DNDi
Drugs for Neglected Diseases *initiative*

CLINICAL TRIAL PROTOCOL

Double-blind, Double-dummy, Phase 2 Randomized, Multicenter, Proof-of-Concept, Safety and Efficacy Trial to Evaluate Different Oral Benznidazole Monotherapy and Benznidazole/E1224 Combination Regimens for the Treatment of Adult Patients with Chronic Indeterminate Chagas Disease.

Short title	BENDITA BEnznidazole New Doses Improved Treatment and Associations
Name of product(s)	E1224 (Fosravuconazole drug substance equivalent to 100 mg of Ravuconazole), Abarax (Benznidazole; N-benzil-2-nitro-1-imidazolacetamide), and respective matched Placebos
Drug Class	Triazole and Nitro-imidazole
Phase	Investigational – Phase 2 trial
Indication	Chronic Indeterminate Chagas Disease
Protocol Number	*DNDi-CH-E1224-003*
EudraCT	*NA*
Sponsor	DND*i*, Chemin Louis Dunant, 15, 1202 GENEVA Switzerland Phone: +41 22 906 9230
Manufacturers	Laboratório Elea, Buenos Aires, Argentina Eisai Co, Ltd., Tokyo, Japan
National Coordinating Investigator	[redacted] Plataforma de Atención Integral de Pacientes con Enfermedad de Chagas. Cochabamba, Bolivia. [redacted] Centro de Salud Internacional, Hospital Clínico de Barcelona CRESIB - Centre de Recerca en Salut Internacional de Barcelona Barcelona, España.
Protocol Version / Date	*Version 5.0 / May 04, 2018*
Protocol Amendment Number / Date	*Protocol Amendment 5 / May 04 2018*

CONTACT DETAILS

- **Name, title, address, and telephone number(s) of the Sponsor's medical expert for the trial**

  [redacted]

  Rua Santa Heloisa, 05 Jardim Botânico, Rio de Janeiro, Brasil.

  [redacted]

- **Name and title of the Investigator(s) who is (are) responsible for conducting the trial, and the address and telephone number(s) of the trial site(s)**

  Please refer to the Appendix 1– Principal Investigators' list

- **Name(s) and addresse(es) of the clinical laboratory(ies) and other medical and/or technical department(s) and/or Institutions involved in the trial**

  Argentina
  Quality Control PCR
  Instituto Nacional de Parasitología [redacted] Buenos Aires, Argentina.
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  Bolivia
  PCR Analysis
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  Clinical Laboratory
  Laboratorio de la Plataforma de Atención Integral de pacientes con Chagas
  Universidad Autónoma Juan Misael Saracho (UJMS), C/España es. California, Tarija, Bolivia

  Laboratorio CEADES (Colectivo de Estudios Aplicados y Desarrollo Social)
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  Clinical Laboratory Genesis
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  Calle Rico Toro, 1054 Cochabamba, Bolivia

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  Universidade Federal de Pernambuco
  Centro de Ciências da Saúde
  Departamento de Ciências Farmacêuticas
  Centro de biodisponibilidade / bioequivalência
  Av. Professor Arthur de Sá, s/n. Cidade Universitária, Recife – PE

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[redacted]

Signature [redacted] 09may2018
Fecha de la firma
(DD/MM/AA)

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Date of Signature
(DD/MM/YY)

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National Coordinating Investigator

Investigator Signature	_____	_____
		Date of Signature (DD/mmm/YY)
Name		
Title		
Institution		
Address		

Investigators Signature Page

I have read this protocol and agree that it contains all necessary details for carrying out this trial. I will conduct the trial as outlined herein and will complete the trial within the time designated.
I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this trial. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the trial.
I will use only the informed consent form approved by the sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) responsible for this trial.
I agree that the sponsor or its representatives shall have access to any source documents from which case report form information may have been generated.

Principal Investigator at each trial site

Investigator Signature _____ _____
Date of Signature (DD/MM/YY)

Name _____

Title _____

Institution _____

_____

Address _____

_____

TABLE OF CONTENTS

ABBREVIATIONS

GLOSSARY OF TERMS

AE	Adverse Event
AESI	Adverse Event of Special Interest
ADR	Adverse Drug Reaction
ALT	Alanine Aminotransferase (SGPT)
AST	Aspartate Aminotransferase
AUC	Area under the Curve
BID	Twice a day
BMI	Body Mass Index
BZN	Benznidazole
BZN/E1224	Benznidazole and E1224
Ca	Calcium
CD	Chagas Disease
CIOMS	Council for International Organizations of Medical Sciences
CL	Clearance
C_{max}	Maximum Concentration
C_{min}	Minimum Concentration
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DALYs	Disability-Adjusted Life Years
DNDi	Drugs for Neglected Diseases initiative
DMC	Data Monitoring Committee
DTU	Direct typing units
EOT	End of Treatment
EKG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
GCP	Good Clinical Practice
GGT	Gamma-glutamyl Transpeptidase
ICH	International Conferences on Harmonization
IEC	Independent Ethics Committee
IRS	Interactive Response System
ITT	Intention to Treat
K	Potassium
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Magnesium
mpk	Milligrams per Kilo
NFX	Nifurtimox
PAHO	Pan American Health Organization
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per Protocol
QD	Once a day
qPCR	Quantitative Polymerase Chain Reaction
QTc	Corrected QT interval
RAV	Ravuconazole
SAE	Serious Adverse Event
SOC	System Class Organ
STD	Standard
$t_{1/2}$	Plasma terminal half-life (t=time)

TBL	Total Bilirubin
TDR	Special Programme for Research and Training in Tropical Diseases
T. Cruzi	Trypanosoma Cruzi
ULN	Upper Limits of Normal
UNDP	United Nations Development Programme
UNICEF	United Nations Children's Fund
Vd	Volume of distribution
VPC	Visual Predictive Checks
WBC	White Blood Cell
WHO	World Health Organization

PROTOCOL SUMMARY

Protocol Title	Double-blind, Double-dummy, Phase 2 Randomized, Multicenter, Proof-of-Concept, Safety and Efficacy Trial to Evaluate Different Oral Benznidazole Monotherapy and Benznidazole/E1224 Combination Regimens for the Treatment of Adult Patients with Chronic Indeterminate Chagas Disease
Phase	Investigational – Phase 2 trial
Indication	Chronic Indeterminate Chagas Disease
Protocol Number	*DNDi-CH-E1224-003*
Background Information and Trial Rationale	The current treatment for Chagas disease has significant limitations, including long treatment durations, safety and tolerability concerns and is currently limited to two nitro-heterocyclic drugs, nifurtimox and benznidazole (BZN). BZN, a nitroimidazole introduced by Roche in 1971, is marketed by Laboratório Farmacêutico do Estado de Pernambuco S/A – LAFEPE and Laboratorio ELEA – Argentina. It is supplied in tablets strengths of 12.5, 50 and 100 mg, administered twice daily at a dose of 5 mg/kg body weight/day for adults and 5-10 mg/kg body weight/day for children for 30-60 days. Notably, the current regimens with BZN for the treatment of adults with CD likely represent the maximum dosing case scenario in terms of dose, duration and schedule of administration (Chagas expert meeting DNDi. January 2014. GVA). Doses and duration of treatment for CD have not been evaluated systematically. Current treatment regimens and dosing intervals have been derived from decades-old patient series and with very limited direct comparisons. Data from recently concluded trials suggest existing opportunities for optimisation of the treatment regimens of BZN. A DNDi-sponsored Phase 2, proof-of-concept clinical trial on E1224 and BZN in adults with chronic indeterminate CD, conducted between 2011 and 2013 in Bolivia, showed that all BZN-treated patients had cleared parasite DNA after 2 weeks of treatment and 81% sustained the parasite clearance at 12 months after treatment. At end-of treatment (EOT, Day 65), E1224 was found to be efficacious in clearing *T. cruzi* parasites when compared to placebo. However, at 12 months less than one third of patients sustained parasite clearance. The trial safety data also indicated a proportion of patients (10-20%) who do not complete treatment in conditions of use, the majority due to adverse drug reactions (ADRs) and the long treatment duration. Taking into consideration the efficacy gap with about 80% sustained response and a tolerability gap, with a proportion of patients (10-20%) who do not complete treatment, two approaches for Chagas treatment optimization are to be pursued: 1) A change in the current adult dosing regimen for BNZ to reduce exposure and improve tolerability while maintaining efficacy; and 2) The development of a combination therapy to improve efficacy while maintaining or improving tolerability. The combination therapy aims to address the efficacy gap and may or may not address tolerability gap. With regards to the dosing regimen, population-pharmacokinetics studies observed BZN plasma concentrations in children were significantly lower than those previously reported in adults (treated with comparable mg/kg doses). At the same time, all children had parasite clearance, few adverse reactions to the drug. Recent population PK data in adults suggested that the current BZN dosing

	regimen (2.5 mg/kg/12 h) may lead to overexposure in the majority of patients. Dosing simulations suggested that a BNZ dose of 2.5 mg/kg/24 h would adequately keep BNZ trough plasma concentrations within the recommended target range for the majority of patients. There are also opportunities for evaluation of fixed dose regimens for adult dosing, rather than mg/kg calculations, with increased ease-of-use and potential for improved compliance in scaling up treatment of CD. Intermittent dosing regimens was also evaluated in murine model of chronic CD and in a pilot follow-up trial with 17 adult patients with chronic CD was, with similar parasitological cure rates to standard treatment. Likewise, several controlled observational trials with BZN, 5 mg/kg/day for 30 or 60 days have shown a reduction in the progression of heart disease serological and sero-negative conversion up to 60% in children and 30% in adults. Different publications showed anti-parasitic efficacy of treatment regimens with 30 and 60 days, and of incomplete treatment of 10 days. Combination therapy is a well-recognized treatment modality in many disease settings, including cancer, cardiovascular disease, and infectious diseases. Several infectious diseases such as tuberculosis, malaria, leprosy, and AIDS only came under control and were effectively treated after introduction of combinations of drugs that utilize different mechanisms of action. E1224 is a water-soluble monolysine salt form of the ravuconazole (RAV) pro-drug (which is a phosphonooxymethyl ether derivative of RAV). It is a broad-spectrum triazole antifungal. Pooled safety data for the monolysine prodrug E1224 from Phase 1 and 2 trials indicated that E1224 was generally well tolerated and exhibited a safety profile quite similar to other azoles. Adverse events occurring in greater than 3% of E1224 recipients, with a dose-dependent pattern and at rates higher than those observed in placebo recipients included nausea, abnormalities in liver enzymes, dizziness, anxiety, and contact dermatitis. Safety evaluations indicated relatively mild, transient, and asymptomatic increases in liver enzymes – completely reversible upon discontinuation of therapy. Phase 1 cardiac safety evaluations showed that E1224 administration did not result in QTc interval prolongation. Experimental data suggest a positive interaction between BZN and azole compounds for the treatment of Chagas disease.[18] A Phase 1 drug-drug interaction trial was designed to assess the pharmacokinetics (PK) and safety interaction of BNZ and E1224 co-administered daily for a total of 54 days (Day 4 to Day 15- E1224 multiple dose 400 mg loading dose once daily for 3 days, followed by maintenance dose 100mg once daily for 9 days (from Day 7 to Day 15); Day 9 BNZ single dose (2.5 mg/kg); and Day 12 to Day 15 BNZ multiple dose (2.5 mg/kg twice daily)). The trial was conducted in Argentina and was concluded in early 2015, with enrolment of 28 healthy male volunteers. Both compounds were well tolerated, in monotherapy and combination. There were no treatment discontinuations or serious adverse events. Transient, minor, non-concomitant increase in bilirubin and liver transaminases occurred in 2 patients in a pattern not suggestive of drug effect. There was no interaction of RAV on BNZ PK and the limited impact of BNZ on RAV PK, suggesting that co-administration of RAV and BNZ may not require any E1224 dosing adaptation. The lack of clinically relevant safety findings provided support for follow-up evaluation of the two compounds in combination. In conclusion, recent scientific advances have provided further impetus to develop new therapeutic approaches for CD using different doses and duration of BZN, as well as combinations directed at multiple therapeutic targets to improve treatment response and tolerability and reduce the potential for development of resistance. This project focuses on the proof-of-concept evaluation of improved treatment

	regimens of BZN, with the assessment of new BZN-sparing regimens in monotherapy and in combination with E1224.
Trial Objectives	General Objective: To determine the efficacy and safety of different dosing regimens of BZN and BZN/E1224 in combination (BZN/E1224) in reducing and clearing *T. cruzi* parasitemia in individuals with the chronic indeterminate CD. Primary Objectives: • To determine the efficacy of different dosing regimens of orally administered BZN and BZN/E1224 in individuals with chronic indeterminate CD, by determining the proportion of patients who convert from positive to negative in serial, qualitative PCR test results (3 negative PCR results) at end of treatment (EOT) and sustain parasitological clearance at 6 months of follow-up, in comparison to placebo. Secondary Objectives: • To measure the reduction in parasite load at weeks 1, 2, 3, 4, 6, 10, 12 and at 4, 6 and 12 months follow-up, as measured by quantitative PCR. • To assess the time to parasite DNA clearance (below the quantitative PCR [qPCR] Limit of Detection [LOD]) for each of the regimens • To assess the sustained parasitological response at week 12, and 12 months for each of the regimens, in comparison with placebo. • To assess the time to sustained clearance of parasitemia for each of the treatment regimens. • To determine the efficacy of the different dosing regimens in individuals with chronic indeterminate CD, by determining the proportion of patients who convert from positive to negative in serial, qualitative PCR test results (3 negative PCR results) at EOT, in comparison with placebo. • To explore if there is a dose-response relationship between the dose of treatment given and parasite clearance in order to determine the minimum effective dose. • To evaluate serological response by conventional serology at 12 months of follow up and non-conventional serology at W12, 4M, 6M, and 12 months of follow up. • To characterize the population pharmacokinetic parameters of orally administered BZN and BZN/E1224 in adults with chronic indeterminate CD. • To evaluate the safety profile of different regimens based on clinical, laboratory measurements, and EKG. • To correlate pharmacokinetic parameters with parasitological response and safety outcomes. • To evaluate the incidence of Serious Adverse Events (SAEs) and/or adverse events leading to discontinuation of treatment.
Trial Endpoints	Primary efficacy assessment parameter: • Parasitological response as determined by serial negative qualitative PCR results (3 negative PCR results, from 3 samples to be collected in the same day) at EOT and sustained parasitological clearance until 6 months follow-up. For efficacy assessments, the EOT of each treatment arm will be defined in accordance to the duration of the treatment regimen. Sustained response will be assessed in all treatment arms using the same number of PCR samples (i.e., EOT; 12 weeks; 4 and 6 months). Secondary efficacy assessment parameters/criteria:

	• Sustained parasitological clearance at 12 weeks and 12 months of follow-up. • Parasite clearance at weeks 1, 2, 3, 4, 6, 10, 12, and at 4, 6, and 12 months follow-up as measured by qualitative PCR. • Change in parasite load over time assessed at weeks 1, 2, 3, 4, 6, 10, 12, and at 4, 6, and 12 months follow-up as measured by quantitative PCR. • Serological response by conventional serology assessed at 12 months of follow up and non-conventional serology assessed at W12, 4, 6, and 12 months of follow up. (changes in titters over time) <u>Primary safety criteria:</u> • Incidence and severity of adverse events (clinical, laboratory measurements, and EKG) • Incidence of SAEs and/or adverse events leading to treatment discontinuation Safety will be assessed through routine monitoring of adverse events, evaluation of haematological and blood chemistry values, regular measurement of vital signs, physical examination, and conduct of EKGs at selected trial visits (according to the trial schedule). <u>Pharmacokinetics (PK) endpoints:</u> • Blood level concentrations will be determined at D0 (pre-dose), after first day of treatment administration (day 1, post-dose), at day 2 and day 3, at steady-state phase (week 2-10) • Population pharmacokinetic parameters will include: AUC, C_{max}, C_{min}, CL, Vd, and $t_{1/2}$. Covariates to be evaluated: age, body mass index, and parasite load at baseline, gender.

Trial Design	Double-blind, double-dummy, randomized, prospective, comparative, placebo-controlled, pharmacokinetic-pharmacodynamic, and proof-of-concept trial design, with seven-parallel groups. The trial will be conducted in 3 sites in Bolivia. The trial will be sufficiently powered to compare the efficacy and safety of each of the dosing regimens of BZN and the combination BZN/E1224, with placebo. Primary efficacy analyses will be performed when all patients complete 6 months follow-up visit. Efficacy and safety database until 6 months will be locked. Analyses of results will be used to guide planning of follow-up Phase 3 clinical study. Sponsor personnel directly involved in the trial, principal investigators and study team site personnel will remain blinded to treatment allocation and results of the analyses. Efficacy and safety will be monitored by an external independent Data Monitoring Committee (DMC) on an ongoing basis. The committee is to include cardiac and liver safety experts, as well as CD clinical expertise. <u>Randomization, Blinding and Code Breaking</u> Each patient will be assigned with a treatment number according to the randomization schedule. The trial drug label will indicate the trial number, trial site and patient treatment numbers (according to local regulations), but will not indicate the actual designation of treatment. Code breaking can only be performed if a life-threatening / emergency condition depends on the information about the treatment allocation. In case of any need of un-blinding, the sponsor (DNDi clinical trial manager or monitor) must be contacted immediately, ideally before doing so.
Main Entry Criteria Screening, Inclusion and Exclusion	**<u>Screening Criteria:</u>** <u>Patients must meet ALL of the following screening criteria to eligible for the trial:</u> • Signed, written informed consent form • Age <u>≥</u>18 to <u>≤</u> 50 years • Weight <u>≥</u> 50 kg to <u>≤</u> 80 kg • Diagnosis of *T. cruzi* infection by: ○ Conventional serology (a minimum of two positive tests [Conventional ELISA, Recombinant Elisa and/or Indirect Immunofluorescence (IIF)]) • Ability to comply with all protocol specified tests and visits and have a permanent address • Patients must be residents of areas free of vectorial transmission (*Triatoma infestans*). For this protocol, it will be accepted the status of Vectorial Transmission Interruption or Consolidation as per the definition of PAHO/WHO, or the Local Health Program. • No signs and/or symptoms of the chronic cardiac and/or digestive form of CD • No acute or chronic health conditions, that in the opinion of the PI, may interfere with the efficacy and/or safety evaluation of the trial drug (such as

acute infections, history of HIV infection, liver, and renal disease requiring treatment)

- No formal contraindication to BZN (according to the Summary of Product Characteristics) and E1224 (according to the Investigator's Brochure)

  Note: The contraindications described for Benznidazol and E1224 are essentially hypersensitivity to the active ingredient or any excipient. In the case of hepatic or renal impairment or blood discrasia, the medication should only be administered under strict medical supervision. During all the treatment period, the blood count will be monitored, with special attention to leucocytes. Subjects will be indicated about the need of no alcohol intake.

- No history of hypersensitivity, allergic, or serious adverse reactions to any of the "azoles" compound, and/or its components
- No history of CD treatment with BZN or NFX at any time in the past
- No history of systemic treatment with itraconazole, ketoconazole, posaconazole, isavuconazole, or allopurinol in the past
- No history of alcohol abuse or any other drug addiction
- No condition that prevents patient from taking oral medication
- No concomitant or anticipated use of drugs that are either sensitive CYP3A4 substrates and/or extensively metabolized by CYP3A4 with a narrow therapeutic range (as per Appendix 2)
- No medical history of Familial Short QT syndrome or concomitant therapy with medications that can shorten the QT interval (as per Appendix 2)
- No family history of sudden death
- No family history of sudden infant death syndrome

Inclusion Criteria:

Following the screening period, patients must also meet ALL of the following inclusion criteria to be eligible for randomization:

- Confirmed diagnosis of *T. cruzi* infection by:
  - Serial qualitative PCR (three samples collected over a single day, at least one of which must be positive) AND
  - Conventional serology (a minimum of two positive tests must be positive [Conventional ELISA, Recombinant Elisa and/or IIF)
- Women in reproductive age must have a negative serum pregnancy test at screening, must not be breastfeeding, and must use a double barrier method of contraception to avoid pregnancy throughout the clinical trial and for 3 months after completion of the trial, in such a manner that the risk of pregnancy is minimized especially during exposure to treatment. Women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an

intrauterine device with a hormonal component are required to use an additional barrier method of contraception for the time period specified

Normal EKG (PR ≤200 msec, QRS <120 msec, and QTc ≥350msec and ≤450 msec interval durations in males and QTc ≤470msec in women) at screening

Exclusion Criteria:

The presence of any of the following will exclude a patient from the trial randomization:

- Signs and/or symptoms of chronic cardiac and/or digestive form of CD
- History of cardiomyopathy, heart failure, or ventricular arrhythmia.
- History of digestive surgery or mega syndromes.
- Any other acute or chronic health conditions that, in the opinion of the PI, may interfere with the efficacy and/or safety evaluation of the trial drug (such as acute infections, history of HIV infection, diabetes, uncontrolled systolic/diastolic blood pressure, liver, and renal diseases requiring medical treatment).
- Laboratory test values considered clinically significant or out of the allowable range at selection period as follows:
    - Total WBC must be within the normal range, with an acceptable margin of +/- 5%.
    - Platelets must be within the normal range up to 550,000/mm^3
    - Total bilirubin must be within the normal range
    - Transaminases (ALT and AST) must be within the normal range, with an acceptable margin of 25% above the upper limit of normality (ULN), ≤ 1.25 x ULN.
    - Creatinine must be within an acceptable margin of 10% above the ULN, ≤1.10 x ULN.
    - Alkaline phosphatase must be within the normal range up to Grade 1 CTCAE (≤ 2.5 x ULN)
    - GGT must be within the normal range up to 2x ULN.
    - Fasting glucose must be within the normal range
    - Electrolytes (Ca, Mg, K) must be within the normal range
- If the results of the blood tests (hematology and biochemistry) are out of the ranges defined above, but within the limits of CTCAE (version 4.03) Grade 1, and the laboratory finding is considered as non-clinically significant, a new sample can be collected for a retest. Only one retest will be allowed within the screening period.

  If the result of the retest is within the margins defined above, the Investigator will review the parameter(s) together with all other medical information available (medical history, clinical examinations, vital signs, etc.) and upon his/her medical judgement will decide if the patient is eligible or not for trial randomization.
- Any condition that prevents the patient from taking oral medication.

<table>
<tr><td></td><td>

Patients with any contra-indication (known hypersensitivity) to any nitroimidazoles, e.g. metronidazole
Patients with history of allergy (serious or not), allergic skin rash, asthma, intolerance, sensitivity or photosensitivity to any drug
Any concomitant use of allopurinol, antimicrobial, or anti-parasitic agents
Any planned surgery likely to interfere with the trial conduction and/or treatment evaluation
Unlikely to co-operate with the trial
Any previous participation in any clinical trial for Chagas Disease treatment evaluation
Participation in another trial at the same time or within 3 months prior to selection (according to local regulations)

</td></tr>
<tr><td>Trial Duration</td><td>
The total duration of patient trial participation will be approximately 13 months.

Screening will occur within 40 days from the signature of the informed consent form.

Once a patient is randomized at Day 1, s/he will have follow-up visits during the treatment phase of the trial at regular intervals at weeks 1-10 (± 3 days) and after 12 weeks, 4, 6 and 12 months from treatment initiation (± 7 days). Patients will be randomly assigned to two PCR samples from D1-D3.

Recruitment is planned to occur over a total period of 10 months, starting after the first patient inclusion. The total trial duration will be 22 months, from recruitment to end of follow-up. In addition, patients will be advised to return on any day during the follow-up period in case of any medical occurrence or adverse event.
</td></tr>
<tr><td>Trial treatments</td><td>
Subjects will be randomly assigned into one of the following balanced groups:

<table>
<tr><td colspan="2">Key</td></tr>
<tr><td>Benznidazole (ELEA 100mg and 50 mg tablets)/</td><td>BZN 100 mg ●
BZN 50mg ■</td></tr>
<tr><td>Placebo (100mg and 50 mg tablets)</td><td>BZN PCB 100 mg ❍
BZN PCB 50mg ❒</td></tr>
<tr><td>E1224 (Eisai 100 mg capsules) /</td><td>E1224 100 mg ♦</td></tr>
<tr><td>Placebo (100 mg capsules)</td><td>E1224 PCB 100 mg ◊</td></tr>
<tr><td>1</td><td>BZN STD Regimen (300 mg - 8 wks):

Benznidazole 150 mg BID for 8 weeks

3 E1224 matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks.</td><td><u>8 weeks</u>
Morning ●■
Evening ●■

<u>D1-D3 (once daily)</u>
◊◊◊
<u>W2-W8 (once weekly)</u>
◊◊◊</td></tr>
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	2	**BZN 300 mg - 4 wks:** Benznidazole 150 mg BID for 4 weeks, to be followed by BZN placebo administered BID to complete 8 weeks 3 E1224 matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks	4 weeks Morning ●■ Evening ●■ 4 weeks Morning ○❒ Evening ○❒ D1-D3 (QD) ◊◊◊ W2-W8 (once weekly) ◊◊◊
	3	**BZN 300 mg - 2 wks:** Benznidazole 150 mg BID for 2 weeks, to be followed by BZN placebo administered BID to complete 8 weeks 3 E1224 matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks.	2 weeks Morning ●■ Evening ●■ 6 weeks Morning ○❒ Evening ○❒ D1-D3 (QD) ◊◊◊ W2-W8 (once weekly) ◊◊◊
	4	**BZN 150 mg - 4 wks:** BZN 150 mg and BZN placebo administered in two separate daily doses for 4 weeks, to be followed by BZN placebo BID to complete 8 weeks 3 E1224 matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks.	4 weeks Morning ●■ Evening ○❒ 4 weeks Morning ○❒ Evening ○❒ D1-D3 (QD) ◊◊◊ W2-W8 (once weekly) ◊◊◊
	5	**BZN 150 mg - 4 wks / E1224 300 mg** BZN 150 mg and BZN placebo administered in two separate daily doses for 4 weeks, to be followed by BZN placebo administered BID to complete 8 weeks Loading dose of E1224 (300 mg QD on days 1-3) followed by 300 mg administered once weekly (starting on week 2) for 7 weeks (total dose: 3000 mg)	4 weeks Morning ●■ Evening ○❒ 4 weeks Morning ○❒ Evening ○❒ D1-D3 (QD) ♦♦♦ W2-W8 (once weekly) ♦♦♦

6	**BZN 300 mg (weekly doses) - 8 wks / E1224 300 mg**: BZN 150 mg BID administered once weekly for 8 weeks (total 8 days of intermittent treatment) and BZN-placebo to be administered BID in the other 6 days of the week Loading dose of E1224 (300 mg QD on days 1-3) followed by 300 mg administered once weekly (starting on week 2) for 7 weeks (total dose: 3000 mg)	Once a week Morning ●■ Evening ●■ BID for 6 week-days Morning ❍❒ Evening ❍❒ D1-D3 (QD) ♦♦♦ W2-W8 (once weekly) ♦♦♦
7	**Placebo:** 4 BZN matched placebo tablets (100 mg and 50 mg) administered BID for 8 weeks 3 E1224-matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks.	8 weeks Morning ❍❒ Evening ❍❒ D1-D3 (QD) ◊◊◊ W2-W8 (once weekly) ◊◊◊

All treatment arms will receive the allocated regimens in fixed doses. The doses of BZN were defined taking as reference the guidelines for the standard treatment of CD, which recommend 5mg/kg/day of BZN for 60 days. Considering an average weight of 60 kg, the fixed dose would be 300 mg daily. With the proposed prescription, patients daily dose will vary as follows:

Patient´s Weight range	**50kg to 80 kg**
Dosing group	**Actual total daily dose (mg/kg/day)**
300 mg - 5.0 mg/kg	3.75 mg/kg to 6 mg/kg
150 mg - 2.5 mg/kg	1.88 mg/kg to 3 mg/kg

The rationale for the selection of the doses and regimens followed the criteria below:

	Group	Objective	Justification / comment
1	BZN STD Regimen 300 mg - 8 wks	Reference	Need to document reference treatment efficacy with ELEA product on a fixed daily dose.
2	BZN 300 mg - 4 wks	Improving safety & maintaining efficacy	Animal data show that standard BZN dose with a 25% reduction in treatment duration showed identical efficacy. Human trials suggest that duration may be reduced.
3	BZN 300 mg - 2 wks	Improving safety & maintaining efficacy	Animal data show that standard BZN dose with a 25% reduction in treatment duration showed identical efficacy. Human trials suggest that duration may be reduced.
	BZN 150 mg	Improving	Paediatric and adult population PK trials

4	- 4 wks	safety & maintaining efficacy	suggest 50% dose scaling may be possible. Human trials suggest that duration may be reduced. Higher risk considering mouse data with one third of dose and full duration.
5	BZN 150 mg - 4 wks E1224 300 mg	Improving both safety & efficacy	Assessment of combination with current dose BZN in short course. Animal data show reduced BZN dose with long duration may lead to a sub-optimal response, thus warranting the addition of E1224.
6	BZN 300 mg - 8 wks, weekly doses E1224 300 mg	Improving both safety & efficacy	Animal and human trial data show that intermittent BZN dose with long duration may lead to a sub-optimal response, thus warranting the addition of E1224.
7	Placebo	Negative control arm	Assessment of sensitivity of PCR in different epidemiological settings and parasite diversity.

BZN will be administered as 100 mg and 50 mg tablets. Placebo will be identically matched to the BZN tablets.

E1224 will be administered as 100 mg capsules. E1224 capsules contain Fosravuconazole drug substance equivalent to 100 mg of RAV. Placebo will be identically matched to the E1224 capsules.

As per recommended in the ABARAX ® package insert, patients will be advised to abstain from alcohol during treatment.
At the trial visits, E1224 will be administered under the supervision of the trial staff. The patient will receive enough BZN until the next scheduled visit. Also, they must bring all remaining trial drugs to check for compliance with prescribed treatment and ensure drug accountability.

Concomitant treatment:

Patients are allowed to receive concomitant therapy for medical occurrences during the course of the trial. However, specific compounds that interact with the trial drugs, increase the liver enzymes, are immunosuppressants or have a known activity against *T. cruzi,* are not allowed during the treatment phase of the trial or up until the 4M visit.

Hormonal contraceptives are allowed during the trial. However, as the contraceptive efficacy is decreased due to interaction with Ravuconazole, a double method is recommended (section **7.6.8**.)

All concomitant medications taken by the patient during the trial, from the date of signature of the informed consent until the last follow up visit, will be recorded in the appropriate section of the Case Report Form.

Rescue treatment:

At the end of the study, patients will receive the information related to what study treatment they took and the PCR results.

All patients treated with placebo will be offered BZN treatment at 5 mg/kg/day, divided in two daily doses for 60 days.

	For all other patients, except those treated with BZN standard regimen, who remain PCR positive at end of trial, BZN treatment will be offered at 5 mg/kg/day, divided in two daily doses for 60 days. For all patients treated with BZN standard regimen who remain PCR positive at end of trial NFX treatment will be offered at doses of 10-15mg/Kg/day, divided in two-three daily administrations for 60 days. Patients who do not tolerate the trial treatment will be withdrawn from the trial and will be offered an alternative treatment with NFX 10-15mg/Kg/day, divided in two-three daily doses for 60 days.
Statistics Sample size considerations	A total of 210 patients will be recruited in the trial. For a comparison of two independent binomial proportions using Pearson's Chi-square statistic with a Chi-square approximation with a two-sided significance level of 0.006 (multiplicity adjustment of 0.05 for 6 comparisons [treated arms] against the control arm), a sample size of 11 patients per arm assuming a balanced design achieves a power of at least 0.8 when the proportions are 0.082 (control arm) and 0.81 (treatment arm). Alpha-adjustment to 0.025 is considered for interim analyses to account for potential inflation of Type I error. With an estimated proportion of 10% patients who would drop-out from the trial, the final sample size needed is 12 patients per arm for one comparison. A sample size of 30 patients per arm will allow a 99% probability of observing at least one event of peripheral neuropathy or paresthesia, transaminase increase, hypersensitivity (13.3%, 15.5%, and 22.2 %, respectively), and 85% probability of observing at least one treatment discontinuation per arm.
Indications for early treatment discontinuation	Efficacy and safety will be reviewed by the established Data Monitoring Committee (DMC), as defined in the DMC charter. An efficacy interim analysis will be performed when 30% of patients completed 12 weeks from treatment initiation. ~~A futility stopping rule is defined by a lack of significant difference to placebo in the sustained parasitological response at 12 weeks. The stopping rule will be applied with no change planned on the sample size.~~ Alpha-adjustment to 0.025 is considered to account for potential inflation of Type I error. Patients will be considered early treatment failures. For safety, upon completion of treatment of 20% of recruited patients, a safety interim analysis will be performed. <u>Liver safety criteria:</u> Discontinuation of treatment should be considered if: • ALT or AST >8xULN (to be reported as Adverse Event of Special Interest) • ALT or AST >5xULN for more than 2 weeks • ALT or AST >3xULN and (Total Bilirubin (TBL) >2xULN or prothrombin time INR >1.5) (Hy Law's criteria = to be reported as a serious adverse event (SAE)) • ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%) Liver enzymes will be repeated as soon as possible after the initial abnormality is identified. After that, the patient should be followed every 96 hours (4 days), or the closest possible interval, until the serum ALT and/or AST falls below 3 X ULN

	or stabilize. All data on liver safety will be presented to the DMC for review. <u>Cardiac safety:</u> Patients will be monitored regularly for vital signs and electrocardiographic abnormalities (see Table 1 – Schedule of Events). Patients will be re-dosed only following on-site physician review of available EKGs (central reading). Specific criteria for treatment discontinuation are described in Section 9 of the trial protocol. Any patient with a delta QTc > 60 msec post dosing will have the trial medication permanently interrupted and will follow the standard trial schedule. For the cases where in addition to the delta QTc > 60 msec post-dosing, cardiac symptoms are present, an EKG should be performed as soon as possible. Echocardiography will be performed on any patients with any clinically significant change in the EKG and in patients with AEs determined to be of cardiac etiology. Holter monitoring will be carried out in patients with symptoms or EKG evidence of arrhythmia. Cardiac safety experts will periodically review all EKGs and present information to the DMC. Central reading of the EKGs will be utilized for the EKG database. In addition, the cardiac safety expert will review all Cardiac System Organ Class (SOC) AEs; and all non-Cardiac SOC, but potential Cardiac AEs. A DMC will review cardiac safety information on an ongoing basis, as prescribed in its Charter.

1. BACKGROUND AND TRIAL RATIONALE

Chagas disease (CD) is a disease caused by *Trypanosoma cruzi (T. cruzi)*, ranking among the world's most neglected diseases. In Latin America, 21 countries are endemic for CD with an estimated 70 million people at risk of contracting the disease[1]. Estimates from the 1980s indicated that some 16 million to 18 million individuals were infected. In the 1990s, after a series of multinational control initiatives, estimates of the number of infected people were revised to 9.8 million in 2001. The estimated burden of disease in terms of disability-adjusted life years (DALYs) declined from 2.7 million in 1990 to 586,000 in 2001. Recent estimates from PAHO (2015) indicate about 6 million infected people[2] and 29,925 new cases of vector transmission per year.

The current treatment for Chagas disease has significant limitations, including long treatment durations, safety and tolerability concerns and is currently limited to two nitro-heterocyclic drugs, nifurtimox and benznidazole (BZN). BZN, a nitroimidazole introduced by Roche in 1971, is now marketed by Laboratório Farmacêutico do Estado de Pernambuco S/A – LAFEPE and Laboratorio ELEA – Argentina. It is supplied in tablets strengths of 12.5 mg, 50 and 100 mg and administered twice daily at a dose of 5 mg/kg body weight/day for adults and 5-10 mg/kg body weight/day for children for 30-60 days. Notably, the current regimens with BZN for the treatment of adults with CD likely represent the maximum dosing case scenario in terms of dose, duration and schedule of administration (Chagas expert meeting DNDi. January 2014. Geneva).

Lack of convincing and consistent data on efficacy and concerns about safety and tolerability profiles have limited the widespread implementation of BZN treatments. Despite being available since the early 70s, treatment recommendations vary significantly from country to country and the comparative evidence-base with the current treatment regimens is limited.

Doses and duration of treatment for CD has not been evaluated systematically. Current treatment regimens and dosing intervals have been derived from decades-old patient series and with very limited direct comparisons. Data from recently concluded trials suggest existing opportunities for optimisation of BZN treatment regimens.

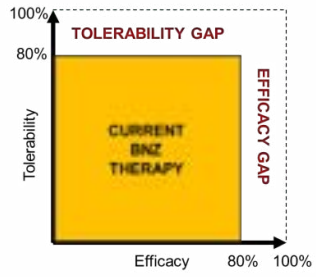
From 2011 to 2013, a DNDi-sponsored Phase 2, proof-of-concept clinical trial on E1224 and BZN in adults with chronic indeterminate CD was conducted in Bolivia assessing different dosing regimen of E1224 given for 4 to 8 weeks and benznidazole standard regimen against placebo. The trial provided clear efficacy and safety information on the two compounds to support further clinical development and access[3]. After one week of treatment, mean qPCR repeated measures analyses showed a significant difference in parasite load vs. placebo in all treatment arms. All BZN-treated patients had cleared parasite DNA after 2 weeks of treatment. At end-of treatment (EOT, Day 65), E1224 was found to be efficacious in clearing *T. cruzi* parasites when compared to placebo. Six months after treatment, 22-33% of patients receiving E1224 low dose and short duration and 60% of those receving high dose E1224 sustained parasite clearance compared with 95.6% of patients treated with BZN Of note, the large majority of treatment failures were identified up to 6 month follow-up visit.

Although E1224 was found to have limited efficacy as a single-treatment agent, it holds promise for use in combination with existing drugs, since it showed strong positive activity during treatment, with a third of patients having a sustained treatment response at the higher dose.

Safety analyses showed that the most common BZN related AEs were nausea (20%), dermatologic AEs (11.1%), hypersensitivity reactions (24.4%), headache (24.4%), and neuropathy (11.1%), with a time-course pattern for development of treatment emergent adverse events. Gastro-intestinal disturbances and hypersensitivity reactions were the first to occur, followed by liver enzyme abnormalities, while most cases of neuropathy occurred after 30 days of treatment. Around 9% of patients in the BZN arm had elevated transaminases and hypersensitivity reactions leading to treatment discontinuation. In the E1224 treatment groups, there was a dose-dependent increase in AEs, including headache (24.4%), nausea (15.6%) and liver enzyme elevations (ALT (22.2%) and AST (17.8%).

These data suggest that BZN is rapidly effective, however there is both an efficacy gap with about 80% sustained response at 12 months and a tolerability gap, with a proportion of patients (10-20%) who do not complete treatment in conditions of use, the majority due to adverse drug reactions (ADRs) and the long treatment duration (see Figure 1).

Figure 1: Current treatment for Chagas with BNZ



Between April 2008 and November 2010 a prospective population-pharmacokinetics cohort trial[4] conducted at the Parasitology and Chagas Service, Buenos Aires Children's Hospital ''R Gutierrez'' enrolled 40 children between 2 and 12 years of age with CD. The observed BZN plasma concentrations in children were significantly lower than those previously reported in adults (treated with comparable mg/kg doses), possibly due to a higher weight-corrected clearance rate (CL/F) in smaller children. The lower blood concentrations were nevertheless associated to parasite clearance in all children, measured by PCR. Unlike adults, children had few adverse reactions to the drug, suggesting that there may be a direct correlation between drug concentrations and incidence of ADRs. A second trial, sponsored by DNDi, testing the pharmacokinetic/pharmacodynamic relationship using the 12.5mg new paediatric dosage form and enrolling a total of 81 children from birth to 12 years of age in multiple paediatric centres in Argentina (PED-CHAGAS network), confirmed this pattern[5].

Recent population PK data in adults further characterized BNZ PK in adults with chronic Chagas disease and suggested that the current BZN dosing regimen (2.5 mg/kg/12 h) may lead to

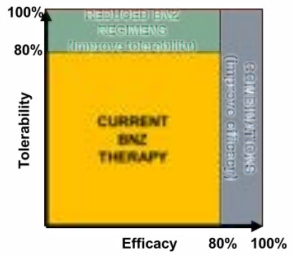
overexposure in the majority of patients. Dosing simulations suggested that a BNZ dose of 2.5 mg/kg/24 h would adequately keep BNZ trough plasma concentrations within the recommended target range for the majority of patients[6]. There are also opportunities for evaluation of fixed dose regimens for adult dosing, rather than mg/kg calculations, with increased ease-of-use and potential for improved compliance in scaling up treatment of CD.

Likewise, several controlled observational trials with BZN, 5 mg/kg/day for 30 or 60 days have shown a reduction in the progression of heart disease serological and sero-negative conversion up to 60% in children and 30% in adults[7,8,9,10,11,12,13]. Different publications showed anti-parasitic efficacy of treatment regimens with 30 and 60 days[14,15] and of incomplete treatment of 10 days[16].

Additional experimental data suggest other opportunities for alternative dosing regimens of BZN. Bustamante *et al.* showed that intermittent BZN administration had similar parasitological cure rates to standard treatment, in a stringent murine model of chronic CD [17]. A pilot follow-up trial was conducted in Argentina by Viotti, Sosa-Estani, and colleagues to evaluate a BZN treatment regimen with intermittent doses of 5 mg/kg/day, taken at 5-day intervals, during 60 days in 17 adult patients with chronic CD. Preliminary results show a high proportion of end of treatment clearance of parasitemia with this regimen, in line with historical data on standard BZN treatment.

Taking into consideration these results, two approaches for Chagas treatment optimization are to be pursued (Figure 2): 1) Changing current adult dosing regimen for BNZ to reduce exposure and improve tolerability while maintaining efficacy; and, 2) Development of a combination therapy to improve efficacy while maintaining or improving tolerability. The former does not address the existing efficacy gap. Combination therapy aims to address the efficacy gap and may or may not address tolerability gap.

Figure 2: Options for improving current treatment



The causative agent of CD, *T. cruzi,* has been called the “cruzi complex” because of the considerable genetic polymorphism observed between parasite populations. The *T. cruzi* genome is quite diverse, with multiple genotypes and phenotypes. Distinct populations correlate with geographic differences in the pathology of the disease and its response to chemotherapy.

Natural resistance of *T. cruzi* to nitro derivatives has been described and is considered to be one of the factors explaining the variable response rates detected in treating CD patients. Acquired

resistance has also been reported while on benznidazole (BZN) therapy. Drug susceptibility varies widely depending upon direct typing units (DTUs), geographic location, host factors, and prior therapy used.

Treatment of virtually every infectious disease begins with empiric therapy intended to cover the broadest possible spectrum of pathogens likely to cause the infection. Therapy is then narrowed once the etiologic pathogen is identified and the drug susceptibility pattern is determined.

In routine treatment of chronic indeterminate CD, identification of the specific etiologic pathogen is almost never possible. Also, there is evidence that more than one parasite lineage can cause an infection in a single patient.

Combination therapy is a well-recognized treatment modality in many disease settings, including cancer, cardiovascular disease, and infectious diseases. Several infectious diseases. such as tuberculosis, malaria, leprosy, and AIDS, only came under control and were effectively treated only after introduction of combinations of drugs that utilize different mechanisms of action.

E1224 is a water-soluble monolysine salt form of the ravuconazole (RAV) pro-drug (which is a phosphonooxymethyl ether derivative of RAV). It is a broad-spectrum triazole antifungal. RAV was evaluated extensively in animal models and in human trials including Phase 2 safety and efficacy trials in oropharyngeal and esophageal candidiasis and onychomycosis, and for prevention of invasive fungal infections in hematopoietic stem cell transplant recipients.

E1224 is rapidly converted to RAV in vivo: data from both preclinical and clinical evaluations of RAV are therefore considered relevant to E1224. E1224 is available as oral formulations (100 capsules). It has completed general toxicology and safety pharmacology trials, as well as five Phase 1 clinical trials: ascending, single and multiple loading and maintenance dose, bioavailability, and food-effect trial; ascending, multiple loading and maintenance dose trial of E1224 administered intravenously over 14 days; effect of E1224 on cytochrome P450 enzymes; and cardiac safety/thorough QTc trial.

Pharmacokinetic trials showed that E1224 is rapidly converted to RAV with a peak concentration at 2.5 to 6 hours post-dose with a long terminal half-life of 7.7 to 10.5 days. RAV accumulated over time (AUC on Day 1 < Day 7 and 14). With a loading and maintenance dose strategy, a steady state level at desired peak concentrations of RAV can be achieved within 7 days of initial dosing.

Safety evaluations indicated relatively mild, transient, and asymptomatic increases in liver enzymes – completely reversible upon discontinuation of therapy. Phase 1 cardiac safety evaluations showed that E1224 administration did not result in QTc interval prolongation. Mean QTcF interval was shortened compared to baseline, with peak effect on QTc interval at 1 and 1.5 hours post-dosing and mean change from baseline of -16.9 to -19.4 msec. QT remained within normal limits and there was no dose-response relationship to these changes at steady-state. Findings resolved spontaneously by 4 hours after dosing. Expert review indicated the unknown relevance of the cardiac safety findings.

Pooled safety data for the monolysine prodrug E1224 from Phase 1 and 2 trials indicated that E1224 was generally well tolerated and exhibited a safety profile quite similar to other azoles. Adverse events occurring in greater than 3% of E1224 recipients, with a dose-dependent pattern and at rates higher than those observed in placebo recipients included nausea, abnormalities in liver enzymes, dizziness, anxiety, and contact dermatitis.

These data indicate that E1224, unlike some other azole antifungal compounds,[18,19,20] does not prolong QTc. Shortening of QTc has been reported for a few compounds[21]. E1224 was generally well tolerated, with the exception of modest increases in liver enzymes (ALT, AST, and alkaline phosphatase).

The results of a pre- and postnatal development study in rats, a 26-week rat toxicity (tox) study, and a 39-week toxicology study in monkeys have shown the following results:

● In the pre- and postnatal development study in rats, the no-observable-adverse-effect level (NOAEL) for F0 maternal general toxicity and reproductive function was 27.4 mg/kg. The NOAEL for F1 development was 8.2 mg/kg. The NOAEL for F1 reproductive function and F2 embryo development was 109.4 mg/kg.

● Results from the 26-week rat tox study (dosed at 0, 10, 30, and 60 mg/kg/day) revealed no death or moribundity related to the test article. There were no changes in clinical signs, body weight, food consumption, ophthalmoscopy, or urinalysis. There were increased adrenal weights in females at ≥30 mg/kg, increased adrenal vacuolation of cortical cells in both sexes at ≥30 mg/kg, and decreased triglycerides in males at 60 mg/kg. Based on these results, the NOAEL was considered to be 10 mg/kg.

● Results from the 39-week toxicity study in monkeys with ravuconazole doses of 1 to 30 mg/kg revealed no death or moribundity related to the test article. No changes were observed in clinical signs or food consumption, though one male monkey in the 30 mg/kg dose group demonstrated gradual body weight decrease throughout the dosing period (-11%, vs pre-dosing). There were no changes in ophthalmoscopy, ECG, urinalysis, or hematology measurements. Gross findings showed enlarged liver and adrenals. The histopathological changes in liver appeared to be related to adaptive hepatocellular hypertrophy. These findings were comparable to those observed in the previous 4- and 13-week toxicity studies at doses of 5 and 30 mg/kg. These were adaptive changes related to hepatic enzyme induction, and no evidence of progression or increased severity was observed after 39 weeks of administration. In the adrenal glands, increased vacuolation of the cortical cells in males at 30 mg/kg and females at 5 and 30 mg/kg; this was an expected class effect of azoles. A 3- to 4-fold increase in triglycerides was noted in the 30 mg/kg dose male monkeys compared to control. Based on these results, NOAEL was considered to be 5 mg/kg for males and 1 mg/kg for females.

Experimental data suggest a positive interaction between BZN and azole compounds for the treatment of Chagas disease[18]. BZN and posaconazole (POS) were administered individually or in

combination in an experimental acute murine infection model. Using a rapid treatment protocol for 7 days, the combined treatments were more efficacious in reducing parasitemia levels than the drugs given alone, with the effects most evident in combinations of sub-optimal doses of the drugs. The curative action of these drug combinations was investigated, using the same infection model and 25, 50, 75 or 100 mg/kg/day (mpk) of BZN in combination with 5, 10 or 20 mpk of POS, given alone or concomitantly for 20 days. The effects of the combination treatments on parasitological cures were higher than the sum of such effects when the drugs were administered separately at the same doses, indicating a positive interaction. Similar results were seen with combination treatment of E1224 and BZN in *T.cruzi* murine infection[22].

A Phase 1 drug-drug interaction trial was designed to assess the pharmacokinetics (PK) and safety interaction of BNZ and E1224 co-administered daily for a total of 54 days (Day 4 to Day 15) E1224 multiple dose 400 mg loading dose once daily for 3 days, followed by maintenance dose 100 mg once daily for 9 days (from Day 7 to Day 15); Day 9 BNZ single dose (2.5 mg/kg); and Day 12 to Day 15 BNZ multiple dose (2.5 mg/kg twice daily). The trial was conducted in Argentina and was concluded in early 2015, with enrolment of 28 healthy male volunteers. Both compounds were well tolerated, in monotherapy and combination. There were no treatment discontinuations or serious adverse events. Transient, minor, non-concomitant increase in bilirubin and liver transaminases occurred in 2 patients in a pattern not suggestive of drug effect. There was no interaction of RAV on BNZ PK and the limited impact of BNZ on RAV PK, suggesting that co-administration of RAV and BNZ may not require any E1224 dosing adaptation. The lack of clinically relevant safety findings provided support for follow-up evaluation of the two compounds in combination.

In conclusion, recent scientific advances have provided further impetus to develop new therapeutic approaches for CD using different doses and duration of BZN, as well as combinations directed at multiple therapeutic targets to improve treatment response and tolerability and reduce the potential for development of resistance.

This project focuses on the proof-of-concept evaluation of improved treatment regimens of BZN, with the assessment of new BZN-sparing regimens in monotherapy and in combination with E1224.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1. Objectives

General objective

To determine the efficacy and safety of different dosing regimens of BZN and BZN and E1224 in combination (BZN/E1224) in reducing and clearing *T. cruzi* parasitemia in individuals with the chronic indeterminate CD.

2.1.1. Primary Objective

To determine the efficacy of different dosing regimens of orally administered BZN and BZN/E1224 in individuals with chronic indeterminate CD, by determining the proportion of patients who convert from positive to negative in serial, qualitative PCR test results (3 negative PCR results) at end-of-treatment (EOT) and sustain parasitological clearance at 6 months of follow-up, in comparison to placebo.

2.1.2. Secondary Objectives

- To measure the reduction in parasite load at weeks 1, 2, 3, 4, 6, 10, 12 and at 4, 6 and 12 months follow-up, as measured by quantitative PCR.
- To assess the time to parasite DNA clearance (below the qPCR LOD for each of the regimens
- To assess the sustained parasitological response at week 12 and 12 months for each of the regimens, in comparison with placebo.
- To assess the time to sustained clearance of parasitemia for each of the regimens.
- To determine the efficacy of the different dosing regimens in individuals with chronic indeterminate CD, by determining the proportion of patients who convert from positive to negative in serial, qualitative PCR test results (3 negative PCR results) at EOT, in comparison with placebo.
- To explore if there is a dose-response relationship between the dose of treatment given and parasite clearance in order to determine the minimum effective dose.
- To evaluate serological response by conventional serology at 12 months of follow up and non-conventional serology at W12, 4M, 6M and 12 months of follow up.
- To characterize the population pharmacokinetic parameters of orally administered BZN and BZN/E1224 in adults with chronic indeterminate CD.
- To evaluate safety profile of different regimens based on clinical, laboratory measurements and EKG.
- To correlate pharmacokinetic parameters with parasitological response and safety outcomes.
- To evaluate the incidence of Serious Adverse Events (SAEs) and/or adverse events leading to discontinuation of treatment.

2.2. Trial Endpoints

2.2.1. Primary Endpoints

Efficacy Endpoint

- Parasitological response as determined by serial negative qualitative PCR results (3 negative PCR results, to be collected in the same day) at EOT and sustained parasitological clearance until 6 months follow-up.

For efficacy assessments, the EOT of each treatment arm will be defined in accordance to the duration of the treatment regimen. Sustained response will be assessed in all treatment arms using the same number of PCR samples (i.e. EOT; 12 weeks; 4 and 6 months).

Safety Endpoints

- Incidence and severity of adverse events (clinical, laboratory measurements and EKG)
- Incidence of SAEs and/or adverse events leading to treatment discontinuation

Safety will be assessed through routine monitoring of adverse events, evaluation of haematological and blood chemistry values, regular measurement of vital signs, physical examination, and conduct of EKGs at selected trial visits (according to the trial schedule).

2.2.2. Secondary Endpoints

Efficacy Endpoints

- Sustained parasitological clearance at 12 weeks and 12 months of follow-up.
- Parasite clearance at weeks 1, 2, 3, 4, 6, 10, 12, and at 4, 6, and 12 months follow-up as measured by qualitative PCR.
- Change in parasite load over time assessed at weeks 1, 2, 3, 4, 6, 10, 12, and at 4, 6, and 12 months follow-up as measured by quantitative PCR.
- Serological response by conventional serology assessed at 12 months of follow up and non-conventional serology assessed at W12, 4, 6, and 12 months of follow up. (changes in titters over time)

Pharmacokinetics Endpoints

- Blood level concentrations will be determined at D0 (pre-dose), after first day of treatment administration (day 1, post-dose), at day 2 and day 3, at steady-state phase (week 2-10).
- Population pharmacokinetic parameters will include: AUC, Cmax, Cmin, CL, Vd, and t1/2.

Covariates to be evaluated: age, body mass index and parasite load at baseline, gender.

3. TRIAL DESIGN AND TRIAL DESIGN RATIONALE

3.1. Trial design

This trial is designed as a double-blind, double-dummy, randomised, prospective, comparative, placebo-controlled, pharmacokinetic-pharmacodynamic and proof-of-concept trial design, with seven-parallel groups.

During this trial, population PK evaluations of Benznidazole and E1224 and pharmacokinetic-pharmacodynamic assessments will be performed.

This trial, that aims to determine whether at least one of seven dosing regimens of orally administered BZN and BZN/E1224 is more appropriate with regards to efficacy and safety when compared to placebo, will be conducted in three sites in Bolivia.

The sites were selected for this Phase 2 trial based on significant disease burden, the necessary expertise, excellence of care of Chagas patients, established infrastructure and previous experience in conducting clinical trials according to GCP.

Patients will be enrolled equally and randomly into each of the seven treatment groups. The trial will be sufficiently powered to provide evidence of superior efficacy of each of the treatment regimens relative to placebo.

Primary efficacy analyses will be performed when all patients complete the 6 months follow-up visit. Efficacy and safety database until 6 months will be locked. Analyses of results will be used to guide planning of a follow-up Phase 3 clinical study. Sponsor personnel directly involved in the clinical trial, principal investigators and study team site personnel will remain unequivocally blinded to treatment allocation and results of the analyses.

Efficacy and safety will be monitored by an external independent DMC, on an ongoing basis. The committee is to include cardiac and liver safety experts, as well as Chagas Disease expertise (see section 11).

3.2. Trial duration and duration of patient participation

The total duration of patient participation in the trial will be approximately 13 months, considering up to 40 days for screening, 8 weeks of treatment, and follow-up visits up to 12 months after treatment initiation.

Following written, voluntary informed consent, patients will initiate a 40-day screening phase. Once a patient is randomized at D1, s/he will have follow-up visits during the treatment phase of the trial at Day 1, 2, 3, (allowable window of + 2 days for day 3 only) and at weekly intervals at week 2 (allowable window of + 3 days), weeks 3-10 (allowable window of ± 3 days), and after 4, 6, and 12 months after treatment initiation (allowable window of ± 7 days).

In addition, patients will be advised to return on any day during the follow-up period if they present any medical occurrence or adverse event.

Patient recruitment is expected to occur within 10 months of recruitment initiation. Therefore, the timeline of First Patient In (FPI) and Last Patient Out (LPO) is 22 months. However, the total trial duration is estimated to be 28 months, from start-up phase to final trial report.

3.3. Rationale of trial design

The selection of a placebo-controlled Phase 2 clinical trial design was based on the requirements for well-controlled proof-of-concept, efficacy and safety data for Benznidazole and Beznnidazole/E1224 for this therapeutic indication.

The choice of the CD target population was largely driven by the unmet medical need for a new, safe and effective treatment for chronic indeterminate CD. Adults with chronic indeterminate CD are recognized as the population with the most urgent requirements for the development of new treatments. Drugs employed in children, with acute and early chronic CD are more often efficacious and better tolerated, with less than 10% of patients presenting serious adverse events.

Moreover, with a decreasing incidence and existing patterns of disease presentation, the highest disease burden is found in patients in the chronic indeterminate phase of the disease. CD is seldom diagnosed in the acute phase, with circulating parasites identifiable by optical microscopy, with the exception of congenital cases, immunosuppressed patients and the small clusters of oral transmission. Carrying out rigorous clinical trials in any of these populations would present ethical and logistical challenges (in light of high response rates to benznidazole in congenital disease), confounding elements (due to the severity of disease and need for frequent concomitant medications in the case of immunosuppressed patients) and feasibility issues (due to the small numbers and dispersed nature of most clusters of oral transmission in the Amazon region).

The efficacy of this indication will be assessed through sustained parasitological response over six months measured by serial negative qualitative PCRs as the primary endpoint. Clinical CD related events occur at a low incidence in this population and would thus require very large clinical trials to assess incidence changes and serological endpoints would require several years to decades in chronic CD. Recently concluded clinical trials provide support for the selected endpoint and timelines for assessment in proof-of-concept.

The secondary endpoints will assess the sustained parasitemia at 12 months and the time to clearance of parasitemia by serial negative qualitative PCR results, comparing each of the six dosing regimens relative to placebo, notably assessment of parasite load over time, conventional and non-conventional serology. Parasite clearance data will be correlated with pharmacokinetic parameters.

The evaluation of cure is considered to be the most complex aspect of treatment assessment in CD. Parasitological cure is difficult to interpret and its evaluation is challenging in

light of the need for total elimination of parasites, not only from the blood but also from tissues where it is not measurable. Assessment of parasitological cure requires long-term follow-up because of the pathogenesis and hysteresis of the disease, the characteristics of the parasite, the host response times, and the consequent clinical manifestations. Such changes occur over long periods of time.

A negative direct blood smear at the end of treatment is generally accepted as evidence of a favorable response in acute CD patients. In chronic disease, serial hemocultures, xenodiagnosis, or PCR have been used to support the serological assessment, and a positive parasitological diagnostic test result indicates treatment failure. Hemoculture and xenodiagnosis are techniques that require proper resources/infrastructure and very skillful and experienced personnel to adequately perform them. With regard to conventional serology, for chronic indeterminate CD patients, seroconversion to negative would occur after 5 to 10 years (although cases with a longer period for conversion to negative have been described). Therefore, for the purposes of clinical development and proof-of-concept, it has been proposed that qualitative and quantitative PCR parasitological tests be used as markers for efficacy in clinical trials of chronic indeterminate CD.

Important considerations have included the significant variability in the clinical sensitivity and specificity of PCR tests observed in early published trials, with differences seen across all phases of the disease and across a number of techniques in use that require standardization. In particular, there has been concern regarding the sensitivity of PCR in chronic CD due to low levels of circulating parasites and natural fluctuations in parasitemia. In a recent comparison of two diagnostic techniques in patients with chronic CD, PCR had high specificity (100%) and moderate sensitivity (70% to 75%).

The latest PCR trials have shown higher sensitivity, with a broad dynamic range for PCR use allowing direct measurements in cases with high parasitic loads such as in immunosuppressed CD patients and congenitally infected newborns, as well as in cases with low parasitic loads, such as patients in the chronic indeterminate phase or who are receiving antiparasitic treatment.

An important advance in recent years has been the trial sponsored by UNICEF/UNDP/ World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) for the standardization and laboratory validation of qualitative PCR testing for *T. cruzi*. Standardized procedures for the qualitative assessment of PCR (standard and real-time) testing with higher analytical sensitivity and specificity, reproducibility, and low levels of intra- and inter-assay variation and high levels of accuracy have been selected.

During expert panel discussions and with support from recent data on DNDi sponsored trials, there is consensus on the value of using serial, sequential blood collections for qualitative and quantitative PCR assays in order to increase PCR test sensitivity and evaluate durability of response. The proposed trial will incorporate the standardized and optimized procedures for

PCR testing. In particular, it would focus on the real-time PCR technique described by Duffy et al and implemented in the recent E1224 Phase 2 clinical trial.

Additionally, the validation of qPCR methods of *T.cruzi* DNA in human blood samples are the object of international efforts aiming to provide an accurate biomarker for diagnosis and treatment monitoring for patients with CD.

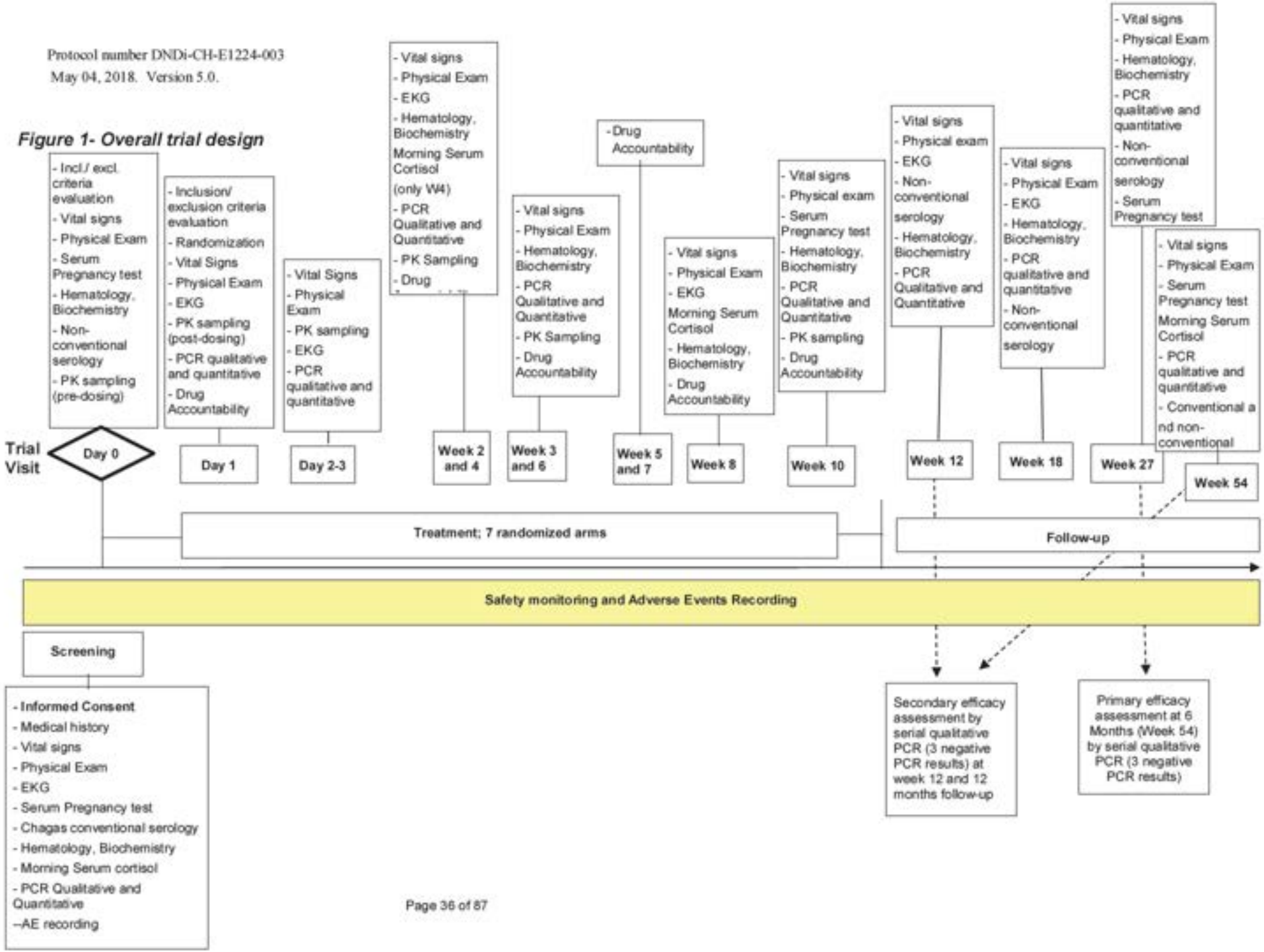
A recent article published by Ramirez, JC *et al*, described an international trial performed by 26 experienced PCR laboratories from 14 countries to assess the performance of duplex quantitative real-time PCR (qPCR) strategies on the basis of TaqMan probes for detection and quantification of parasitic loads in peripheral blood samples from CD patients. Two methods were studied: Satellite DNA (SatDNA) qPCR and kinetoplastid DNA (kDNA) qPCR. Both methods were challenged against 156 blood samples provided by the participant laboratories, including samples from acute and chronic patients with varied clinical findings, infected by oral route or vectorial transmission. Analyses of clinical samples revealed a high concordance in terms of sensitivity and parasitic loads determined by both SatDNA and kDNA qPCRs. All the laboratories participants of this clinical trial participated in this qPCR validation trial[23].The current trial will obtain information on pharmacokinetics and pharmacodynamics of alternative regimens of Benznidazole and the combination of Benznidazole and E1224 in CD to inform further clinical development. Sampling for population pharmacokinetic analysis of drug concentrations over time will be obtained in the present trial, and correlation is planned of pharmacokinetic parameters with different efficacy and safety outcomes.

The rationale for the selection of the doses and regimens are based on the following criteria:

	Group	Objective	Justification / comment
1	BZN STD Regimen 300 mg - 8 wks	Reference	Need to document reference treatment efficacy with ELEA product on a fixed daily dose.
2	BZN 300 mg - 4 wks	Improving safety & maintaining efficacy	Animal data show that standard BZN dose with a 25% reduction in treatment duration showed identical efficacy. Human trials suggest that duration may be reduced.
3	BZN 300 mg - 2 wks	Improving safety & maintaining efficacy	Animal data show that standard BZN dose with a 25% reduction in treatment duration showed identical efficacy. Human trials suggest that duration may be reduced.
4	BZN 150 mg - 4 wks	Improving safety & maintaining efficacy	Pediatric and adult population PK trials suggest 50% dose scaling may be possible. Human trials suggest that duration may be reduced. Higher risk considering mouse data with one third of dose and full duration.
5	BZN 150 mg - 4 wks E1224 300 mg	Improving both safety & efficacy	Assessment of combination with current dose BZN in short course. Animal data show reduced BZN dose with long duration may lead to a sub-optimal response, thus warranting the addition of E1224.
6	BZN 300 mg -	Improving both	Animal and human trial data show that intermittent

	8 wks, weekly doses E1224 300 mg	safety & efficacy	BZN dose with long duration may lead to a sub-optimal response, thus warranting the addition of E1224.
7	Placebo	Negative control arm	Assessment of sensitivity of PCR in different epidemiological settings and parasite diversity.

Figure 1- Overall trial design



4. SELECTION OF PATIENTS

Adult patients ≥18 and ≤50 years and weight ≥50 kg to ≤80 with serologic tests confirming a diagnosis of *T. cruzi* infection will be selected to participate in the trial.

The following screening criteria are designed to select patients for whom the protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular patient. Eligibility criteria may not be waived by the investigator. Any questions regarding a patient's eligibility should be discussed with DNDi's medically qualified trial manager prior to a patient's enrollment.

4.1. Screening criteria

- Signed, written informed consent form
- Age ≥18 to ≤50 years
- Weight ≥50 kg to ≤80 kg
- Diagnosis of *T. cruzi* infection by:
    - Conventional serology (a minimum of two positive tests [Conventional ELISA, Recombinant Elisa and/or Indirect Immunofluorescence (IIF)])
- Ability to comply with all protocol specified tests and visits and have a permanent address
- Patients must be residents of areas free of vectorial transmission (*Triatoma infestans*). For this protocol, it will be accepted the status of Vectorial Transmission Interruption or Consolidation as per the definition of PAHO/WHO, or the Local Health Program.
- No signs and/or symptoms of the chronic cardiac and/or digestive form of CD
- No acute or chronic health conditions, that in the opinion of the PI, may interfere with the efficacy and/or safety evaluation of the trial drug (such as acute infections, history of HIV infection, liver and renal disease requiring treatment)
- No formal contraindication to BZN (according to the Summary of Product Characteristics) and E1224 (according to the Investigator's Brochure)

  Note: The contraindications described for Benznidazol and E1224 are essentially hypersensitivity to the active ingredient or any excipient. In the case of hepatic or renal impairment or blood discrasia, the medication should only be administered under strict medical supervision. During all the treatment period, the blood count will be monitored,

with special attention to leucocytes. Subjects will be indicated about the need of no alcohol intake.

- No history of hypersensitivity, allergic, or serious adverse reactions to any of the "azoles" compound, and/or its components
- No history of CD treatment with BZN or NFX at any time in the past
- No history of systemic treatment with itraconazole, ketoconazole, posaconazole, isavuconazole, or allopurinol in the past
- No history of alcohol abuse or any other drug addiction (as specified in the Study Manual of Operations)
- No condition that prevents patient from taking oral medication
- No concomitant or anticipated use of drugs that are either sensitive CYP3A4 substrates and/or extensively metabolized by CYP3A4 with a narrow therapeutic range (as per Appendix 2)
- No medical history of Familial Short QT syndrome or concomitant therapy with medications that can shorten the QT interval (as per Appendix 2)
- No family history of sudden death
- No family history of sudden infant death syndrome

4.2. Inclusion criteria

Following the screening period, patients must meet ALL of the following inclusion criteria to be eligible for randomization:

- Confirmed diagnosis of *T. cruzi* infection by:
    - Serial qualitative PCR (three samples collected over a single day, at least one of which must be positive) AND
    - Conventional serology (a minimum of two positive tests must be positive [Conventional ELISA, Recombinant Elisa and/or IIF)
- Women in reproductive age must have a negative serum pregnancy test at screening, must not be breastfeeding, and must use a double barrier method of contraception to avoid pregnancy throughout a clinical trial and for 3 months after completion of the trial, in such a manner that the risk of pregnancy is minimized especially during exposure to

treatment. Women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device with a hormonal component are required to use an additional barrier method of contraception for the time period specified.

- Normal EKG (PR ≤200 msec, QRS <120 msec, and QTc ≥350 msec and ≤450 msec interval durations in males and QTc ≤470 msec in women) at screening.

4.3. Exclusion criteria

The presence of any of the following will exclude a patient from trial randomization:

- Signs and/or symptoms of chronic cardiac and/or digestive form of CD
- History of cardiomyopathy, heart failure, or ventricular arrhythmia.
- History of digestive surgery or mega syndromes.
- Any other acute or chronic health conditions that, in the opinion of the PI, may interfere with the efficacy and/or safety evaluation of the trial drug (such as acute infections, history of HIV infection, diabetes, uncontrolled systolic/diastolic blood pressure, liver, and renal disease requiring medical treatment).
- Laboratory test values considered clinically significant or out of the allowable range at selection period as follows:
    - Total WBC must be within the normal range, with an acceptable margin of +/- 5% (3,800 – 10,500/mm3).
    - Platelets must be within the normal range up to 550,000/mm3
    - Total bilirubin must be within the normal range
    - Transaminases (ALT and AST) must be within the normal range, with an acceptable margin of 25% above the upper limit of normality (ULN), ≤1.25 x ULN.
    - Creatinine must be within an acceptable margin of 10% above the ULN, ≤1.10 x ULN.
    - Alkaline phosphatase must be within the normal range up to Grade 1 CTCAE (≤ 2.5 x ULN)
    - GGT must be within the normal range up to 2x ULN.
    - Fasting glucose must be within the normal range
    - Electrolytes (Ca, Mg, K) must be within the normal range
- If the results of the blood tests (hematology and biochemistry) are out of the ranges defined above, but within the limits of CTCAE (version 4.03) Grade 1, and the laboratory

finding is considered as non-clinically significant, a new sample can be collected for a retest. Only one retest will be allowed within the screening period.

- If the result of retest is within the margins defined above, the Investigator will review the parameter(s) together with all other medical information available (medical history, clinical examinations, vital signs, etc.) and upon his/her medical judgment will decide if the patient is eligible or not for trial randomization.
- Any condition that prevents the patient from taking oral medication
- Patients with history of allergy (serious or not), allergic skin rash, asthma, intolerance, sensitivity or photosensitivity to any drug
- Patients with any contra-indication (known hypersensitivity) to any nitroimidazoles, e.g. metronidazole.
- Any concomitant use of allopurinol, antimicrobial, or anti-parasitic agents.
- Any planned surgery likely to interfere with the trial conduction and/or treatment evaluation
- Unlikely to co-operate with the trial
- Any previous participation in any clinical trial for Chagas Disease treatment evaluation
- Participation in another trial at the same time or within 3 months prior to selection (according to local regulations)

5. SCHEDULE OF EVENTS

Period	Screening	Baseline	Treatment									Follow-up				
Visit	Day -40 to -1	D0[f] Pre-dose	W1			W2	W3	W4	W5	W6	W7	W8	W10	W12	4M 18w	6M 27w	12M 54 w
			D1[f] Post Dose	D2	D3												
Trial Procedures																	
Informed Consent	X																
Randomization			X														
Medical History	X																
Prior/Con Meds	←																→
Inclusion/Exclusion		X															
Vital Signs [a]	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X
Physical Exam	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X
EKG	X		X	X	X	X		X				X		X	X		
Serum Pregnancy Test	X	X											X			X	X
Conventional Serology	X																X
Non- conventional Serology [b]		X												X	X	X	X
Laboratory [c]	X	X				X	X	X		X		X	X	X	X	X	
Morning serum cortisol	X							X				X					X
PCR Qualitative and Quantitative [d]	X		(X)[e]	(X)[e]	(X)[e]	X	X	X		X			X	X	X	X	X
Adverse Events	←																→
Blood PK Samples		X	X	X	X	X	X	X		X			X				
Drug Accountability			X			X	X	X	X	X	X	X	X				

a Vital Signs: axillary temperature, blood pressure and pulse rate;
b Non- conventional Serology: Lytic Antibodies.
c Laboratory: CBC. Biochemistry: ALT; AST; total, direct, and indirect bilirubin; GGT; a kaline phosphatase; creatinine, fasting glucose, calcium, magnesium, potassium, morning serum cortisol. Prothrombin Time (INR) will be assessed in case of ALT or AST >3xULN. The serum remained from the lab analysis (2 mL) will be stored for future biomarkers assays.
d PCR samples: 15mL, collected in 3 tubes of 5 mL;
e Patients will be sampled at 2 randomly time-points to be collected between D1-3;
f D0 and D1 may be at the same day if all results necessary for patients' inclusion/ exclusion criteria evaluation are available. In this case, vital signs and PK sampling described at D1 should be done after treatment administration. Eligibility criteria should be evaluated before randomisation and dosing.

6. ENROLMENT PROCEDURES

Patients presenting at the trial sites will undergo the routine assessments, including complete medical history, physical examination, CD conventional serology, hematology and biochemistry tests.

Adult patients ≥18 and ≤50 years with serology tests confirming a diagnosis of *T. cruzi* infection and fulfilling screening criteria will be invited to participate in the trial through the informed consent process. In order for the patient to be enrolled in the trial, the informed consent form must be obtained from the patient by the Principal Investigator or the delegated trial team member prior to any trial specific procedure.

The participating site must complete a Patient Screening/Enrolment Log to reflect screening number, enrolment status, date of enrolment, trial enrolment number, and reason for not enrolling, whenever applicable. Enrolment and screening procedures should occur up to 40 days or less (i.e., –D40 to D0) from the intended initiation of trial therapy. Patients fulfilling entry criteria will go through the randomisation procedures. Only patients randomized into the trial will receive trial medications and will be assigned a trial identification number.

A patient will have fulfilled participation in the clinical trial after completion of the last protocol-specified contact, i.e. the 12-month visit.

7. TREATMENTS

7.1. Investigational Products

Investigational products supplied for this trial will include:

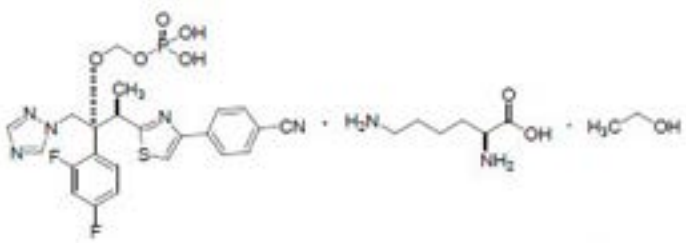
E1224 100-mg capsules or matching placebos in blister packaging, supplied by Eisai Pharmaceuticals.

E1224 capsules are size #3 capsules containing 100 mg of E1224 drug substance (ravuconazole equivalent dose) comprising 125.1 mg fosravuconazole, 33.4 mg L-lysine, and 10.5 mg ethanol. The colors of the cap and body are red and yellow, respectively, and each of them has the word “Eisai” printed on them. The capsule content is prepared by blending E1224 drug substance and excipients.

Developmental Code: E1224 (monolysine salt ethanolate of fosravuconazole)

INN: fosravuconazole (active moiety)

The chemical structure of E1224 is shown below:



fosravuconazole L-lysine ethanol

Molecular formula: $C_{23}H_{20}F_2N_5O_5PS \cdot C_6H_{14}N_2O_2 \cdot C_2H_6O$
Molecular weight: 739.73 (the molecular weight of active moiety is 547.47)

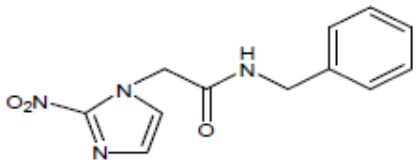
Abarax® (BZN 100 mg and 50 mg tablets or matching placebos), supplied by Laboratorios ELEA, S.A., Argentina.

Each double-scored tablet of Abarax® 50 mg contains 50 mg of Benznidazole. Excipients: corn starch, lactose monohydrate, sodium croscarmellose, magnesium stearate, cellulose microcrystalline PH 102, FD & C Blue 2 lake.

Each double-scored tablet of Abarax® 100 mg contains 100 mg of Benznidazole. Excipients: corn starch, lactose monohydrate, sodium croscarmellose, magnesium stearate, and cellulose microcrystalline PH 102.

Benznidazole (N-Benzyl 2-Nitro-1-Imidazolacetamide), which is Abarax® pharmaceutical active ingredient, is obtained by chemical synthesis, whose molecular formula is C12H12N4O3 empirical and molecular weight of 260.25.

Its chemical molecular structure is as follows:



7.2. Doses and treatment regimens

Patients will be randomly assigned into one of the following balanced groups:

Key		
Benznidazole (Abarax ELEA 100mg and 50 mg tablets)/		**BZN 100 mg ●** **BZN 50mg ■**
BZN Placebo (100mg and 50 mg tablets)		**BZN PCB 100 mg ❍** **BZN PCB 50mg ❒**
E1224 (Eisai 100 mg capsules) / **E1224 Placebo (100 mg capsules)**		**E1224 100 mg ♦** **E1224 PCB 100 mg ◊**
1	**BZN 300 mg - 8 wks (STD Regimen):** Benznidazole 150 mg BID for 8 weeks 3 E1224 matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks.	8 weeks Morning ●■ Evening ●■ D1-D3 (once daily) ◊◊◊ W2-W8 (once weekly) ◊◊◊
2	**BZN 300 mg - 4 wks:** Benznidazole 150 mg BID for 4 weeks, to be followed by BZN placebo administered BID to complete 8 weeks 3 E1224 matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks	4 weeks Morning ●■ Evening ●■ 4 weeks Morning ❍❒ Evening ❍❒ D1-D3 (QD) ◊◊◊ W2-W8 (once weekly) ◊◊◊
3	**BZN 300 mg - 2 wks:** Benznidazole 150 mg BID for 2 weeks, to be followed by BZN placebo administered BID to complete 8 weeks 3 E1224 matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks.	2 weeks Morning ●■ Evening ●■ 6 weeks Morning ❍❒ Evening ❍❒ D1-D3 (QD) ◊◊◊ W2-W8 (once weekly) ◊◊◊

4	**BZN 150 mg - 4 wks:** BZN 150 mg and BZN placebo administered in two separate daily doses for 4 weeks, to be followed by BZN placebo BID to complete 8 weeks 3 E1224 matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks.	4 weeks Morning ●■ Evening ❍❒ 4 weeks Morning ❍❒ Evening ❍❒ D1-D3 (QD) ◊◊◊ W2-W8 (once weekly) ◊◊◊
5	**BZN 150 mg - 4 wks / E1224 300 mg** BZN 150 mg and BZN placebo administered in two separate daily doses for 4 weeks, to be followed by BZN placebo administered BID to complete 8 weeks Loading dose of E1224 (300 mg QD on days 1-3) followed by 300 mg administered once weekly (starting on week 2) for 7 weeks (total dose: 3000 mg)	4 weeks Morning ●■ Evening ❍❒ 4 weeks Morning ❍❒ Evening ❍❒ D1-D3 (QD) ♦♦♦ W2-W8 (once weekly) ♦♦♦
6	**BZN 300 mg (weekly doses) - 8 wks / E1224 300 mg**: BZN 150 mg BID administered once weekly for 8 weeks (total 8 days of intermittent treatment) and BZN-placebo to be administered BID in the other 6 days of the week Loading dose of E1224 (300 mg QD on days 1-3) followed by 300 mg administered once weekly (starting on week 2) for 7 weeks (total dose: 3000 mg)	Once a week Morning ●■ Evening ●■ QD for 6-week days Morning ❍❒ Evening ❍❒ D1-D3 (QD) ♦♦♦ W2-W8 (once weekly) ♦♦♦
7	**Placebo:** 4 BZN matched placebo tablets (100 mg and 50 mg) administered BID for 8 weeks 3 E1224-matched placebo capsules QD on days 1-3, followed by 3 placebo capsules administered once weekly (starting on week 2) for 7 weeks.	8 weeks Morning ❍❒ Evening ❍❒ D1-D3 (QD) ◊◊◊ W2-W8 (once weekly) ◊◊◊

All treatment arms consider the regimens in fixed doses. The doses of Benznidazole were defined taking as reference the guidelines for the standard treatment of CD, which recommend 5 mg/kg/day of BZN for 60 days. Considering an average weight of 60 kg, the fixed dose would be 300 mg daily. With the proposed prescription, patients' daily doses will vary as follows:

Patients' Weight range	**50kg to 80 kg**
Dosing group	**Actual total daily dose (mg/kg/day)**
300 mg - 5.0 mg/kg	3.75 mg/kg to 6 mg/kg
150 mg - 2.5 mg/kg	1.88 mg/kg to 3 mg/kg

At the weekly visits, E1224 or E1224–matched placebos will be administered under the supervision of trial staff. The patient will receive enough BZN or BZN matched placebos until the next scheduled visit. Also, they must bring all remaining trial drugs to check for compliance with prescribed treatment and ensure drug accountability.

If a patient misses a dose or vomits within 30 minutes of undertaking the treatment, the dose may be re–administered.

As per recommended in the ABARAX ® package insert, patients will be advised to abstain from alcohol during treatment.

Rescue treatment:

At the end of the study, patients will receive the information related to what study treatment they took and the PCR results.

All patients treated with placebo will be offered BZN treatment at 5 mg/kg/day, divided in two daily doses for 60 days.

For all other patients, except those treated with BZN standard regimen, who remain PCR positive at end of trial, BZN treatment will be offered at 5 mg/kg/day, divided in two daily doses for 60 days.

For all patients treated with BZN standard regimen who remain PCR positive at end of trial NFX treatment will be offered at doses of 10-15 mg/Kg/day, divided in two-three daily administrations for 60 days.

Patients who do not tolerate the trial treatment will be withdrawn from the trial and will

be offered an alternative treatment with NFX 10-15 mg/Kg/day, divided in two-three daily doses for 60 days.

7.3. Drugs labelling, packaging

Minimum information to be included on the trial medication label will be:

• Name of Sponsor

• Trial protocol number

• Treatment number

• Pharmaceutical dosage form, route of administration, quantity of dosage units

• “For clinical trial use only”

• Expiry date and storage conditions

Any additional, country-specific requirements need to be identified and applied.

7.4. Accountability

The clinical supplies will be shipped to the coordinating trial site at each country and then, distributed to each site accordingly.

Trial specific forms to track and register the trial drugs will be used for the accountability. Adequate records on the trial medications receipt, use, return, loss, or other disposition will be documented and maintained by the trial site. The investigator or the pharmacist or a designated person must complete in real time all the documents provided by the sponsor for the treatment management. The trial specific forms will be considered as the source document.

Trial monitors will be in charge of checking the drug accountability regularly during the monitoring visits.

The trial drugs must not be used for any other purpose other than the trial. Under no circumstances the investigator or site staff may supply the trial medication to other investigators or health care services, or allow the medication to be used other than as directed by this protocol without prior authorization from DNDi.

All patients must be asked to bring back the trial medication at each visit for accountability and treatment compliance verification. The remaining treatments will be later collected by the trial monitor. After checking, the treatments will be packaged in an inviolable

primary container and stored in the research site or in the coordinator center until the destruction is authorized by the sponsor.

7.5. Storage

Stability trials performed by Eisai showed that E1224 100-mg capsules were stable at 30°C/75% RH for 12 months and 40°C/75% RH for 6 months. Therefore, provisional shelf life for E1224 100-mg capsules is 24 months at controlled room temperature. The shelf life of the 100-mg capsules will be extended based on the results of future stability data.

BZN tablets are stable at room temperature (15 – 30º C).

Therefore, the trial drug can be shipped at normal conditions and must be kept at room temperatures, at 30º C. The trial drugs must be kept in a locked room at each of the trial sites, with restricted access only by the authorized trial personnel and/or pharmacist.

Site storage conditions and temperature must be monitored by the site personnel for adherence to product specifications. Records must be done at least once daily and kept in the investigator trial file or in the pharmacy file.

7.6. Blinding and procedures for unblinding

This is a double-blinded trial. Patients, investigators and sponsor staff directly involved with the clinical trial will not be aware of the treatment allocation and randomization list. Double-blinding will be adopted for all trial arms and the placebo arm.

Primary efficacy analyses will be performed when all patients complete 6 months follow-up visit. Analyses of results will be used to guide planning of a follow-up Phase 3 clinical study. Analysis is to be performed by an independent, external statistician. In order to allow administrative decisions, the results of the primary endpoint will be disclosed unblinded (by treatment arm) to the Joint Development Committee. No individual patient data will be shared with the Committee. In addition, data is to be shared with the Data Monitoring Committee.

The Joint Development Committee is composed of the DNDi Project Leader (DNDi Head of Chagas Program or designated person), DNDi Medical Director, and members from the Eisai team. On occasions the JDC meetings are attended by other members of staff from these organisations. Under no circumstances will, the Principal Investigator, trial site staff and study personnel from the Sponsor managing the trial have access to the results of the primary efficacy analysis prior to the end of the study. They will remain unequivocally blinded until completion of 12 months follow-up and final database lock. The study statisticians will not have access to unblinded data before conduct of the analyses at the end of the study.

Randomization procedures will be done via the IRS (Interactive Response System), according to a predefined list. The specific procedures will be detailed in the IRS guideline. The treatment groups will be allocated on Day 1 based on a balanced block randomization.

Each patient will be assigned with an identification code that will correspond to the trial kit number allocated to the patient. The label will indicate the trial number and number of the kit, but will not indicate the treatment designation.

All objective measurements in this trial (in terms of efficacy and safety) will be performed blinded to the treatment allocation. EKGs will be read centrally and without knowledge of the patient's treatment group. PCR and laboratory information will be provided coded, without the name of the patient to allow blinding during sample processing and analysis.

Code breaking will be performed via the IRS. The investigator or person designated must contact the IRS and do the specific procedure as described in the IRS manual.

Breaking the code of any trial patient should only be performed by the investigator or by an authorized person if absolutely necessary to find out the treatment administered. The breaking of the code can only be performed when the result of a life threatening medical emergency may depend on the knowledge of the treatment the received by the patient. The DNDi clinical trial manager or the trial monitor must be immediately informed about the need of unblinding, ideally before doing so. An emergency code-break form will be filled out with information on patient number, date of code-break, identification and signature of person breaking the code, identification of person requesting the code break, reasons for breaking the code and investigator's signature.

A Serious Adverse Event form should also be filled out if the reason for code-breaking meets the criteria.

In addition, there will be situations whereby unblinding may not necessarily justify patient withdrawal from the trial. In such cases, these patients should also continue with trial visits and assessments as planned.

7.7. Concomitant treatment

Patients may receive concomitant therapy for medical occurrences during the course of the trial. However, specific drugs that interact with the trial drugs, have the potential to shorten the QT interval, elevate liver enzymes, are either sensitive CYP3A4 substrates and/or extensively metabolized by CYP3A4 with a narrow therapeutic range, are immunosuppressants or are known to have activity against *T. cruzi* may not be taken during the treatment phase of the trial or up to the 4M visit. These specific drugs are listed in the *Appendix 2*.

The use of hormonal contraceptives is allowed, however, the interaction with E1224 suggests the potential for decreased efficacy. The use of any oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device with a hormonal component should be registered as concomitant treatment. Additionally, as addressed in section 7.6.8, women will be required to add an additional method, such as barrier.

All concomitant medications taken by the patient during the trial, from the date of signature of the informed consent until the last follow up visit, will be recorded in the appropriate section of the Case Record Form.

8. TRIAL ASSESSMENTS

8.1. Timing of Assessments

As described in Section 5, Schedule of events, trial assessments will be done at Screening (Days –D -40 to D -1), Day 0 (baseline), Day 1, Day 2, Day 3 (allowable window of + 2 days for day 3 only), at weekly intervals at week 2 (allowable window of + 3 days), 3, 4, 6, 8, 10, and 12 (allowable window of ± 3 days), and after 4, 6, and 12 months from treatment initiation (allowable window of ± 7 days). On the weeks 5 and 7, patients will go directly to the pharmacy to return and take their treatments (E1224 will be administered on site).

The primary efficacy assessment will be done at EOT, week 12 and at 4, and 6months of follow-up by serial qualitative PCR (3 samples to be collected in the same day). Secondary efficacy assessments will be performed at weeks 1, 2, 3, 4, 6, 10, 12, and at 4, and 12 months.

PK assessments will be done at Day 0 (pre-dose), Day 1 (post 1st dose), Day 2, Day 3, week 2, 3, 4, 6 (steady-state phase), and at week 10 (elimination phase).

Safety assessments will be done at Day 1 (post-dose), at D2, D3, W2, W3, W4, W6, W8, W10, W12, and at 4, 6, and 12 months, and at any unscheduled visit.

8.2. Screening and Baseline Assessments

During screening, and after obtaining Informed Consent, the following assessments will be done in order to evaluate patient eligibility for the trial:

- Complete medical history with an emphasis on CD;

- Demographic data and history of medications;
- Physical examination, body weight and height and vital signs;
- Chagas Disease serology: serum sample will be collected at screening for conventional CD serology. Patient must have at least 2 positive tests (among Conventional ELISA, Recombinant ELISA or IIF) to be eligible;
- Real-time PCR for qualitative and quantitative assessment (q-PCR) for all patients. In addition to positive serological tests, the patient must at least have one positive qualitative PCR test to be eligible for this trial;
- Clinical safety laboratory evaluations: CBC, ALT, AST, total and direct bilirubin, GGT, alkaline phosphatase, creatinine, fasting glucose, Ca, Mg and Kwill be assessed at screening and repeated at Day 0.
- Morning serum cortisol: a morning blood sample will be taken for cortisol levels at screening visit.

  The following parameters must be within the lab ranges defined below:

  - Total WBC must be within the normal range, with an acceptable margin of +/- 5%
  - Platelets must be within the normal range up to 550,000 / mm^3
  - Total bilirubin must be within the normal range
  - Transaminases (ALT and AST) must be within the normal range, with an acceptable margin of 25% above the upper limit of normality (ULN), $\leq$1.25 x ULN.
  - Creatinine must be within the normal range, with an acceptable margin of 10% above the ULN, $\leq$1.10 x ULN.
  - Alkaline phosphatase must be within the normal range up to Grade 1 CTCAE (< 2.5 x ULN)
  - GGT must be within the normal range up to 2x ULN.
  - Fasting glucose must be within the normal range
  - Electrolytes (Ca, Mg, K) must be within the normal range

If blood samplings (haematology and biochemistry) are out of the ranges defined above, but within the limits of CTCAE Grade 1, and the investigator consider this laboratory finding as not clinically significant, one new sample can be collected from the patient and retested. The retest could be done only one time within the screening period.

If the result of retest is within the margins defined above, the Investigator will review the parameter(s) together with all other medical information available (medical history, clinical examinations, vital signs, etc.) and upon his/her medical judgement will decide if the patient is eligible or not for trial randomization.

- Serum pregnancy test: Women of reproductive potential will undergo a serum pregnancy test at screening. The test must be negative for the woman to be considered eligible for this trial;
- EKG: 1 examination will be obtained at screening and must be normal for patient eligibility, i.e. PR ≤200 msec; QRS <120 msec; and QTc ≥ 350 msec and ≤450 msec interval durations in males, and QTc ≤470 msec in women. Any clinically significant abnormalities found on the electrocardiogram will automatically lead to patient exclusion from this trial.
    - Please refer to item 7.5.2 (Sampling Assessment Schedule) for detailed information on required sampling volume.

At baseline (Day 0) the following assessments will be performed before the first dose of medication is administered, in order to obtain baseline safety data and data for further comparative analyses and multiple parameter correlations:

- Physical exam
- Vital signs: axillary temperature, blood pressure and pulse rate.
- Serum pregnancy test: Women of childbearing potential will undergo a second serum pregnancy test so as to ensure they are not pregnant at treatment onset. Any positive test at baseline will automatically exclude the patient from this clinical trial.
- Blood sample for non-conventional serology and laboratory assessments
- PK sample, pre-dose

Concomitant medications at baseline will be recorded in the appropriate CRF.

Patient's eligibility criteria evaluation will be performed at day 0/day 1 (before dosing), or when the investigator has access to all results necessary for patient's inclusion/exclusion evaluation. After patient eligibility is confirmed, randomization will occur and patient will receive the first dose of medication at the clinic. On day 1, vital signs, EKG (1 examination) and PK sampling will also be performed after treatment administration. D0 and D1 may be at the same day if all results are available for patient's eligibility assessment. In this case, site should pay attention to perform examinations pre and post-dose accordingly (see Schedule of Events in section 5).

Patients will be observed for occurrence of adverse events during the whole trial. If any AE occurs, the patient will receive medical assistance, and the event will be recorded in the appropriate CRF.

8.3. Assessment of Efficacy

Efficacy of trial treatment will be assessed by comparing the parasitological response, determined by serial qualitative PCR (3 negative PCR results to be collected in the same day) assessed at EOT and sustained parasitological clearance until 6 months of follow-up. Follow-up will be continued until 12 months for complementary assessment of response.

For the assessment of efficacy, the EOT of each treatment arm will be defined accordingly to its corresponding duration. For efficacy assessments, the EOT of each treatment arm will be defined in accordance to the duration of the treatment regimen. Sustained response will be assessed in all treatment arms using the same number of PCR samples (i.e., EOT; 12 weeks; 4, and 6 months). The primary efficacy outcome is evaluated until 6 months of follow-up.

As this is a double-blind trial, this will not interfere on the schedule of events, since all patients will take all samples planned at each visit.

In addition, a qualitative, quantitative, and multi-criteria efficacy assessment of Benznidazole and BZN/E1224 will be performed, correlating incidence of and time to parasitological clearance with changes in *T. cruzi* serology and with safety and PK parameters, as described in the following sections.

8.3.1. Assessments performed

A blood samples (15mL) will be collected in the same day at screening, 2 randomly times between Days 1, 2 and 3 (allowable window of + 2 days for day 3 only), at weeks 2 (allowable window of + 3 days), 3, 4, 6, 10 and 12 (allowable window of ± 3 days), and at 4, 6, and 12 months follow-up (allowable window of ± 7 days) for PCR analysis (qualitative and quantitative PCR). Blood samples collected for PCR shall be immediately added to a tube containing one volume (5mL) of a solution of Guanidine/ClH 6M EDTA 0.2M pH 8,0 Buffer (GEB) (Schijman, 2003). Samples with guanidine buffer can remain at room temperature without exposure to sunlight or heat.

PCR samples will be shipped to their respective molecular biology laboratory where PCR assays will be performed according to the site country:

Samples from Bolivia: Universidad Mayor San Simón, Cochabamba.

The real-time PCR technique that will be used is the one described and optimised by Duffy *et al*. Results will be expressed qualitatively for the assessment of the primary efficacy endpoint and quantitatively for secondary analyses of parasite load.

In order to guarantee the quality, integrity and standardization of the qPCR procedures between the two laboratories, Quality Assurance mechanisms were developed such as:

Reproducibility evaluation: Blind analysis of 10% of samples from the trial, taken randomly and sent to two laboratories of molecular biology quarterly. The results of these samples will be re-sent to an independent laboratory in a pre-established time to a DNDi ad-hoc committee for analysis of concordance compared to the results obtained from the two laboratories participating in the trial.

Qualitative concordance: Detectable or undetectable values of each sample following the reporting criteria according to pre-established standardized analysis PCR evaluation process. It is expected to achieve ≥ 0.75 kappa index for concordance of all samples analyzed.

Quantitative concordance:

The degree of concordance must match the coefficient of variation of the method according to the parasitic load. The limit of quantification of the method was estimated as 1,531 CL Brener par. eq./mL GEB (equivalent to 1.85 log10 par. eq./10 mL). By definition this limit was chosen to present a coefficient of variation of 20% of the results expressed in logarithm, as recommended by the document EP17-A (NCCLS, 2004) from the precision calculation of the method published in Duffy et al, 2012. Thus, values less than 1.85 log par.eq /10 mL are not quantifiable, .values 2 log par/10 mL have a coefficient of variation of 6% and values 4 log par/ 10 mL show a coefficient of variation of 1.72%. The parasite load corresponding to the CV of each sample can be estimated by Sigma Plot (SPSS, Chicago, IL).

PCR samples taken at different moments during the treatment period will allow a comparative assessment of the time to clearance of parasitemia for each of the treatment regimens versus that of placebo. Sustained parasitological clearance will be evaluated at EOT until 6 months follow-up visit as primary endpoint, and until 12 months as secondary endpoint.

The real-time PCR assays will allow assessment of change in parasite load over time and of comparative reduction in parasite load values at weeks 1, 2, 3, 4, 6, 10, 12, and at 4, 6, and 12 months follow-up. It will also allow for correlation between parasite load at baseline with the incidence and time of parasitological response and pharmacokinetic parameters.

Other measurements will also be performed, as follows:

- For conventional serology blood sample will be collected at screening and 12 months after treatment initiation. For non-conventional serology, blood sample will be collected at D0, at week 12 and at 4, 6 and, 12 months after treatment initiation.

- Samples will be collected for future biomarkers assessment. No further assessments will be done prior to local Ethics Committee(s) authorization(s). This information will also be available in the informed consent form.

The trial manual of operations will provide full details regarding procedures for sampling (tubes types and amount, aliquots volume), handling, storage, and shipment of the samples for laboratory evaluation. Quality assurance systems will be in place and described. Training of personnel in each of the activities described above will take place before initiation of this clinical trial. All parasitological and other laboratory assessments will be performed blinded to treatment allocation.

8.4. PK Assessments

Blood samples for PK purpose will be taken from either arm. They will be collected either by finger-prick, cannulation or by venepuncture of a forearm vein(s).

For RAV and benznidazole, whole blood samples will be collected into filter paper (dried blood spots – DBS).

The blood samples will be transported to the Bioanalytical laboratory.

Blood samples will be analyzed for BZN and RAV using validated bioanalytical methods that will be described in a separate document.

RAV concentrations will be assessed using a LC/MS/MS method using dried blood spots. The limit of quantification is 20 ng/mL.

For BZN, a LC/MS/MS method for benznidazole analysis in dried blood spots will be used. The limit of quantification is 50 ng/mL.

Blood level concentrations over time will be determined during the absorption, steady-state and elimination pharmacokinetics phases.

The PK parameters to be modelled are AUC, Cmax, Cmin, CL, Vd, and t1/2.

Blood samples will be collected from each patient at the following time-points:

- Day 0, pre-dose
- Day 1, after administration of 1st dose
- Day2, Day3

- Steady-state samples on weeks 2, 3, 4, and 6
- Week 10

Overall, a total of 9 blood samples will be collected for PK analyses.

The time of trial drug administration and blood draws will be recorded in the appropriate sections of the CRF.

Whenever possible, the PK sample will be an aliquot from blood draws collected for other reasons, such as safety laboratory assessments.

The PK data obtained will be correlated with parasitological response (qualitative and quantitative), other secondary efficacy data and safety outcomes.

8.5. Assessment of Safety

Safety and tolerability of all treatments will be assessed through routine monitoring of adverse events. At each trial visit, the patients will be enquired about current adverse events or any events observed during the period previous to the visit. Evaluation of hematology and blood chemistry values, regular measurement of vital signs and physical examinations will be made at each scheduled follow-up visit, as summarized in section 5.

Cardiac Safety:

- EKGs will be performed at selected trial visits (Days 1, 2, 3 and at weeks 2, 4, 8, 12, and 4 months) in order to identify possible electrocardiographic abnormalities. If clinically significant abnormalities are judged by the investigators at these visits, unscheduled EKG should be performed until normalization.
- Patients will be re-dosed only following on-site physician review of available EKGs (central reading). Specific criteria for treatment discontinuation are described in Section 9 of the trial protocol.
- Any patient with a QTc, post dosing <310 msec or with a QTc, post dosing <350 msec accompanied of cardiac symptoms, shall be withdrawn (Specific criteria for treatment discontinuation are described in sections 9.1 and 9.2). In those cases, a full cardiac assessment should be performed, including EKG, Echocardiogram and Holter.
- Any patient with **a delta QTc >60 msec post dosing** will have the trial medication permanently interrupted and will follow the standard trial schedule. For the cases where in addition to the delta QTc >60 msec post-dosing, cardiac symptoms are present, an EKG should be performed at unscheduled visits.

- Echocardiography will be performed in all patients with any clinically significant change in the EKG and in all patients with AEs determined to be of cardiac etiology.
- Holter monitoring will be carried out in patients with cardiac symptoms or EKG with evidence of arrhythmia.
- Cardiac safety experts will periodically review all EKGs and present information to the DMC. Central reading of the EKGs will constitute the EKG database. In addition, the cardiac safety expert will review all Cardiac System Organ Class (SOC) AEs; and all non-Cardiac SOC that have the potential to be related to Cardiac AEs.

  Liver Safety:

  As part of the liver risk assessment plan, treatment discontinuation should be considered if:

- **ALT or AST >8xULN** (to be reported as Adverse Event of Special Interest=**AESI**)
- **ALT or AST >5xULN for more than 2 weeks**
- **ALT or AST >3xULN and (TBL >2x ULN or prothrombin time INR >1.5) (Hy Law's criteria** = to be reported as a serious adverse event (**SAE))**
- **ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)**
- Liver enzymes will be repeated as soon as possible after the initial abnormality is identified. After that, the patient should be followed every 96 hours (4 days), or the closest possible interval, until the serum ALT and/or AST falls below 3 X ULN or stabilizes. If a patient develops **symptoms of liver injury** (fatigue, nausea, right upper quadrant pain, dark urine, or jaundice), s/he will be instructed to immediately contact the trial site for evaluation.
- All data on liver safety will be presented to the DMC for review.
- An expert review will be conducted at each of the planned interim analysis to assess the need to continue the same frequency of monitoring of liver enzymes.

Adrenal Safety

- Confirmed abnormal low morning cortisol test results will be reported as an AESI. More details are described in **section 8.6.3**.

Also, patients will be advised to return to the clinic on any day during the follow-up period if they present any medical occurrence, as to allow for AE assessments at any unscheduled visits.

All safety assessments will be performed by a trial team member, who is blinded to the treatment allocation.

AEs will be recorded in the appropriate section of the CRF (see details on Section 8.6.). A DMC will review safety information on an ongoing basis (see section 11).

8.5.1. Laboratory examinations

Laboratory parameters of hematology and biochemistry will be measured at screening, Day 0, weekly during treatment duration (weeks 2, 3, 4, 6, and 8), at weeks 10 and 12 and at 4, and 6 months follow up and at any unscheduled visit if clinically indicated.

Clinical safety laboratory evaluations include CBC, ALT, AST, total direct and indirect bilirubin, GGT, alkaline phosphatase, and creatinine, fasting glucose, Ca, Mg and K. Prothrombin Time (INR) will be assessed in case of ALT or AST >3xULN.

In addition, a morning blood sample will be taken for serum cortisol levels at screening, weeks 4, 8 and at 12 months. Normal laboratory parameters are detailed in Common Terminology Criteria for Adverse Events (CTCAE v 4.03) for the laboratory/metabolic category.

8.5.2. Sampling assessments schedule

The schedule below indicates the estimated blood volume sample to be collected per visit.

Table 1: Sample collection and blood volume per visit:

Sample type	Blood volume (mL)

Sample type	Blood volume (mL)

Biochemistry, Pregnancy and serum cortisol	5x 12 = 60
CBC	3x 11 = 33
Conventional and Non- Conventional Serology	6x 3 = 18
Glucose	2x 11 = 22
Clinical laboratory evaluations (total)	**133mL**
PK samples for Ravuconazole /Benznidazole determination	1x 09 = 09
PK samples (total)	**09mL**
PCR (Qualitative, Quantitative) **PCR samples (total)**	12x 15 = 180 **180mL**
Total	**322mL**

8.6. Adverse event definitions and reporting

8.6.1. Adverse Event definition

An adverse event will be defined as any untoward medical occurrence in a clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

It can therefore be any unfavorable and unintended sign, symptom, (i.e. an abnormal laboratory or EKG finding) or disease temporarily associated with the use of a medicinal product, whether or not considered to be causally related to the medicinal product.

Definition of an adverse event includes worsening (in severity or frequency) of any pre-existing conditions (“medical history”) before first administration of the trial drug and abnormalities of procedures or laboratory results which are assessed as clinically significant.

Abnormal laboratory (hematology and biochemistry) results will be reported as adverse events if the abnormality occurs or worsens after start of the trial treatment, and if they are considered clinically significant adverse changes by the Investigator or are greater than CTC Grade 1, unless they are associated with an already reported clinical AE.

Laboratory/procedures abnormalities (or worsening in severity or frequency of pre-existing abnormalities) assessed as "*clinically significant*" if they meet AT LEAST ONE of the following conditions:

- The abnormality suggests a disease and/or organ toxicity AND this abnormality was not present at the screening visit or is assessed as having evolved since the screening visit
- The abnormality results in discontinuation of the study drug
- The abnormality requires medical intervention or concomitant therapy

When reporting an abnormal laboratory/procedure result, a clinical diagnosis should be recorded rather than the abnormal value itself, if available (for example, "anaemia" rather than "decreased red blood cell count").

The investigator or appropriate site personnel will examine any patient experiencing an AE as soon as possible. The investigator will do whatever is medically necessary for the safety and well-being of the patient. The patient will remain under observation as long as a patient is receiving trial drug, and for 10 months following the last day of drug administration, or longer if medically indicated in the opinion of the investigator.

All AEs observed or reported following administration of investigational treatment and will be followed until resolved, until assessed as chronic or medically stable or until the patient participation in the trial ends.

All adverse events identified will be recorded in the appropriate AE section of the CRF using standard medical terminology in order to avoid the use of vague, ambiguous or colloquial expressions. Serious and/or unexpected adverse events will be reported by telephone or e-mail to DNDi (see details in Section 8.6.5).

8.6.2. Serious Adverse Event

An Adverse Event or Reaction which:

- results in death,

i.e. causes or contributes to the death.

- is life-threatening,

in this context refers to an AE/AR in which the patient was at risk of death at the time of the AE/AR; it does not refer to an AE/AR that hypothetically might have caused death if more severe.

- requires in-patient hospitalization or prolongation of existing hospitalization,

i.e. the AE requires at least an overnight admission or prolongs a hospitalization beyond the expected length of stay. Hospital admissions for surgery planned before study entry, for social reasons, for any elective surgery (i.e. plastic surgery) or for normal disease management (including treatment adjustment) are NOT to be considered as SAE according to this criterion (i.e. if the protocol or the standard management of the disease under study requires planned hospitalization).

- results in persistent or significant disability or incapacity,

i.e. the AE resulted in a substantial disruption of the subject's ability to conduct normal activities.

- is a miscarriage, congenital anomaly / birth defect,

i.e. an adverse event outcome in a child or foetus of a subject exposed to the Investigational Medicinal Product (or marketed medicinal product (Note: to be only added for marketed drug)) before conception or during pregnancy.

- is an important medical event, i.e. is medically significant.

Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious events/reactions, such as important medical events that might not be immediately life-threatening or result in death or hospitalization but might jeopardise the patient or might require intervention to prevent one of the other outcomes listed above. "Examples of such events are intensive treatment in an emergency room, or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization or development of dependency or abuse."

Any suspected transmission via a medicinal product of an infectious agent.

- Liver function abnormalities reaching any Hy Law criteria

Liver function abnormalities reaching any **Hy Law criteria (ALT or AST > 3 x ULN in combination with total bilirubin elevation >2 x ULN or prothrombin time INR > 1.5**) will be considered as serious AEs if there is no evidence of obstructive disease and no other reason can be found to explain the combination of aminotransferase and total bilirubin elevation. Subjects who experience these liver abnormalities should permanently be discontinued from treatment. Liver enzymes should be followed until resolution or until return to baseline levels. Other possible causes of similar observed injury include viral hepatitis A, B, or C, pre-existing or acute liver disease, or other drugs and should be investigated in case of such occurrence.

8.6.3. Adverse Event of Special Interest (AESI):

An adverse event of special interest (AESI) is defined as a non-serious adverse event of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the Sponsor may be appropriate. Such an event may require further investigation in order to characterize and understand it**- Abnormal low morning cortisol**

Confirmed abnormal low morning cortisol test results will be reported as an AESI; Confirmation of abnormal result will be done by repeating the test after 2-7 days of an initial abnormal result. If repeat test for confirmation is not done or feasible, the initial abnormal value will be considered confirmed and reported as an AESI. Subjects with abnormal results may continue treatment with close monitoring and will be referred to a specialist or can be withdraw based on medical judgment

- Liver function abnormalities > 8*ULN

Liver enzymes will be repeated as soon as possible after the initial abnormality is identified. After that, the patient should be followed every 96 hours (4 days), or the closest possible interval, until the serum ALT and/or AST falls below 3 X ULN or stabilizes. If a patient develops symptoms of liver injury (fatigue, nausea, right upper quadrant pain, dark urine, or jaundice), s/he will be instructed to immediately contact the trial site for evaluation.

AESIs are not SAEs per se (from a regulatory point of view) but the Sponsor shall be informed in an expedited manner within same timelines (irrespective of the grade of the AESI) as required for SAEs but using the AESI form. Shall they meet any seriousness criteria (8.6.2) they should then comply for associated safety reporting requirements and be reported on the SAE form.

8.6.4. Eliciting Adverse Event information

The investigator is required to report all directly observed adverse events and all adverse events spontaneously reported by the trial patient using concise medical terminology. In addition, during each trial visit patient will be interviewed with the use of a checklist and undergo a physical exam for adverse event evaluation.

8.6.5. Adverse Event reporting period

The adverse events reporting period for this trial begins

- Upon administration of the first dose of trial medication at Day 1 for non-serious events;

- Upon patient enrolment in the trial (after signature of informed consent) for serious events

and ends at the end of patient participation in the trial.

All adverse events that occur during the adverse event reporting period specified in the protocol must be reported in the CRF, whether or not the event is considered treatment related.

In addition, any serious adverse event that occurs subsequent to the adverse event reporting period that the investigator assesses as related to the investigational medication should also be reported as an adverse event.

8.6.6. Adverse Event reporting requirements

Information on adverse events must be evaluated by a physician. Each adverse event is to be classified by the investigator as serious or non-serious. This classification will determine the reporting procedure for the event.

All serious adverse events (SAE) are to be reported immediately (within 24 hours of awareness of the SAE by the investigator) to an email to be informed at Study Manual in copy to the DNDi clinical trial manager, using the SAE report form. This includes a description of the event, onset date and type, duration, severity, relationship to trial drug, outcome, measures taken and all other relevant clinical and laboratory data. The initial report is to be followed by submission of additional information (follow-up SAE form) as it becomes available. Any follow-up reports should be submitted as soon as possible and if possible within 5 working days.

Serious adverse events should also be reported on the clinical trial adverse event case report form (CRF). It should be noted that the form for reporting of SAE (SAE form) is not the same as the adverse event section of the CRF. Whereas the same data are collected, the two forms must be completed in a consistent manner, and the same medical terminology should be used. Any other additional reporting requirement will be addressed in the trial manual.

Adverse Events of Special Interest shall be informed to the Sponsor in an expedited manner within same timelines (irrespective of the grade of the AES) as required for SAEs, but using the AESI form. If AESI meet any seriousness criteria (8.6.2), they should then comply for associated safety reporting requirements and be reported on the SAE form.

Non-serious adverse events are to be reported in the CRF. In the CRF, a given adverse event will be recorded only one time per patient, and the severity recorded will be the maximum level reached. If several distinct episodes of the same condition occur, their number will be recorded in the CRF.

In addition to immediately reporting SAEs to DNDi, Investigators are responsible for reporting SAEs occurring at their site to their Independent Ethics Committee (IEC), and any periodic safety reporting, following the local requirements of their institution.

8.6.7. Grading of Adverse Event severity

Severity is a clinical determination of the intensity of an AE. The severity for an AE should be graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE, version 4.03). In case of AEs that are not described in the CTCAE v 4.03, the investigator will use the terminology MILD, MODERATE, or SEVERE to describe the maximum severity of the adverse event as follows:

MILD	Does not interfere with patient's usual functions
MODERATE	Interferes to some extent with patient's usual functions
SEVERE	Interferes significantly with patient's usual functions

This information on AE grading will be entered in the adverse event section of the CRF.

It is to be noted the distinction between severity and seriousness of adverse events. A severe adverse event is not necessarily a serious event.

8.6.8. Adverse Event causality assessment

For both serious and non-serious adverse events, the investigator is required to assess the potential relationship between the adverse event and the trial drug, i.e. to determine whether there exists a reasonable possibility that the trial drug caused or contributed to the adverse event.

To help investigators with the decision binary tree in the evaluation of causality, the CIOMS VI group recommends that investigators be asked to consider the following before reaching a decision:

- Medical history
- Lack of efficacy/worsening of existing condition
- Trial medications
- Other medications (concomitant or previous)
- Withdrawal of trial medication, especially following trial discontinuation/end of trial medication
- Erroneous treatment with trial medication (or concomitant)
- Protocol related procedure

The relationship of an AE to investigational treatment is assessed and determined by the investigator after careful consideration of the event in terms of biological plausibility, possible unrelated causes, any pre-existing medical conditions or concomitant medications, temporal relationship between administration of investigational treatment and the onset (or worsening) of the event, and known patterns of response to trial medications in general.

The causality assessment of an AE to the investigational medicinal product will be rated as follows:

- **Not related:** There is no reasonable possibility of causal relationship.
- **Related:** There is at least a reasonable possibility of a causal relationship between an adverse event and an investigational medicinal product. This means that there are facts (evidence) or arguments to suggest a causal relationship.

The decision to suspend, and resume treatment or to permanently interrupt treatment due to an adverse event will be left to the trial clinician in charge.The clinician should take in consideration the Assessment of Safety defined for this protocol (**section 8.5**) and the Rules for permanently interrupting trial treatment (**section 9.2**).

8.6.9. Exposure in utero

In this trial, women of reproductive age must have a negative serum pregnancy test at screening, must not be breastfeeding, and must use a double barrier method of contraception to avoid exposure to the study drugs during pregnancy throughout the clinical trial and **up** for 3 months after completion of the trial, in such a manner that the risk of pregnancy is minimized especially during exposure to treatment and until complete elimination. Women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device with a hormonal component are required to use an additional barrier method of contraception for the time period specified.

In addition to the serum pregnancy tests performed at screening and Day 0, an additional pregnancy test will be carried out at week 10, and at 6 and 12 months follow-up visit.

If any trial subject becomes or is found to be pregnant while receiving any of the study treatment drugs (or during study post-treatment follow-up period + 3 months), the medication must be interrupted and investigator must submit the event on a '**Clinical Study Pregnancy Report Form**' together with the first day of last menstruation period and the expected date of birth. This must be done irrespective of whether an adverse event has occurred. The information submitted should include the anticipated date of delivery.

The investigator will follow the patient until completion of the pregnancy or until pregnancy termination (i.e., induced/spontaneous abortion). The investigator will provide pregnancy outcome information in a '**Clinical Study Pregnancy Outcome Report Form'**.

Note: A pregnancy is not an SAE. Any unfavorable outcome meeting a seriousness criterion, i.e., in the case of unfavorable pregnancy outcome (miscarriage, stillbirth), birth defect occurs or congenital abnormality shall be reported using the SAE form (in addition to the **Pregnancy Outcome Form**).

In the case of a live birth, a pediatrician should assess the infant at the time of birth and submit a **Child Surveillance form**. Newborns of study subjects exposed to study drugs during pregnancy will be followed up until the age of 2 years.

Because of the risk of development of lenticular opacity, (cataract) found in animal studies with E1224, an ophthalmologist will also assess the infant of study subjects exposed to study drugs during pregnancy from the time of birth until the child reaches 24 months of age. The principal investigator must gather information on the child's health status from birth to 24 months of age by the child's treating physicians (pediatrician, ophthalmologist). Investigator must inform and advise the mother to ensure the monitoring of the child's health status, as well as complete the safety information of the child's health status in the corresponding form (**Child Surveillance Form**).

8.6.10. Adverse event follow-up

All adverse events should be followed until they are resolved or the investigator assesses them as chronic or stable or the patient participation in the trial ends (i.e., until a final report is completed for that patient).

In addition, all serious adverse events and those non-serious events assessed by the investigator as possibly related to the investigational drug must continue to be followed even after the patient participation in the trial is over. Such events should be followed until they resolve or until the investigator assesses them as "chronic" or "stable."

9. WITHDRAWAL CRITERIA

A patient should be withdrawn from the trial treatment if, in the opinion of the investigator, it is medically necessary, or if it is the wish of the patient.

If a patient does not return for a scheduled visit, every effort should be made to contact the patient.

In any circumstance, every effort should be made to document patient outcome, if

possible.

If the patient withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data, with the exception of safety data, which should be collected if possible and in accordance with patient consent.

If a patient withdraws from the trial, the reason must be noted on the CRF.

If a patient is withdrawn from the trial because of a treatment limiting adverse event, thorough efforts should be made to clearly document the outcome of AE.

9.1. Rules in case of treatment suspension or temporarily interruption

Treatment discontinuation does not imply withdrawal from the trial. In such cases, the treatment might be discontinued for a few days; therefore, the treatment will be considered incomplete or delayed. Treatment can be resumed, according to the assessment of the trial investigator in charge of the patient. Please check the instructions for safety assessment and follow-up as per section 8.5. These patients should continue with trial visits and assessments as planned, but the reasons for treatment discontinuation must be recorded in the appropriate source documentation and CRF.

9.2. Rules for permanently interrupting trial treatment

The Investigator will interrupt permanently the treatment based on the following:

- ALT or AST >8x ULN (to be reported as Adverse Event of Special Interest (AESI))
- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and (TBL >2x ULN or INR >1.5; Hy law's criteria; to be reported as a Serious Adverse Event (SAE)
- ALT or AST >3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)
- QTc post dosing <310 msec or <350 msec accompanied of cardiac symptoms
- QTc> 500 msec at any time during treatment
- QTc post dosing >60msec
- Adverse event or any other condition which, in the investigator's opinion, would put the patient at undue risk by continuing the trial treatment
- Major protocol deviation incompatible with the continuation/participation on the trial
- Any condition that the investigators considers medically necessary to interrupt the treatment, such as:
    - Significant leukopenia (<2,500 cells/mm3)
    - Severe gastrointestinal symptoms
    - Severe allergic dermopathy

- Peripheral sensitive neuropathy
- Pregnancy

Regardless of the reasons for trial treatment interruption, the investigator will make all necessary arrangements to ensure that the patient receives the appropriate treatment for the relevant medical condition.

Rescue treatment will be offered for these patients upon trial completion.

9.3. Patient withdrawal from the trial

The following will be considered reasons to indicate patient withdrawal from the trial:

- Withdrawal of consent by the patient/legal representative
- Trial termination by the Sponsor
- Any condition that the investigators considers medically necessary to withdraw patient from the trial

If the patient withdraws the consent, no further evaluations should be performed and no attempts should be made to collect additional data, with the exception of safety data, which should be collected if possible.

All patients withdrawn will be clinically evaluated by the investigator and if appropriate treated with rescue medication. Patient care will continue according to national treatment guidelines.

If a patient withdraws from the trial, the reason must be noted in the CRF. Data obtained prior to the withdrawal of a patient will still be included in the PK, efficacy, and safety analyses.

9.4. Lost to follow-up

If a patient does not return for a scheduled visit, all necessary measures should be taken to contact the patient and document the patient's outcome, and before declaring him "lost to follow-up", the investigator must ensure every effort to contact him/her and to establish the reason for the discontinuation of treatment. All contact attempts should be documented accordingly.

In order to minimise loss to follow-up detailed contact information including address and mobile phone number for the patient and /or a relative will be taken as part of the screening assessments. This information will remain at the site. If the patient does not self-present to the site and a phone number is not available then the field team will follow-up with the patient or their family directly. A patient will only be considered lost to follow-up after the 12 month follow-up visit.

9.5 Patient replacement

Patients withdrawn from this trial will not be replaced.

10. DATA ANALYSIS AND STATISTICAL METHODS

The Statistical Analysis Plan will be written after the protocol finalization and definitely finalized before breaking the blind mode, prior to the first database lock. These specifications will detail the implementation of all statistical analyzes planned according to the main features reported in the protocol.

10.1. Sample size determination

A total of 210 patients will be recruited in the trial.

For a comparison of two independent binomial proportions using Pearson's Chi-square statistic with a Chi-square approximation with a two-sided significance level of 0.006 (multiplicity adjustment of 0.05 for 6 comparisons [treated arms] against the control arm), a sample size of 11 patients per arm assuming a balanced design achieves a power of at least 0.8 when the proportions are 0.082 (control arm) and 0.81 (treatment arm).

With an estimated proportion of 10% patients who might drop-out from the trial, the final sample size needed is 12 patients per arm for one comparison.

A sample size of 30 patients per arm will allow a 99% probability of observing at least one event of peripheral neuropathy or paresthesia, transaminase increase, hypersensitivity (13.3%, 15.5%, and 22.2 %, respectively) and an 85% probability of observing at least one treatment discontinuation per arm.

10.2. Definition of trial populations included in the analysis

The primary efficacy analyses will be performed on the Intention-to-treat (ITT) data set, defined as all randomized patients by their assigned treatment arms.

Additionally, efficacy analyses will be performed on a per-protocol (PP) dataset. The per-protocol dataset will be defined as patients receiving randomized treatment, who meets entry criteria, have not permanently discontinued treatment administration and had no major protocol deviation. Eligibility and evaluability criteria will be further detailed in the Statistical Analysis Plan.

All safety analyses will be performed on the All Treated Set, defined as all patients who received at least one dose of the treatment.

Based on actual deviations, the criteria for any patient exclusion from the different data sets will be specified and updated as necessary prior to the break of the blind.

Patient Disposition

At the end of the trial the following will be described:

- Number of patients screened;
- Number of patients assessed for eligibility excluded from the trial and reasons for exclusion;
- Number of patients who have been enrolled in the trial (ITT set);
- Number of patients randomized into each trial arm;
- Number (%) of patients who have received at least one dose of trial medication(s) (safety population – All Treated Set), per trial arm;
- Number (%) of patients who have received a full course of treatment and completed EOT assessments (treatment completers), per trial arm;
- Number (%) of patients who have completed the trial per trial arm (trial completers, PP set);
- Number (%) of patients who completed the trial visits at Days 0, 1, 2, 3, weeks 2, 3, 4, 5, 6, 7, 8, 10, and 4, 6, and 12 months follow-up visits, total and per trial arm;
- Number (%) of patients lost to follow-up and reasons of lost, total and per trial arm;
- Number (%) of patients who have withdrawn from the trial, per trial arm and reasons for withdrawal.
- Number (%) of patients excluded from analysis per trial arm and reasons for exclusion;
- Number (%) of patients with at least one protocol violation and nature of protocol violation (major, minor)

10.3. Baseline

The following baseline characteristics of the trial population will be described generally and per centre/country.

- Age distribution
- Gender distribution
- Weight
- BMI
- Nationality and country(ies) of residence;
- Parasite load measured through real-time quantitative PCR;
- Antibody titers assessed by conventional and selected non-conventional serology, per type of assay;

10.4. Treatment Compliance

This is a double blind trial and compliance will be considered according to treatment course. During the trial, the treatment compliance will be monitored through weekly follow-up visits, as summarized in section 5, and all information registered in the appropriate section of the CRF.

At the weekly visits, E1224 or E1224–matching placebos will be administered under the supervision of trial staff. The patient will receive enough BZN or BZN matching placebos until the next scheduled visit. Also, they must bring all remaining trial drugs to check for compliance with prescribed treatment and ensure drug accountability.

Treatment compliance will be described in the trial report, describing the number and percentage of patients who completed and who did not complete a full course of treatment per trial arm, and the reasons for treatment drop-out per trial arm.

For patients who do not complete a full treatment course, mean time of exposure to trial drug will be described per trial arm.

10.5. Efficacy Analysis

The primary efficacy endpoint is parasitological response, defined as sustained parasitological clearance until6 months of follow-up. It is determined by serial qualitative PCR results (3 negative PCR results to be collected in the same day).

Hypothesis: Benznidazole or Benznidazole/E1124 improved regimens treatment leads to a statistically significant better sustained parasitological clearance rate at 6 months of follow-up than placebo in patients with chronic indeterminate CD.

The primary analysis will be the comparison of the sustained parasitological clearance of each treatment arm versus that of the placebo arm. Six main final comparisons will be performed. Hochberg procedure will be used to keep the global type I error at the 5% level and cope with the multiplicity issue. With sustained clearance of parasitemia as the parameter of interest, a comparison with placebo will be carried out. An alpha adjustment to 0.025 is considered for the interim analyses to account for potential Type I inflation.

Primary efficacy analyses will be performed when all patients complete 6 months follow-up visit. Analyses of results will be used to guide planning of a follow-up Phase 3 clinical study. Analysis is to be performed by an independent, external statistician. In order to allow administrative decisions, the results of the primary endpoint will be disclosed unblinded (by treatment arm) to the Joint Development Committee. No individual patient data will be shared with the Committee. In addition, data is to be shared with the Data Monitoring Committee.

The Joint Development Committee is composed of the DNDi Project Leader (DNDi Head of Chagas Program), DNDi Medical Director, and members from Eisai team. On occasion the JDC meetings are attended by other members of staff from these organisations (such as, DNDi Research and Development Director and Head of Chemistry, Manufacturing and Controls). Under no circumstances will the Principal Investigator, trial site staff and study personnel from the Sponsor managing day-to-day activities of the trial have access to the results of the primary efficacy analysis prior to the end of the study. They will remain unequivocally blinded until completion of 12 months follow-up and final database lock. The study statisticians will not have access to unblinded data before conduction of the analyses at the end of the study. Study database will be cleaned and locked in preparation of analyses of primary efficacy endpoint. As data accrues following 6 months assessment, it is to be included to constitute the final study database for clinical trial data analyses. Other secondary efficacy analyses will further characterize differences in therapeutic response within this trial. For all secondary comparisons of proportions between active arms versus placebo, an exact test will be performed.

For efficacy assessments, the EOT of each treatment arm will be defined according to the duration of the arm dosing regimen.

The current primary efficacy endpoint includes the actual EOT time-point for each of the different treatment groups, thus the sustained parasitological response is to be assessed in a standardised way across the different arms independent of treatment duration, i.e:

Table 3. Sustained response considering variable EOT for the different treatment groups

Group		**EOT**	**Negative serial PCR at the following timepoints**
1	BZN 300 mg - 8 wks	W10	W10, W12, 4M, 6M*, 12M
2	BZN 300 mg - 4 wks	W6	W6, W12, 4M, 6M*, 12M
3	BZN 300 mg - 2 wks	W4	W4, W12, 4M, 6M*, 12M
4	BZN 150 mg - 4 wks	W6	W6, W12, 4M, 6M*, 12M
5	BZN 150 mg - 4 wks / E1224 300 mg	W10	W10, W12, 4M, 6M*, 12M
6	BZN 300 mg (weekly doses) - 8 wks / E1224 300 mg	W10	W10, W12, 4M, 6M*, 12M
7	Placebo	W10	W10, W12, 4M, 6M*, 12M

*Primary efficacy analyses

For quantitative PCR analyses, means and 95% confidence intervals will be presented by group and visit. Time to sustained parasite clearance will be assessed using Kaplan–Meier survival analysis with log rank test for significance. Repeated measures analysis will also be performed.

Latent class analyses will be used to evaluate the association between the parasitological clearance and serological response.

Multivariate analysis will be performed to define early and late predictors of a sustained parasitological response.

Detailed analysis plan per question of interest will be provided in the Statistical Analysis Plan (SAP). Information on interim analysis is provided in section 9.8 and also further detailed in the SAP.

10.6. Safety Analysis

Safety analyses will include all patients who have received at least one dose of the trial medication.

The proportion of patients with SAE and/or AEs leading to treatment discontinuation will be described per trial arm and by System Organ Class (using preferred terms defined by MedDRA 13.1), according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE, version 4.03).

The proportion of patients presenting at least one AE will be described. Patients with multiple events with the same preferred terms will be counted once. The maximum severity grade for each preferred term and body system will be summarized. If multiple events with the same preferred terms are recorded for a patient, the event with the maximum grade will be included in the analysis. In addition, a narrative for each of the SAEs and AESIs will be developed detailing all aspects related to the medical event.

AEs not leading to treatment discontinuation will also be described per trial arm using the same classification as presented above.

Incidence rate and 95% confidence interval will be presented per trial arm will be presented for SAE, AESI, and AEs per category and most frequent AEs. Otherwise only descriptive statistics will be presented.

Safety laboratory parameters (haematology and biochemistry) will also be described individually per trial arm, showing the proportion of patients by degree of elevation relative to ULN and to baseline values, and blood levels changes over time. Shift tables will be presented. A listing of patients experiencing lab parameters elevation will be included.

EKG abnormalities will be described per treatment arm as the proportion of patients per type of EKG finding and changes over time. The cardiac safety data will be compared between the different treatment groups and placebo.

Safety data will be correlated to efficacy and treatment compliance data and to PK parameters.

10.7. Analysis of other endpoints

Descriptive statistics (i.e. mean, median, SD, variation coefficient, etc) will be used to describe the analyzed variables.

The PK parameters assessed in the population will be AUC, Cmax, Cmin, CL, Vd, and t1/2.

Population pharmacokinetics modelling will be performed using non-linear mixed-effects modelling as implemented in the software NONMEM® VI (NONMEM Users Guides, (1989-2006) (Beal, S.L., Sheiner L.B., Boeckmann, A.J. (Eds.) Icon Development Solutions, Ellicott City, Maryland, USA). This program uses mixed (fixed and random) effects non-linear regression to estimate population parameter means with inter-individual and intra-individual (i.e., residual) variability.

To determine the basic structural pharmacokinetic parameters, a stepwise procedure will be used to find the model that best fits Benznidazole/ E1224 data in blood.

The influence of covariates such as age, body mass index and parasite load at baseline will be assessed by graphical visual inspection of the individual estimates of the pharmacokinetics parameters vs. the covariate plots. Potentially or known influential covariates will be incorporated sequentially into the pharmacokinetics model. The typical value of a given parameter (e.g., CL) will be modelled as linearly dependent on each covariate (e.g. body weight, age, etc). Categorical covariates will be coded as indicator variable 0/1.

Inter-individual variations in PK parameters will be described using an exponential error model with normally distributed inter-individual random variability with mean zero and variance $\omega 2$. An exponential error model will be used to describe the intra-patient (residual) variability.

Parameter estimation and model selection: The data will be fitted using the first-order conditional method (FOCE INTER in NONMEM). Model selection will be based on the likelihood ratio test, pharmacokinetics parameters point estimates, and their respective confidence intervals, goodness-of-fit plots, and visual predictive checks (VPC). A model will be considered as a statistically significant improvement over a previous model if it produces a decrease in objective function of >10.8 for one additional parameter (Chi-Square; $P < 0.001$).

Diagnostic plots will be done in R (R Development Core Team 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna,

Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.)

The PK parameters (AUC and Cmax) will be correlated with parasitological response (sustained parasitological response and fractional reduction of parasitemia), and safety outcomes at Day 1(post dose), 2, 3, and weeks 2, 3, 4, 5, 9, and 10.

10.8. Interim analysis

Two interim analyses and a primary outcome analysis will be performed.

One Interim analysis ~~for futility,~~ will be performed when 30% of the patients complete 12 weeks from treatment initiation and another, for safety, upon completion of treatment of 20% of recruited patients. The primary outcome analysis will occur when all patients have completed the 6-month follow-up visit.

The interim analyses results will not be disclosed, under any condition, to Sponsor personnel, the Principal Investigators and trial personnel at the trial site managing day-to-day activities of the trial prior to the end of the trial. They will remain unequivocally blinded until completion of 12 months of follow-up and final database lock. As discussed in section 10.5, the Joint Development Committee (including key sponsor management staff not linked with clinical trial operations) and Data Monitoring Committee will have access to primary outcome (efficacy and safety) results by treatment arm at the time of completion of 6 months follow-up in order to guide planning of a follow-up Phase 3 clinical study. Sponsor personnel directly involved in the trial, principal investigators and study team site personnel will remain blinded to treatment allocation and results of the analyses. The trial statisticians will not have access to unblinded data before conduct of analysis at the end of the trial. Interim analyses will be performed by an independent statistician~~. A futility stopping rule is defined as no difference from placebo in sustained parasitemia clearance at 12 weeks. The stopping rule will be applied with no change planned on the sample size of the different study arms. Patients will be considered as early treatment failures.~~

The interim analysis results/outcome will not interfere on the trial procedures, unless they meet the criteria defined for the harm ~~and futility~~ stopping rule. If the stopping rules are not applied, all patients will be required to complete the 12 months visits follow-up as planned by this protocol. In order to account to Type I error inflation, an alpha adjustment is considered to 0.025. A total sample size of 11 patients per arm assuming a balanced design achieves a power of at least 0.8 when the proportions are 0.082 and 0.81. With a proportion of 10% patients who would drop-out the trial, the final total sample size needed is 12 patients per arm.

No other changes in the final analysis defined by this protocol and Statistical Analysis Plan will be necessary.

10.9. Missing, unused, and spurious data

The adequate procedure to handle with missing, unused, and spurious data will be described in detail in the statistical analysis plan, prior to the code break.

10.10. Deviations from the original statistical plan

In case of any deviation from the original statistical plan, a justification and description will be detailed in a protocol amendment and/or in the final report, as appropriate.

11. DATA MONITORING COMMITTEE

A DMC, composed of a minimum of three members independent of the investigator and sponsors, will be set up prior to trial initiation. The DMC will monitor the trial in order to ensure that harm is minimized and benefits maximized for the trial patients. The DMC will review efficacy and safety data on an ongoing basis and at pre-determined intervals, review all information related to the occurrence of SAEs, AESIs and AEs leading to treatment discontinuation, and issue recommendations about the trial if the existing benefit/risk of the patients in the trial seems compromised. The data and intervals will be agreed prior to or soon after the trial initiation and documented in the DMC Charter. It is to be noted that data on liver and cardiac safety will be presented to the DMC for review. The DMC members will include a liver specialist, a cardiac safety specialist and a member with CD expertise.

12. QUALITY ASSURANCE AND QUALITY CONTROL PROCEDURES

12.1. Investigator's file

The investigator must maintain adequate and accurate records to enable the conduct of the trial to be fully documented and the trial data to be subsequently verified. These documents include Investigator's Site File, patient clinical source documents and screening/enrolment logs. The Investigator's Site File will contain the protocol/protocol amendments, CRF and query forms, IEC and regulatory approval with correspondence, sample informed consent, drug accountability records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence.

12.2. Case report forms (CRFs)

Data will be collected by laboratory technicians, medical doctors, clinical officers and nurses authorized by the investigator. It will be supervised by the Investigator and signed by the investigator or by an authorized staff member. Trial-specific information will be entered into an electronic Case Report Form (CRF). Data that are derived should be consistent with the source documents or the discrepancies should be explained. All CRF data should be anonymized, ie, identified by trial patient number only.

The investigator should ensure the accuracy, completeness, legibility, and timely

completion of all data reported to the sponsor in the CRFs and any other additional information that is required. The investigator is responsible for keeping all consent forms, screening forms, CRF and the completed patient identification code list in a secure location.

12.3. Source documents

The verification of the CRF data must be by direct inspection of source documents. Source documents include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, EKG, special assessment reports, signed informed consent forms, consultant letters, and patient screening and enrolment logs.

The investigator must maintain source documents (such as laboratory and consultation reports, history and physical examination reports), for possible review and/or audit by DNDi and/or Regulatory Authorities. The Investigator / designee will record the date of each patient's visit together with a summary of their status and progress in the trial.

12.4. Record Retention

The investigator must keep all essential documents until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 15 years have elapsed since the formal discontinuation of clinical development of the investigational product. Trial documents should be retained for a longer period however, if required by the applicable regulatory requirements or by an agreement with DNDi. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained. After that, these documents may be destroyed with prior permission from DNDi, patient to local regulations.

Should the investigator wish to assign the trial records to another party or move them to another location, DNDi must be notified in advance.

12.5. Monitoring

Clinical Monitors will conduct regular monitoring visits following the monitoring plan, during which he/she will review and source data verify the Informed Consent Forms, medical records, laboratory results, imaging assessments, Case Report Forms, drug dispensing logs, and protocol violations.

The investigators will permit representatives of DNDi and/or designated clinical monitors to verify all CRFs, medical records, laboratory work sheets and to assess the status of drug storage, dispensing and retrieval at any time during the trial. Direct access to the corresponding source documents for each patient will be made granted provided that patient confidentiality is maintained in accord with local regulations. The review is for the purpose of verifying the adherence to the protocol and to ensure the trial is conducted according to GCP.

It is important that the investigators and other trial site staff are available during these visits.

The monitoring visits provide DNDi with the opportunity to evaluate the progress of the trial, verify the accuracy and completeness of CRFs, resolve any inconsistencies in the trial records, as well as to ensure that all protocol requirements, applicable regulations, and investigator's obligations are being fulfilled. Four visit types are planned: pre-trial, trial start, during the trial, and trial end. Visits may also be performed by regulatory authorities.

It will be the clinical monitor's responsibility to inspect the CRF at regular intervals throughout the trial, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The investigator agrees to cooperate with the clinical monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

12.6. Audits and Inspections

The trial site may also be patient to quality assurance audits by DNDi or designated representatives and/or to inspection by regulatory authorities or Independent Ethics Committees (IEC).

The investigators will permit representatives of DNDi and/or designated clinical auditors to inspect all CRFs, medical records, laboratory work sheets and to assess the status of drug storage, dispensing and retrieval at any time during the trial. The corresponding source documents for each patient will be made available provided that patient confidentiality is maintained in accord with local regulations.

It is important that the investigators and their relevant personnel are available for possible audits or inspections.

12.7. Data Management

A CRF must be completed for all patients that have given informed consent. The current clinical trial will use a validated electronic CRF. The trial data will be stored in a computer database maintaining confidentiality in accordance with national data legislation.

All entries into the CRF are the responsibility of the investigator or a qualified designated staff member. The investigator will attest in writing at the beginning of the trial that his/her electronic signature is the legally binding equivalent of a written signature.

Data will be continuously reviewed by the clinical monitor. Data queries will be generated, documented and resolved on an ongoing basis during the trial.

Six-month efficacy and safety database will be locked when all patients complete 6 months follow-up assessment (after all data queries generated, documented and resolved, as appropriate). Twelve-month efficacy and safety database will be locked upon completion of all

12 months study visits, then constituting the final clinical trial database for final analyses.

12.8. Confidentiality of Trial Documents and Patients Records

The investigator must assure that patients' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor, patients should not be identified by their names, but exclusively by an identification code. The investigator should keep a patient enrolment list showing codes, names, and addresses. The investigator should maintain documents for submission to sponsor authorized representative, and patient's signed written consent forms, in strict confidence.

13. PROTOCOL AMENDMENTS

The Principal investigator will ensure that the trial protocol is strictly adhered to throughout, and that all data are collected and recorded correctly on the CRF.

All protocol modifications must be documented in writing. Any protocol amendment must be approved and signed by the sponsor and the Principal investigator and is to be submitted to the appropriate IEC for information and approval in accordance with local requirements, and to regulatory agencies if required. Approval by IEC (and Regulatory Authority, if applicable) must be received before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial patients, or when the change involves only logistical or administrative aspects of the trial (e.g. change in clinical monitor(s), change of telephone number(s)).

The protocol amendment can be initiated by either sponsor or by any Principal investigator.

The investigator will provide in writing the reasons for the proposed amendment and will discuss with the clinical trial manager and sponsor.

14. EARLY TERMINATION OF THE TRIAL

Both the sponsor and the investigator reserve the right to terminate the trial at any time prior to inclusion of the intended number of patients, but they intend to exercise this right only for valid scientific or administrative reasons. Should this be necessary, both parties will arrange the procedures on an individual trial basis after review and consultation. In terminating the trial, the sponsor and the investigator will assure that adequate consideration is given to the protection of the patient's interest.

Reasons for early termination by the sponsor(s) may include but not be limited to:

- Low enrollment rate
- High frequency of protocol violations
- Inaccurate or incomplete data

- Unsafe or unethical practices
- Following the recommendation of the DMC or IEC
- Administrative decision

Reasons for early termination by the investigator may be:

- Insufficient time or resource to conduct the trial
- Lack of eligible patients

Stopping rules will be clearly defined for~~both futility and~~ safety.

- For safety, a harm stopping rule will be defined on safety criteria and depending on the DMC judgment, as defined in the DMC charter. The DMC will review AEs and Lab data to decide whether the arm should be stopped or not
- ~~Regarding efficacy, with sustained clearance of parasitemia at 12 weeks as the parameter of interest, a comparison with placebo will be undertaken.~~Alpha-adjustment to 0.025 is considered to account for the type I error inflation.

In the event that a trial is terminated either by the sponsor or by the investigator, the investigator has to:

- Complete all CRFs to the greater extent possible
- Return all test articles, CRF, and related trial materials to the sponsor who provided them
- Answer all questions of the sponsors or their representatives related to data of patients enrolled at the site prior to trial termination
- Ensure that patients enrolled in the trial who had not yet reached a follow up time point are followed up with the necessary medical care
- Provide in writing the reasons for his/her decision to the regulatory bodies and the sponsor

15. ETHICS

The protocol for this trial has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki and ICH guidelines for Good Clinical Practice (ICH Harmonised Tripartite Guideline - Guideline for Good Clinical Practice E6 (R1) - current step 4 version dated 10 June 1996)). DNDi assures that it will comply with all applicable state, local and regional laws for protecting the rights and welfare of human patients. This protocol and any protocol amendment (s) will be reviewed/approved by an IEC before its

implementation.

It is the responsibility of the National Coordinating Investigator/Investigator to apply for review to the IEC of the country where the trial takes place regarding local rules and regulations. Written approval from all involved IECs must be obtained before implementation of any protocol-specified intervention /investigation provided to the patient.

Any modifications made to the protocol after receipt of the IEC approval must also be submitted by the investigator in writing to the IEC in accordance with local procedures and regulatory requirements.

15.1. Informed consent process

Inclusion in the trial will occur only if the patient gives written informed consent. It is the responsibility of the investigator/designee to obtain voluntary written informed consent from each individual participating in this trial, after adequate presentation of aims, methods, anticipated benefits, and potential hazards of the trial. The written informed consent document will be translated into the local language or a language understood by the patient (s). The patient will be given time to discuss the information received with members of the community or family before deciding to consent. The patient will be asked to provide written and signed consent.

If the patient is illiterate or unable to write, s/he will include a mark in the form and a literate witness must sign (this person should have no connection to the research team and the sponsor, and, if possible, should be selected by the participant).

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the trial.

15.2. Ethical aspects of patient inclusion and trial procedures

Only eligible patients for Chagas therapy according to national guidelines and for whom Benznidazole standard treatment is indicated will be enrolled in this trial. The management of their disease and therapy will follow national guidelines. In comparison with current BZN standard treatment, the new therapeutic approaches for CD using different doses and duration of BZN, as well as combinations, target an improvement in treatment response and tolerability and reduce the potential for development of resistance.

Participants on this trial may experience discomfort during examination and blood sampling.

The total volume of blood collected during the trial is about 322 mL for all evaluations (around 20 table spoons). The amount of blood drawn does not cause anaemia or problems related to the body's defence mechanisms. The maximal quantity during 1 visit is 28mL (equivalent to 2 table spoons).

15.3. Ethical aspects of trial treatments

Clinical data demonstrated that E1224 has potential for the specific treatment of *T. cruzi* infections at EOT and with a proportion of patients sustaining parasitological response until 6 months. Phase 1 trials in healthy volunteers receiving E1224 suggest that the investigational drug has a satisfactory safety profile. A Phase 1 drug-drug interaction trial which assessed the interaction of BNZ and E1224 showed that both compounds were well tolerated, in monotherapy and combination. The lack of clinically relevant safety findings provided support for follow-up evaluation of the two compounds in combination. Likewise, experimental data suggest a positive interaction between BZN and azole compounds for the treatment of Chagas disease.

A placebo comparator has been selected for this trial. Due to the chronic nature of Chagas Disease and slow progression of organs impairment there is no evidence of a significant risk for adult patients recruited to the placebo group within the timelines of trial evaluation. All patients will be offered treatment at the end of the trial. This arm will provide a control for the therapeutic efficacy assessment of Benznidazole and BZN/E1224 alternative regimens. In addition, it will allow a more complete evaluation of PCR as an outcome measure for CD clinical trials.

15.4. Patient costs

Patients will be reimbursed for travel to and from the trial site and in case of any lost day(s) of work, but will not receive any payment for trial participation. Any treatment for trial-related injuries that is required during the trial period will be provided free of charge to the patient.

16. INSURANCE AND LIABILITY

DNDi will provide insurance against claims arising from the trial, except for claims that arise from malpractice and/or negligence. In addition, the DNDi will address the costs of treatment of trial patients in the event of trial-related injuries in accordance with the applicable regulatory requirements.

17. REPORTING AND PUBLICATION

This clinical trial will be registered with a recognized clinical trial registry and a final clinical trial report will be generated.

The results of this trial may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to DNDi prior to submission.

In accord with standard editorial and ethical practice, the sponsor will generally support publication of multicenter trials only in their entirety and not as individual center data. Any formal publication of the trial in which input of DNDi personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate DNDi personnel. Authorship will be determined by mutual agreement.

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Appendix 1 – PRINCIPAL INVESTIGATORS' LIST

Investigator list is now a separate document.

Appendix 2 – CONCOMITANT TREATMENT

CYP3A4 Substrates Not Studied *in vitro* or *in vivo* but Likely to Result in Significant Drug Interactions

Drug or Drug Class	Recommendations
Terfenadine, Astemizole, Pimozide, Cisapride, Quinidine	Increased plasma concentrations of these drugs can lead to QT prolongation with rare occurrences of torsade de pointes. Coadministration with E1224 is contraindicated
Ergot Alkaloids	E1224 may increase the plasma concentration of ergot alkaloids (ergotamine and dihydroergotamine) which may lead to ergotism. Co-administration with E1224 is contraindicated.
Sirolimus	E1224 may substantially increase the plasma concentration of sirolimus. Co-administration with E1224 is contraindicated
Vinca Alkaloids	E1224 may increase the plasma concentrations of vinca alkaloids (e.g., vincristine and vinblastine) which may lead to neurotoxicity. Therefore, it is recommended that the dose adjustment of the vinca alkaloid be considered.
Tacrolimus	Frequent monitoring of tacrolimus whole blood trough concentrations should be performed upon initiation, during co-administration, and at discontinuation of E1224 treatment, with tacrolimus doses adjusted accordingly. Consider a 33% reduction in dose upon start of E1224 therapy
Benzodiazepines metabolized through CYP3A4	E1224 is likely to increase the plasma concentrations of benzodiazepines and lead to a prolonged sedative effect. It is recommended that dose adjustment of the benzodiazepine be considered during co-administration
HMG-CoA reductase inhibitors (statins) metabolized through CYP3A4	It is recommended that dose reduction of statins be considered during co-administration with E1224. Increased statin concentrations in plasma can be associated with rhabdomyolysis.
HIV Protease Inhibitors metabolized through CYP3A4	Although *in vitro* and *in vivo* testing failed to show an interaction between ravuconazole and nelfinavir, subjects should be frequently monitored for drug toxicity during the coadministration of E1224 and HIV protease inhibitors
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) metabolized through CYP3A4	E1224 may inhibit the metabolism of some NNRTIs. Subjects should be frequently monitored for drug toxicity during the coadministration of E1224 and other NNRTIs (eg, nevirapine and delavirdine)
Calcium Channel Blockers metabolized through CYP3A4	Frequent monitoring for adverse events and toxicity related to calcium channel blockers is recommended during co-administration with E1224. Dose reduction of calcium channel blockers may be needed.

This list is not an exhaustive list. For the complete list, please refer to the Study Manual of Operations.