomes SC. The survival rate of treated mice at Months 6 and 8 was 84% and 79%, respectively, compared to 75% in untreated infected mice and 6% in uninfected untreated mice. The heart weight was decreased by ~13 to 22% in treated mice compared to untreated mice.
Tulahuen (Meckert et al. 1988)	BALB/c mice were infected IP with 25 trypomastigotes and followed for parasitemia weekly by direct microscopic examination as well as by subinoculation of naïve mice; mice which became parasitemic during Month 1 were treated with NFX (100 mg/kg/day for 30 days by the intragastric route) or vehicle at Month 3 PI. Two months after the end of 30-day treatment, no parasites could be recovered by subinoculation into suckling mice. No mortality was observed in either the untreated or treated mice. Anti-*T. cruzi* antibodies were detected by conventional IFA and HA test in the sera of all treated and untreated mice; however, complement-dependent lysis of the parasite or ADCC activity could not be detected after treatment, although they were present in the sera of most of the untreated mice (Table 124). There was no difference in cell-mediated response in treated and untreated mice. A group of infected treated and untreated mice were re-infected with either 25 or 2500 trypomastigotes. Mice re-infected with 2500 trypomastigotes developed higher parasitemia increased mortality compared to untreated mice; however, when re-infected with 25 trypomastigotes, treated and untreated animals showed similar parasitemia and mortalities. Overall, the study suggests that that treatment with NFX leads to a loss of resistance to re-infection with a large number of trypanosomes; however, it is maintained when challenged with a few parasites.
4 clinical isolates from Brazil (Berilo and Januária, Minas Gerais State) 501, 452, 728 (TcII) and 1337 (TcVI) N=4 (de Oliveira et al. 2017)	Swiss mice were infected with 10^4 blood trypomastigotes (previously maintained in mice) and oral treatment with 100 mg/kg NFX started on Day 90 PI; otherwise, experimental design was similar to that summarized above for the acute infection model. Like for the acute infection model, the cure was based on hemoculture, PCR, and conventional serological test (IgG anti-*T. cruzi*) and nonconventional serology (flow cytometric anti-live trypomastigotes antibodies – IgG antibody by FC- ALTA), at Days 90, 180 and 360 after treatment. The development of parasitemia infected with different isolates varied; parasitemia in 1337 infected mice was low. Similarly, the growth rate was shown to vary in vitro in axenic cultures as well as coculture with Vero cells. The duration of prepatent and patent periods also varied. The results show that parasitemia was reduced in NFX treated mice by Day 90. Cure rates were higher and cardiac inflammatory infiltration lower when mice were treated in the acute phase compared to chronic phase. NFX treatment during the chronic phase did not induce any changes in cardiac inflammation.

***T. cruzi* Strains/Clones (Reference)**	**Study Summary**
	Collagen deposition was similar in the acute and chronic phase. None of the mice died. All mice remained seropositive by both conventional and nonconventional assays on Days 90, 180 and 360 PI.
	Rabbits
Ernestina (Teixeira et al. 1990)	New Zealand white rabbits were infected SC with 10^6 trypomastigotes/kg body weight. Parasitemia was monitored by xenodiagnosis and repeated every 15 days for 6 months and every month thereafter; xenodiagnosis was performed using 20 first instar nymphs of the reduviid bug (*Dipetalogaster maximus*). A positive diagnostic test was indicated by the presence of the parasite in the feces of bug fed on the rabbit's blood; feces were examined 30 and 60 days after exposure. Treatment with NFX (8 mg/kg/day for 60 days) was administered IP. The time of initiation of treatment was not specified. Complete necropsies were performed on each rabbit and all organs and tissues were processed for histopathological evaluations. Parasitemia in infected rabbits lasted for 5 months, after which time the parasites disappeared spontaneously. Compared to untreated rabbits, treatment with NFX reduced the duration of parasitemia by 63%. Myocarditis was reported in NFX treated infected rabbits. Lymphomas were reported in treated infected and noninfected rabbits administered NFX.
	Dogs
Ecuador Tulahuen [Study report PH-2451 (Haberkorn and Gönnert 1970) and Haberkorn and Gönnert (Haberkorn and Gonnert 1972)]	Beagle dogs were infected IP with 50,000 trypanosomes of the Ecuador strain and parasitemia followed by microscopic examination and inoculation of naïve mice at regular intervals. Parasitemia by microscopy was reported 11 days PI that lasted up to 25.6 (±6.7) days; the maximal parasite density was 253 (range 80-520)/3 mcL blood. Body temperature increased 1-3 days before parasitemia and during parasitemia. Lyophilized blood from each animal, collected once a month was tested for antibodies by CFT, HA and IFA tests. Xenodiagnosis was also conducted. ***Subacute infection:*** Treatment was started in 7 dogs (4 male, 3 female) 46 days after inoculation with the Ecuador strain; the authors state that at this time the last positive microscopic demonstration of trypanosomes had been made at least a week previously in 8 of the 14 infected animals. Only 4 animals, one of which was from the group intended for treatment, showed parasitemia on the day of treatment. At the time of beginning of treatment, the body temperature in all animals was normal again. Lampit (gelatine capsules) was administered daily; 30 mg/kg for 15 days followed by 20 mg/kg daily for 75 days. Blood from dogs, collected a 1½ month after treatment, i.e., 6 months PI, was able to induce infection in naïve mice; however, during the 1½ year postobservation, blood from none of the dogs tested positive in mice. By the IFA test, 11 of 14 infected dogs were positive one-month PI; all dogs were negative at the end of study i.e., 7 months PI. The untreated 7 dogs remained seropositive until the end of the study. By the CFT and HA test, 4 months after infection, the untreated dogs were seropositive. The treated dogs were seronegative by the end of the experiment. By the CFT, the treated dogs were seropositive before that of the control animals; this may be due to massive decomposition of parasites by NFX leading to a rapid formation of antibodies than in the untreated controls. One month later the CFT was positive in only one animal and at Month 5, after

T. cruzi Strains/Clones (Reference)	Study Summary
	the end of treatment, the CFT remained negative until the end of the experiment in all dogs. The results in the dogs showed corresponding serological behavior comparable to that observed in untreated subjects or in the subjects treated towards the end of the acute phase of the infection. There was no change in liver and kidney function. In the electrocardiograms obtained 21- and 25-months PI, 3 animals showed marked changes and one animal showed slight changes. One of the dogs was noninfected and untreated; another was infected untreated; and for the 3rd whose treatment had started 1½ years PI, there was a slight increase in ST segment. ***Chronic infection:*** Of the 7 untreated dogs from the above subacute infection study, 4 were treated with Lampit (20 mg/kg/day for 15 days followed by 16 mg/kg for 105 days) 1½ years PI, for 120 days. No parasites were detected by subinoculation of naïve mice from the blood of dogs for the duration of the study. In the chronic phase of the infection, the treated dogs did not become seronegative. The postobservation period, however, lasted only four months.

Abbreviations: ADCC, antibody dependent cell-mediated cytotoxicity; BZN, benznidazole; CFT, complement fixation test, HA, hemagglutination test; IFA, indirect fluorescent antibody test; IP, intraperitoneal; NFX, nifurtimox; PBMC, peripheral blood mononuclear cells; PI, postinfection; SC, subcutaneous; WT, wild type

Table 124. Anti-*T. cruzi* Humoral Immune Response in Mice

Chagasic mouse group	No. positive/total no. of mice			ADCC (% of lysis ± SD) with effector cells:		
	Hemagglutination[a]	Immunofluorescence[b]	Complement-dependent lysis[c]	Polymorphonuclear leukocytes	Blood mononuclear leukocytes	Spleen mononuclear cells
Nontreated	13/14	13/14	12/14	69 ± 8[d]	58 ± 12[e]	57 ± 13.5[f]
Nifurtimox treated	9/11	10/13	0/13	17 ± 10.4[d]	17.6 ± 13[e]	13.6 ± 9.9[f]

[a] Titer higher than 1/40.
[b] Titer higher than 1/32.
[c] Positive, Lysis higher than 30%.
[d] $P < 0.0005$ (Student's t test).
[e] $P < 0.05$ (Student's t test).
[f] $P < 0.025$ (Student's t test).

18.6.1.4. Drug Resistance

A potential for development of resistance to nifurtimox was measured in vitro and in vivo.

18.6.1.4.1. In Vitro

Neal et al. (Neal et al. 1989) reported development of resistance by the epimastigotes of the X10 strain (clone 1) of *T. cruzi* in vitro; increasing concentrations of nifurtimox up to 20 mcM (5.7 mcg/mL) were added and the growth of the parasites measured by incorporation of ^{3}H thymidine. The results showed about a 3-fold increase in the IC_{50} values (7 mcM to 20 mcM i.e., 2.0 and 5.7 mcg/mL, respectively). The resistance was reversed by the P-glycoprotein (Pgp) inhibitor, verapamil (1 mcM) and the nifurtimox IC_{50} was 2.4 mcM (0.7 mcg/mL); verapamil had no activity against *T. cruzi* at up to 8-fold higher concentration.

Similar observations were reported by Wilkinson et al. (Wilkinson et al. 2008) with the epimastigotes of clone MHOM/BR/78/Sylvio - X10/6 in the presence of 10 mcM (2.9 mcg/mL) nifurtimox incubated for 2 months at 27°C and subcultured; this was repeated every 3-4 weeks for 6 more months in the presence of nifurtimox; the resistant parasites were cloned. The IC_{50} value increased by 4-fold and the rate of growth of the parasites was 2-times faster in the presence of drug compared to the parental strain; unlike the parental strain, resistant clones continued to grow beyond 1 week.

Neal and van Bueren (Neal and van Bueren 1988) reported a difference in the time to development of resistance by the epimastigotes of 3 strains of *T. cruzi* (Sonya clone, Colombiana, and Y) grown continuously in the presence of nifurtimox, at an initial concentration of ~10% suppressive value for the parent strain. After 5 to 10 successive subcultures, the drug concentration was increased. The results in Table 125 show that the rate of development of resistance to nifurtimox, at a concentration of 3 mcM (i.e., 0.9 mcg/mL, respectively) was similar for the Sonya clone and Y strain and about 2-fold higher for the Colombiana strain; however, at a higher nifurtimox concentration (10 mcM i.e., 2.9 mcg/mL), the time to reach resistance was higher for the Y strain, compared to the Sonya clone.

Table 125. Rate of Development of Resistance to Nifurtimox by Cultures of *T. cruzi*

Strain	Nifurtimox Concentration (mcM)	Time to Reach Resistance (Days)
Y	3	17
	10	108
Sonya clone	3	18
	10	31
Colombiana	3	32

In another experiment, epimastigotes obtained from mice infected with the Sonya clone after 28 days of treatment with 100 mg/kg/day of nifurtimox or 120 mg/kg/day of benznidazole were resistant to nifurtimox in vitro; the 50% suppressive concentrations were 30-fold higher for parasites subcultured from treated mice compared to untreated mice. Epimastigotes from mice treated with benznidazole were also less sensitive to nifurtimox suggesting cross-resistance.

18.6.1.4.2. In Vivo

Haberkorn and Gonnert (1972) reported a low potential for development of resistance in mice infected with the Tulahuen strain. Briefly, mice were treated with 10 mg/kg nifurtimox between Days 2 and 5 postinfection. Inoculation from one passage to the next was made from those animals that responded the least, or not at all, to treatment; the nifurtimox dose was increased to 25, 50, 100, and 250 mg/kg. The parasites were considered resistant when all treated animals, at the respective doses, showed the same course of infection (i.e., parasitemia and survival time) as the untreated controls. No nifurtimox resistant strain was selected over a period of two years under the experimental conditions tested. Tulahuen strain is considered to be resistant and difficult to treat in mice (for details see Section 20.6.1.3). Strains, other than Tulahuen strain, was not tested under the same experimental conditions.

Filardi and Brener (Filardi and Brener 1987) reported the activity of nifurtimox against 47 strains of *T. cruzi* (frozen in liquid nitrogen in the cryobank, WHO Collaborating Centre for the Cryopreservation of American Trypanosoma - maintained at the Laboratory of Chagas Disease in the Centro de Pesquisas Rene Rachouin in Belo Horizonte). Of the 47 strains, 26 were isolated from humans, 7 from the intradomiciliary vector *Triatoma infestans*, 8 from the sylvatic vector *Panstrongylus megistus*, 1 from a domestic cat, and 5 from wild reservoirs. Albino mice were infected with 10^4 *T. cruzi* blood forms and treated orally from Day 4 postinfection for 20 days, with either 100 mg/kg/day of nifurtimox or benznidazole. Blood was examined microscopically for living flagellates starting 10 days after treatment and performed 2 to 5 times over a period of 15 days. Hemoculture was performed 30 to 45 days after the end of treatment on treated and surviving mice. Anti-*T. cruzi* antibodies were measured by IFA test using formalin-fixed amastigotes and trypomastigotes grown in Vero cell cultures. The results show that the cure rates varied from 0 to 100% (Table 126). There appears to be a higher sensitivity of the strains from Rio Grande do Sul (Brazil) and Argentina (Figure 39C) compared to other strains (Figure 39 B and D). Strains from animal reservoirs also showed a wide range of drug sensitivity (Table 126 and Figure 39). The authors used an arbitrary level of 50% cure as a criterion for discriminating naturally resistant- and sensitive- *T. cruzi* strains; 9 strains (19.1%) were considered as resistant to nifurtimox; of the 9 nifurtimox resistant strains, 6 (66.7%) were resistant to benznidazole as well. It is unclear if the cure rates were based on hemoculture and/or IFA. The authors state that agreement between hemoculture and IFA tests was 92.9%; 2.6% of the treated mice gave negative hemoculture and positive IFT.

Table 126. Percentages of Cure in Mice Inoculated With *T. cruzi* Strains From Different Origins and Geographical Areas, Submitted to Specific Treatment With Nifurtimox and BZN

T. cruzi strain	Geographical area	Origin	Benznidazole % cure	Nifurtimox % cure
Noel	Minas Gerais	Acute case	0	0
Gilmar	"	"	100	100
J	"	"	100	100
Generoso	"	"	81	81
JAT	"	"	86	100
JM	"	"	80	73
AAS	"	"	40	60
Basileu	"	"	100	100
Silvio	"	"	69	31
MVC	"	"	100	100
VL10	"	Chronic (Ind.)*	27	15
PNM	"	Chronic (Dig.)*	93	100
ABC	"	Chronic (Card.-Dig.)*	40	47
7557	"	Chronic (Card.)*	93	100
Berenice	"	Chronic (Ind.)	93	80
JA	Goiás	Chronic (Card.-Dig.)	87	93
Ernane	"	Chronic (Card.-Dig.)	79	100
SMJ	"	Chronic (Card.)	87	100
Y	São Paulo	Acute case	47	47
DM	Rio Grande do Sul	Chronic (Card.-Dig.)	93	100
S. Felipe	Bahia	Chronic	64	92
Luna	Argentina	Acute case	100	100
Ibarra	"	Chronic	100	100
CA1	"	Chronic (Card.)	93	93
Romano	"	Acute case	100	100
Colombiana	Colombia	Chronic	7	0
CL	Rio Grande do Sul	*Triatoma infestans*	100	93
FL	"	"	93	100
MR	"	"	100	93
Buriti	"	"	100	100
Guaraizinho	"	"	93	93
Barra Seca	"	"	100	100
YuYu	Bahia	"	0	0
SC-16	Santa Catarina	*Panstrongylus megistus*	47	54
SC-17	"	"	60	21
SC-18	"	"	80	60
SC-19	"	"	80	50
SC-20	"	"	73	47
SC-25	"	"	93	71
SC-7	"	"	100	100
SC-1	"	"	75	86
A-88	Santa Catarina	*Didelphis azarae azarae*	100	60
Chaninha	Minas Gerais	*Felis domesticus*	79	100
EXR 426/1	São Paulo	*Didelphis marsupialis*	100	100
EXR 442/7	"	*D. marsupialis*	93	100
EXR 424/3	"	*Philander opossum*	50	100
Mão Pelada	Minas Gerais	*Procyon cancrivorus nigripes*	100	100

*Ind.=indeterminate form, Dig.=digestive form, Card.-Dig.=Cardiac-digestive form, Card.=cardiac form.

Figure 39. Percentage of Cure in Groups of Mice Inoculated With Different *T. cruzi* Strains and Treated With Nifurtimox or Benznidazole

A: Distribution of drug sensitivity of all 47 strains tested;
B: Strains isolated from humans in Minas Gerais, Brazil;
C: Strains isolated from humans or domestic vectors in Rio Grande do Sul (Brazil) and Argentina;
D: Strains isolated from Goias, Sao Paulo and Bahia (Brazil);
E: *T. cruzi* strains isolated from wild animal reservoirs and vectors in Minas Gerais, Santa Catarina and Sao Paulo (Brazil)

18.6.1.4.3. Mechanism of Drug Resistance

Role of P-glycoprotein (Efflux Pump)

Neal et al. (Neal et al. 1989) reported reversal of resistance in the presence of verapamil, a Pgp inhibitor (for details see Section 20.6.1.4.1) suggesting a role of the efflux pump in inducing resistance.

Role of Type I Nitroreductase

Wilkinson et al. (Wilkinson et al. 2008) reported that an 800-kbp band, representing Type I *NTR,* present in the parent strain was missing in the resistant clone generated in vitro. Trypanosomes with reduced NTR enzyme levels were resistant to nifurtimox, whereas cells overexpressing the enzyme were hypersensitive. NTR is essential for differentiation of noninfectious *T. cruzi* epimastigotes into metacyclic trypomastigotes, the life cycle stage infective to humans. Overall, the study shows that reduced expression of Type I NTR results in drug resistance.

Similar observations were made using *T. brucei* parasites (Wilkinson et al. 2008; Hall et al. 2010; Hall et al. 2011).

Role of Glutathione

Moncada et al. (Moncada et al. 1989) reported that resistance to nifurtimox may be due to higher glutathione concentration within the *T. cruzi* parasites. Studies were conducted using the epimastigotes of the LQ strain and Tulahuen strains of *T. cruzi*. Buthionine sulfoximine inhibited glutathione synthesis leading to increased activity of both nifurtimox and benznidazole against the *T. cruzi* parasites (for details see Section 20.6.1.1.1).

18.6.1.5. Cross-Resistance

Studies (Filardi and Brener 1987; Neal and van Bueren 1988; Wilkinson et al. 2008) in vitro and in vivo (animal models) suggest cross-resistance between nifurtimox and benznidazole (for details see Section 20.6.1.4). Similar observations were reported by Mejia-Jaramillo et al. (Mejía-Jaramillo et al. 2011; Mejia et al. 2012) using a benznidazole resistant strain generated in vitro from the epimastigotes of a sensitive Gal61 (DTU I) strain of *T. cruzi*; the resistant strain had an altered nitroreductase (*NTR*) gene (Wilkinson et al. 2008; Wilkinson and Kelly 2009; Hall et al. 2010; Hall et al. 2011).

Campos et al. (Campos et al. 2014) reported that benznidazole resistant clones, generated in vitro from the epimastigotes of the Y strain of *T. cruzi*, were cross-resistant with nifurtimox. Each clone had acquired a stop-codon-generating mutation in the gene which encodes the *TcNTR* that is responsible for activating nitroheterocyclic prodrugs. In addition, one clone had lost a copy of the chromosome containing *TcNTR*. There were no significant differences in the rate of amastigote replication or the extent of infectivity between the parental parasites and any of the drug-resistant clones.

18.6.2. Clinical Study 16027

The diagnosis of subjects at the time of enrollment was based on serological testing, done at the site laboratories, by Recombinant-ELISA and Lysate-ELISA. However, the clinical specimens, collected at different visits, were stored and analyzed by the four different serological tests and RT-qPCR in the central laboratory. Serological testing was conducted in batches, i.e., the tests were done once all samples of an individual patient collected at different visits, were received at the central laboratory. Concentration test was performed at the site laboratories.

18.6.2.1. Chagatest ELISA Recombinante v3.0 (Wiener Lab, Rosario, Argentina)

The Applicant used a 3rd generation Chagatest ELISA recombinante v3.0 (Wiener Lab, Rosario, Argentina). The assay is a qualitative test and the results are expressed as seropositive or seronegative based on the cut-off defined as the average of the negative control OD readings plus 0.300 OD. A copy of the package insert for the test available in Argentina, an approval summary of 510(k) safety and effectiveness information for the FDA cleared Chagatest ELISA recombinante v3.0 (Wiener lab, USA), a verification report for the testing conducted in the central laboratory, as well as quality control parameters implemented during testing of clinical trial specimens were provided.

Information From the Test Package Inserts (Argentina and USA)

The available information, in the Wiener Lab package inserts of the test from Argentina and the US (FDA cleared test), is summarized in Table 127. Briefly, anti-*T.cruzi* antibodies are detected against a cocktail of 6 recombinant *T. cruzi* antigens obtained using recombinant DNA techniques from proteins of the epimastigote and trypomastigote forms of *T. cruzi* corresponding to highly conserved zones among different strains. As anti-human immunoglobulins conjugated with peroxidase are used as a probe, both IgG and IgM are detected in the assay.

Although the Wiener Lab package insert is different from that of the US cleared test by the same manufacturer, the antigens as well as the cut-off used for characterizing a specimen as positive or negative are the same. Some of the differences include listing of interfering substances in the package insert of the test from Argentina and not in the US FDA cleared test. Additionally, the specificity values are slightly different; it is unclear if these differences are typographical errors or based on testing of different samples. The package insert for the FDA cleared test includes results of testing of different dilutions of two samples; the Argentina test does not include results of testing of different dilutions of any sample.

Table 127. Chagatest ELISA Recombinante v3.0—A Summary of the Information in the Wiener Test Package Inserts (Marketed in Argentina and the US) as Well as Verification in the Central Laboratory

Package insert (Wiener Lab, Argentina)	Additional Information From FDA Package Insert and 510k Approval Summary
Inactivated serum or plasma samples are added to microtiter plates with removable strips containing immobilized *T. cruzi* recombinant antigens [1, 2, 13, 30, 36 and SAPA]. If the sample contains specific antibodies, antigen-antibody complexes are formed that remain bound to the support. The unbound fraction is eliminated by washing and anti-human immunoglobulin antibodies conjugated with peroxidase are added and incubated. If the antigen-antibody complexes were produced in the first step of the process, the conjugate will be bound. The unbound conjugate is washed and enzymatic substrate added for the development of blue color. The reaction is stopped by adding sulfuric acid, and the color changes to yellow. OD readings are measured at 450 nm (spectrophotometer) or 450/620-650 nm (bichromatic). Reaction color is stable for 30 minutes; the results should be read within this period. Known interfering substances: hemolysis, hyperlipemia or other causes of turbidity may	Two reactive specimens, serially diluted in *T. cruzi* antibody nonreactive serum, were tested by 3 methods [Wiener Lab Chagatest ELISA rec. v. 3.0, Chagas' EIA 1 (Meridian Diag.) and Wiener Lab IHA (commercial Latin American Indirect Hemagglutination Assay kit)]. One specimen exhibited detectable reactivity in the Wiener Lab Chagatest ELISA rec v.3.0 at the sixth dilution; the other exhibited detectable reactivity at the fourth dilution. The results are shown in table below.

Package insert (Wiener Lab, Argentina)	Additional Information From FDA Package Insert and 510k Approval Summary
cause erroneous results. These samples must be clarified by centrifugation. The cut-off defined as average negative control readings plus 0.300 OD. Sensitivity: 100% in samples with positive xenodiagnosis and serology. 99.3% in samples with positive serology (HAI, IIF, another ELISA). Specificity: 98.7% in samples with negative xenodiagnosis and negative serology, 100% specificity in samples negative by serologic testing (IHA, IFA, another ELISA). Comparative analytical reactivity: No information included. A negative result does not exclude the possibility of exposure or infection by *T. cruzi*. Any reactive result must be verified by other technique such us IFA, CFT, IHA, DA, etc. It is stated that “Mind the "Fatala Chaben" Institute recommendations, according to which the immunodiagnosis of the infection should be performed with a minimum of two of the following methods: IFA, IHA, ELISA, (latex) particles agglutination, validated by the Centro Nacional de Referencia.” No information supporting the quantitative use of the assay based on OD was included.	**Table I: Wiener Lab Chagatest ELISA rec.v.3.0: Performance on Serial Dilutions of 2 Reactive Specimens, Comparison With Chagas' EIA 1 and Wiener Lab Indirect Hemagglutination Assay (IHA).** **Specimen Nr.1 (706007)** DILUTION: Wiener lab Chagatest ELISA rec v.3.0 — Absorbance 450/650 nm; s/c ratio. Chagas' EIA 1 — Absorbance 405 nm; S/c ratio. Wiener lab IHA — Result Neat[a]: >3.000; >9.87; 1.414; 2.45; + 1:2: >3.000; >9.87; 1.283; 2.22; + 1:4: >3.000; >9.87; 1.133; 1.96; + 1:8: >3.000; >9.87; 0.974; 1.69; + 1:16: 2.341; 7.70; 0.735; 1.27; + 1:32: 1.278; 4.20; 0.593[b]; 1.03; + 1:64: 0.469[b]; 1.54; 0.313; 0.54; + 1:128: 0.089; 0.29; 0.214; 0.37; + 1:256: 0.005; 0.02; 0.121; 0.21; ±[b] 1:512: 0.004; 0.01; 0.088; 0.15; - cutoff: 0.304; 0.577 [a] Serial dilutions in normal human serum, seronegative for *T. cruzi* [b] Highest dilution considered reactive. *s/c ratio, signal/cut-off ratio* **Specimen Nr.2 (907012)** DILUTION: Wiener lab Chagatest ELISA rec v.3.0 — Absorbance 450/650 nm; s/c ratio. Chagas' EIA 1 — Absorbance 405 nm; s/c ratio. Wiener lab IHA — Result Neat[a]: >3.000; >9.87; 1.090; 1.89; + 1:2: >3.000; >9.87; 0.834; 1.44; + 1:4: 2.964; 9.75; 0.704[b]; 1.22; + 1:8: 1.751; 5.76; 0.530[c]; 0.92; +[b] 1:16: 0.777[b]; 2.55; 0.381; 0.66; - 1:32: 0.308[c]; 1.01; 0.287; 0.50; - 1:64: 0.006; 0.02; 0.215; 0.37; - 1:128: 0.004; 0.01; 0.109; 0.19; - 1:256: 0.004; 0.01; 0.081; 0.14; - 1:512: 0.004; 0.01; 0.039; 0.07; - cutoff: 0.304; 0.577 [a] Serial dilutions in normal human serum, seronegative for *T. cruzi* [b] Highest dilution considered reactive. [c] Equivocal *s/c ratio, signal/cut-off ratio*

Package insert (Wiener Lab, Argentina)	Additional Information From FDA Package Insert and 510k Approval Summary
Verification in the [redacted] (b) (4) Lab: • Accuracy: 1.5% CVs reported based on 20 runs of positive and negative controls. • Sensitivity and Specificity: Not determined. • Agreement: For comparing agreement with other ELISAs (Lysate and F29), samples from 70* and 67* patients, respectively, were tested, however, no details of the of patient history were included; 42/70 (60%) specimens tested positive by the Recombinant-ELISA. Overall discordance among the 3 ELISAs was in 11/67 (16.4%) patients. • Discordance with: - ELISA Lisado (lysate-ELISA): 4 (5.7%) showed discordant results: ▪ 3 negative by Recombinant-ELISA were positive by Lysate-ELISA. ▪ 1 positive by Recombinant-ELISA was negative by the Lysate-ELISA. - F29 ELISA: Based on testing of samples from 67 patients, 9 (13.4%) showed discordant results; all 9 were positive by Recombinant-ELISA and negative by F29-ELISA. *The Applicant states that 69 samples were tested by the Recombinant- and Lysate-ELISA and 66 by F29-ELISA. However, on Pages 7-9 of the verification report, the results for 70 patients by the Recombinant-ELISA and Lysate-ELISA and 67 by the F29-ELISA were listed.	

Abbreviations: CFT, complement fixation test; DA, direct agglutination; ELISA, enzyme-linked immunosorbent assay; IFA, indirect fluorescent antibody test; IHA, indirect hemagglutination; OD, optical density

Verification in the Central Laboratory

The verification protocol for the qualitative method, of the 3 ELISAs, was consistent with the Clinical and Laboratory Standards Institute (CLSI) guidelines EP12 A2 - User Protocol for Evaluation of Qualitative Test Performance, 2nd Edition and the User Manual of the Ep Evaluator - V.11. Briefly, accuracy, based on 20 runs of positive and negative controls was adequate (CVs 1.5%). Based on testing of specimens from approximately 70 patients (no details of the patient history were included), 60% were positive by the Recombinant-ELISA; agreement with Lisado-ELISA and F29-ELISA was 95% and 86%, respectively. For details see Table 127.

Verification of the Recombinant-ELISA with the IHA test was not performed.

Quality Control During Testing of Clinical Trial Specimens in the Central Laboratory

In each run, the central laboratory processed approximately all samples from 15 patients, plus positive controls x 3 and negative controls x 3. The Applicant did not provide positive and negative controls for each run when clinical samples were tested. However, an example of the results of the positive and negative control of a batch run of February 21, 2018 was provided (Table 128) for the 15 patients [redacted] (b) (6)). Positive and negative controls of the other batch runs were stated to be similar.

Table 128. The Results of the Positive and Negative Control of a Batch Run From February 21, 2018

Batch run date: 21FEB2018						
	Recombinant ELISA			**Total Purified Antigen ELISA**		
NC results	0.007	0.001	0.003	0.024	0.027	0.027
NC average	0.003			0.026		
Cut off	0.303			0.226		
PC results	3	3.066	3.033	2.295	2.355	2.472
PC average	3.033			2.374		
NC … negative control; PC … positive control						

18.6.2.2. Chagatest ELISA Lisado (Wiener Lab, Rosario, Argentina)

Information From the Test Package Insert (Argentina)

Chagatest ELISA lisado (Lysate-ELISA) is a qualitative test that detects IgG antibodies against the cytoplasmic and membrane antigens of *T. cruzi*, from highly preserved areas of the parasite in serum or plasma specimens. The assay is a qualitative test and the results are expressed as seropositive or seronegative based on the cut-off defined as the average of the negative control OD readings plus 0.200 (for details see Table 129). Chagatest ELISA lisado is not cleared by the FDA.

Verification in the Central Laboratory

The verification protocol for the qualitative test was the same as that of the Recombinant-ELISA test summarized in Section 20.6.2.1. The accuracy based on 20 runs of positive and negative controls was adequate (CVs 3.5%). Based on testing of specimens from approximately 70 patients (no details of the of patient history were included), 63% were positive by the Lysate-ELISA; agreement with Recombinant-ELISA and F29-ELISA was 95% and 85%, respectively. For details see Table 127.

Verification of the Lysate-ELISA with the IHA test was not performed.

Table 129. Chagatest ELISA Lisado—A Summary of the Information in the Wiener Lab Test Package Insert as Well as Verification in the Central Laboratory

Package insert (Wiener Lab, Argentina):

- Antigen coated to microtiter plates and incubated with diluted test samples and controls; plates washed and incubated with conjugate (monoclonal antibodies goat-antihuman IgG antibody labelled with peroxidase) and plates washed. After enzyme substrate reaction, readout in a spectrophotometer. The OD is measured bichromatically at a wavelength 450/620-650 nm or at 450 nm.
- Heat inactivation may affect the result.
- Presence of interfering substances, such as autoantibodies, drugs, etc. may cause false positive reaction. No interference observed with bilirubin up to 21 mg/dL, ascorbic acid up to 50 mg/dL, triglycerides up to 1500 mg/dL or hemoglobin up to 300 mg/dL.

- Samples containing particles should be clarified by centrifugation.
- Reaction color is stable for 10 minutes. Thus, results should be read within this period.
- Run validation: run was valid if the following two conditions were met simultaneously:
  - The readings of at least 2 out of the 3 negative controls (NC) should be ≤0.150 OD.
  - The corrected mean reading of the positive controls should be 0.600 OD.
  - The difference between the OD average of the positive and negative controls should be ≥1.200.
- The cut-off:
  - Defined as the average of the negative control OD readings plus 0.200.
  - Grey zone: cut-off ±10%.
- Sensitivity: 100% assuming a prevalence of 100% in the presence of specific IgG antibodies detectable by other techniques (IFA, IHA, DA).
- Specificity: 99.2–99.6%.
- Any reactive result must be verified by another technique. It is stated that "Remember the criteria recommended by the Fatala Chabén Institute, whereby immunodiagnosis of infection must be performed with 2 of the following methods at a minimum: IFA, IHA and ELISA, duly validated by the National Reference Center."

Verification in the (b) (4) Lab:

- Accuracy: 3.5% CVs reported based on 20 runs of positive and negative controls.
- Sensitivity and Specificity: Not determined.
- Agreement: For comparing agreement with other ELISAs [Lysate-ELISA and F29-ELISA, samples from 70* and 67* patients were tested, however, no details of the of patient history was included; 44/70 (62.9%) specimens tested positive by the Lisado-ELISA. Overall, discordance among the 3 ELISAs was in 11/67 (16.4%) patients; discordance with
  - Recombinant-ELISA: 4 (5.7%) showed discordant results:
    - 3 positive by lysate-ELISA were negative by recombinant-ELISA.
    - 1 negative by lysate-ELISA was positive by the recombinant-ELISA.
  - F29-ELISA: Based on testing of samples from 67 patients, 10 (14.9%) showed discordant results; all 10 samples were positive by lysate-ELISA and negative by F29-ELISA.
- *The Applicant states that 69 samples were tested by the recombinant-ELISA and lysate-ELISA and 66 by F29-ELISA. However, on Pages 7-9 of the verification report, the results for 70 patients by the recombinant-ELISA and lysate-ELISA and 67 by the F29-ELISA were listed.

Abbreviations: DA, direct agglutination test; ELISA, enzyme-linked immunosorbent assay; IFA, Indirect fluorescent antibody test; IHA, indirect hemagglutination test; OD, optical density

Quality Control During Testing of Clinical Trial Specimens in the Central Laboratory

As for the Recombinant-ELISA, the central laboratory processed nearly all samples from 15 patients, plus positive controls x 3 and negative controls x 3 in each run. The Applicant did not provide positive and negative control results for all the runs. However, an example of the results of the positive and negative control of a batch run of February 21, 2018 was provided (Table 128). Positive and negative controls of the other batch runs were stated to be similar.

18.6.2.3. Chagatest HAI (Wiener Lab, Rosario, Argentina)

The Chagatest HAI detects agglutinating antibodies against the cytoplasmic and membrane antigens of *T. cruzi* coated on the sensitized red blood cells (RBCs). As the nonspecific antibodies such as heterophile antibodies (capable of agglutinating RBCs) can be present in infected and uninfected patients, their presence should be evaluated by incubating the serum with nonsensitized RBCs; the interfering antibodies are eliminated by treating them with 2-mercaptoethanol. Seropositive results are based on a smooth film of RBCs covering 50% or more of the bottom of wells; sera with titers ≥1/16 are considered seropositive for anti-*T cruzi* antibodies. The information from the package insert of the test commercially available in Argentina is summarized in Table 130; the test is not cleared by the FDA.

Table 130. Chagatest HAI Test—A Summary of the Information in the Wiener Lab Test Package Insert

- Sheep RBCs sensitized with *T. cruzi* cytoplasmic antigens have the characteristic of being able to agglutinate sensitized RBCs.
- Nonsensitized RBCs: suspension of 1% goat RBCs, for the control of heterophilic antibodies.
- Heat inactivated serum samples (samples should be fasting) used; plasma cannot be used.
- IHA antigen: Once reconstituted, is stable for 2 months stored in refrigerator (2-10ºC). Do not freeze.
- Known interfering substances: Hemolysis, hyperlipemia (chylomicronemia) may cause erroneous results.
- In sera with heterophilia, the heterophile antibodies can be adsorbed onto unsensitized RBCs.
- Instability:
  - When all the sera dilutions are reactive, it might be an indication of self-agglutination of the IHA antigen. Verify this by using a well of the microtitration plate only to mix the IHA antigen and IHA serum diluent, without the sample. If agglutinations persists, the reagent is deteriorated. Discard.
  - The absence of reactivity in all the sera dilutions: might be an indication of deterioration of the reagents. Process the sample with known positiveness.
- Serial dilutions of patient's serum are added to a microtiter plate with the RBCs carrying *T. cruzi* antigens.
- Assay performance comparison with reference methods - IFA and CFT:
  - Endemic populations: 98% of IHA titers <1/8 and 95% of those ≥1/8 were confirmed by reference methods.
  - Non-endemic populations: 100% of healthy individuals showed titers <1/8 determined by IHA.
  - In 100% of individuals with positive serology confirmed by reference methods and parasitosis confirmed by xenodiagnosis and/or hemoculture, ≥1/32 titer determined by Chagatest IHA.
- The highest serum dilution presenting agglutination should be compared to the titer of positive and negative control.
- Interpretation of results:
  - *Reactive*: Smooth film of cells covering 50% or more of the bottom of wells; sera with titers ≥1/16 considered reactive for anti-*T cruzi* antibodies.
  - *Nonreactive*: Button-shaped sedimentation or little ring with regular borders.

- Reference values: It is stated that "Among immunological techniques, IHA is considered a reliable method for the determination of specific antibodies. However, the results, as those of any other serological method, only constitutes an auxiliary information for the diagnosis. It is for this reason that reports should be considered in terms of probability; in this case, higher or lower probability of *T. cruzi* parasitosis.
- Verify any Reactive result using an additional technique. The "Fatala Chaben" Institute recommendations -according to which the immunodiagnosis of the infection should be performed with a minimum of two of the following methods: IFA, ELISA, (latex) particles agglutination, validated by the Centro Nacional de Referencia."

Abbreviations: CFT, complement fixation test; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescent test; IHA, indirect hemagglutination; RBC, red blood cell

No verification report for the HAI test, like for the ELISAs, based on testing in the central laboratory, was provided.

18.6.2.4. F29-ELISA

The Applicant used a nonconventional ELISA for detecting antibodies in serum against the F29 antigen, a 29 kDa flagellar of *T.cruzi* and other trypanosomes. The F29-ELISA was used for detecting antibodies on available serum samples; F29-ELISA is not commercially available in any country. The studies (Engman et al. 1989; Porcel et al. 1996) show that the recombinant F29 antigen from epimastigotes, cloned and expressed in *E. coli*, is a flagellar calcium-binding protein encoded by several highly conserved among different *T. cruzi* isolates. F29 contributes to the rapid motility of the trypanosomes and plays a role either in flagellar structure or in Ca^{2+} metabolism.

The Applicant included a version 1.3 SOP for the testing done at *Instituto Nacional de Parasitologıa* (INP) Dr Mario Fatala Chaben. The antigen concentration used for plate coating is 5 mcg/mL. The various steps of the method used were similar to that summarized above for Lysate-ELISA except that peroxidase-labeled gamma globulin was used as a probe and OD was determined at 492 nm. The cut-off of 0.170 OD was determined based on testing of 50 sera from healthy individuals; this was based on the formula Average OD ±3 SD. Data supporting performance of the assay were not provided.

Verification in the Central Laboratory

It appears that performance of the assay was not assessed in the central laboratory. However, a comparison of the F29-ELISA with Recombinant-ELISA as well as Lysate-ELISA was performed. For details see Sections 20.6.2.1 and 20.6.2.2 above.

Comparison between F29-ELISA and IHA was not assessed.

Quality Control During Testing of Clinical Trial Specimens in the Central Laboratory

No information provided.

18.6.2.5. Historical Control Studies—Comparison of Serological Tests With Those Used By the Applicant for Study 16027

The Applicant compared cure rates for Study 16027 with information available for the placebo groups in 2 published studies (de Andrade et al. 1996; Sosa Estani et al. 1998). The information relevant for the comparison of the serological tests, used by the Applicant for Study 16027 with those of the two published studies, is summarized:

Sosa-Estani et al. (1998)

IHA, IFA, conventional ELISA, and nonconventional ELISA (F29-ELISA) tests were performed. It appears that the testing was performed at the Instituto Nacional de Parasitologia Dr. Mario Fatala Chaben. None of the tests were commercially available. The available information for the assays in the publication, the cross-referenced publications, and the SOP for the F29-ELISA, provided by the Applicant, is summarized.

<u>Conventional ELISA</u>

The authors cross-referenced a publication by Voller et al. (Voller et al. 1975) for the conventional ELISA used. Briefly, the antigen used was a soluble extract derived from the Peru strain of *T. cruzi*; details such as the concentration of the antigen or the stage of the parasite from which antigen was prepared were not specified. Rabbit-anti-human immunoglobulin labeled with alkaline phosphatase was used as a probe. OD was measured at 400 nm. The cut-off for characterizing patients as seropositive was >0.4 OD. All 26 patients were positive by xenodiagnosis as well as ELISA. Of the 44 patients in the placebo-group, 2 (4.5%) became seronegative at Month 48. Cure rates at Month 12 were not specified. There was no significant change in OD up to Month 48 in the placebo group of patients.

<u>F29-ELISA</u>

The Applicant used an SOP from Instituto Nacional de Parasitologia Dr. Mario Fatala Chaben which appears to be same as that used in the published study. Based on the publication, none of the children (age 6 -12 years) in the placebo-group were seroconverted to a negative result up to Month 48 of follow-up. However, based on the dataset provided by the Applicant, 1/31 (3.2%) and 4/36 (11.1%), became seronegative at Months 12 and 48, respectively.

<u>IHA</u>

The authors cross-referenced a publication by Cerisola et al. (Cerisola et al. 1971); English translation of the publication was not available for review. Cure rates based on IHA were not specified; however, there was no significant change in IHA titer in the placebo group of patients up to Month 48 of follow-up.

IFA

Testing by IFA was not performed for Study 16027.

de Andrade et al. (1996)

IHA, conventional ELISA, IFA, and nonconventional ELISA (AT-ELISA) tests were performed.

Conventional ELISA

The authors cross-referenced a publication by Voller et al. (Voller et al. 1975) that is same as that cross-referenced by Sosa-Estani et al. (Sosa Estani et al. 1998). Cure rates were based on an index of ≥1.2; however, cure rates were not included. There was an initial decrease in OD up to Month 5 followed by a gradual increase in the placebo group of patients up to Month 36.

IHA

The authors cross referenced publications by Camargo et al. (Camargo et al. 1973) and de Andrade et al. (de Andrade et al. 1992). The cut-off used for characterizing patients as seropositive was a titer ≥1:16; however, cure rates were not specified. There was an initial decrease in IHA titer up to Month 5 followed by a gradual increase in the placebo group of patients up to Month 36.

IFA

Testing by IFA was not performed for Study 16027.

Nonconventional AT-ELISA

Testing by AT-ELISA was not performed for Study 16027.

18.6.2.6. Concentration Test for Detection of *T. cruzi* Parasites

The Applicant provided details of the concentration method used for microscopic examination of parasites in whole blood (heparinized or EDTA containing), in 11 site laboratories (for details see Table 131).

Samples were processed immediately after collection.

Table 131. Summary of Procedure (SOP) and Readings Among Different Laboratories

Laboratory	Details of the Method
Biochemistry: (b) (4)	*Capillary tube procedure*: • 6 heparinized capillary tubes were loaded and centrifuged at 5,000 rpm for 45 seconds. Each tube, where a separation of plasma and pools of RBCs observed, was cut below its interface (where the band of WBCs can be observed) and the layer of WBCs placed between the slide and coverslip.

Laboratory	Details of the Method
	• Six coverslips per specimen were observed under the microscope at 40x. *Results:* • Positive: Mobile flagellate forms of trypomastigotes of *T. cruzi*. • Negative: No forms of *T. cruzi* trypomastigotes observed. Each positive sample of trypomastigotes was filmed and stored by date of completion and patient name.
(b) (4)	*INP(*) Micromethod (Eppendorf tube):* • 0.5 mL of blood was collected in a commercial lithium heparin microcontainer or if not available in a 1.5 mL Eppendorf tube with 1 drop of heparin (0.05 mL) and mixed by inversion and centrifuged for 1 minute at 3,000 rpm. • Using a 50 µL micropipette or Pasteur pipette, one drop from the interface, rich in WBCs was collected and placed between the slide and coverslip and observed immediately with 400x magnification. • A minimum of four preparations per patient were performed and observed for no less than 30 minutes each before considered negative. • Positive samples: the presence of trypomastigotes with their characteristic movement was observed. Note: Samples must be processed immediately following collection. (*) "Dr. Mario Fatala Chaben" National Parasitology Institute
(b) (4)	*Eppendorf tube:* • Added one drop of heparin and 0.5 mL of blood to Eppendorf tubes, mixed by inversion and centrifuged for one minute at 3,000 rpm. • One drop rich in WBCs from the interface was observed between the slide and coverslip under the microscope with 400x magnification. • A minimum of two preparations were taken and observed for no less than 15 minutes each before they could be considered to be negative.
	Same as for (b) (4)
	Microhematocrit (MicroStrout) technique in the Parasitology and Coprology Division, Specialized Biochemistry Ward of the central laboratory: • Blood samples with EDTA were collected in tubes. Loaded 8 nonheparinized hematocrit capillary tubes and centrifuged in microcentrifuge. Marked and cut six of the capillary tubes, just below the WBC line. • Placed between slide and coverslip. Observed using optical microscope, viewing all fields at 100 and 400X.
	The Micromethod, recommended by the "Dr. Mario Fatala Chaben" Institute, a center of reference for pathology. • 0.5 mL blood in a pediatric blood collection tube was mixed by inversion and centrifuged for one minute at 3,000 rpm. • A drop taken from the interface was observed between the slide and coverslip under an optic microscope with 40x magnification.

Laboratory	Details of the Method
	• A minimum of two preparations were completed and observed for no less than 15 minutes each before they could be considered negative.
(b) (4)	*Micromethod in Eppendorf tube:* • Placed one drop of heparin and 0.5 mL of venous blood in a 1.5 mL capacity Eppendorf tube or similar tube and mixed by inversion and centrifuged at 3,000 rpm for one minute. • One drop from the cell-plasma interface, rich in WBCs was observed between the slide and coverslip at 400x magnification. • A minimum of 6 preparations were made, each of which was observed for no less than 15 minutes before they could be considered negative.
	• A minimum of three consecutive samples; five samples from different days were taken; 0.5 mL heparinized Eppendorf tube was inverted several times to yield a homogenous uncoagulated sample and centrifuged for one minute at 3,000 rpm. Removed fibrin. • A sample from part of the interface, tapped several times and placed one drop on a slide (four drops must be taken, i.e., four preparations), placed coverslip, and observed under the microscope at 40x. • There was a 30-minute observation period for each drop or preparation for each patient.
	Tube Micromethod: • In an Eppendorf tube containing 30 µL of sodium heparin, added 0.5 mL blood from a ≤2 year old child, mixed by inversion, and centrifuged for 2 minutes at 3,000 rpm. For a ≥3 y old child 1 mL blood was added to EDTA containing tube. • 15 µL from the WBC layer was observed between slide and coverslip (x3).
	Micromethod (Capillary Tube Technique) • Centrifuged at (8,000 to 12,000 rpm) for five minutes and tubes placed vertically until read; reading was performed immediately using a laboratory-made support which consists of a standard slide to which two of its faces and one of its longer edges was attached with a piece of masking tape. • The support and capillary tube were read using a microscope; focus was on the area of the dividing line of the buffy coat (WBCs and platelets) and blood plasma with 10x lens. • Carefully observed the region with the 40x lens, rotating the tube to a 45° angle, until the entire circumference of the capillary tube could be observed. *Reading Results:* A **positive** diagnosis was made when one or more active mobile trypomastigotes were detected in the region dividing the milky layer (buffy coat) and the blood plasma in one or more of the four capillary tubes. Results were documented as shown below:

Laboratory	Details of the Method
	Concentration of Active Mobile Trypomastigotes in Capillary Tubes Parasites/Capillary Tube: Concentration of Parasites in Crosses 1-5: + 6-10: ++ 11-30: +++ >30: ++++ If the samples were not read immediately, for any reason, the following should have been done: • The samples kept at 4°C (lower part of the refrigerator) away from light and stored vertically until reading. • It was advisable to not read the capillary tubes after 8-12 hours from when the sample was taken.
(b) (4)	Appears to be same as above.

18.6.2.7. Real Time Quantitative PCR

The multiplex real time quantitative PCR (RT-qPCR) assay was performed, in the central laboratory, on whole blood samples (collected in Guanidine-EDTA) at baseline as well as during treatment and follow-up visits. The assay was based on the amplification of a 166 bp segment in the satellite (Sat) DNA of *T. cruzi* by the method of Ramirez et al. (Ramírez et al. 2015); a multiplex RT-qPCR assay and TaqMan probes were used for quantification of *T. cruzi* SatDNA in blood samples (Duffy et al. 2013). Some of the parameters of the RT-qPCR assay tested in the central laboratory include analytical sensitivity near the cut-off, clinical sensitivity and specificity, and reproducibility. For other aspects of the performance of the assay such as determination of the cut-off and analytical specificity, the Applicant refers to the publications. The information available from the published papers, verification report is summarized below.

A: Published Information

Ramirez et al. (Ramírez et al. 2015) reported performance of the RT-qPCR assay based on testing in 26 PCR testing laboratories from 14 countries. Briefly, DNA was extracted from blood samples treated with guanidine hydrochloride. The 3 primers used to amplify a 166 bp segment

in the satellite DNA of *T. cruzi* and the internal amplification control were same as those published by Duffy et al. (Duffy et al. 2013) and shown in Table 132.

Table 132. Primers Used to Amplify 166 bp Segment of *T. cruzi* Satellite DNA and Internal Amplification Control

Target	Oligonucleotide	Sequence	Final Concentration (μM)
T. cruzi satellite DNA	Cruzi 1	5′-ASTCGGCTGATCGTTTTCGA-3	0.75
	Cruzi 2	5′ -AATTCCTCCAAGCAGCGGATA-3	0.75
	Cruzi 3	5′ -Fam-CACACACTGGACACCAA-NFQ-MGB-3′	0.05
IAC	IAC Fw	5′ -ACCGTCATGGAACAGCACGTA-3′	0.1
	IAC Rv	5′ -CTCCCGCAACAAACCCTATAAAT-3′	0.1
	IAC Tq	5′ -VIC-AGCATCTGTTCTTGAAGGT-NFQ-MGB-3′	0.05

IAC: Internal amplification control. S: C/G.
doi:10.1371/journal.pntd.0002000.t001

Amplification was performed in duplicate in a thermocycler SmartCycler (Cepheid, USA). Internal (TaqMan Human RNase P detection reagent; Applied Biosystems) and external (known positive or negative samples) controls were tested. The results, based on the information available in the publications, are summarized:

- Analytical sensitivity:
  - 0.0625 fg/mcL DNA for stocks* representative of TcIa, TcII, TcIII, TcV, and TcVI
  - 0.25 fg/mcL DNA for stocks belonging to TcId (epimastigotes of Sylvio X10 strain) and TcIV (epimastigotes of CL-Brener strain)
  - 1 fg/mcL DNA for the TcIe stock*

  *Note: The *T. cruzi* strains and the stage of the parasite used for preparing spiked samples was not specified.
- **Lower limit of detection (LLOD):** LLOD determined for TcVI DTU in boiled and nonboiled samples was 0.46 and 0.70 parasite equivalent (par eq)/mL, respectively.
- **Precision:** CV for nonboiled samples spiked with TcVI stock at a concentration closer to LLOD, or higher concentrations of 10 and 1000 par eq/mL, respectively were 46.6%, 6% and 1.72%, respectively; the CV of 46.6% at the concentration closer to LLOD is very high suggesting an increased possibility of variability in measurements closer to LOD.
- **Lower limit of quantitation (LLOQ):** 1.53 par eq/mL; the value is derived from a 20% threshold value of the CVs obtained for determining precision.
- **False positivity:** No DNA detected using 1000 pg/mcL DNA of *Trypanosoma rangeli* and *Leishmania* spp. parasites. Other pathogens or host factors that may interfere with the performance of the assay leading to false-positive findings were not tested.

- **Cut-off:** It appears that a patient was considered RT-qPCR positive when the cycle threshold (Ct) value was <40 as the cycling parameter included running the samples for 40 cycles for 15 seconds.
- **Verification of clinical sensitivity:** All 11 patients with **acute Chagas disease** (10 seropositive and 1 seronegative) had DNA from DTUs TcI or TcIV and were positive by RT-qPCR; the seronegative patient had 3.71 Log_{10} par eq/mL and presented with cardiac symptoms.

  Of the 145 patients with **chronic Chagas disease**, 70 were asymptomatic and 75 with cardiac and/or digestive syndromes; 117 (80.6%) were PCR positive. By genotyping, the chronic CD patients were stated to be infected with all *T. cruzi* DTUs, except TcIII and TcIV.

  Acute Chagas disease patients had approximately 2-fold higher parasitic load (median, 4.03; interquartile range, 2.72 to 7.20 Log10 par eq/10 mL) compared to **chronic Chagas disease** patients (1.90; 1.61 to 2.46 Log10 par eq/10 mL) with the use of SatDNA qPCR (*P*=0.0086). However, there was no difference in parasitic loads of asymptomatic and symptomatic chronic **Chagas disease** patients (asymptomatic persons: 2.17; 1.65 to 2.48 Log10 par eq/10 mL); symptomatic patients: 1.77; 1.45 to 2.13 Log10 par eq/10 mL).

Reviewer Comments: *It is unclear whether the central laboratory used for the testing of clinical specimens took part in an external quality assurance (EQA) program to assess the agreement of RT-qPCR performance among the laboratories.*

B: Verification in the Central Laboratory

The studies conducted by the Applicant, in the central laboratory, are summarized:

- **Analytical Verification:** The DNA standards for the construction of the quantification curves of *T.cruzi* parasitic load were provided by the *Instituto de Investigaciones en Ingeniería Genética y Biología Molecular* "*Dr. Héctor N. Torres*" (INGEBI-CONICET-ARGENTINA). The curve was created based on processing of 5 standards (0.1, 1, 10, 100, 1000 par eq/ml). Each concentration was assayed in duplicate and the Ct value determined for each concentration (Table 133). On the basis of the Ct values of each concentration, a calibration curve was performed plotting Ct as a function of the concentration logarithm. The linearity of the curve and the calculation of the different parameters determined. The Ct value was <40 at all the concentrations tested.

Table 133. DNA Standard—Spiked Samples

Standard assigned values		Results obtained by Laboratory			
Parasite equivalent/ ml	Log 10 parasite equivalent/10 ml	CP *	Parasite equivalent/ ml	Parasite equivalent/ 10ml	Log 10 parasite equivalent/10 ml
0.1	0	33.19	0.11	1.10	0.041
0.1	0	33.45	0.09	0.87	-0.062
1	1	30.66	0.93	9.30	0.968
1	1	30.25	1.28	12.80	1.107
10	2	27.32	9.76	97.60	1.989
10	2	27.25	10.20	102.00	2.009
100	3	23.58	100.00	1000.00	3.000
100	3	23.57	101.00	1010.00	3.004
1000	4	19.94	973.00	9730.00	3.988
1000	4	19.86	1020.00	10200.00	4,009

**CP: Crossing Point*

- **Accuracy:** The accuracy under repeatability conditions as well as the intermediate/total accuracy was lower at 1 par eq/mL compared to 10 par eq/mL (Table 134).

Table 134. Accuracy—Based on Testing in the Central Laboratory

1 Parasite Equivalent/mL

Parasite equivalent/ ml					
RUN Nº	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	0.654	1.200	0.597	1.210	1.260
2	0.924	1.130	1.370	0.656	0.602
3	0.510	0.618	0.363	0.795	0.753
Parasite equivalent/ 10 ml					
RUN Nº	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	6.540	12.000	5.970	12.100	12.600
2	9.240	11.300	13.700	6.560	6.020
3	5.100	6.180	3.630	7.950	7.530
	Log 10 parasite equivalent/10 ml				
RUN Nº	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	0.816	1.079	0.776	1.083	1.100
2	0.966	1.053	1.137	0.817	0.780
3	0.708	0.791	0.560	0.900	0.877

10 Parasite Equivalents/mL

Parasite equivalent/ ml					
RUN Nº	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	10.30	12.30	11.20	11.20	9.83
2	8.82	11.50	11.50	9.93	9.88
3	10.50	12.30	13.70	9.13	10.70
Parasite equivalent/ 10 ml					
RUN Nº	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	103.000	123.000	112.000	112.000	98.300
2	88.200	115.000	115.000	99.300	98.800
3	105.000	123.000	137.000	91.300	107.000
	Log 10 parasite equivalent/10 ml				
RUN Nº	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1	2.013	2.090	2.049	2.049	1.993
2	1.945	2.061	2.061	1.997	1.995
3	2.021	2.090	2.137	1.960	2.029

CV% at 1 and 10 Parasite Equivalent(s)/mL

ACCURACY:	CV% 1 Parasite equivalent/ 10 ml	CV% 10 parasite equivalents/ 10 ml
Accuracy in repeatability conditions	18.50%	2.16 %
Intermediate or total accuracy	16.50%	2.30%

Source: NDA

- **LLOD:** The LLOD was 0.1 par eq/mL. This was based on the testing of the commercial standard (0.1 par eq/mL), processed 17 times, each in duplicate or triplicate for 6 days. The accuracy in repeatability conditions and the intermediate/total accuracy was lower at 1 par eq/mL compared to 10 par eq/mL (Table 133).
- **LLOQ:** The established LLoQ was stated to be 1.53 par eq/mL (same as in the publication) with a tolerance of 20% (data not shown).

- **Upper limit of quantitation (ULOQ):** Not determined.
- **Verification of clinical sensitivity:** The clinical sensitivity and specificity of the assay was verified in the central laboratory based on testing of 20 samples from patients with serological and molecular diagnosis and 20 samples from healthy individuals with negative serology and without the risk of getting Chagas disease in acute form; however, the details of the patients, from whom samples were obtained, such as patient history, any comorbidities, other infections, or previous treatments, results of previous testing by any of the parasitological tests were not specified. The results were grouped into 2 categories: positive (detectable), negative (nondetectable); there was 100% agreement (sensitivity and specificity) with serological findings (Table 135). The serological test(s) used for comparison were not specified.

Table 135. Verification of Clinical Sensitivity

INFECTED PATIENTS (PCR AND ELISA POSITIVE)				NON INFECTED PATIENTS (ELISA NEGATIVE)			
N°	SAMPLE	1° PCR (CP)*	2° PCR (CP)*	N°	SAMPLE	ELISA	PCR
1	(b) (6)	27.2	26,4	1	(b) (6)	NEGATIVE	NEGATIVE
2		31.8	32.7	2		NEGATIVE	NEGATIVE
3		23.6	24.6	3		NEGATIVE	NEGATIVE
4		30.5	30.8	4		NEGATIVE	NEGATIVE
5		21.6	21.9	5		NEGATIVE	NEGATIVE
6		29.4	30.6	6		NEGATIVE	NEGATIVE
7		16.1	16.6	7		NEGATIVE	NEGATIVE
8		29.6	30.3	8		NEGATIVE	NEGATIVE
9		30.7	31.6	9		NEGATIVE	NEGATIVE
10		30.3	30.9	10		NEGATIVE	NEGATIVE
11		31.7	31.9	11		NEGATIVE	NEGATIVE
12		31.4	31.0	12		NEGATIVE	NEGATIVE
13		21.9	22.4	13		NEGATIVE	NEGATIVE
14		30.0	31.1	14		NEGATIVE	NEGATIVE
15		30.2	30.5	15		NEGATIVE	NEGATIVE
16		20.3	21.3	16		NEGATIVE	NEGATIVE
17		25.0	26.6	17		NEGATIVE	NEGATIVE
18		27.0	28.2	18		NEGATIVE	NEGATIVE
19		21.7	22.4	19		NEGATIVE	NEGATIVE
20		23.0	24.5	20		NEGATIVE	NEGATIVE

*CP: Crossing Point

Source: NDA

Quality Control During Testing of Clinical Trial Specimens in the Central Laboratory

Clinical samples for RT-qPCR were processed on an on-going basis as soon as they were received at the central laboratory. The testing period was between Jun 2016 to Aug 2018. No information supporting quality control measures implemented was provided.

18.6.2.8. Results—Additional Analysis

Serological and parasitological test results in a subset of PP set patients that were seropositive by the F29-ELISA or RT-qPCR at baseline i.e., prior to treatment are shown in Table 136 and Table 137, respectively.

Table 136. Seronegative or Parasitologically Negative Patients, by Different Tests at Baseline, During Treatment, and Follow-Up Visits, Subset of PP Population, and Based on F29 Positive Findings at Enrollment, Study 16027

			Treatment – Day 30 or 60: Visit			Follow-Up Day: Visit	
Test	**Treatment Arm**	**Baseline: Visit 1**	**Day 7: Visit 3**	**Day 30: Visit 6**	**Day 60: Visit 8**	**Month 6: Visit 10**	**Month 12: Visit 11**
IHA*	30-day (n=63)	0 (0.0)	ND	ND	ND	ND	2 (3.2)
	60-day (n=122)	1 (0.8)	ND	ND	ND	ND	6 (4.9)
Recombinant-ELISA	30-day (n=63)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.2)	2 (3.2)
	60-day (n=122)	0 (0.0)	0 (.0.0)	0 (0.0)	0 (0.0)	6 (4.9	7 (5.7)
Lysate-ELISA	30-day (n=63)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.6)	1 (1.6)
	60-day (n=122)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (4.9)	6 (4.9)
F29-ELISA**	30-day (n=63)	0 (0.0)	9 (14.3)	5 (7.9)	8 (12.7)	12 (19.0)	18 (28.6)
	60-day (n=122)	0 (0.0)	12 (9.8)	15 (12.3)	19 (15.6)	32 (26.2)	42 (34.4)
RT-qPCR	30-day (n=63)	23 (36.5)	50 (79.4)	62 (98.4)	62 (98.4)	62 (98.4)	60 (95.2)
	60-day (n=122)	40 (32.8)	91 (74.6)	117 (95.9)	119 (97.5)	118 (96.7)	119 (97.5)

All values are expressed as n (%).
! Nifurtimox tablets administered three times daily for 30 or 60 days. Treatment initiated on Day 1 (Visit 2).
* IHA testing performed at only at Visit 1 and Visit 11.
** All subjects were positive, by F29 ELISA, at baseline.
Patients with a missing value were imputed as seropositive.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; ND, not done; PP, per protocol; RT-qPCR, realt-ime quantitative polymerase chain reaction

Table 137. Seronegative or Aparasitemic (Parasite Negative) Patients, by Different Tests at Baseline, During Treatment, and Follow-Up Visits, Subset of PP Population and Based on RT-qPCR Positive Findings at Enrollment, Study 16027

			Treatment – Day 30 or 60			Follow-Up: Visit	
Test	**Treatment Arm**	**Baseline: Visit 1**	**Day 7: Visit 3**	**Day 30: Visit 6**	**Day 60: Visit 8**	**Month 6: Visit 10**	**Month 12: Visit 11**
IHA*	30-day (n=51)	0 (0.0)	ND	ND	ND	ND	5 (9.8)
	60-day (n=100)	0 (0.0)	ND	ND	ND	ND	5 (5.0)
Recombinant-ELISA	30-day (n=51)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (11.8)	6 (11.8)
	60-day (n=100)	0 (0.0)	0 (.0.0)	0 (0.0)	0 (0.0)	6 (6.0)	7 (7.0)
Lysate-ELISA	30-day (n=51)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (9.8)
	60-day (n=100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (6.0)	6 (6.0)
F29-ELISA	30-day (n=51)	11 (21.6)	15 (29.4)	13 (25.5)	12 (23.5)	17 (33.3)	20 (39.2)
	60-day (n=100)	20 (20.0)	24 (24.0)	26 (26.0)	29 (29.0)	31 (31.0)	42 (42.0)
RT-qPCR**	30-day (n=51)	0 (0.0)	31 (60.8)	47 (92.2)	49 (96.1)	49 (96.1)	46 (90.2)
	60-day (n=100)	0 (0.0)	61 (61.0)	95 (95.0)	96 (96.0)	95 (95.0)	96 (96.0)

All values are expressed as n (%).
! Nifurtimox tablets administered three times daily for 30 or 60 days. Treatment initiated on Day 1 (Visit 2).
* IHA testing performed at only at Visit 1 and Visit 11.
** All subjects were positive, by RT-qPCR, at baseline.
Patients with a missing value were imputed as seropositive
Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHA, indirect hemagglutination assay; ND, not done; PP, per protocol; RT-qPCR, realt-ime quantitative polymerase chain reaction

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/s/

GREGORY F DIBERNARDO
08/05/2020 07:30:06 PM

SHALINI ANAND
08/05/2020 09:31:43 PM

JAMES S WILD
08/05/2020 10:37:00 PM

TERRY J MILLER
08/06/2020 09:07:13 AM

HANAN N GHANTOUS
08/06/2020 09:13:47 AM

ABHAY JOSHI
08/06/2020 09:21:16 AM

KELLIE S REYNOLDS
08/06/2020 09:30:04 AM
I am signing for the clinical pharmacology team leader, Zhixia (Grace) Yan, and myself, the clinical pharmacology division director.

JASON N MOORE
08/06/2020 09:32:30 AM

JIHYE AHN
08/06/2020 09:37:49 AM
Signing on behalf of Justin Earp, PhD.

SHUKAL BALA
08/06/2020 09:40:44 AM

AVERY C GOODWIN
08/06/2020 09:42:16 AM

MUKILAN NATARAJAN
08/06/2020 09:52:05 AM

PETER W KIM
08/06/2020 09:53:24 AM

JIE CONG
08/06/2020 09:58:54 AM

KAREN M HIGGINS
08/06/2020 10:34:47 AM

TSAE YUN D LIN
08/06/2020 10:38:04 AM

ABIMBOLA O ADEBOWALE
08/06/2020 11:07:34 AM

MAUREEN P DILLON PARKER
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SUMATHI NAMBIAR
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JOHN J FARLEY
08/06/2020 11:21:55 AM