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.