Public Meeting on Chagas Disease Patient-Focused Drug Development

April 28, 2015

Welcome

Soujanya Giambone, MBA
Office of Strategic Programs
Center for Drug Evaluation and Research
U.S. Food and Drug Administration

April 28, 2015

Agenda

- **Setting the Context**
  - Opening Remarks
  - Overview of FDA's Patient-Focused Drug Development Initiative
  - Background on Chagas disease and Therapeutic Options
  - Overview of Discussion Format
- **Discussion Topic 1**: Disease symptoms and daily impacts that matter most to patients
- **Discussion Topic 2**: Patients' perspectives on current approaches to treating Chagas disease
- **Lunch**
- **Scientific Discussion**
- **Open Public Comment**
- **Closing Remarks**

Opening Remarks

John Farley, MD MPH

Deputy Director, Office of Antimicrobial Products
Center for Drug Evaluation and Research
U.S. Food and Drug Administration

April 28, 2015

FDA's Patient-Focused Drug Development Initiative

Theresa Mullin, PhD
Director, Office of Strategic Programs
Center for Drug Evaluation and Research
U.S. Food and Drug Administration

April 28, 2015

Patient-Focused Drug Development under PDUFA V

- **FDA is developing a more systematic way of gathering patient perspective on their condition and available treatment options**
  - Patient perspective helps inform our understanding of the context for the assessment of benefit-risk and decision making for new drugs
  - Input can inform FDA's oversight both during drug development and during our review of a marketing application

- **Patient-Focused Drug Development is part of FDA commitments under the fifth authorization of the Prescription Drug User Fee Act (PDUFA V)**
  - FDA will convene at least 20 meetings on specific disease areas in Fiscal Years (FY) 2013 - 2017
  - Meetings will help develop a systematic approach to gathering patient input

Identifying Disease Areas for the Patient-Focused Meetings

- **In September 2012, FDA announced a preliminary set of diseases as potential meeting candidates**
  - Public input on these nominations was collected. FDA carefully considered these public comments and the perspectives of our drug review divisions at FDA

- **FDA identified a set of 16 diseases to be the focus of meetings for FY 2013-2015**
  - Another public process has been initiated to determine the disease set for FY 2016-2017

Disease Areas to be the focus of meetings for FY 2013-2015

FY 2013

- Chronic fatigue syndrome
- HIV
- Lung cancer
- Narcolepsy

FY 2014

- Sickle cell disease
- Fibromyalgia
- Pulmonary arterial hypertension
- Inborn errors of metabolism
- Hemophilia A, Hemophilia B, von Willebrand disease, and other heritable bleeding disorders
- Idiopathic pulmonary fibrosis

FY 2015

- Female sexual dysfunction
- Breast cancer
- **Chagas disease**
- Functional gastrointestinal disorders (May 11, 2015)
- Alpha-1 antitrypsin deficiency
- Parkinson's disease and Huntington's disease

Tailoring Each Patient-Focused Meeting

- **Each meeting focuses on a set of questions that aim to elicit patients' perspectives on their disease and on treatment approaches**
  - We start with a set of questions that could apply to any disease area; these questions are taken from FDA's benefit-risk framework and represent important considerations in our decision-making
  - We then further tailor the questions to the disease area of the meeting (e.g., current state of drug development, specific interests of the FDA review division, and the needs of the patient population)
- **Focus on relevant current topics in drug development for the disease at each meeting**
  - E.g., focus on HIV patient perspectives on potential "cure research"
- **We've learned that active patient involvement and participation is key to the success of these meetings.**

"Voice of the Patient" Reports

- Following each meeting, FDA publishes a Voice of the Patient report that summarizes the patient testimony at the meeting, perspectives shared in written docket comments, as well as any unique views provided by those who joined the meeting webcast.

- These reports serve an important function in communicating to both FDA review staff and the regulated industry what improvements patients would most like to see in their daily life.

- FDA believes that the long run impact of this program will be a better, more informed understanding of how we might find ways to develop new treatments for these diseases.

Overview of Chagas Disease and Available Treatment Options

Maria Allende, MD

Medical Officer, Division of Anti-infective Products
Center for Drug Evaluation and Research
U.S. Food and Drug Administration

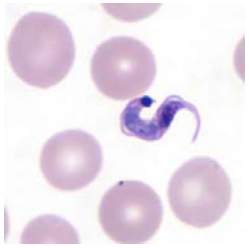
April 28, 2015

Chagas disease overview

- What is Chagas disease?
- Why is it called Chagas disease?
- Who can get Chagas disease?
- Symptoms
- Diagnosis
- Treatments available: nifurtimox and benznidazole
- Side effects of medications

What is Chagas Disease?

- A disease spread by contact with feces of an infected insect (triatomine) called “kissing bug”, “vinchuca” or “barbeiro”
- The infected insect carries the agent of the disease, a parasite called *Trypanosoma cruzi*
- The disease can cause serious heart illness
- It can also affect swallowing and digestion



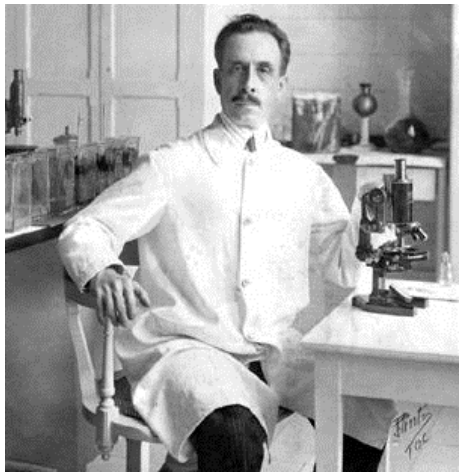
Trypanosoma cruzi
in human blood

Disease ***vector*** (carrier) of the parasite

What is Chagas Disease? - continued

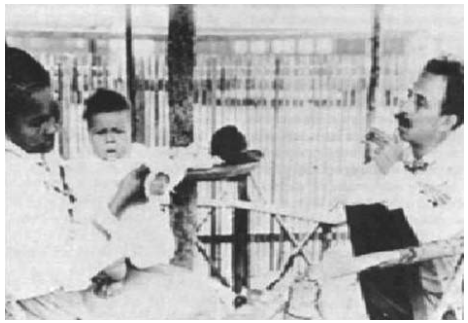
- There are two phases of Chagas disease: the *acute phase* and the *chronic phase*
- Acute phase: few weeks/months after infection
- Chronic phase: years and decades after infection
- Both phases can be symptom free (most common form) or can be life threatening
- Spontaneous cures are extremely rare, infections last for life without treatment
- Certain people are at higher risk of more serious disease: those with weakened immune system (AIDS, treatment after kidney transplant)

Why is it called "Chagas" disease?



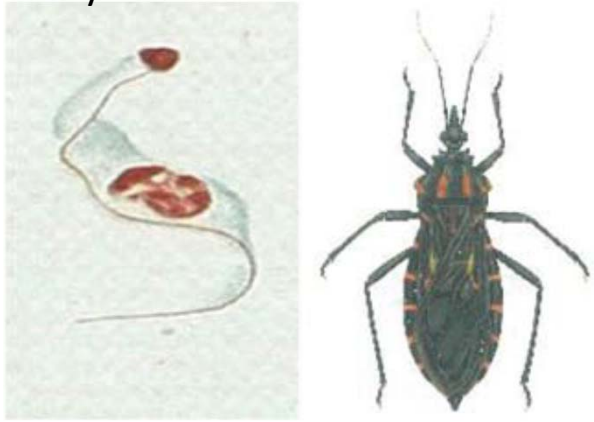
Dr. Carlos Chagas, a Brazilian physician, discovered the disease in 1909. He discovered the triatomine vector and the parasite, which he called *Trypanosoma cruzi.* He was the first to describe the disease in humans and the parasite cycle in nature.

The disease is also called **"American Trypanosomiasis"**



Dr Chagas with the first patient, Berenice, a 2 year old girl from Minas Gerais, Brazil.

Dr. Chagas injected the blood from Berenice, into Guinea pigs which died 6 days later, with large amounts of *Trypanosoma cruzi,* confirming the cause of the disease.



Trypanosoma cruzi (causative agent) and ***barbeiro*** *or* ***vinchuca*** (triatomine vector, carrier), from original 1909 article

Also called "Chagas-Mazza" disease

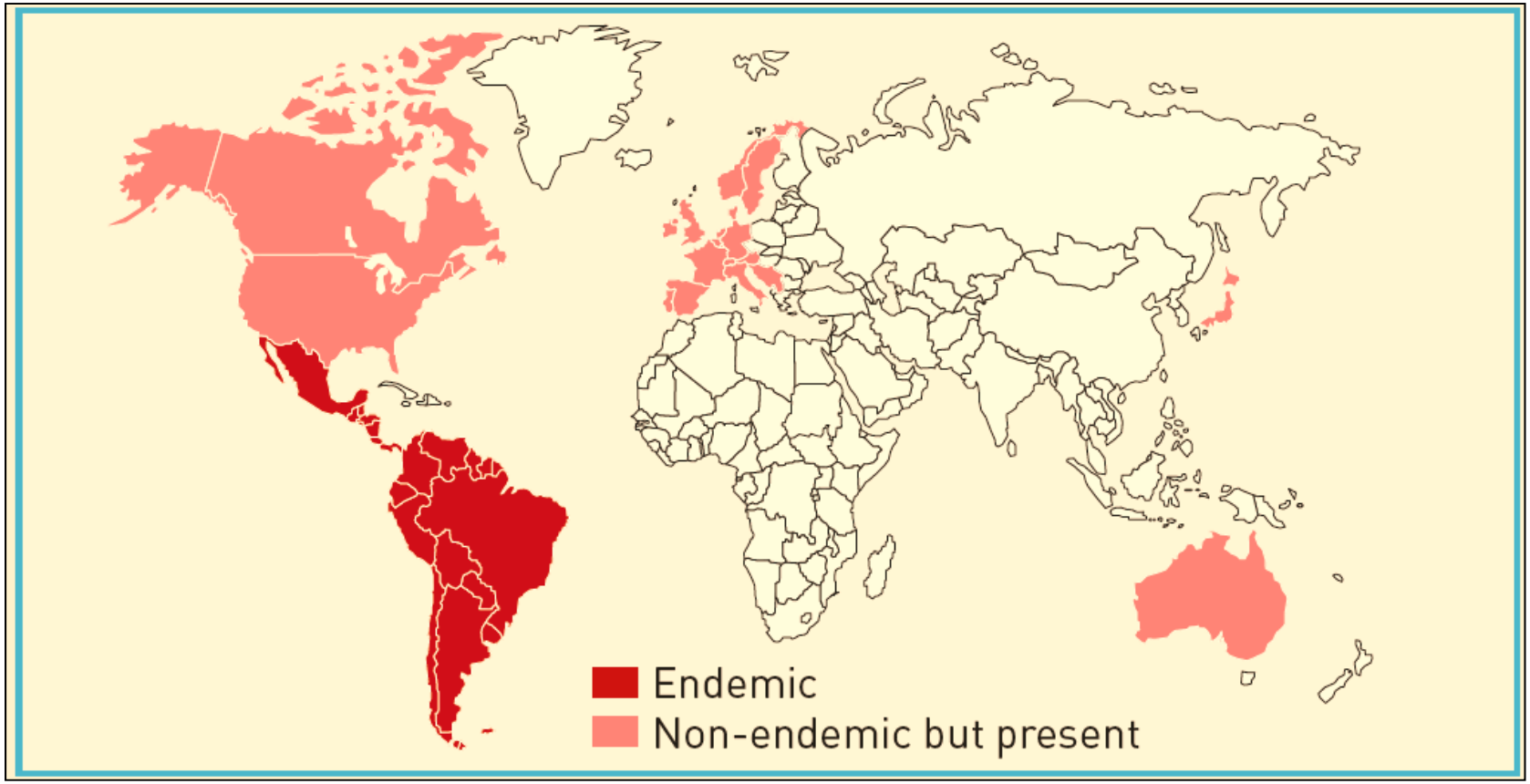
- Dr. Salvador Mazza's contributions:
  - Documented widespread cases in northern Argentina beginning in 1926 with the discovery of dogs infected with *Trypanosoma cruzi*
- Dr. Mazza died from a laboratory infection with *Trypanosoma cruzi*, while working with patient's blood

Who can get Chagas disease?

- Especially those who have lived in rural areas in Latin America, in contact with infected bugs
- Also the disease can be spread from:
  - Mother to baby (congenital)
  - Organ transplant
  - Blood transfusion

- Less common transmission:
  - laboratory accident, contaminated food/drink
- The disease is <u>not</u> spread through casual person to person contact

Chagas Disease spread around the world



Source: Drugs for Neglected Diseases Initiative (DNDi), www.dndi.org

Symptoms

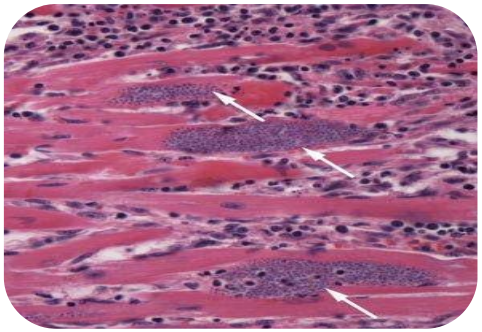
- Days after the contact (acute phase),

some may have:

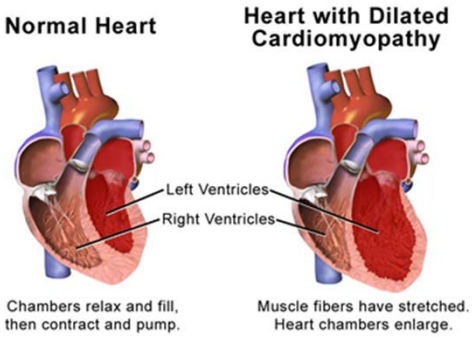
  - Fever and body aches
  - Swelling of the eyelid or at the bite site
  - Weakness and inflammation of the heart (myocarditis) and inflammation of the brain in a few patients

- Most people have no symptoms and years later, about a third of them may develop the chronic phase:
  - Heart failure (enlarged heart, not pumping blood well, causing difficulty breathing/leg swelling)
  - Irregular heart beats that can cause sudden death and risk of stroke
  - Problems with digestion and bowel movements

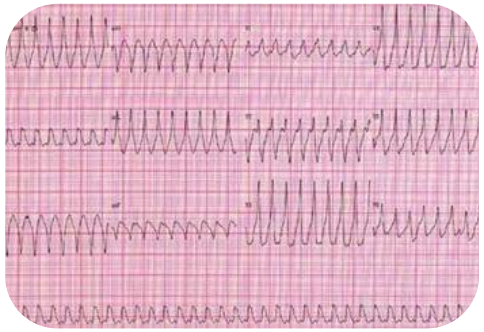
Possible complications of Chronic Chagas disease *(occurring in about 30% of those infected)*



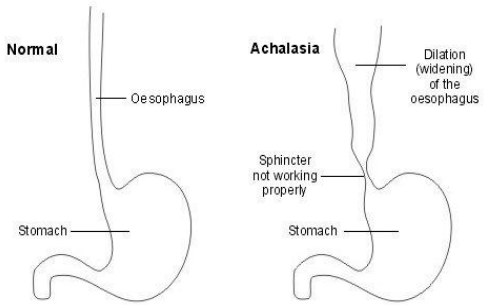
Heart tissue with inflammation and infection



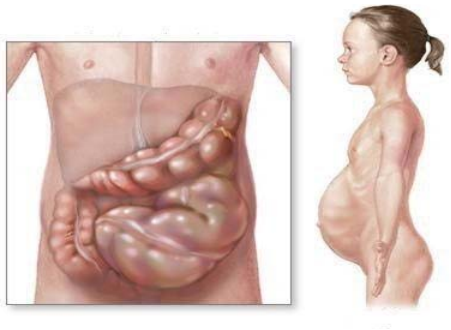
Enlarged heart



Severely irregular heart beats (arrhythmias)



Dilation of the esophagus (achalasia)



Dilation of the intestine (Megacolon)

Diagnosis

- There are several blood tests approved by FDA for diagnosis of Chagas disease
- No test predicts who will or will not be sick
- The tests are done at the CDC (doctors send the patient's blood sample to CDC through the State Health Department)
- Blood Banks and organ donor programs in the U.S. screen for Chagas disease
  - Some people find out they have Chagas disease when they try to donate blood

Treatment of Chagas Disease

- **Antiparasitic** treatment, to kill the parasite (antiparasitic drugs)
- **Symptomatic** treatment, to manage the symptoms and signs of infection (cardiac drugs and pacemakers)

Treatment of Chagas Disease

- There are no treatments currently approved by the FDA
- Two drugs available (oral tablets only), exclusively through the CDC, at a doctor's request:
  - Nifurtimox
  - Benznidazole
- Treatment consists of daily doses taken by mouth for 60 days

Treatment of Chagas Disease

- CDC and WHO recommend treatment in the acute (shortly after infection) cases and young, with or without symptoms. These include:
  - Babies infected from their mothers, children and adolescents
  - Women who can get pregnant
  - Patients with weakened immune systems (AIDS, treatments after kidney transplant)
  - Patients less than 50 years of age, without severe symptoms of heart disease
- Reported efficacy is higher (60-90%) when treatment is given shortly after infection occurs, especially in young patients up to 18 years of age

Treatment of Chagas Disease

- Treatment is optional in:
  - Patients older than 50 years of age, without severe symptoms of heart disease
- Treatment is not currently recommended for:
  - Pregnant women
  - Patients with severe kidney or liver disease
  - Patients with severe heart disease (a study is ongoing)

Commonly reported side effects

- Nifurtimox: decrease or loss of appetite, weight loss, nausea/vomiting, headache, sleeping problems, dizziness, seizures, changes in sensation and/or tingling or numbness in arms or legs
- Benznidazole: allergic skin rashes, changes in sensation and/or tingling and numbness in arms or legs, decrease or loss of appetite, nausea/vomiting, headache, dizziness
- *With either drug, side effects improve after stopping treatment*

Things to remember…

- Chagas disease can be transmitted from mother to child (congenitally) even through more than one generation
- Also transmitted through blood transfusion or organ transplants
- Chagas disease has an acute and chronic phase
- In both phases, most people do not have symptoms
- Infections usually last for life without treatment
- About a third of all infected people get life threatening cardiac disease, many years after infection
- In a small number of people, acute disease can be life-threatening
- No drug is approved in the U.S. but treatment is available through a CDC program

Acknowledgements

- **Office of Antimicrobial Products**
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  Soujanya Giambone MBA
  Sayyedeh Mariani, BA

  Special thanks to all panelists, including Rodolfo Viotti, MD, who was not able to come but contributed to this workshop

Thank You, Gracias, Obrigada !

To my first mentors, Drs. Jorge Bernabó, Diana Zoruba and José Leguizamón, from the **Hospital Municipal de Vicente López**, province of Buenos Aires, Argentina, who first taught me about Chagas disease and whose expertise, compassion and dedication continues to inspire me today.

To my patients, past, present and future, on whose behalf we hope to one day eradicate this disease.

Lionel Messi, Chagas campaign champion

Overview of Discussion Format

Soujanya Giambone, MBA
Office of Strategic Programs
Center for Drug Evaluation and Research
U.S. Food and Drug Administration

April 28, 2015

Discussion Overview

Topic 1: The symptoms that matter most to you

- What worries you most about your disease?
- Which symptoms have the most significant impact on your life?
- How do these symptoms affect your ability to do specific activities?
- How have your symptoms changed?

Topic 2: Current approaches to treating Chagas disease

- What are you doing to treat Chagas disease?
- What are the biggest downsides to your treatments?
- What would you look for in an “ideal” treatment?

Discussion Format

- **We will first hear from a panel of patients and caregivers**
  - The purpose is to set a good foundation for our discussion
  - They reflect a range of experiences with Chagas disease

- **We will then broaden the dialogue to include patients and patient representatives in the audience**
  - The purpose is to build on the experiences shared by the panel
  - We will ask questions and invite you to raise your hand to respond
  - Please state your name before answering

Discussion Format, continued

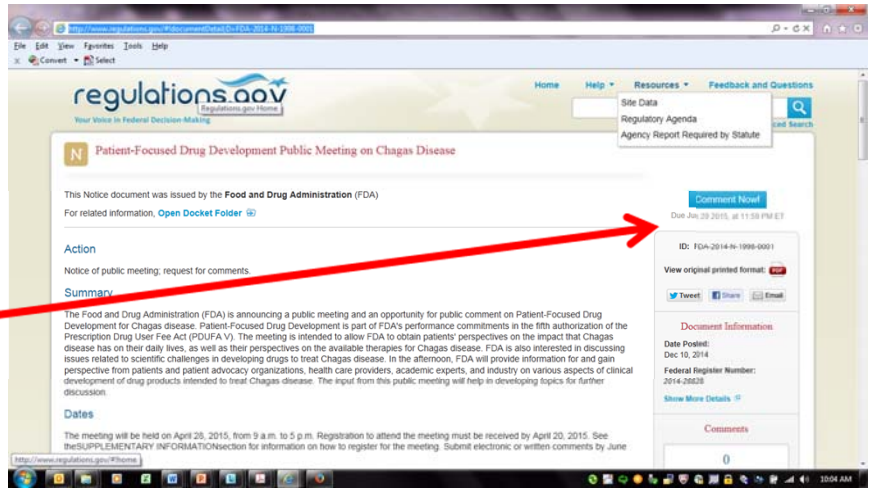
- **Web participants can add comments through the webcast**
  - Although they may not all be read or summarized today, your comments will be incorporated into our summary report
  - We'll occasionally go to the phones to give you another opportunity to contribute

Send us your comments!

- **You can send us comments through the "public docket"**
  - The docket will be open until June 29, 2015
  - Share your experience, or expand upon something discussed today
  - Comments will be incorporated into our summary report
  - Anyone is welcome to comment

Visit:
http://www.regulations.gov/#!documentDetail;D=FDA-2014-N-1998-0001

Click Comment Now!



Resources at FDA

- FDA Office of Health and Constituent Affairs
  - Contact: PatientNetwork@fda.hhs.gov, (301) 796-8460
  - Liaison between FDA and stakeholder organizations
  - Runs the Patient Representative Program
    - Patient Representatives advise FDA at Advisory Committee meetings
- CDER Office of Center Director
  - Professional Affairs and Stakeholder Engagement (PASE)
  - Contact: Mary Ghods, mary.ghods@fda.hhs.gov
  - Facilitates communication and collaboration between CDER and patient and healthcare professional stakeholders and others on issues concerning drug development, drug review and drug safety.

Discussion Ground Rules

- We encourage patients to contribute to the dialogue– caregivers, advocates, and healthcare providers are welcome too
- FDA is here to listen
- Discussion will focus on symptoms and treatments
  - Open Public Comment Period is available to comment on other topics
- The views expressed today are personal opinions
- Respect for one another is paramount
- Let us know how the meeting went today; evaluations at registration desk

Discussion Topic 1

Disease symptoms and daily impacts that matter most to patients

Soujanya Giambone
Facilitator

Panel Participants

- Candace Stark
- Maira Gutierrez
- Lorena Medrano
- Carlos Toba Beza
- Rachel Marcus
- Maria Abrigo (Phone)

Topic 1 Discussion: Disease symptoms and daily impacts that matter most to patients

- What **worries you most** about your condition?
- Of all the symptoms that you experience because of your condition, which **1-3 symptoms** have the most significant impact on your life?
- Are there **specific activities** that are important to you but that you cannot do at all or as fully as you would like because of your condition?
- How have your condition and its symptoms **changed over time**?
- Do your symptoms come and go? If so, do you know of anything that makes your symptoms better or worse?

BREAK

Discussion Topic 2

Patients' perspectives on current approaches to treating Chagas Disease

Soujanya Giambone
Facilitator

Topic 2 Discussion: Patients' perspectives on current approaches to treating Chagas disease

- **What are you currently doing** to help treat your condition?
  - What specific symptoms do your treatments address?
  - How has your treatment regimen changed over time, and why?

- What are the most significant **downsides to your current treatments**, and how do they affect your daily life?

- What specific things would you look for in an **ideal treatment** for your condition?

Scenario 1

- Imagine you are just diagnosed with Chagas disease.
  - You have no symptoms.
  - You may have had the disease for 2-3 decades.
  - 3 out of 10 patients who have no symptoms may develop symptoms that will lead to sudden death from heart conditions (usually around the age of 40)
- Drug X is developed to treat patients with Chagas disease
  - Patients will need to take Drug X for 60 days.
  - Drug X has been shown to cure 7 out of 10 patients that do not have symptoms of Chagas disease
  - Drug X causes nausea, vomiting or tingling or numbness in arms or legs in many patients. In rare cases, it causes non-fatal, reversible side effects such as seizures.

Would you consider this treatment: For yourself? For your teenage child?

Scenario 2

Imagine that…

- You have been invited to participate in a clinical trial to study an experimental treatment for Chagas disease
- Early research in animals and people shows that this treatment may cure the disease in some people
- The purpose of the study is to better understand how well this treatment works and its safety
- The study will enroll 50 adults who have been diagnosed with Chagas disease but do not show symptoms

Scenario 2

Imagine that…

- This clinical study lasts 2 years and clinic visits will occur every 2 months for the first year, and once every 4 months in the second year

- Some visits may involve blood tests

- More common side effects of this therapy may include nausea, vomiting, and weight loss.

- Rarer but more serious side effects may include changes in sensation and nerve damage and skin rash

What thoughts and questions

come to mind as you hear this scenario?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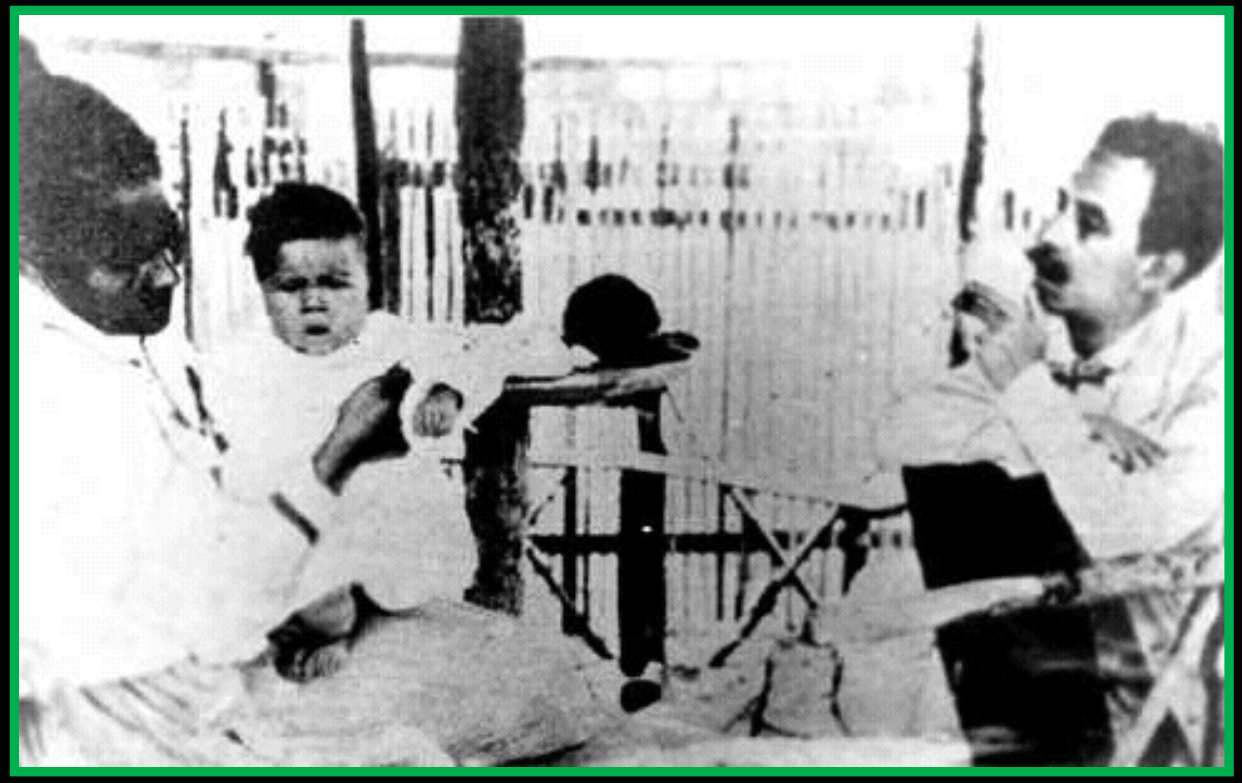
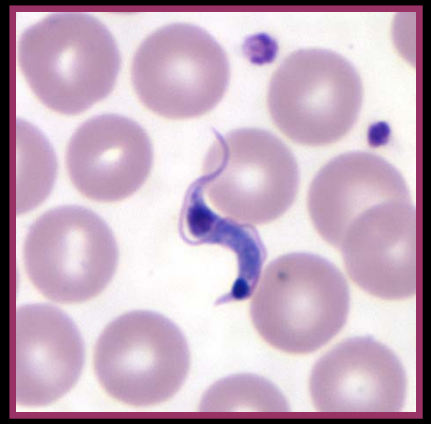
Lunch Break

Scientific Discussion

Chagas disease epidemiology and natural history



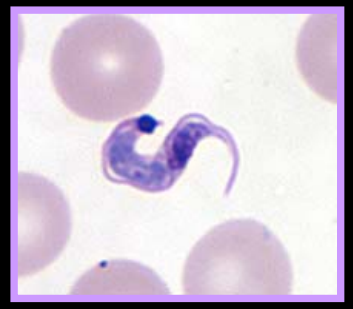


UCSF
University of California
San Francisco

Caryn Bern, MD, MPH

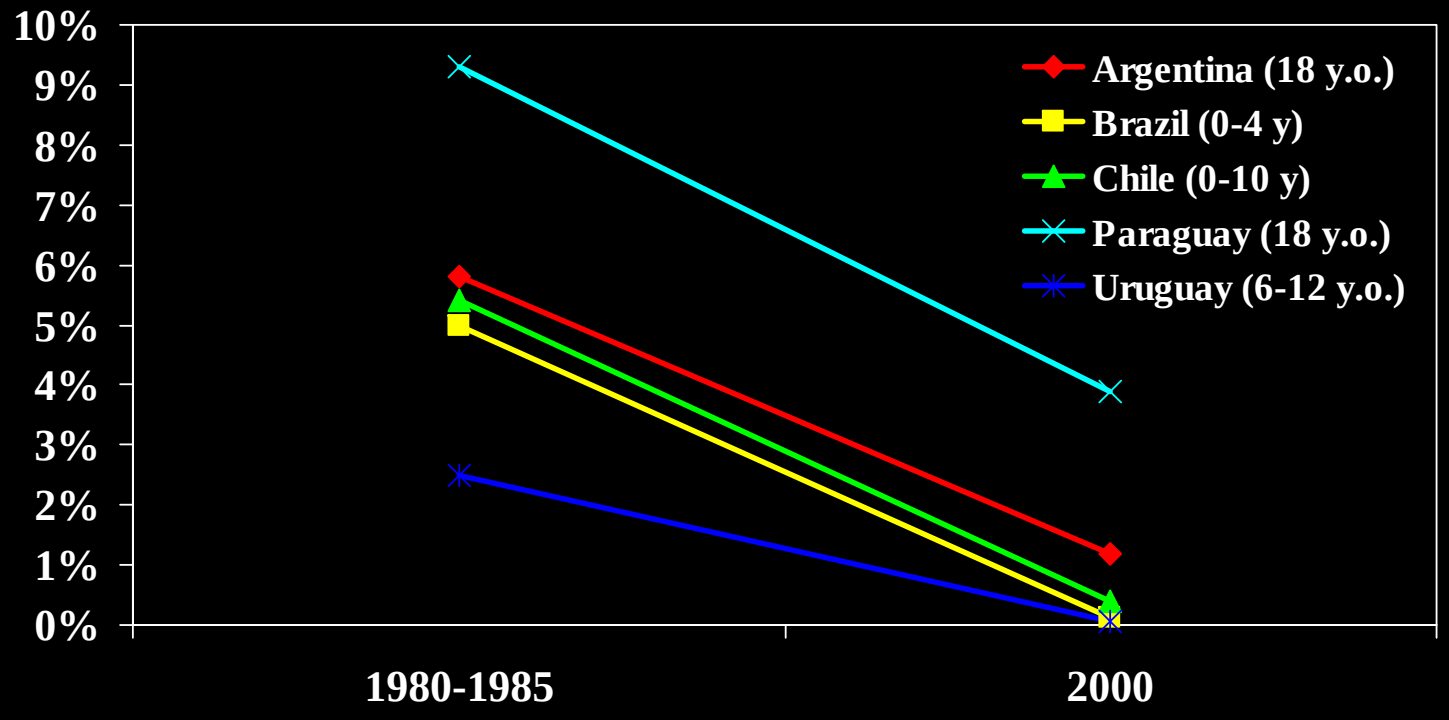
April 2015

T. cruzi transmission routes



- Congenital
- Transfusion
- Transplant
- Oral

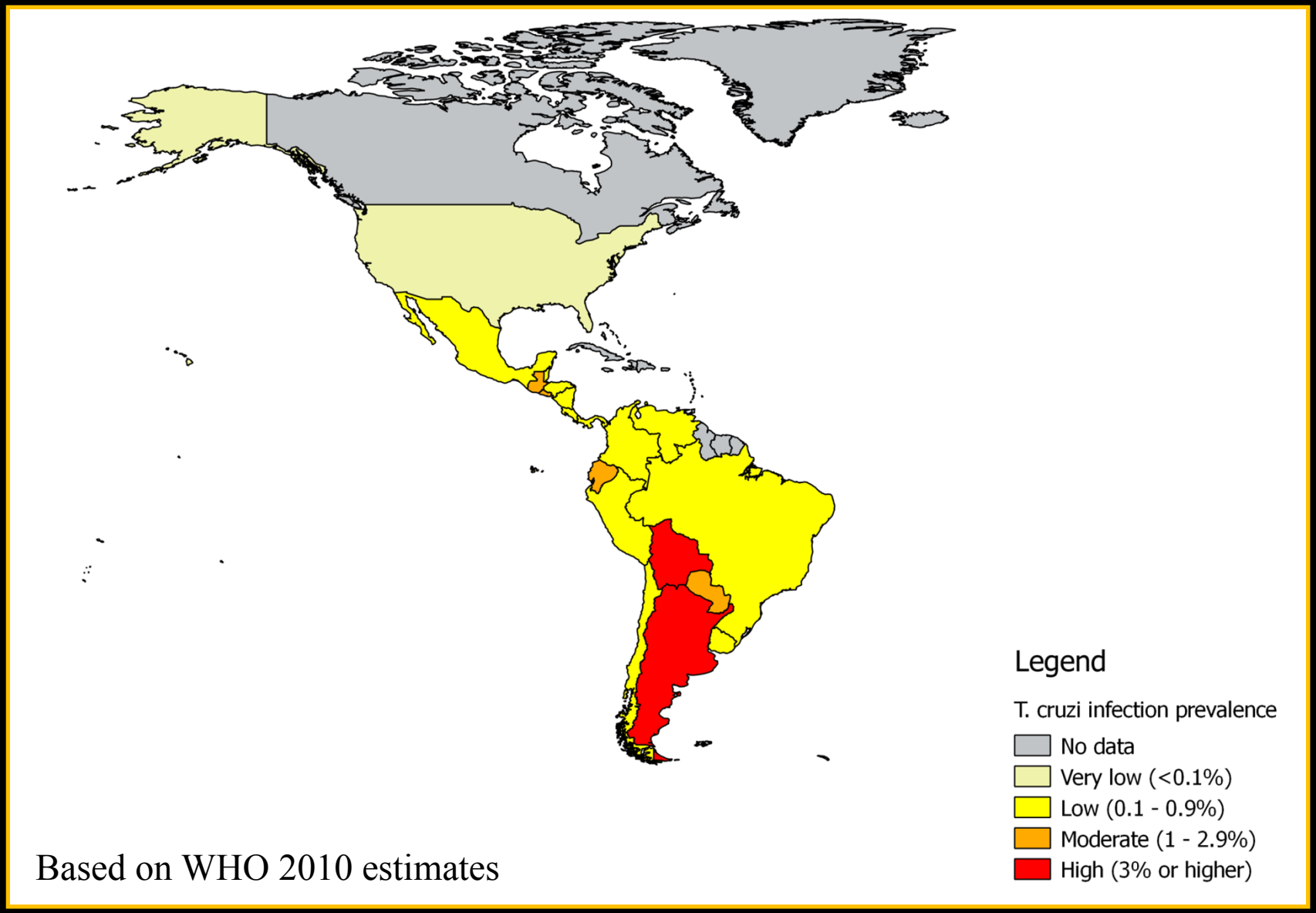
T. cruzi seroprevalence in sentinel population groups from 1980s to 2000



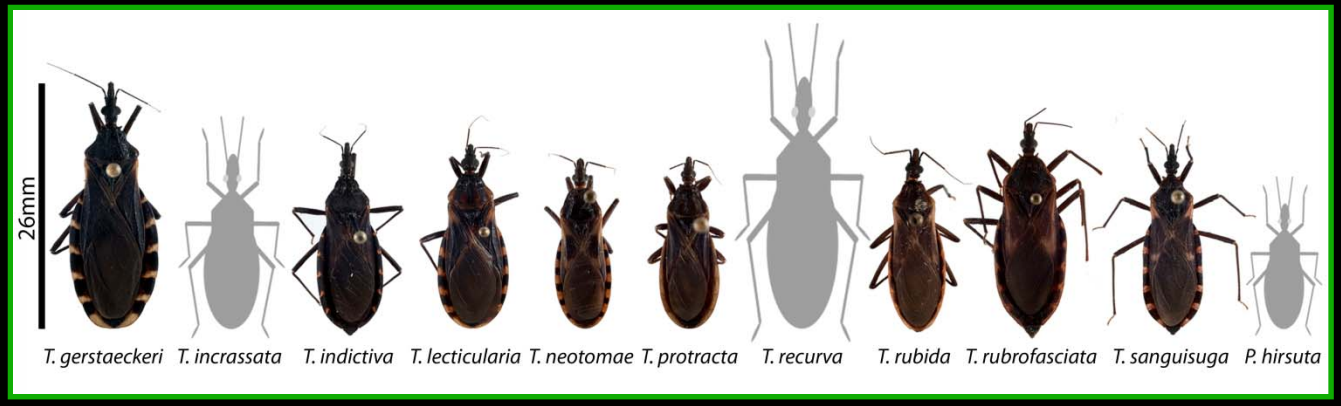
Estimated prevalence / incidence
1990: 18 million / 500,000
2010: 5.7 million / 39,000

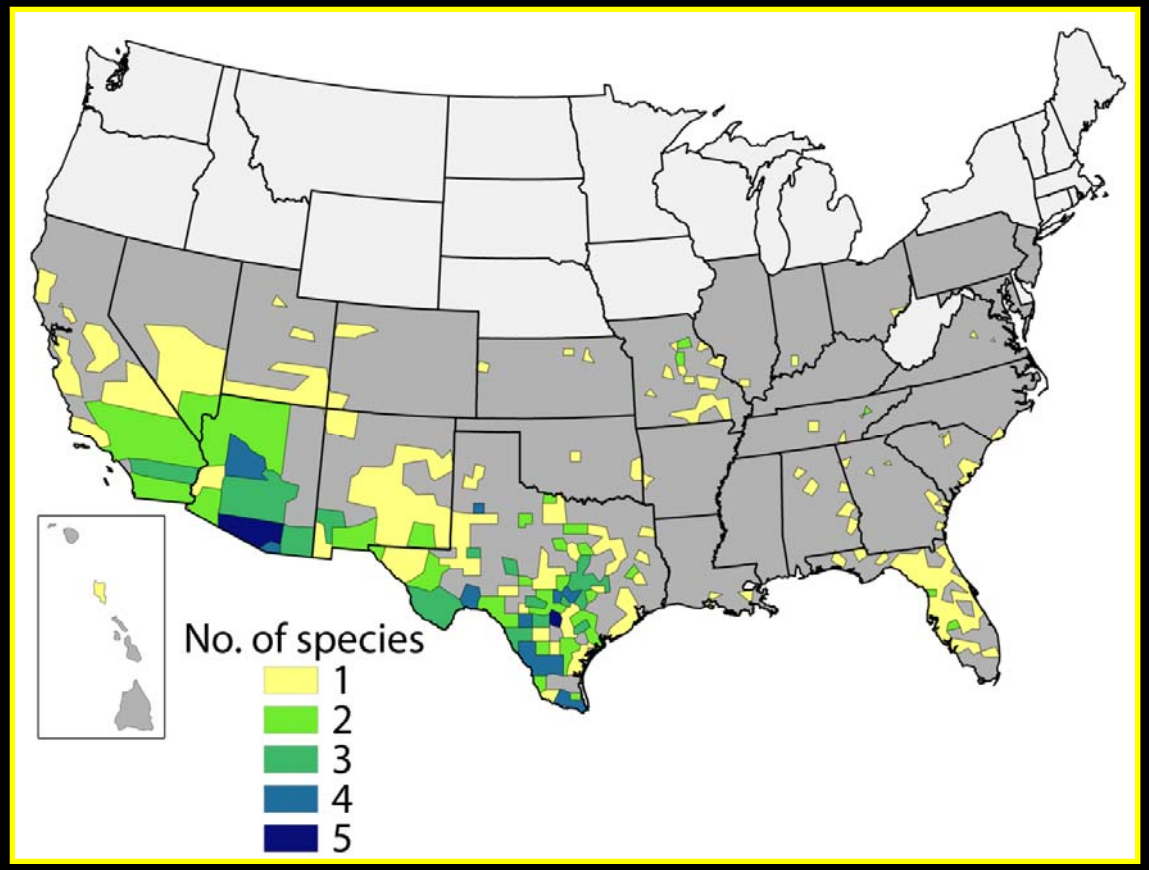
Moncayo Ann Trop Med Parasitol 2006; WHO Weekly Epi Rec 2015

Estimated *T. cruzi* infection prevalence by country



Based on WHO 2010 estimates



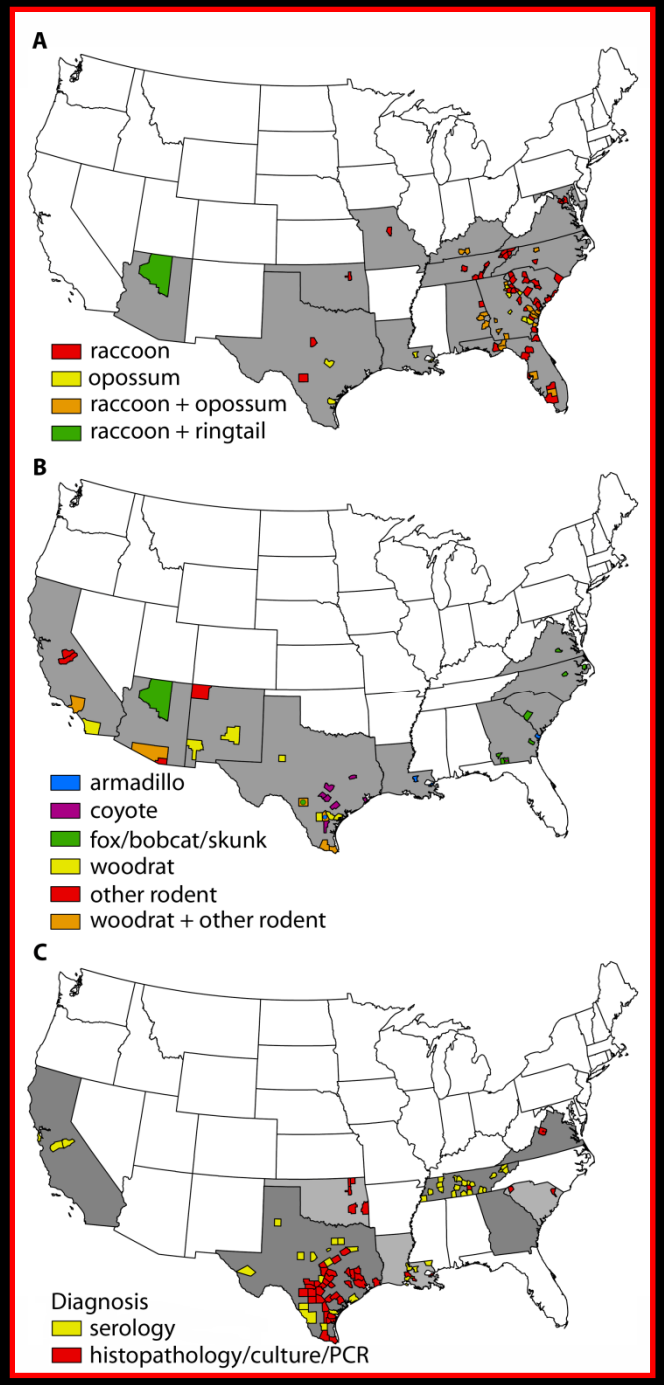


The US is an endemic [enzootic] country

11 vector species

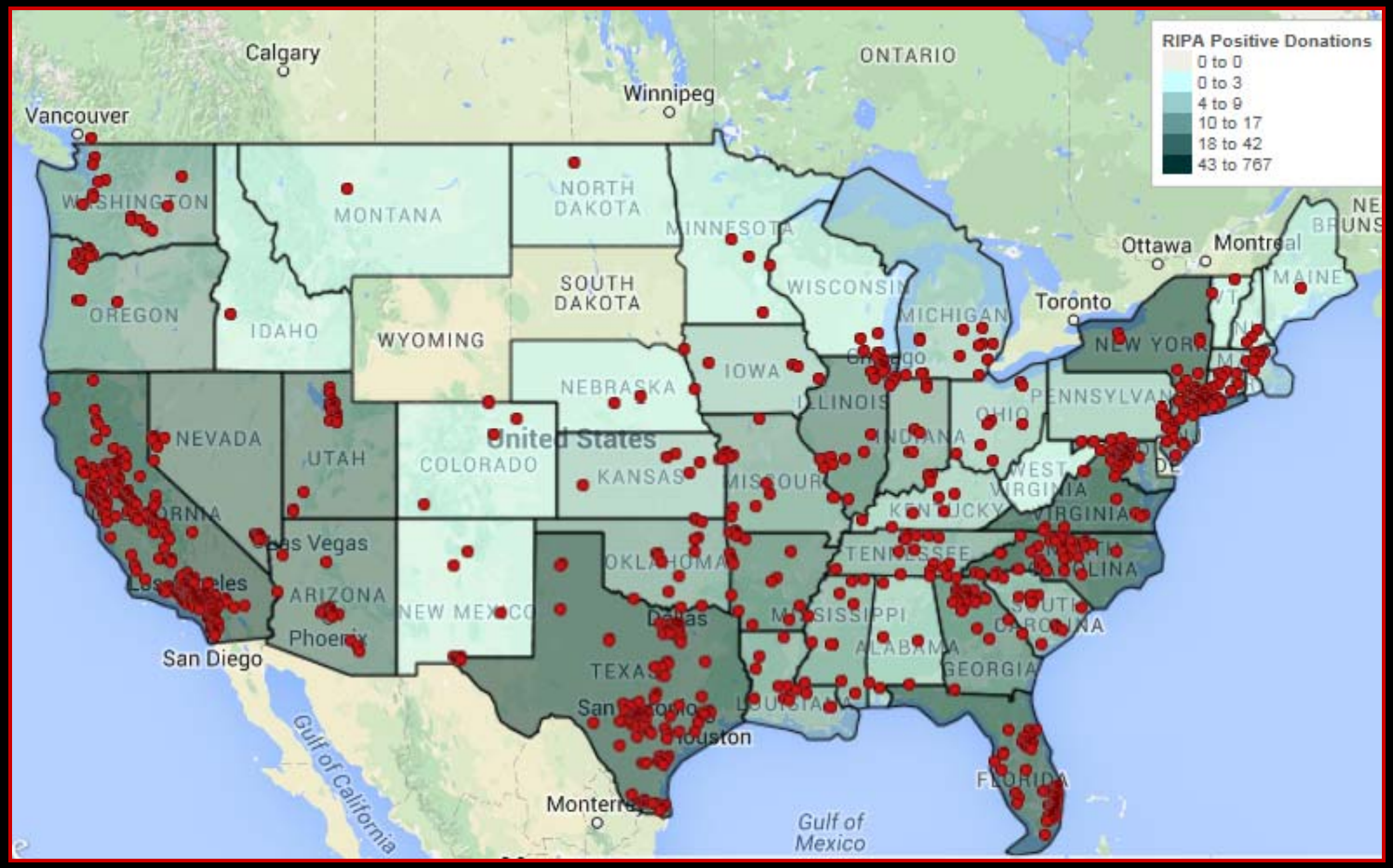
Bern et al Clin Micro Rev 2011

Many infected reservoir hosts



Confirmed *T.cruzi*-positive blood donations

Jan 1, 2007 – Apr 23, 2015, N = 2,043



Source: AABB Biovigilance program

Chagas disease in the United States

- Locally-acquired Chagas disease burden undefined
  - 7 autochthonous vector-borne human infections documented since 1955 (TX [4], CA, TN, LA)
  - Extrapolation from study of 16 blood donors apparently infected in US suggests prevalence of 1 in 354,000 donors
- 23 million people born in Chagas disease-endemic countries of Latin America live in the U.S.
  - Estimated ~300,000 infected immigrants, based on *T. cruzi* infection prevalence in countries of origin
  - In case series, 13 - 16% of non-ischemic cardiomyopathy in Latin American immigrants attributed to *T. cruzi*

Bern et al Clin Micro Rev 2011; Cantey Transfus 2012; Bern & Montgomery CID 2009; Kapelusznik CID 2013; Traina ASMTH abstract 2009.

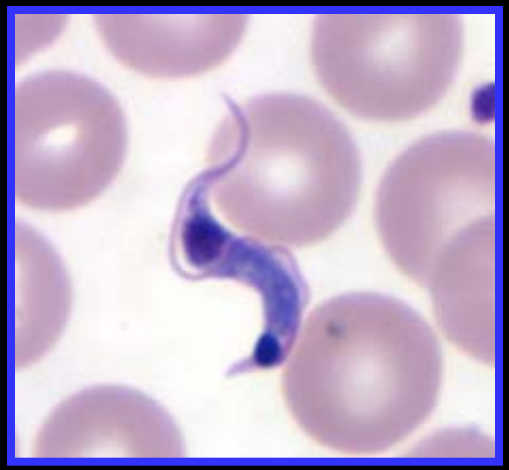
1-2 weeks

Acute phase
of Chagas disease

*1-2 weeks**

Acute phase of Chagas disease

- < 1% diagnosed, most mild
- May have signs at portal of entry (chagoma, Romaña's sign)
- Fever, systemic symptoms, hepato-splenomegaly, atypical lymphocytosis
- Acute meningoencephalitis and myocarditis rare, but associated with high mortality
- Patent parasitemia
  - Parasites may be visible on wet prep of heparinized blood or buffy coat, Giemsa-stained smears
  - PCR-based assays have high sensitivity



**Transfusion- and transplant-associated cases may have incubation period up to 120 days*

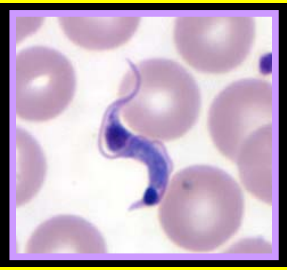
Congenital *T. cruzi* infection

- Similar to acute *T. cruzi* infection
- Median 6% (1-10%) of infants of infected women
- Most mild or asymptomatic
  - Rarely meningoencephalitis, myocarditis, respiratory distress syndrome, fetal hydrops
- Early diagnosis by microscopy or PCR
  - Microscopy of concentrated cord or neonatal blood sensitivity <50% in one specimen
  - PCR sensitivity ~75% in one specimen
  - Parasitemia rises after birth, peaks 30-90 days
  - Transferred maternal IgG until 8-9 months

Torrico et al AJTMH 2004, Bern et al CID 2009, Oliveira et al 2010

1-2 weeks

Acute phase
of Chagas disease



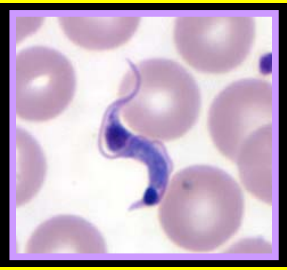
8 weeks

Chronic phase

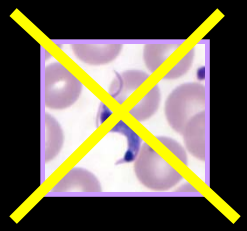
- Parasitemia level falls steeply ~ 8 weeks post infection
- Acute symptoms (if any) resolve spontaneously

1-2 weeks

Acute phase
of Chagas disease



8 weeks

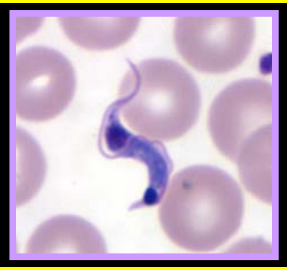


Chronic phase

- Blood smear negative, PCR sensitivity variable (20 to 90%)
- Diagnosis relies on serology
  - ELISA, IFA, TESA-blot
  - confirmed by positive results on at least 2 different tests
- Infectious to vector, congenitally, via transplant or transfusion
- Can reactivate if immunosuppressed

1-2 weeks

Acute phase
of Chagas disease



8 weeks

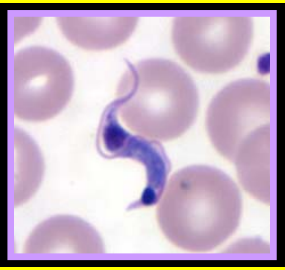
Chronic phase

Indeterminate form

- No cardiac or GI signs or symptoms, normal EKG
  - may have subtle abnormalities on echocardiogram, autonomic testing; prognostic significance unknown
  - some experts require negative barium studies as well
- Lifelong infection in absence of treatment

1-2 weeks

Acute phase
of Chagas disease



8 weeks

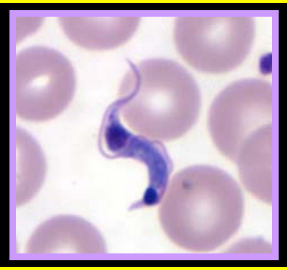
Chronic phase

Indeterminate form
No signs or symptoms of Chagas disease

70 - 80% remain indeterminate throughout life

1-2 weeks

Acute phase
of Chagas disease



8 weeks

Chronic phase

Indeterminate form
No signs or symptoms of Chagas disease

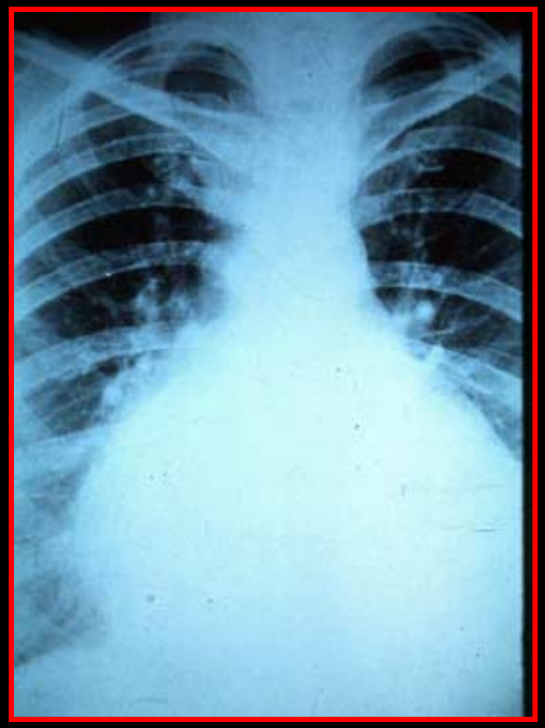
20 - 30% progress
over years - decades

70 - 80% remain
indeterminate
throughout life

Determinate forms
- Chagas cardiomyopathy &/or
- Gastrointestinal disease

Chagas cardiomyopathy

- Conduction system defects
  - Earliest sign, especially RBBB, LAFB
  - Later, high grade AV blocks
- Brady- and tachyarrhythmias
  - Sinus node dysfunction, bradycardia
  - Multifocal ventricular extrasystoles
  - Sustained and non-sustained ventricular tachycardia
- Apical aneurysm, thrombus, strokes
- Dilated cardiomyopathy and congestive heart failure



Rassi et al Clin Cardiol 2000

Box. Classification Schemes to Grade Presence and Severity of Chagas Cardiomyopathy

Modified Kuschnir Classification[25]

0: Normal ECG findings and normal heart size (usually based on chest radiography)

I: Abnormal ECG findings and normal heart size (usually based on chest radiography)

II: Left ventricular enlargement

III: Congestive heart failure

Brazilian Consensus Classification[20]

A: Abnormal ECG findings, normal echocardiogram findings, no signs of CHF

B1: Abnormal ECG findings, abnormal echocardiogram findings with LVEF >45%, no signs of CHF

B2: Abnormal ECG findings, abnormal echocardiogram findings with LVEF <45%, no signs of CHF

C: Abnormal ECG findings, abnormal echocardiogram findings, compensated CHF

D: Abnormal ECG findings, abnormal echocardiogram findings, refractory CHF

Modified Los Andes Classification[26]

IA: Normal ECG findings, normal echocardiogram findings, no signs of CHF

IB: Normal ECG findings, abnormal echocardiogram findings, no signs of CHF

II: Abnormal ECG findings, abnormal echocardiogram findings, no signs of CHF

III: Abnormal ECG findings, abnormal echocardiogram findings, CHF

Classification Incorporating American College of Cardiology/American Heart Association Staging[27,28]

A: Normal ECG findings, normal heart size, normal LVEF, NYHA class I

B: Abnormal ECG findings, normal heart size, normal LVEF, NYHA class I

C: Abnormal ECG findings, increased heart size, decreased LVEF, NYHA class II-III

D: Abnormal ECG findings, increased heart size, decreased LVEF, NYHA class IV

Abbreviations: CHF, congestive heart failure; ECG, electrocardiogram; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

Classification schemes for severity of Chagas cardiomyopathy

Characteristic ECG findings

ECG findings

Common
- Right bundle-branch block
- Incomplete right bundle-branch block[a]
- Left anterior fascicular block
- 1° AV block
- 2° AV block, Mobitz type I or II
- Complete AV block
- Bradycardia, sinus node dysfunction
- Ventricular extrasystoles, often frequent, multifocal, or paired
- Ventricular tachycardia, nonsustained or sustained

Less common but clinically significant when present
- Atrial fibrillation or flutter
- Left bundle-branch block
- Low QRS voltage
- Q waves

Signs of cardiac insufficiency

- Cardiomegaly
- Clinical CHF
- LV end-diastolic volume
- LV ejection fraction

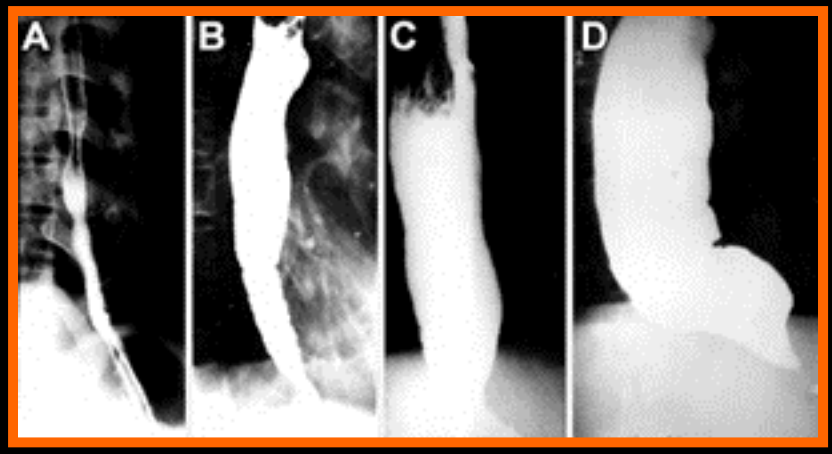
Mortality in Chagas heart disease

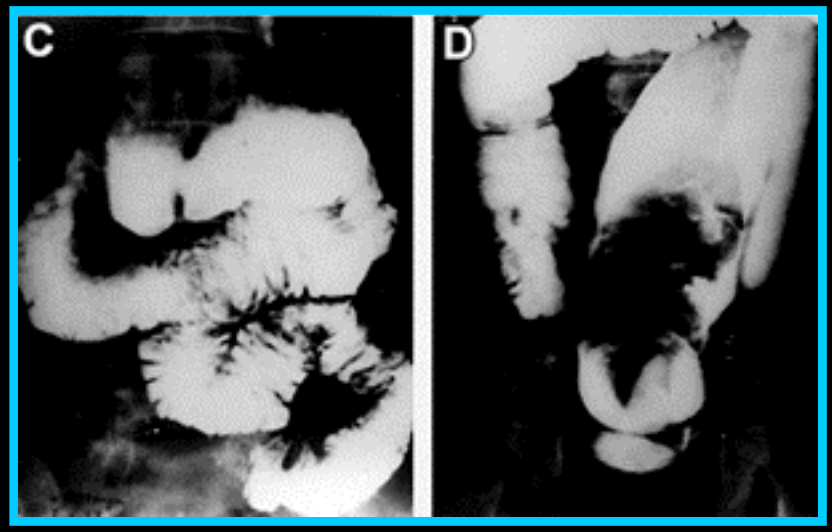
- Signs of poor prognosis
  - Complex ventricular arrhythmias
  - Global or segmental wall motion abnormalities
  - Sustained or non-sustained ventricular tachycardia
  - Increased LVEDD, decreased LV ejection fraction
- Sudden death can occur early or late in course
  - Ventricular arrhythmias, complete AV block, emboli
- Mortality from intractable CHF in advanced disease
  - LVEF< 30% associated with <30% survival over 2-4 years

Rassi NEJM 2006

Gastrointestinal Chagas disease

- Esophagus: Dysphagia, odynophagia, weight loss, regurgitation, aspiration, megaesophagus
- Colon: Chronic severe constipation, fecaloma, megacolon
- Parasite strain differences?
  - Seen in Argentina, Bolivia, Paraguay, Uruguay, Brazil
  - Very rare in Central America, northern South America
- Treatment largely surgical





De Oliveira Am J GE 1998; Miles Lancet 1981

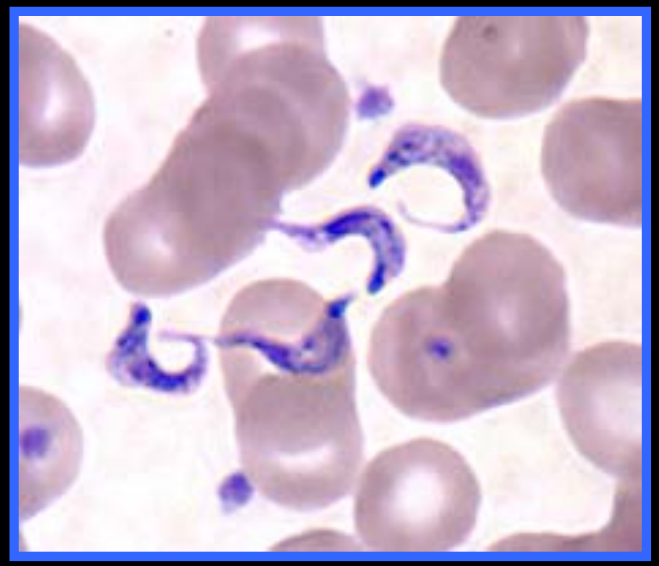
Photos: www.fiocruz.br/chagas

Transplant-derived acute *T. cruzi* infection

- *T. cruzi* transmission risk varies by organ
  - Kidney 13% (2/15) in US cohort; 19% (3/16) in Argentina
  - Liver 20% (2/10) in US cohort
  - Heart 75% (3/4) in US cohort

Chagas in Transplant Working Group recommendations:

- Kidney, liver can be used; use of heart contraindicated
- Serial monitoring with PCR
  - Presumptive treatment not recommended
  - Good outcomes with early detection and treatment

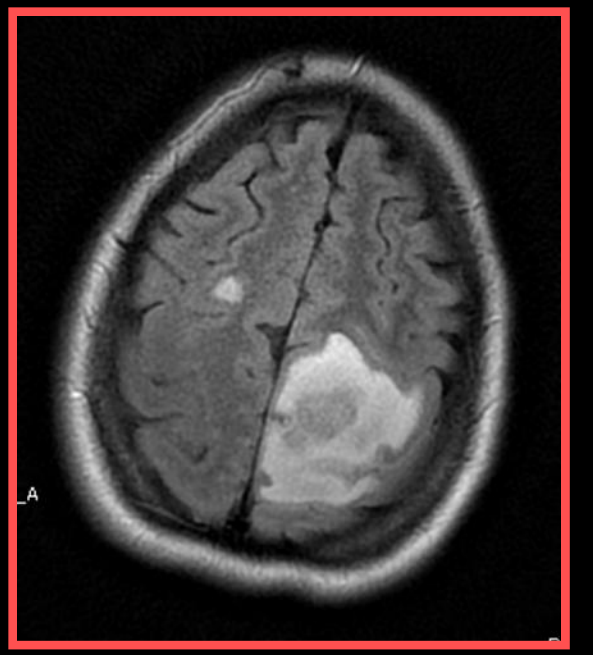


Huprikar 2013; Riarte 1999; Chin-Hong (Chagas in Transplant Working Group) 2011

Reactivation in immunosuppressed hosts with pre-existing chronic *T. cruzi* infection

Two major settings for reactivation

- *T. cruzi*-infected patient who receives solid organ or bone marrow transplant
  - Acute myocarditis, skin lesions, inflammatory panniculitis
  - Good prognosis with monitoring and prompt treatment
- HIV/AIDS patients
  - CNS disease most common: mass lesion, meningoencephalitis; 80% mortality
  - Acute myocarditis 2nd most common
  - Role of and indications for antitrypanosomal prophylaxis unresolved



Kransdorf Am J Transpl 2013; Sartori Ann Trop Med Para 2007

Annals of Internal Medicine | ARTICLE

Long-Term Cardiac Outcomes of Treating Chronic Chagas Disease with Benznidazole versus No Treatment

A Nonrandomized Trial

Rodolfo Viotti, MD; Carlos Vigliano, MD; Bruno Lococo, MD; Graciela Bertocchi, MD; Marcos Petti, MD; María Gabriela Alvarez, MD; Miriam Postan, MD, PhD; and Alejandro Armenti, MD

Background: Benznidazole is effective for treating acute-stage Chagas disease, but its effectiveness for treating indeterminate and chronic stages remains uncertain.

Objective: To compare long-term outcomes of patients with nonacute Chagas disease treated with benznidazole versus outcomes of those who did not receive treatment.

Design: Clinical trial with unblinded, nonrandom assignment of patients to intervention or control groups.

Setting: Chagas disease center in Buenos Aires, Argentina.

Patients: 566 patients 30 to 50 years of age with 3 positive results on serologic tests and without heart failure.

Measurements: The primary outcome was disease progression, defined as a change to a more advanced Kuschnir group or death. Secondary outcomes included new abnormalities on electrocardiography and serologic reactivity.

Median follow-up 9.8 years

Results: Fewer treated patients had progression of disease (12 of 283 [4%] vs. 40 of 283 [14%]; adjusted hazard ratio, 0.24 [95% CI, 0.10 to 0.59]; $P = 0.002$) or developed abnormalities on electrocardiography (15 of 283 [5%] vs. 45 of 283 [16%]; adjusted hazard ratio, 0.27 [CI, 0.13 to 0.57]; $P = 0.001$) compared with untreated patients. Left ventricular ejection fraction (hazard ratio, 0.97 [CI, 0.94 to 0.99]; $P < 0.002$) and left ventricular diastolic diameter (hazard ratio, 2.45 [CI, 1.53 to 3.95]; $P < 0.001$) were also associated with disease progression. Conversion to negative results on serologic testing was more frequent in treated patients than in untreated patients (32 of 218 [15%] vs. 12 of 212 [6%]; adjusted hazard ratio, 2.1 [CI, 1.06 to 4.06]; $P = 0.034$).

Limitations: Nonrandom, unblinded treatment assignment was used, and follow-up data were missing for 20% of patients. Loss to follow-up was more common among patients who were less sick. Two uncontrolled interim analyses were conducted.

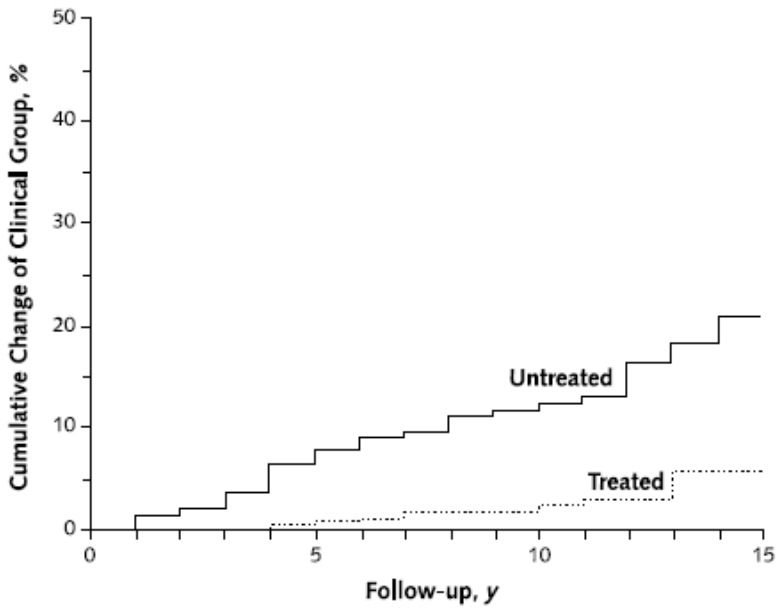
Conclusions: Compared with no treatment, benznidazole treatment was associated with reduced progression of Chagas disease and increased negative seroconversion for patients presenting with nonacute disease and no heart failure. These observations indicate that a randomized, controlled trial should now be conducted.

Ann Intern Med. 2006;144:724-734. www.annals.org

For author affiliations, see end of text.

Treated group had significantly lower rate of progression than the untreated group

Figure 2. Kaplan–Meier curves of cumulative percentage of patients who changed clinical group.



12/283 (4.2%) treated vs 40/283 (14.1%)
aHR 0.24 (95% CI 0.10, 0.59); P =0.002

Baseline group	Progression to higher group	
	Untreated	Treated
0	7.2%	3.3%
I	18.7%	4.1%
II	46.4%	10.0%

More severe baseline status had higher rate of progression

Mortality

Three of 283 treated patients [1.1%] vs 12 of 283 untreated patients [4.2%] died; in models adjusted for LV ejection fraction, adjusted hazard ratio was 0.2 (CI, 0.03 to 1.2; P =0.085).

Trend toward decreased mortality in treated group

Treated women significantly less likely to transmit to their infants than untreated women

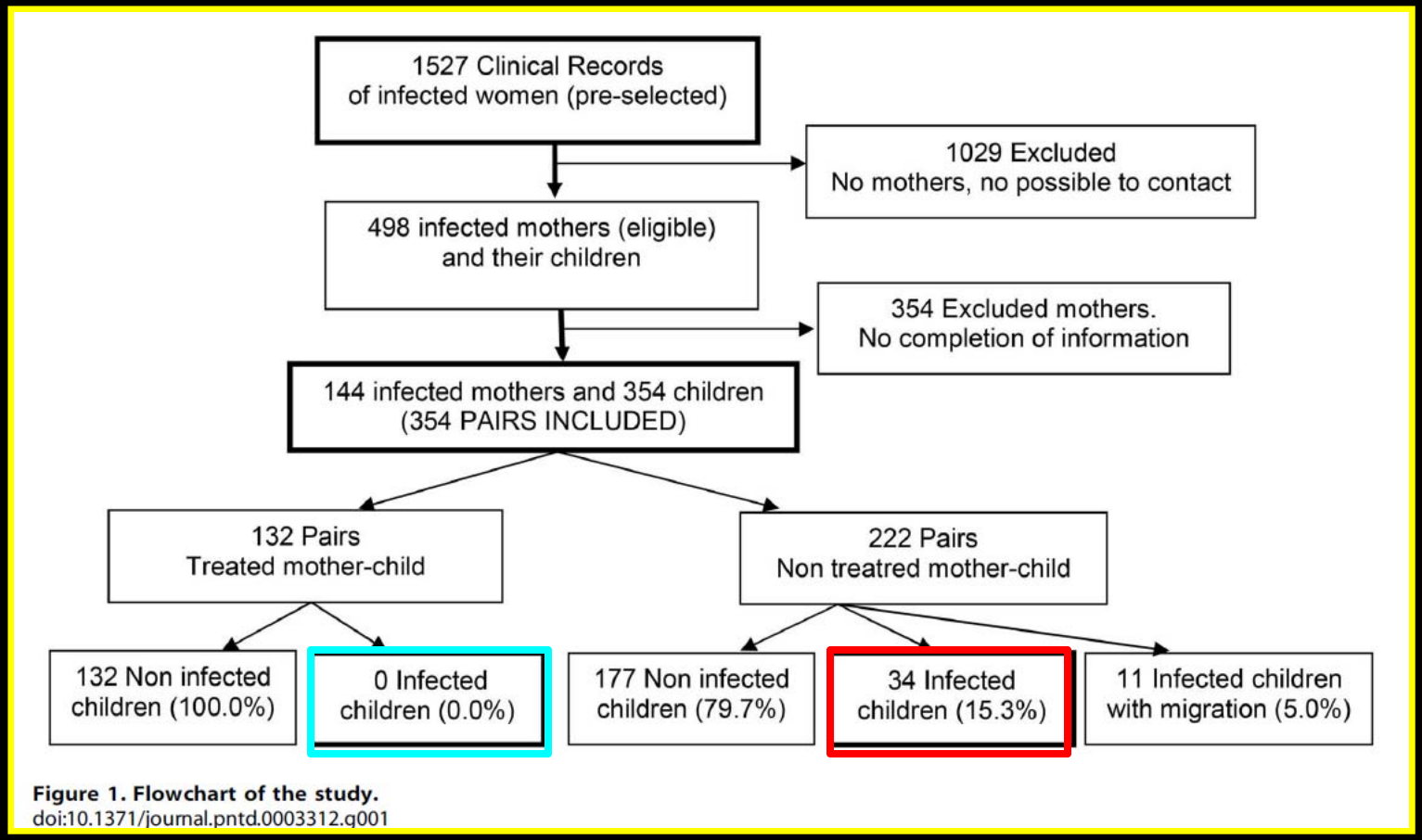


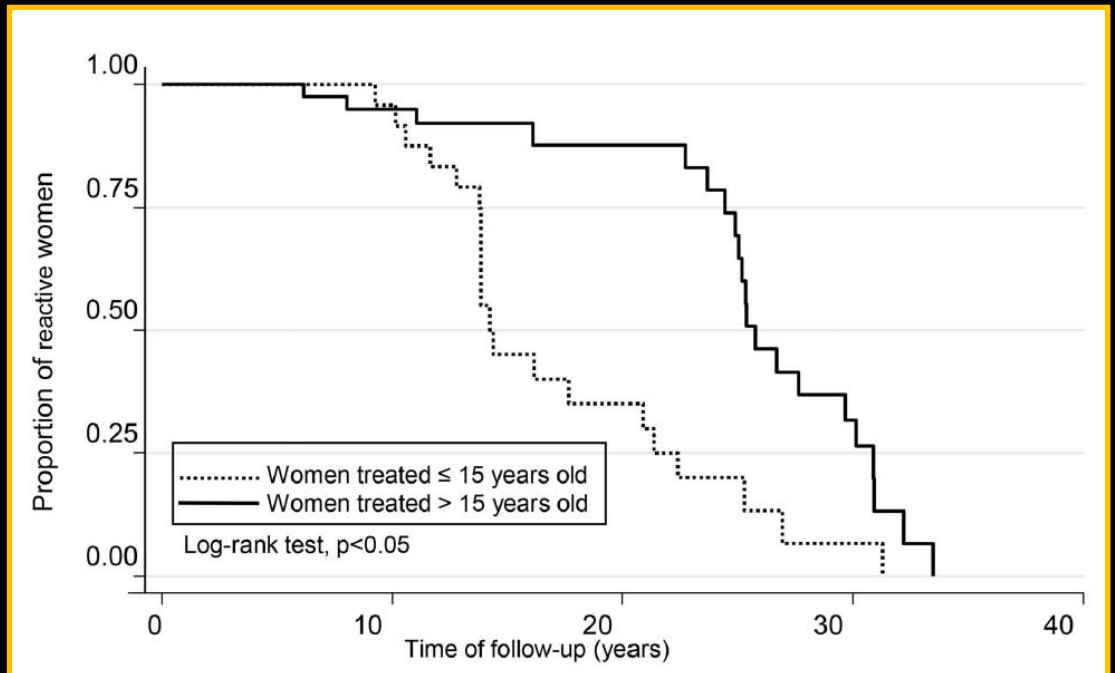
Figure 1. Flowchart of the study.
doi:10.1371/journal.pntd.0003312.g001

RR 0.04 [95% CI 0.012, 0.166]

Fabbro PLoS NTD 2014

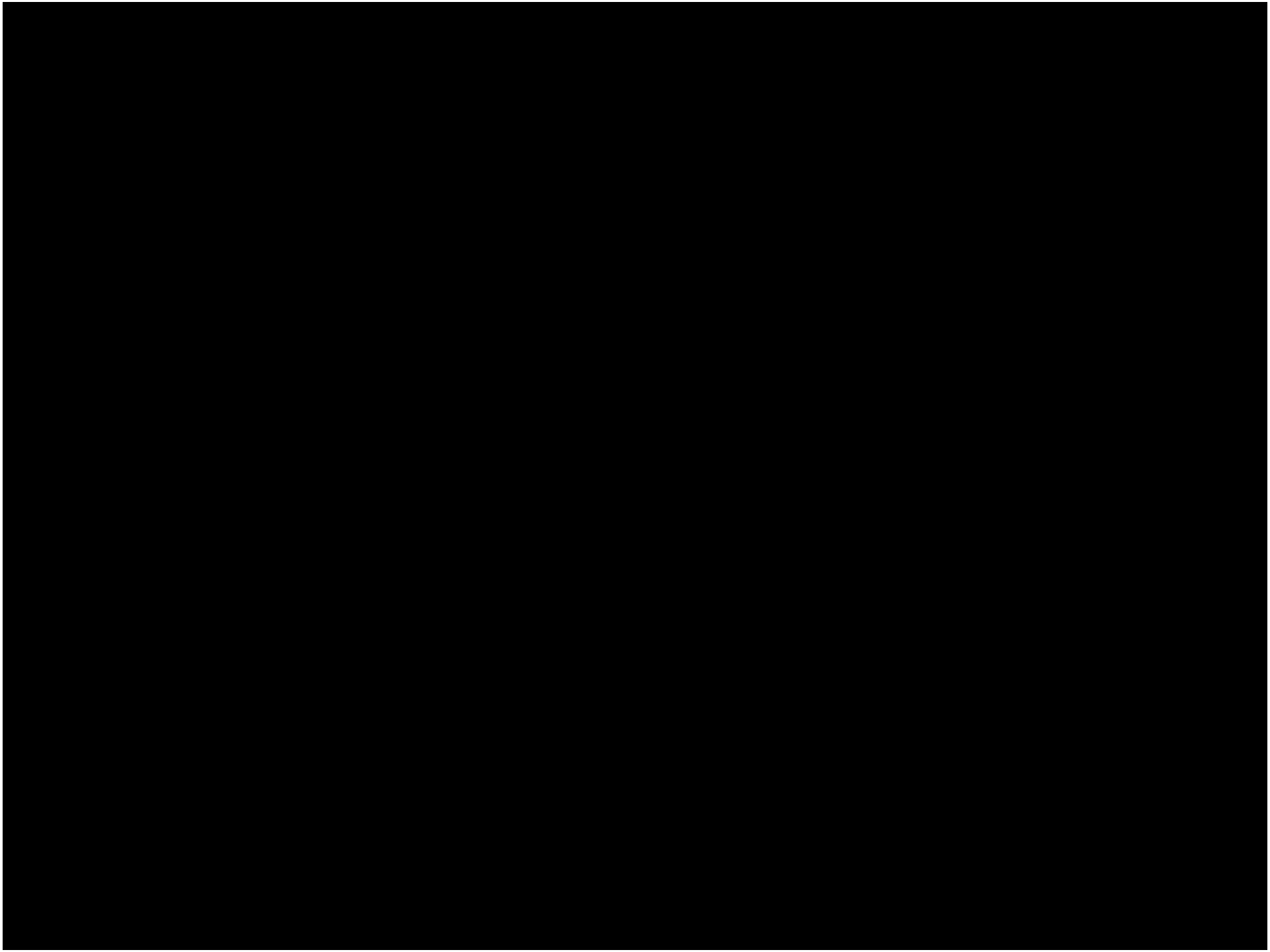
Conventional serology after treatment

- Viotti 2006: negative seroconversion in 15% of treated vs 6% of untreated; median time 11.7 years
- Observational data (Fabbro 2007): up to 40% of treated (at 30 years) vs 0% of untreated
- Observational data (Fabbro 2014): seroreversion more rapid (less slow!) in those treated as children



4. Kaplan-Meier curve showing serological reactivity rate by age during follow-up in 71 treated women.
371/journal.pntd.0003312.g004

Lassance, MG, Brazil 1909



Potential outcomes for studies of drug treatment of chronic *T. cruzi* infection

Outcome	Advantages	Disadvantages
IgG serology	Widely accepted as most rigorous, widely available	Takes >5 to 40 years; positive results ≠ failure
Fall in IgG titers	Widely available	No independent basis for cut-offs, high biological variability
Lytic antibodies	Supported by pediatric RCT data, respond more rapidly than conventional serology	Takes months to several years; direct assays challenging
Serial qPCR	Rapid response, sensitive indicator of treatment failure	Dependent on lab, blood volume; negative results ≠ cure
Cardiac progression	Clinical outcome of most interest	Takes years to decades; requires large sample size

Review Considerations for New Drugs in the United States

Chagas Disease Public Meeting on Patient-Focused Drug Development

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Deputy Director for Safety
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CDER, FDA

April 28, 2015

*No conflicts of interest

Outline of the Presentation

- Adequate and Well-Controlled Trials
- Endpoints
- Regulatory Approvals

Adequate and Well-Controlled Trials

- Trials designed to show that a new drug is safe and effective for treatment
  - Effective: the benefit that patients experience (cure, improvement)
  - Safe: the risk of side effects
- FDA and clinicians weigh the benefits and risks of new drugs for treatment

Adequate and Well-Controlled Trials

Drugs approved must meet the statutory standards for effectiveness and safety

- Section 505(d) of the FD&C Act
- Section 115(a) of the Modernization Act allows for one trial

Substantial evidence from adequate and well-controlled clinical trials

- 21 CFR 314.126

Adequate and Well-Controlled Trials

Placebo concurrent control

- A test drug is compared with an inactive preparation designed to resemble the test drug
- Success = test drug is better than placebo
  - Success = statistical inference testing shows robust evidence of efficacy

Adequate and Well-Controlled Trials

Dose-comparison concurrent control

- Two or more doses of the test drug are compared
- Success = one dose of the test drug is better than a different dose of the test drug

Adequate and Well-Controlled Trials

No treatment concurrent control

- A test drug is compared with no treatment
- Usually patients are randomized to test drug or to no treatment
- Success = test drug is better than no treatment

Adequate and Well-Controlled Trials

Active treatment concurrent control

- A test drug is compared with a known effective therapy (active control)
- Success = test drug is better than known effective therapy, or test drug is similar (non-inferiority)
  - Treatment effect over placebo of the active control drug must be known for non-inferiority

Adequate and Well-Controlled Trials

Historical control

- A test drug is compared to experience historically derived (natural history)
- Success = test drug is better than the historical experience
- Usually reserved for rare circumstances
  - e.g., historical experience = high mortality

Outline of the Presentation

- Adequate and Well-Controlled Trials
- Endpoints
- Regulatory Approvals

Endpoint Definitions

The methods of assessment of subjects' responses are well-defined and reliable. The protocol for the study and the report of results should explain the variables measured, the methods of observation, and criteria used to assess response.

–21 CFR 314.126(b)(6)

Endpoint Definitions

…a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives...

– Federal Register/Vol. 57, No.73/April 15, 1992

Endpoint Definitions

A characteristic or variable that reflects how a patient feels, functions, or survives. Clinical endpoints are distinct measurements or analyses of disease characteristics observed in a study or a clinical trial that reflect the effect of a therapeutic intervention. Clinical endpoints are the most credible characteristics used in the assessment of benefits and risks of a therapeutic intervention in randomized clinical trials.

Biomarkers Definitions Working Group:

- Clin Pharmacol Ther 2001;69:89-95
- Also used in a 2011 IOM Report "Committee on Qualification of Biomarkers and Surrogate Endpoints in Chronic Disease"

Types of Endpoint Measures

- Clinician-reported outcomes
- Patient-reported outcomes (PRO)
- Biomarkers

Clinician-Reported Endpoint Measures

- Assessment of the patient's health condition based on direct clinician observations and interpretation
- Advantages as efficacy endpoints
  - Standardized
  - Reproducible and consistent
  - Well-defined and reliable
- Example: reduction in lesion size by at least 20% within 2-3 days for acute bacterial skin infections

Patient-Reported Endpoint Measures: PRO

- Any report of the status of the patient's health condition coming directly from the patient, without interpretation by clinicians, about how the patient functions or feels in relation to a health condition and its treatment
- Example: PRO used in inhaled antibacterial drug trials in cystic fibrosis

Biomarker Endpoint Measures

- Biomarker: *A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to an intervention.*
  - Biomarkers Definitions Working Group 2001 & IOM Report 2011
  - Usually used as a surrogate endpoint
  - Rarely used as a primary efficacy endpoint measurement

Biomarker Endpoint Measures

A surrogate endpoint, or "marker", is a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives and that is expected to predict the effect of therapy.

- Federal Register/Vol. 57, No.73/April 15, 1992
- Accelerated Approval 21 CFR 314.500 (subpart H): *reasonably likely to predict clinical benefit*

Biomarker Endpoint Measures

Examples of biomarker endpoints

- HIV viral load
- TB culture conversion to no growth
- Serologic tests for antibody to *T. cruzi*

Outline of the Presentation

- Adequate and Well-Controlled Trials
- Endpoints
- Regulatory Approvals

Regulatory Approvals

- Standard approval
  - Adequate and well-controlled trials show that a drug is safe and effective on the basis of clinically meaningful endpoint
  - Examples:
    - Drugs for treatment of skin infection (ABSSSI) approved on the basis of reduction in lesion size
    - Drugs for treatment of HIV/AIDS approved on the basis of reduction in HIV viral load (a biomarker ***validated*** as a primary efficacy endpoint)

Regulatory Approvals

- Accelerated approval
  - Adequate and well-controlled trials show that a drug is safe and effective on the basis of a surrogate marker
    - Surrogate is reasonably likely to predict benefit
  - Additional trials confirm the clinical benefit
  - Example:
    - Drugs for treatment of tuberculosis approved on the basis of the surrogate of TB culture to no growth

Summary

- Adequate and well-controlled trials
  - Substantial evidence of efficacy and safety
  - Several types of trial designs
- Endpoints
  - A measure of patient feels, functions, survives: patient-reported or clinician-reported
  - Biomarker is usually a surrogate marker reasonably likely to predict clinical benefit
- Regulatory approvals
  - Standard approval; accelerated approval

RECENT, ONGOING, AND PLANNED CLINICAL TRIALS FOR CHAGAS DISEASE

ISABELA RIBEIRO, MD

DNDi
Drugs for Neglected Diseases *initiative*

April 28, 2015

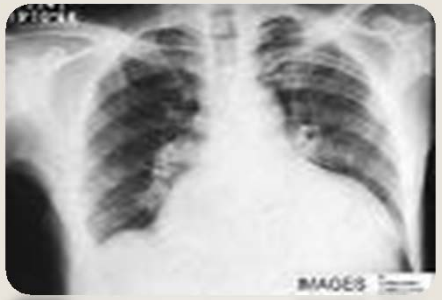
US FDA - Chagas Disease Public Meeting on Patient-Focused Drug Development

Chagas Disease - an unmet medical need

- Most common parasitic disease in the Americas
- Leading cause of infectious myocarditis worldwide
- Two drugs available: nifurtimox and benznidazole
  - Developed and registered in 1960-1970's
- < 1% of those infected receive treatment
  - Safety and tolerability issues
  - Long treatment period (1-2 months)







Evaluation and Treatment of Chagas Disease in the United States

A Systematic Review

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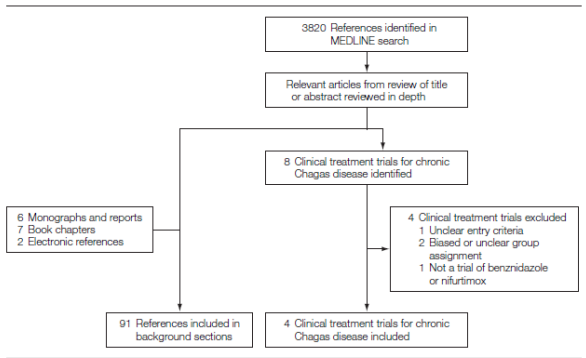


Table 1. Prospective Controlled Trials of Benznidazole or Nifurtimox for Chronic Chagas Disease in the Published Literature

Source	Chagas Form	Study Design	Age, y	Length of Treatment, d	Comparison Groups	Sample Size, No.	Primary Outcome of Interest, %	Major Adverse Events or Adverse Effects >5%
de Andrade et al,[87] 1996[a]	Indeterminate (n = 120) Early Chagas heart disease (n = 9)[b]	Randomized, double-blinded	7-12	60			Negative seroconversion at 36 mo by AT-ELISA	Maculopapular rash and pruritus
					Benznidazole, 7.5 mg/kg per d	64	58	12.5
					Placebo	65	5	3.1
Sosa Estani et al,[88] 1998	Indeterminate	Randomized, double-blinded	6-12	60			Negative seroconversion at 48 mo by F29-ELISA	Intestinal colic
					Benznidazole, 5 mg/kg per d	55	62	NR
					Placebo	51	0	NR
							Xenodiagnosis-positive at 48 mo	
					Benznidazole, 5 mg/kg per d	55	5	NR
					Placebo	51	51	NR
Coura et al,[89] 1997[c]	Indeterminate with ≥2 of 3 pretreatment xeno-diagnoses positive[d]	Randomized but apparently not double-blinded	Adults[d]	30	Benznidazole, 5 mg/kg per d	26	Posttreatment xeno-diagnosis positive 1.8	NR
					Nifurtimox, 5 mg/kg per d	27	9.6	NR
					Placebo	24	34.3	NR
Viotti et al,[90] 2006[d]	Indeterminate and nonsevere determinate	Alternate assignment to benznidazole or no treatment; nonrandomized, unblinded	Mean, 39.4	30			Progression	Severe allergic dermatitis prompting discontinuation
					Benznidazole, 5 mg/kg per d	283	4.2	13.0
					No treatment	283	14.1	NR
					Benznidazole, 5 mg/kg per d	283	Mortality 1.1	NR
					No treatment	283	4.2	NR

Abbreviations: AT-ELISA, Antigen trypomastigote chemoluminescent enzyme-linked immunosorbent assay; CI, confidence interval; ECG, electrocardiogram; HR, hazard ratio (mortality adjusted for ejection fraction); F29-ELISA, flagellar calcium binding protein F29-antigen–based enzyme-linked immunosorbent assay; IFA, indirect immunofluorescence assay; IHA, indirect hemagglutination; NR, not reported.

[a] Efficacy, 55.8% (95% confidence interval, 40.8%-67.0%) by intention-to-treat analysis based on AT-ELISA results.

[b] All children were asymptomatic but 9 had right bundle-branch block on ECG; no difference in distribution in treatment vs placebo groups.

[c] Neither age nor clinical findings reported in article; presumed to have the indeterminate form.

[d] Chagas cardiac disease Kuschnir grades I or II; those with grade III, defined by presence of heart failure, were excluded. Distribution at study entry: 63.6% Kuschnir 0, 26.1% grade I, 10.2% grade II. See Box for definition of Kuschnir grades. Median follow-up, 9.8 years.

Journal of Antimicrobial Chemotherapy (2009) **64**, 1139–1147
doi:10.1093/jac/dkp357
Advance Access publication 9 October 2009

JAC

Use of benznidazole to treat chronic Chagas' disease: a systematic review with a meta-analysis

José A. Pérez-Molina[1]*, Ana Pérez-Ayala[1], Santiago Moreno[2], M. Carmen Fernández-González[2], Javier Zamora[3] and Rogelio López-Velez[1]

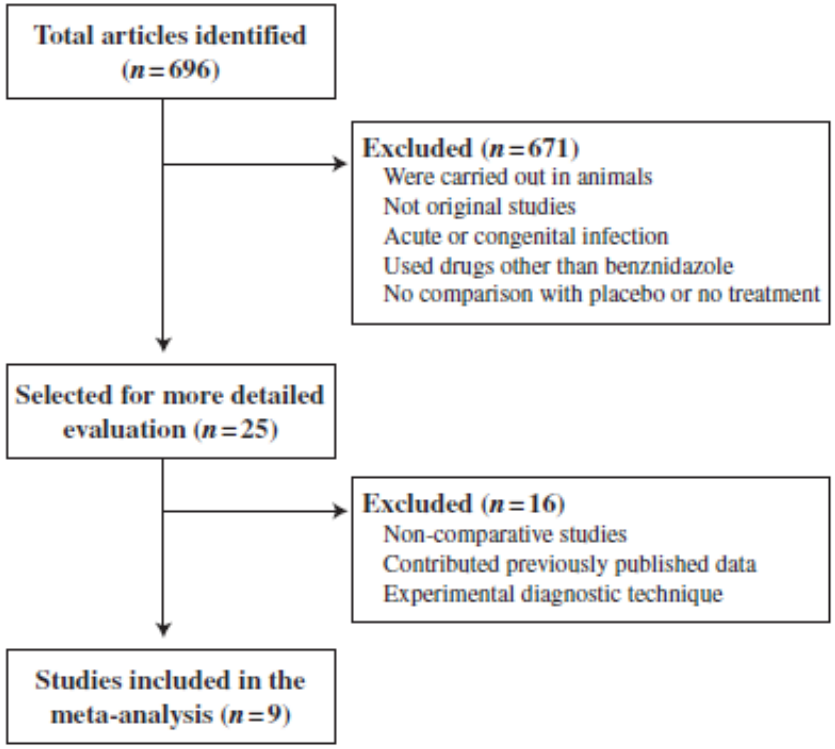
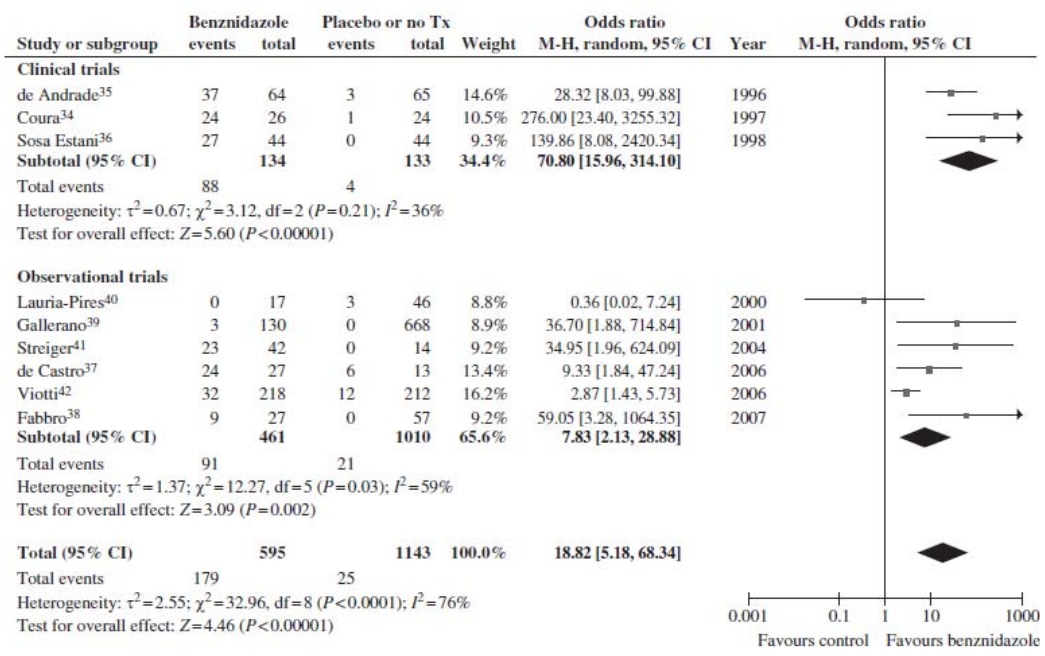


Figure 1. Flow diagram for selected studies.



Randomised trial of efficacy of benznidazole in treatment of early *Trypanosoma cruzi* infection

Ana Lucia S Sgambatti de Andrade, Fabio Zicker, Renato Mauricio de Oliveira, Simonne Almeida e Silva, Alejandro Luquetti, Luiz R Travassos, Igor C Almeida, Soraya S de Andrade, João Guimarães de Andrade, Celina M T Martelli

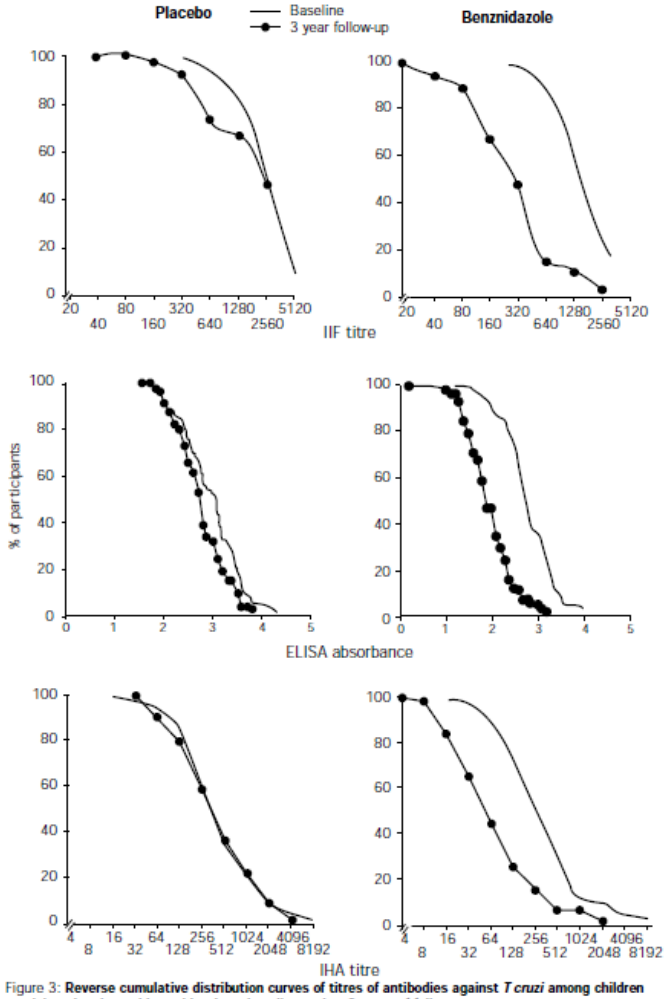


Figure 3: **Reverse cumulative distribution curves of titres of antibodies against *T cruzi* among children receiving placebo and benznidazole at baseline and at 3 years of follow-up.**
n=58 for benznidazole, 54 for placebo group. IIF= indirect immunofluorescence; IHA=indirect haemagglutination.

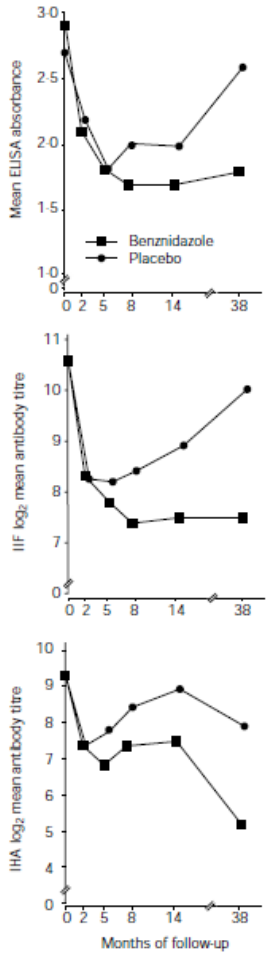


Figure 4: ***T cruzi* serological response in benznidazole and placebo groups by time**
Error bars indicate 95% CI. IIF=indirect immunofluorescence; IHA=indirect haemagglutination.

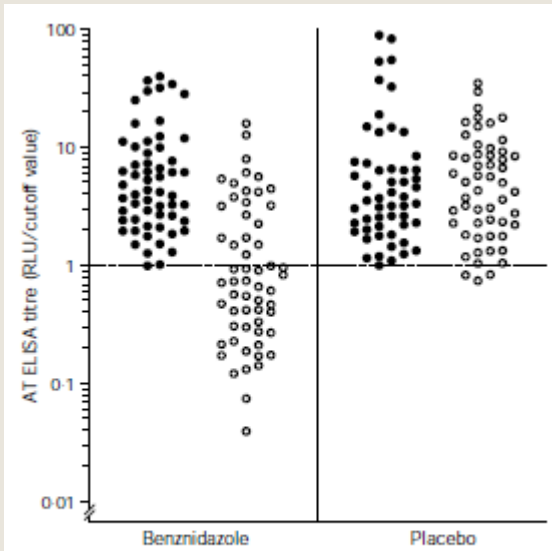


Figure 2: **AT ELISA results at trial entry (●) and at end of follow-up (○) for 58 benznidazole-treated and 54 placebo-treated children who completed trial treatment**
Broken horizontal line=cut-off; values below this indicate seronegativity.

Am. J. Trop. Med. Hyg., 59(4), 1998, pp. 526–529

EFFICACY OF CHEMOTHERAPY WITH BENZNIDAZOLE IN CHILDREN IN THE INDETERMINATE PHASE OF CHAGAS' DISEASE

SERGIO SOSA ESTANI, ELSA LEONOR SEGURA, ANDRES MARIANO RUIZ, ELSA VELAZQUEZ, BETINA MABEL PORCEL, AND CRISTINA YAMPOTIS

Centro Nacional de Diagnóstico e Investigación de Endemo-Epidemias/Administración Nacional de Laboratorios e Institutos de Salud (ANLIS) Dr. Carlos G. Malbrán, Buenos Aires, Argentina; Instituto Nacional de Parasitología Dr. Mario Fatala Chaben/ ANLIS, Secretaría de Salud, Ministerio de Salud y Acción Social de la Nación, Buenos Aires, Argentina; Hospital San Roque, Ministerio de Salud de la Provincia, Embarción Salta, Argentina

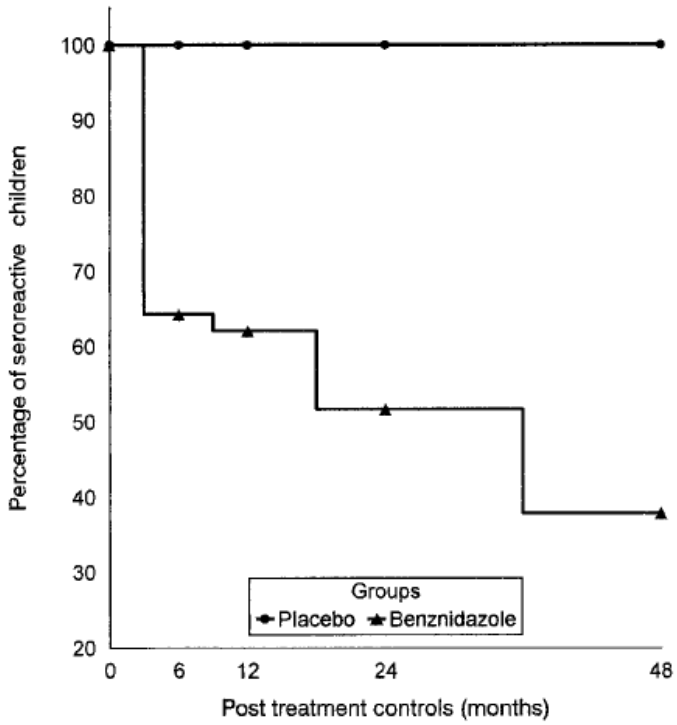


FIGURE 1. Decrease in the percentage of children with reactive serology against *Trypanosoma cruzi* (indeterminate phase of Chagas' disease) by enzyme immunoassay using the F29 protein after treatment with benznidazole or placebo in Salta, Argentina, 1991–1995.

Serologic follow-up of children treated with benznidazole or placebo to 48 months post-treatment in Salta, Argentina, 1991–1995*

		IHA				IFA				EIA			
Treatment	n	Mean	SD	Test		Mean	SD	Test		Mean	SD	Test	
Benznidazole													
Pretreatment	51	7.98	1.82	7 DF	1 DF	7.05	1.12	7 DF	1 DF	0.467	0.099	7 DF	1 DF
End of treatment	47	7.68	2.14		NS	6.57	1.58		NS	0.433	0.110		NS
3 months	45	7.26	2.33		NS	6.27	1.28		$P<0.01$	0.409	0.112		$P<0.01$
6 months	45	7.00	2.53		$P<0.05$	6.11	1.57		$P<0.001$	0.371	0.115		$P<0.001$
12 months	48	7.00	2.27		$P<0.05$	5.87	1.56		$P<0.001$	0.369	0.107		$P<0.001$
18 months	47	6.53	2.62		$P<0.001$	5.80	1.82		$P<0.001$	0.358	0.120		$P<0.001$
24 months	46	6.80	2.26		$P<0.01$	5.32	2.03		$P<0.001$	0.330	0.098		$P<0.001$
48 months	44	5.93	2.11	$P<0.001$	$P<0.001$	5.65	2.18	$P<0.001$	$P<0.001$	0.343	0.094	$P<0.001$	$P<0.001$
Placebo													
Pretreatment	50	8.00	1.16	7 DF	1 DF	6.80	1.22	7 DF	1 DF	0.472	0.095	7 DF	1 DF
End of treatment	45	8.11	1.21		NS	6.80	1.07		NS	0.492	0.090		NS
3 months	44	8.11	1.10		NS	6.54	1.15		NS	0.489	0.098		NS
6 months	39	7.87	1.34		NS	6.61	1.60		NS	0.477	0.101		NS
12 months	47	8.08	1.26		NS	6.40	1.13		NS	0.476	0.113		NS
18 months	48	7.93	1.17		NS	6.47	1.16		NS	0.464	0.108		NS
24 months	49	7.77	1.22		NS	6.34	1.54		NS	0.479	0.104		NS
48 months	44	7.47	0.95	NS	$P<0.05$	6.97	2.21	$P<0.05$	$P<0.05$	0.501	0.115	NS	NS

* IHA = indirect hemagglutination assay; IFA = indirect immunofluorescence assay; EIA = enzyme immunoassay; Test = analysis of variance or Kruskal-Wallis test; df = degrees of freedom; NS = not significant ($P > 0.05$). The IFA and IHA values are means ($\log_2$ of two-fold dilutions of serum samples). The EIA values are mean optical densities.

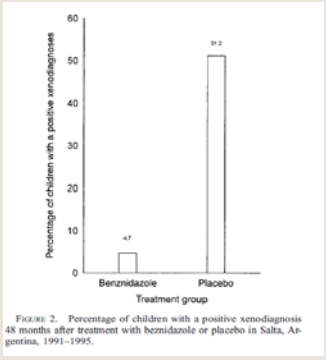


FIGURE 2. Percentage of children with a positive xenodiagnosis 48 months after treatment with beznidazole or placebo in Salta, Argentina, 1991–1995.

Chagas Disease Clinical Trials - 2008

- Two randomised clinical trial of BZN in adults
  - TRAENA (started in 03/1999 – 12/2012)
  - BENEFIT (11/2004 – ongoing)
- Decades with no new clinical trials for new treatment options in Chagas disease
- R&D and access stalled by existing knowledge gaps

  - Relevance of animal models
  - Limited data on:
    - the importance of different parasite strains to human disease
    - Coexistence of infection
    - Mechanisms of resistance
  - PK/PD in Chagas largely unknown
  - No consensus on reference treatment
  - Lack of early test of cure
  - Limited sensitivity of PCR test

Focused approach
Balancing Gaps and the Urgent Medical Need

- Clear need of new treatment options for patients with chronic Chagas disease (adults and older children)
- Decision to proceed to clinical development and generation of scientific information → fill existing gaps and inform future drug development
- PCR: selected as the primary endpoint for clinical trials after extensive expert consultation
  - Standardised methodology with multi-centre evaluation
  - Serial and sequential PCR examination
  - Rationale for selection: plausible biological rationale (link parasite persistence and chronic heart inflammation), animal models, human data from acute Chagas disease (children, reactivation), observational studies in humans
- Early regulatory consultation and agreement on endpoints, trial design and development strategy
- Generation of PK/PD data in humans – using different biomarkers and parasite genotyping– for new candidates and benznidazole

Clinical Trials - Chronic Chagas Disease
A lot of progress over recent years

- **Benznidazole in children**
  - **Pop PK study in children 0-12 years – results ASTMH and ESPID 2013/2014**
  - **Pop PK in children 2-12 years – publication 2014**

Azoles for Chagas Disease

- **Posaconazole and Benznidazole in adults**
  - **CHAGASAZOL - Hospital Val Hebron – Barcelona - publication 2014**
  - **STOP-CHAGAS – Merck-sponsored, multi-country clinical trial - ongoing**

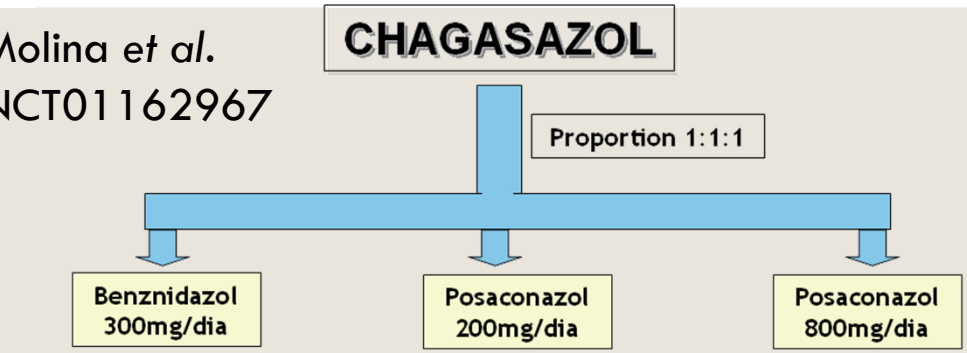
- **E1224 and Benznidazole in adults**
  - **Phase 2, PoC E1224 - Bolivia - results ASTMH 2013**

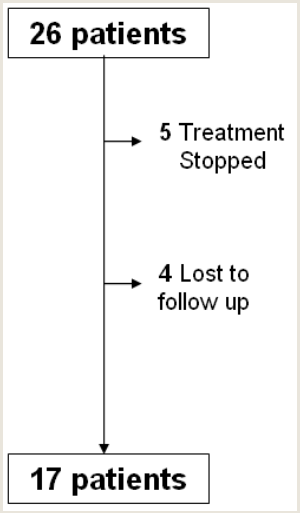
Fexinidazole for Chagas disease

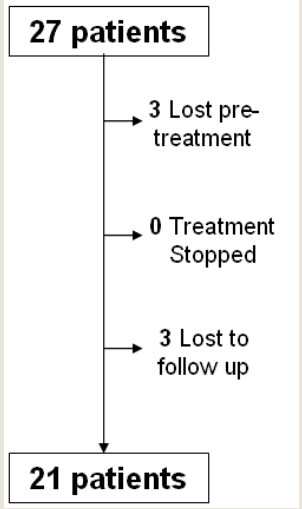
- **Phase 2, PoC FEXI in adults – Bolivia - ongoing**

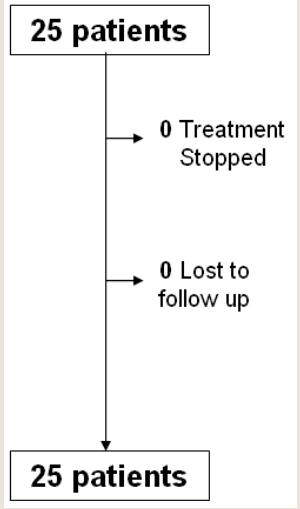
Azole Class Clinical Trial Results - ICTMM

Molina *et al.*
NCT01162967







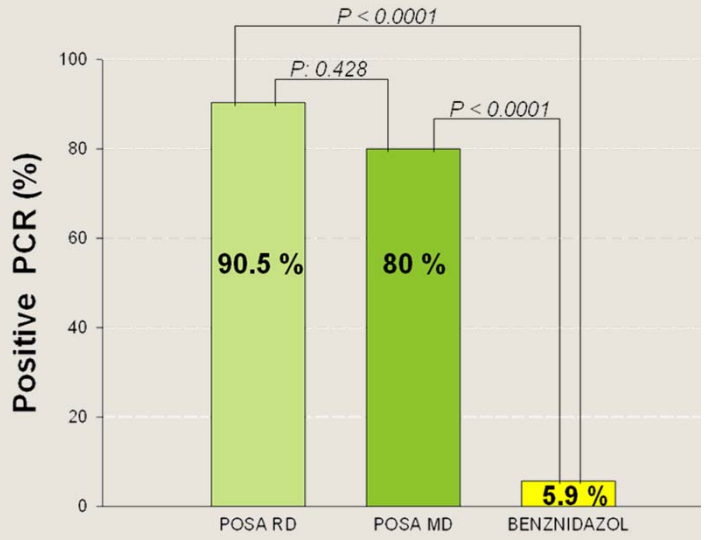


PCR **TREATMENT:** D0 D7 D14 D28 D45 D60

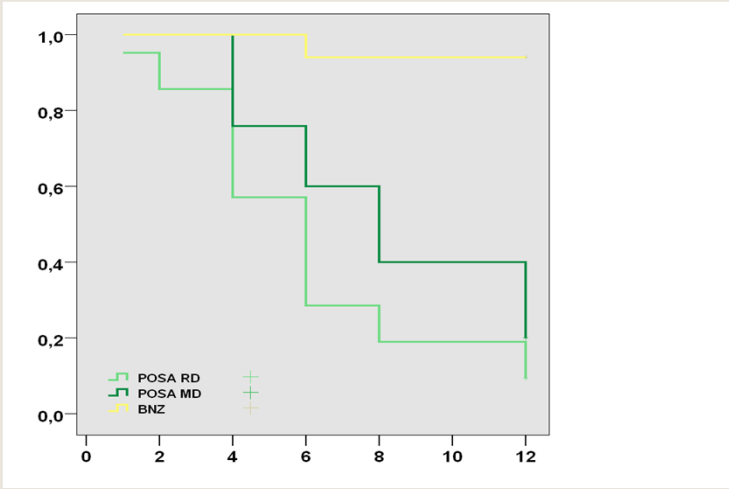
FOLLOW UP: M4 M6 M8 M12

TWICE / 10ML

<40: Positive

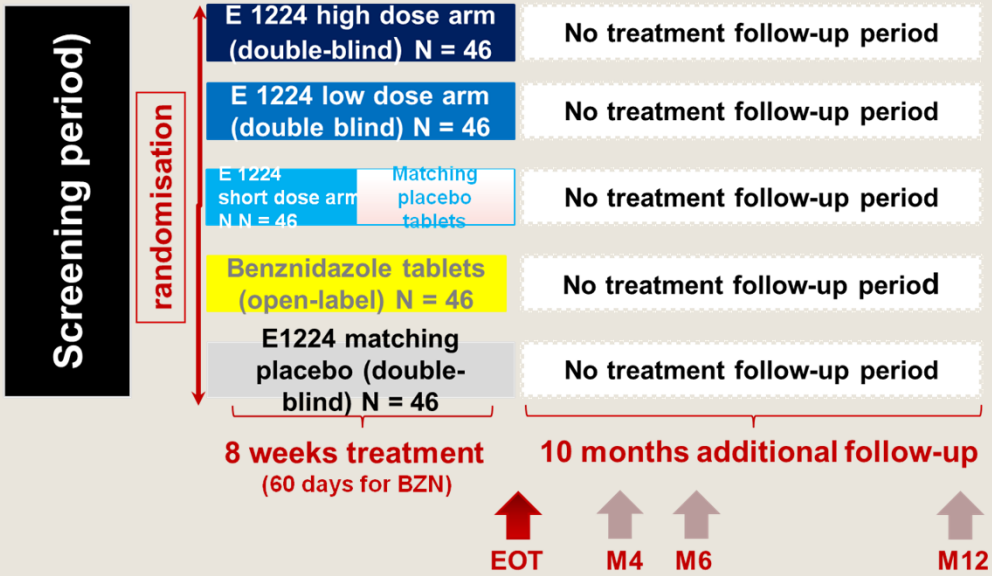


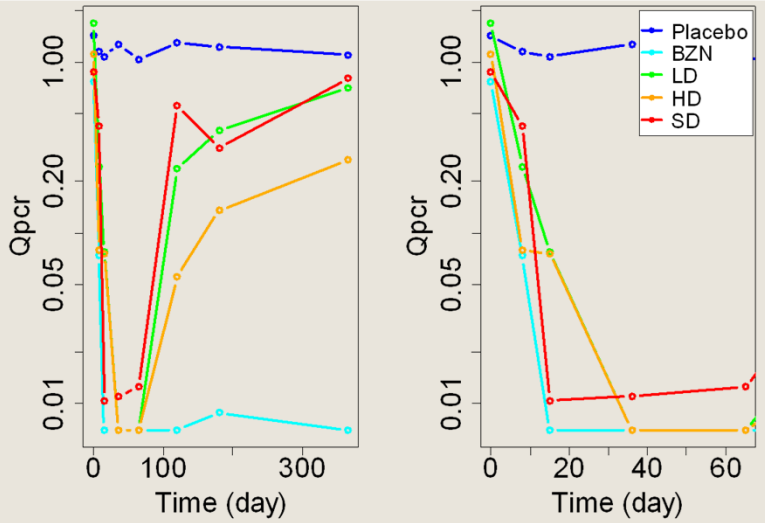
Cumulative probability of failure



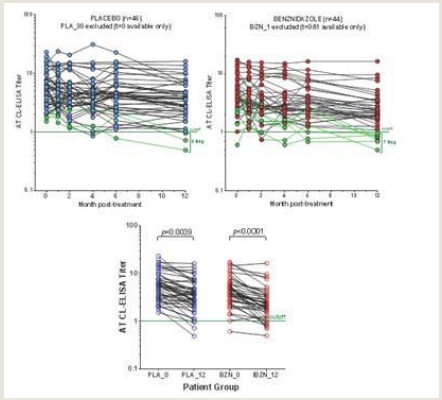
E1224 - Phase II PoC Study

DNDi-CH-E1224-001
NCT01489228





Efficacy based on repeated PCR and candidate biomarkers, parallel evaluation of serology



Day 65 (EOT)

			Placebo (N=47)	LD (N=48)	SD (N=46)	HD (N=45)	BZN (N=45)	All (N=231)
Parasite clearance at D65		N	47	48	46	45	45	231
		Missing	0	0	0	0	0	0
	No	n (%)	35 (74.5)	5 (10.4)	5 (10.9)	11 (24.4)	4 (8.9)	60 (26.0)
	Yes	n (%)	12 (25.5)	43 (89.6)	41 (89.1)	34 (75.6)	41 (91.1)	171 (74.0)

12 Month Follow-up

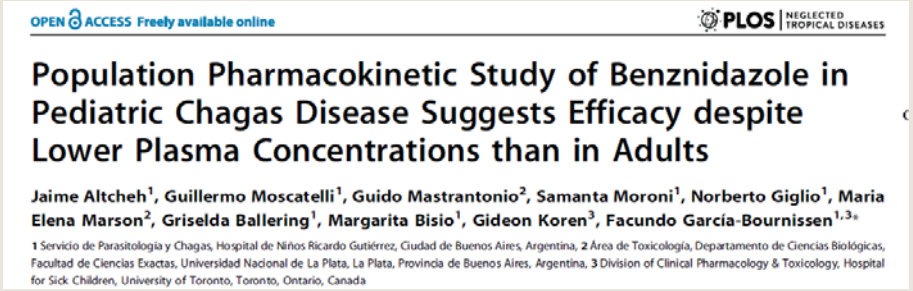
			(N=47)	(N=48)	(N=46)	(N=45)	(N=45)	(N=231)
Sustained clearance At 12 months	No	n (%)	43 (91.5)	44 (91.7)	41 (89.1)	32 (71.1)	8 (19.0)	168 (72.7)
	Yes	n (%)	4 (8.5)	4 (8.3)	5 (10.9)	13 (28.9)	37 (81.0)	63 (27.3)

ISGlobal Instituto de Salud Global Barcelona
CEADES
cooperación española

DNDi Drugs for Neglected Diseases *initiative*

Population Pharmacokinetics of Benznidazole in Children With Chagas Disease

- **2 open-label, single-arm, prospective Pop PK studies**
  - **NCT01549236 40 Children 2 – 12 years old 40**
    Age: 7.3 years (range 2.1 – 12)
  - **NCT00699387** 81 **Children 1d – 12 years old**
    Age: >2a : 40; < 2a: 41 (8 newborn)
- **Samples for PK were obtained at randomly pre-assigned times**
- **Benznidazole in plasma was measured by HPLC, HPLC-MS-MS**
- **PopPK modeling was performed with NONMEM software (non linear mixed effects analysis)**



OPEN ACCESS Freely available online — PLOS | NEGLECTED TROPICAL DISEASES

Population Pharmacokinetic Study of Benznidazole in Pediatric Chagas Disease Suggests Efficacy despite Lower Plasma Concentrations than in Adults

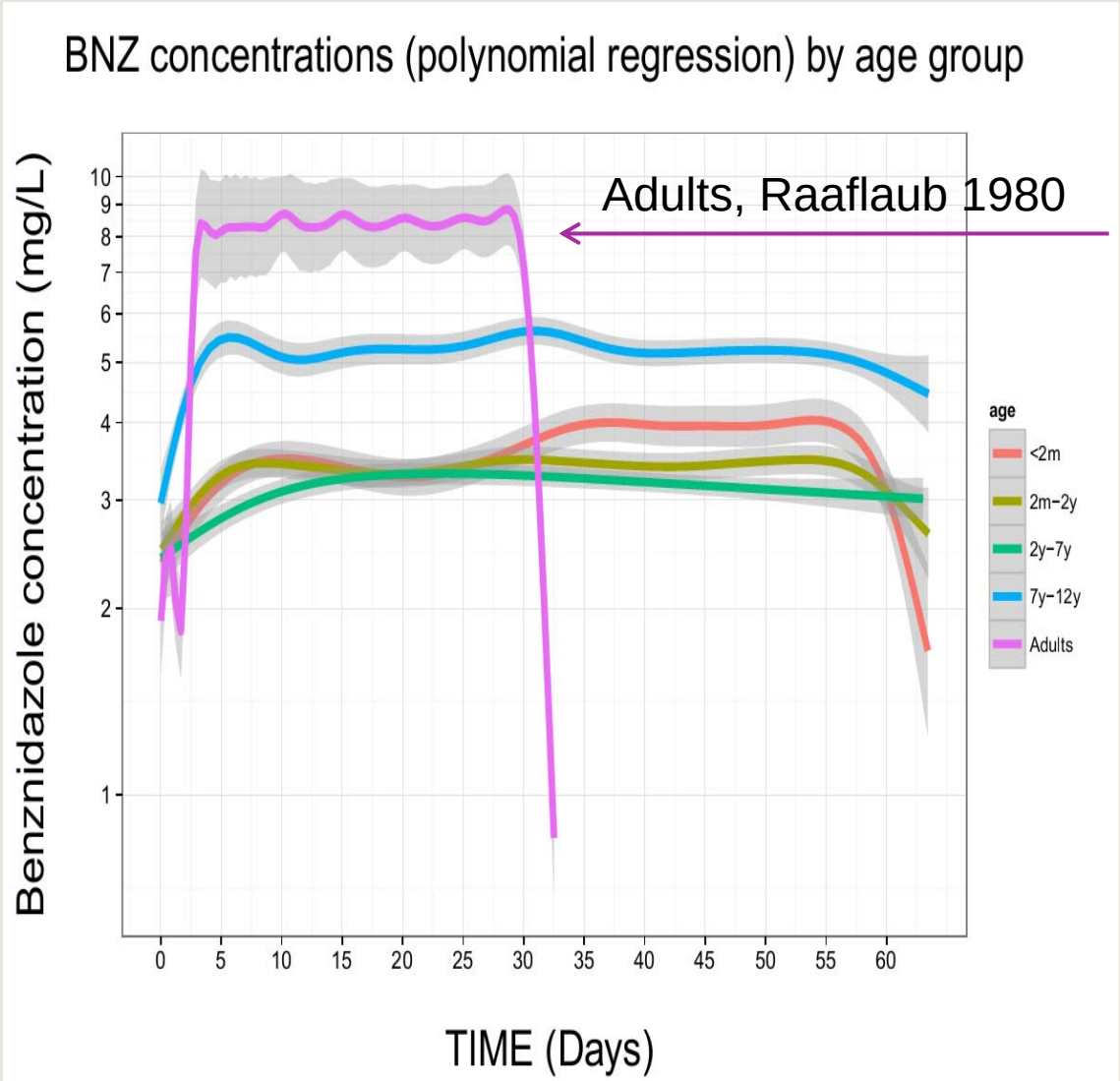
Jaime Altcheh[1], Guillermo Moscatelli[1], Guido Mastrantonio[2], Samanta Moroni[1], Norberto Giglio[1], Maria Elena Marson[2], Griselda Ballering[1], Margarita Bisio[1], Gideon Koren[3], Facundo García-Bournissen[1,3]*

1 Servicio de Parasitología y Chagas, Hospital de Niños Ricardo Gutiérrez, Ciudad de Buenos Aires, Argentina, 2 Área de Toxicología, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Provincia de Buenos Aires, Argentina, 3 Division of Clinical Pharmacology & Toxicology, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada

Pediatric network
PEDCHAGAS

DNDi
Drugs for Neglected Diseases *initiative*

Population Pharmacokinetics of Benznidazole in Children With Chagas Disease

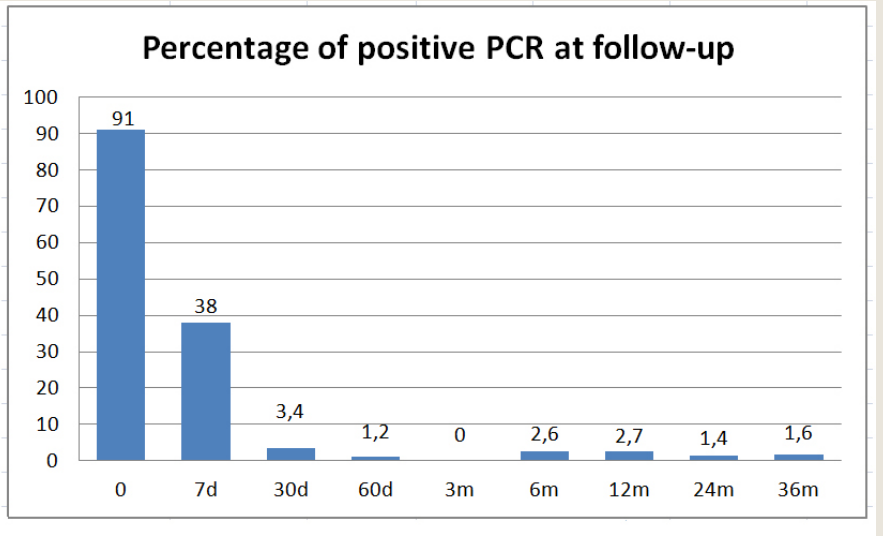


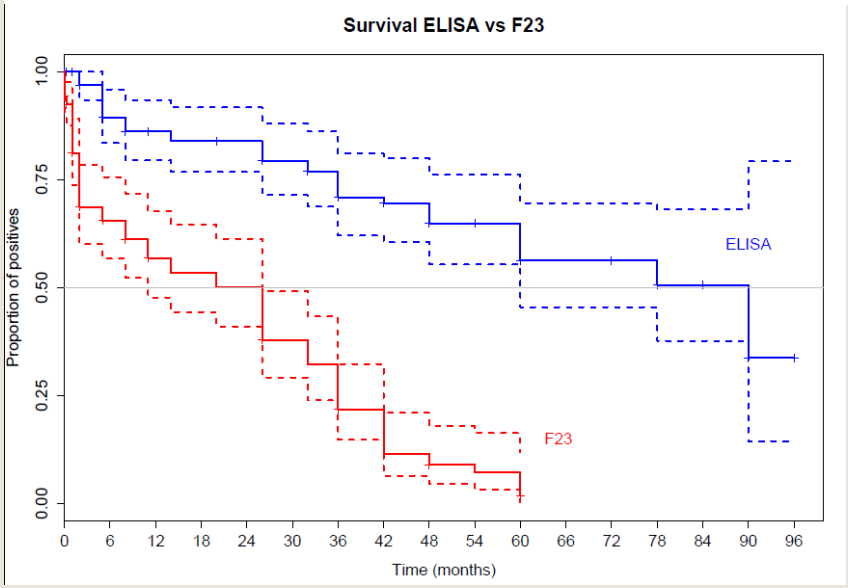
- 100% PCR negative at EOT
- Have we been overdosing adults?...

Pediatric network
PEDCHAGAS

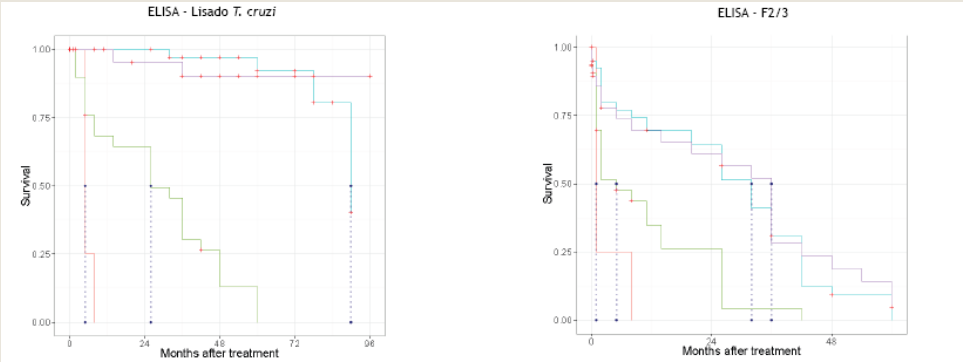
Paediatric cohorts

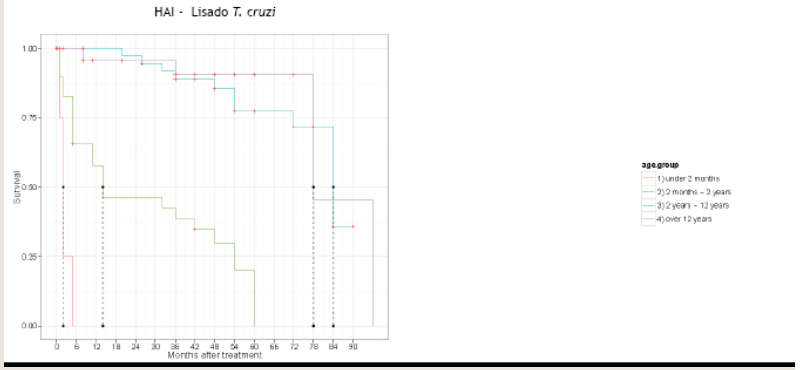
tiempo	n	+	%	IC
0	100	91	91	83,8- 95,2
7d	92	35	38	28,8-48,3
30d	89	3	3,4	1,2-9,4
60d	85	1	1,2	0,2-6,4
3m	80	0	0	0-4,6
6m	76	2	2,6	0,7-9,1
12m	75	2	2,7	0,7-9,2
24m	69	1	1,4	0,3-7,8
36m	64	1	1,6	0,3-8,3

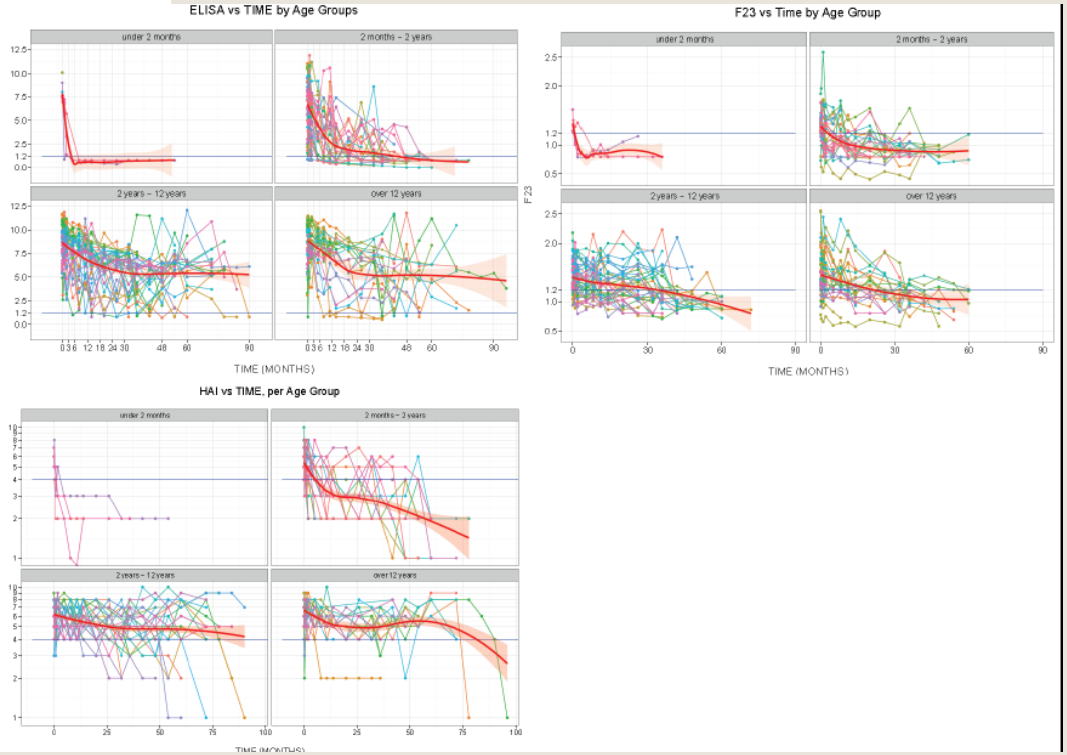




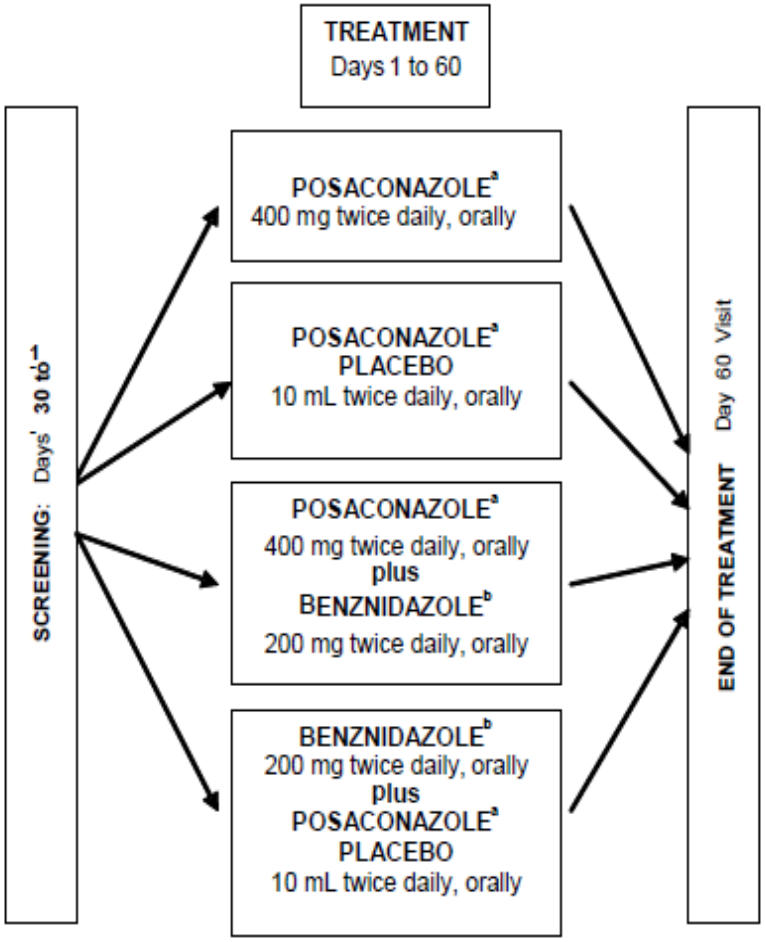
Paediatric cohorts

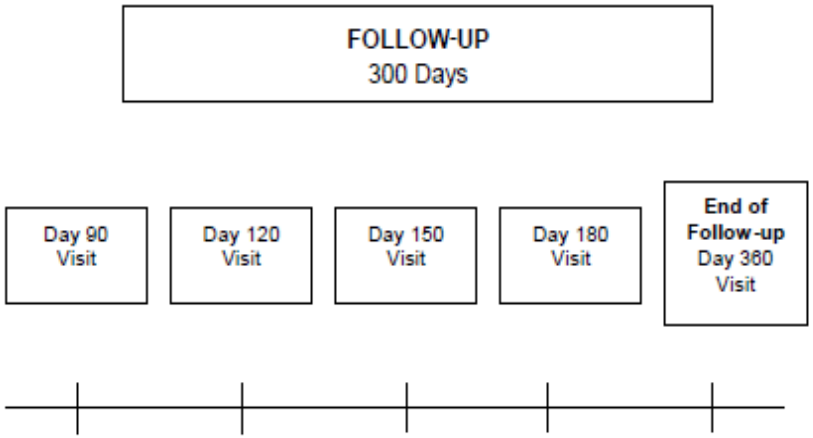






STOP-CHAGAS - A Study of the Use of Oral Posaconazole (POS) in the Treatment of Asymptomatic Chronic Chagas Disease - NCT01377480





- Multi-centre, multi-country, randomised, double-blinded placebo-controlled study
- Status:
  - 120 patients randomised
  - Enrollment and follow-up concluded
  - Top level report: Q2 2015

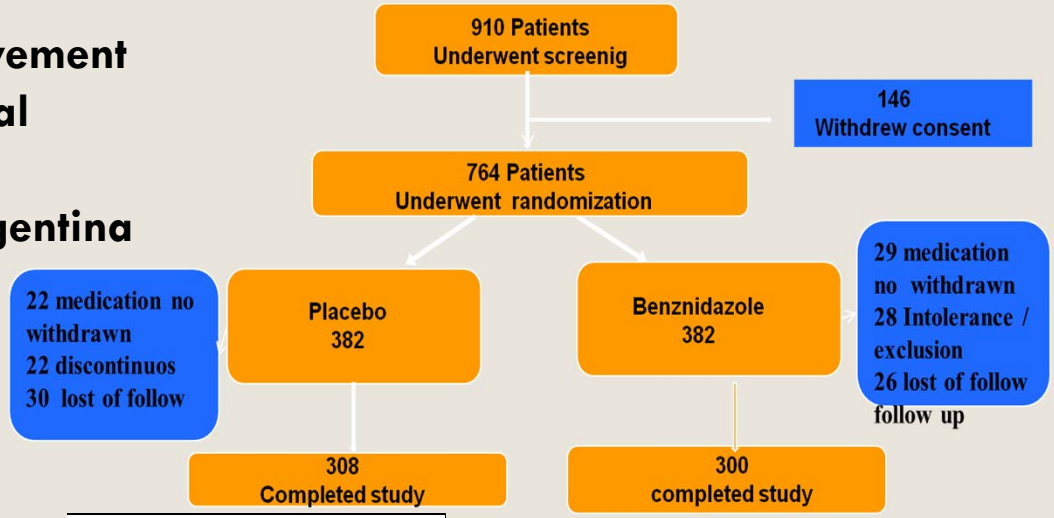
PI : Morillo CA

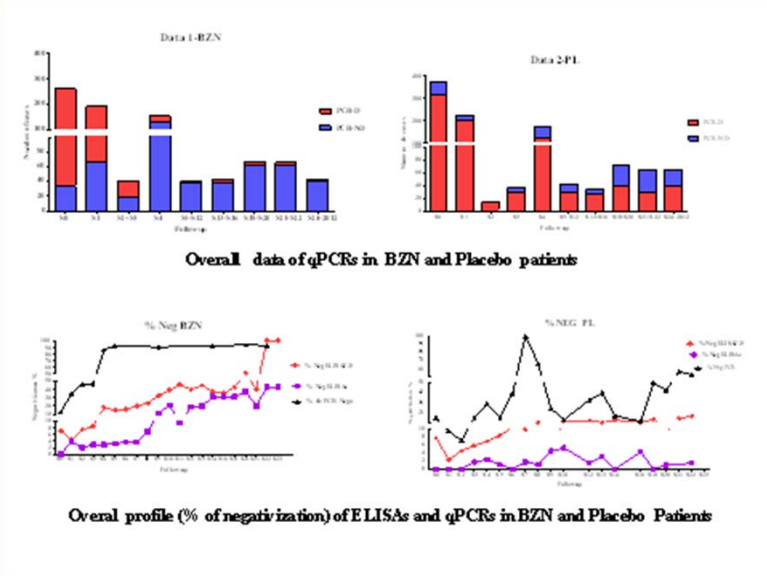
Steering Committee: S Sosa-Estani, A Avezum, S Yusuf, S Chrolavicious.

DNDi Drugs for Neglected Diseases *initiative*

TRAENA - Treatment with benznidazole in adult chronic Chagas disease patients

- Adults with chronic Chagas disease – indeterminate and with cardiac involvement
- Randomized, double-blind, clinical trial
- PI: Dr Adelina Riarte
- INP Fatala-Chaben, Buenos Aires, Argentina





Sustained PCR response 12 months			
	NO	YES	Total
PLB	112 33.84 68.71 82.96	51 15.41 31.29 26.02	163 49.24
BZN	23 6.95 13.69 17.04	145 43.81 86.31 73.98	168 50.76
Total	135 40.79	196 59.21	331 100.00
Frequency Missing = 61			

INSTITUTO NACIONAL DE PARASITOLOGIA DR. MARIO FATALA CHABEN

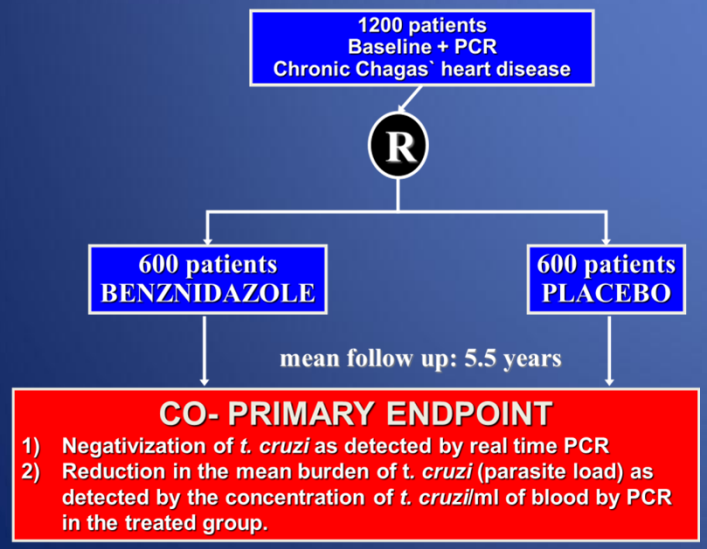
CONICET

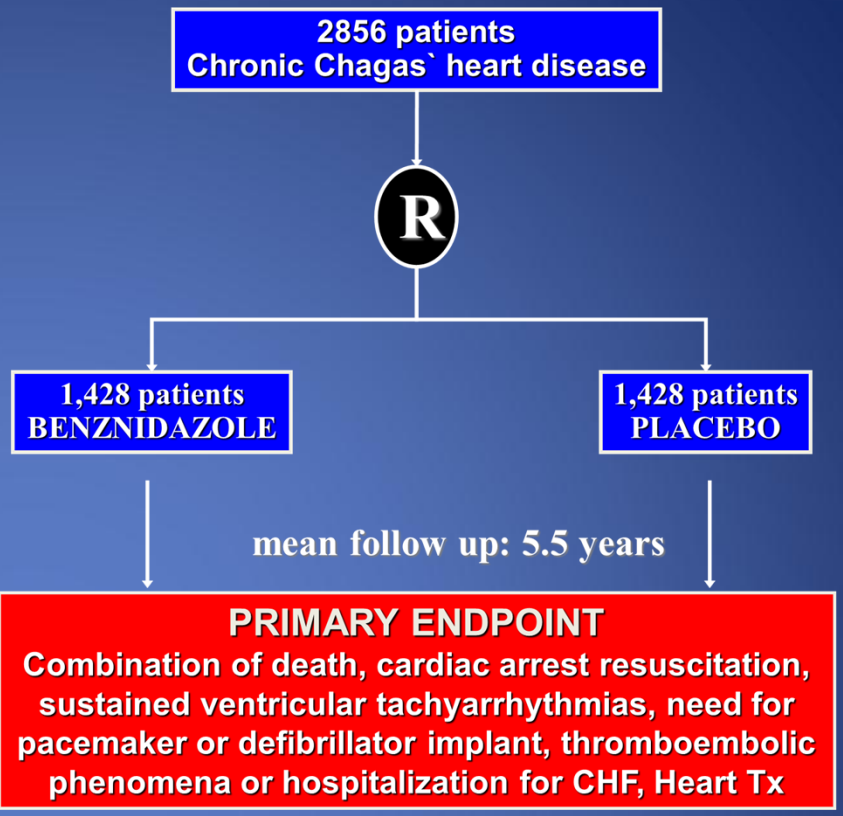
DNDi Drugs for Neglected Diseases *initiative*

BENznidazole **E**valuation **F**or **I**nterrupting **T**rypanosomiasis

BENEFIT

- **Randomized, double-blind, clinical trial**
- **Adults with chronic Chagas disease with cardiac**
- **PIs: Dr Carlos Morillo, Dr. Marin Neto**





Last Follow-up Visits – April 2015 – 1.5% LTFU

	# of Sites	Total Patients Randomized
Argentina	19	559
Bolivia	1	357
Brazil	24	1360
Colombia	5	502
El Salvador	1	78
TOTAL	50	2856

Medical History

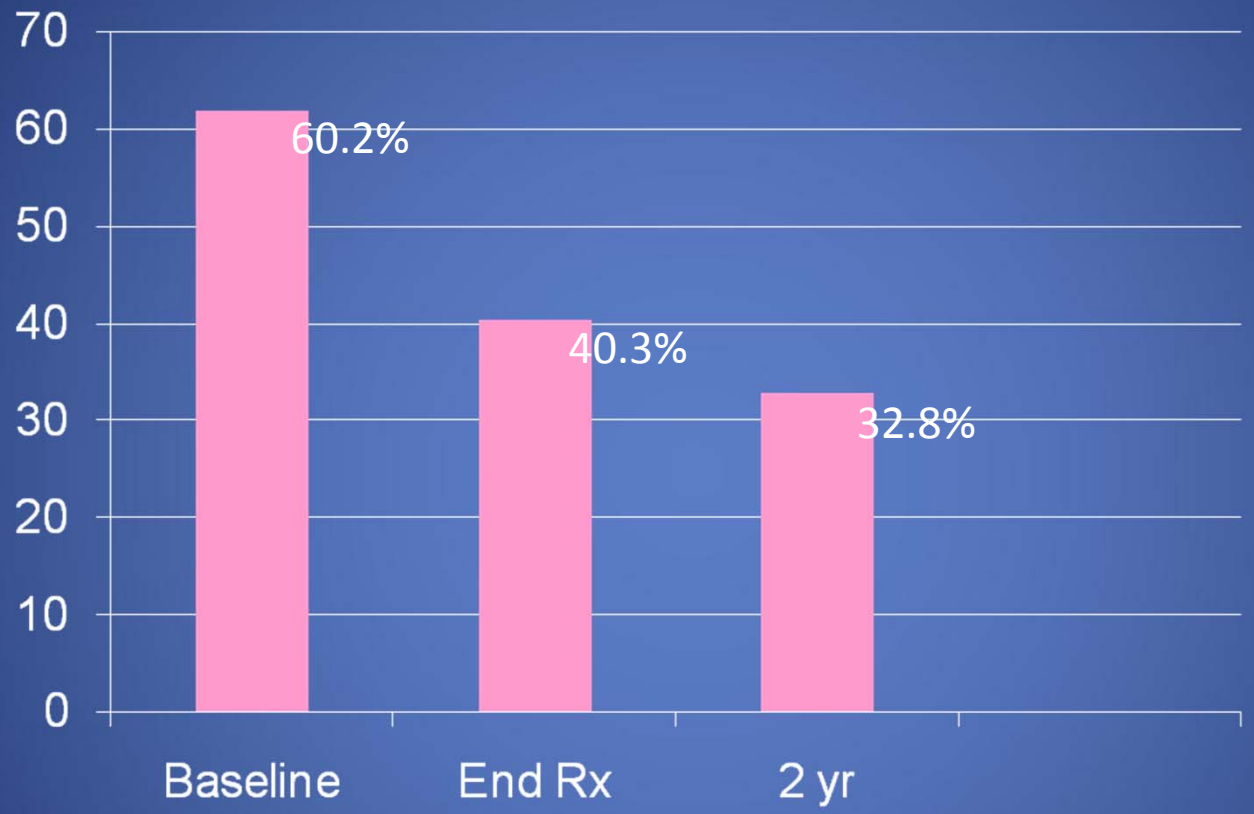
	Data Received on 2770 pts %
NYHA Class	
I	74.6
II	22.8
III	2.6
Previous Heart Failure	9.5
Resuscitated Cardiac Arrest	1.3
Sustained VT	2.8
Internal Cardiac Defibrillator	2.5
Atrial Fibrillation	7.0
Pacemaker	14.2
Stroke/TIA	4.4
Syncope	8.5

Study Drug Compliance

as of October 17, 2011

	Total Pts Randomized n	Study Drug Interrupted (at the end of treatment) %	Pts ≥ 75% compliance %		
			11 day	21 day	40-80
Argentina	559	16.6	91.6	89.1	87.7
Bolivia	357	2.5	98.8	99.0	97.2
Brazil	1360	12.3	91.6	90.6	88.8
Colombia	502	7.9	92.7	92.0	91.4
El Salvador	78	5.6	98.7	98.7	95.8
OVERALL	2856	11.2	92.8	91.7	90.2
Drug interrupted			6.3	8.2	11.2
Drug Restarted			3.3	4.3	3.0

BENEFIT PCR



COUNTRY	# Pts RANDOMIZED	BASELINEC OLLECTED	ANALYZED (%)	POSITIVE (%)	End of treatment COLLECTED	ANALYZED (%)	POSITIVE (%)	3rd Sample COLLECTED	ANALYZED (%)	POSITIVE (%)
OVERALL	2856	1932	1123 (58.1)	676(60.2)	1629	965 (59.2)	389 (40.3)	996	641 (64.4)	210 (32.8)

Fexinidazole
Proof-of-Concept Dose Ranging Study

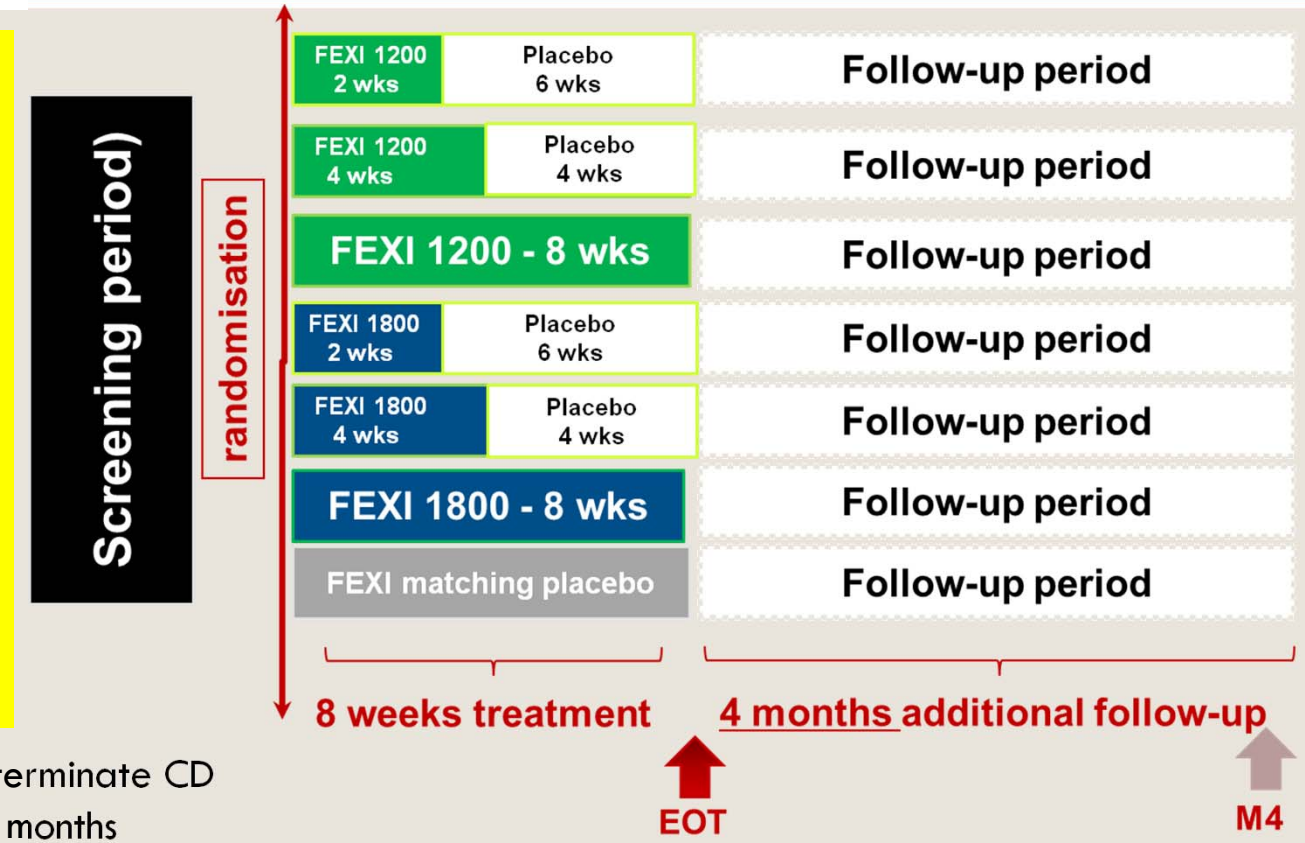
Study initiated:
July 2014

Study recruitment temporary interruption: Oct 17, 2014

Study recruitment interruption:
December 11, 2014

Target for Top Line Report (TLR):
August 2015

180 ICF signed
47 patients randomised
LVLP planned April 2015



- 140 adults with chronic indeterminate CD
- PCR sustained response at 6 months
- Stopping rules: futility and safety

Risk Management:

- Timelines for recruitment
- Safety monitoring

ISGlobal Instituto de Salud Global Barcelona
CEADES
cooperación española
DNDi Drugs for Neglected Diseases *initiative*

Improved Treatment Regimens of Benznidazole BZN New Regimen and BZN / E1224 Combination

Principal Investigators: Faustino Torrico, Joaquim Gascón, Rodolfo Viotti, Sergio Sosa Estani

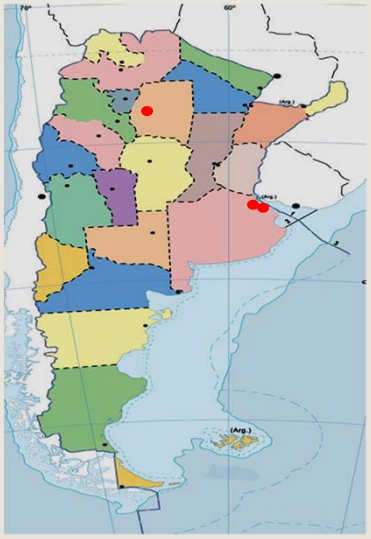
Sites: Bolivia and Argentina:

Plataforma de Atención Integral a los Pacientes con Enfermedad de Chagas
CEADES Bolivia/IS Global/CRESIB

Hospital Eva Peron, Buenos Aires
INP Fatala-Chaben, Buenos Aires
Centro de Chagas, Santiago del Estero

INGEBI/CONICET, Buenos Aires, Argentina

Study Initiation Date: 15/10/2015



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Future Clinical Trials
Chronic Chagas Disease

- **Benznidazole in children**
  - ELEA/Chemo –sponsored, Mundo Sano Foundation
  - Assessment of efficacy and safety of BZN in children
  - Historical placebo-control
  - Design under finalisation

- **New Benznidazole Treatment regimens in adults**
  - DNDi-sponsored, collaboration with Eisai, ELEA and Mundo Sano Foundation
  - Assessment of efficacy and safety of BZN as monotherapy and E1224 combination in adults 18-50 years

- **BERENICE project**

- **Nifurtimox in children**
  - Bayer –sponsored
  - Assessment of efficacy and safety of Nifurtimox in children
  - Historical placebo-control
  - Design under finalisation

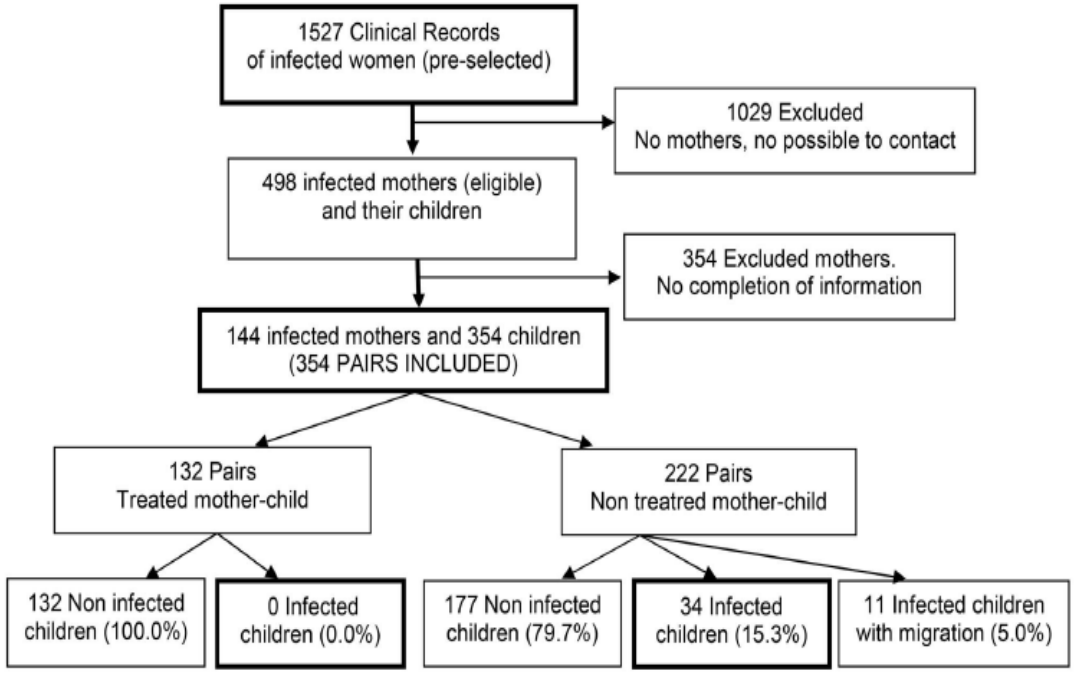
OPEN ACCESS Freely available online

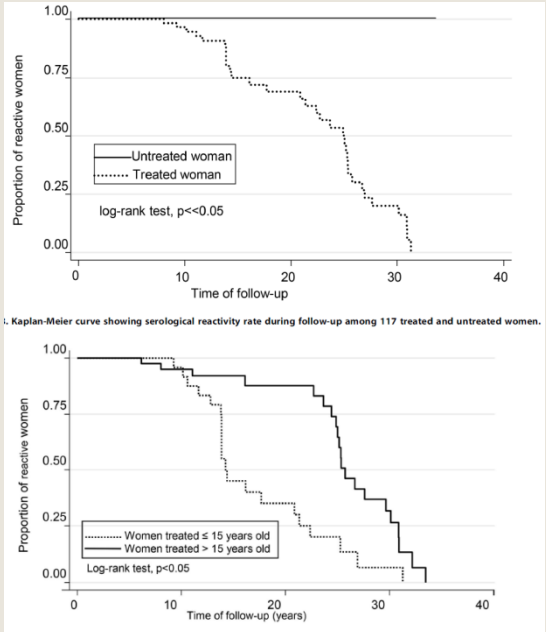
PLOS | NEGLECTED TROPICAL DISEASES

Trypanocide Treatment of Women Infected with *Trypanosoma cruzi* and Its Effect on Preventing Congenital Chagas

Diana L. Fabbro[1], Emmaria Danesi[2], Veronica Olivera[1], Maria Olenka Codebó[3], Susana Denner[1], Cecilia Heredia[2], Mirtha Streiger[1], Sergio Sosa-Estani[2,3]*

1 Centro de Investigaciones sobre Endemias Nacionales (CIEN) - Facultad de Bioquímica y Ciencias Biológicas- Universidad Nacional del Litoral, Santa Fe, Argentina, **2** Centro Nacional de Diagnóstico e Investigaciones Endemo-epidemicas, Administración Nacional de Laboratorios e Institutos de Salud (ANLIS), Buenos Aires, Argentina, **3** Instituto Nacional de Parasitología (INP), "Dr Mario Fatala Chaben", Administración Nacional de Laboratorios e Institutos de Salud (ANLIS) Malbrán, Buenos Aires, Argentina





. Kaplan-Meier curve showing serological reactivity rate during follow-up among 117 treated and untreated women.

Mem Inst Oswaldo Cruz, Rio de Janeiro: 1-3, 2015

Prevention of congenital Chagas through treatment of girls and women of childbearing age

Guillermo Moscatelli/+, Samanta Moroni, Facundo García-Bournissen, Griselda Ballering, Margarita Bisio, Héctor Freilij, Jaime Altcheh

Department of Parasitology and Chagas, Ricardo Gutiérrez Children's Hospital, Buenos Aires, Argentina

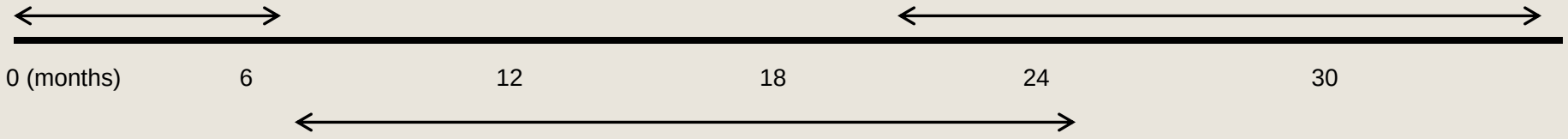
Non-Human Primate Study

Validity of PCR and Other Biomarkers for Assessing Parasitological Cure in Chagas Disease

- **Primary Aim:** To determine if multiple, sequential blood PCR assays for *T. cruzi* DNA post-treatment can consistently differentiate parasitological cure from treatment failure
- 64 cynomolgous macaques infected with T. cruzi in the field from natural sources
- **Biomarkers under-evaluation: multiplex real-time qPCR, multiplex serodiagnostic assay, lytic antibodies, hemocultures, whole transcriptome biomarker**

Confirm health and infection status; pre-treatment sample collection; acclimation and taste-testing; PK to determine dosing

Immunosuppression; determination of infection status; sample testing and data analysis



60 day course of treatment (staged); sample collection at 8 week intervals up to 52 weeks post-treatment (7 post-treatment blood and tissue samples)

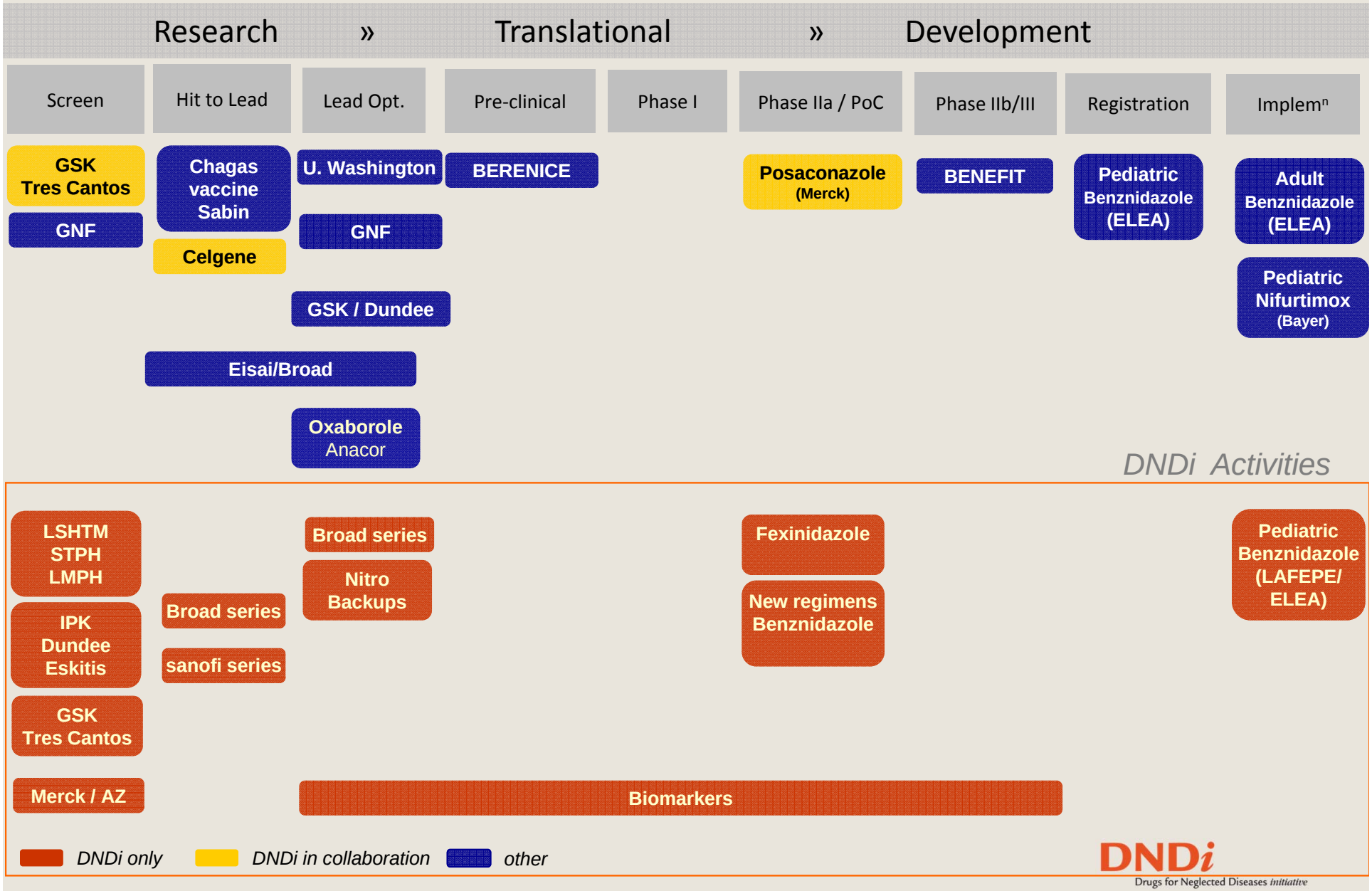
Treatment groups

Total N=56 animals to be enrolled
- Vehicle (n=8)
- Benznidazole standard dose (n=16)
- Benznidazole low dose (n=16)
- E1224 standard dose (n=16)

Main analysis:
Kappa value PCR+ after treatment and presence of infection estimated at 0.9182 (95% CI 0.8074, 1)

- 80% power to detect Kappa > 0.7

Chagas Landscape 2015



Conclusions

- **Significant impact of recent clinical trial data (adults and children) on the overall Chagas disease R&D landscape**
  - **Additional push for scaling up diagnosis and treatment of Chagas disease, improved access to available drugs and formulations**
- **Work towards new treatments for the chronic form of Chagas Disease**
  - **PKPD for new treatments in Chagas disease**
  - **POC studies for reduced BNZ, combination and Fexinidazole**
- **Need for clear regulatory framework for registration of new treatments for adults with chronic Chagas disease**

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via the 4th Sector Health Project implemented by Abt Associates, Inc.

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Acknowledgements

- **Chagas Clinical Research Platform**
- **Principal Investigators and collaborators on the reported trials**
  - Sergio Sosa Estani
  - Jaime Altcheh
  - Facundo Garcia Bounissen
  - Alejandro Schijman
  - Faustino Torrico
  - Joaquim Gascón
  - Adelina Riarte
  - Carlos Morillo
- **DNDi R&D Chagas Team**
  - Fabiana Barreira
  - Bethania Blum
  - Jayme Fernandes
  - Erika Correia
  - Cristina Alonso Vega
  - Fabiana Alves

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Panel Discussion

Sumathi Nambiar, MD PhD
Division Director, Division of Anti-infective Products
Center for Drug Evaluation and Research
U.S. Food and Drug Administration

Afternoon Panel Discussion

- Populations who could be enrolled in a clinical trial
  - *What are the populations (e.g. stage of disease) for which a clinical trial could be feasible and acceptable?*
- Acceptable control groups
  - *Are there any situations for which a placebo control would be acceptable?*


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Protecting and Promoting Public Health
www.fda.gov

BREAK

Use of Serology to Assess the Efficacy of Drugs for Chagas Disease

Louis V. Kirchhoff, MD, MPH

Professor, Departments of Internal Medicine (Infectious Diseases) and Epidemiology
University of Iowa

Chagas Disease Public Meeting on Patient-Focused Drug Development

FDA, Silver Spring, MD, April 28, 2015

General Issues for Evaluating Drugs for Chagas Disease

1. Evaluating drugs for Chagas disease is a major challenge, but not uniquely so
2. Following clinical parameters is not useful
3. Parasitologic cure is the goal but determining that it has been achieved is difficult
4. Parasitologic assays lack sensitivity
5. Serologic assays are excellent for diagnosis in donors and clinical settings (pre-treatment)
6. Variability and delay in the fall of anti-*T. cruzi* antibody titers after treatment make assessment of drug efficacy a difficult and prolonged process

Recruitment of Study Subjects with Chronic Chagas Disease

1. Younger persons who have been infected for fewer years are more curable
2. Avoid reinfection after treatment
3. Perform screening and confirmatory serologic assays
4. Need to avoid including subjects who are false positives in serologic assays
    a. Option 1: Include persons with a broad range of titers
    b. Option 2: Include only persons with “robust” titers
    c. Options 3 & 4: Include only PCR+ persons in a. or b.

Serology as an Approach for Detecting Parasitologic Cure

1. Logistical issue: freeze multiple aliquots of serum from each blood draw to allow head-to-head testing of all samples at each time point
2. Long-term goal is to detect after treatment an early pattern of declining antibody reactivity or a lack of detectable antibodies that is indicative of parasitologic cure
3. Options for targets:
   a. Broad *T. cruzi* lysate [epimastigotes (e.g. Ortho ELISA) vs. trypomastigotes as sources]
   b. Mixtures of single or chimeric recombinant proteins (e.g., Wiener Rec Chagatest; Abbott Prism, Architect, and ESA assays)
   c. Whole parasites or single native antigen [e.g., IIF; (trypomastigotes in CoML assay; gp160; and t-GPI-mucins as targets of "lytic antibodies")]
   d. Different approaches: parasite or human biomarkers as indicators of infection status (e.g. mass spectrometry, APOA-1, FN1; PCR)

POLYMERASE CHAIN REACTION

Tool for treatment monitoring in Chagas disease

Standardization and Validation issues

Patient-Focused Drug Development

meeting on Chagas Disease

Silver Spring, Maryland April 2015

Alejandro Gabriel Schijman

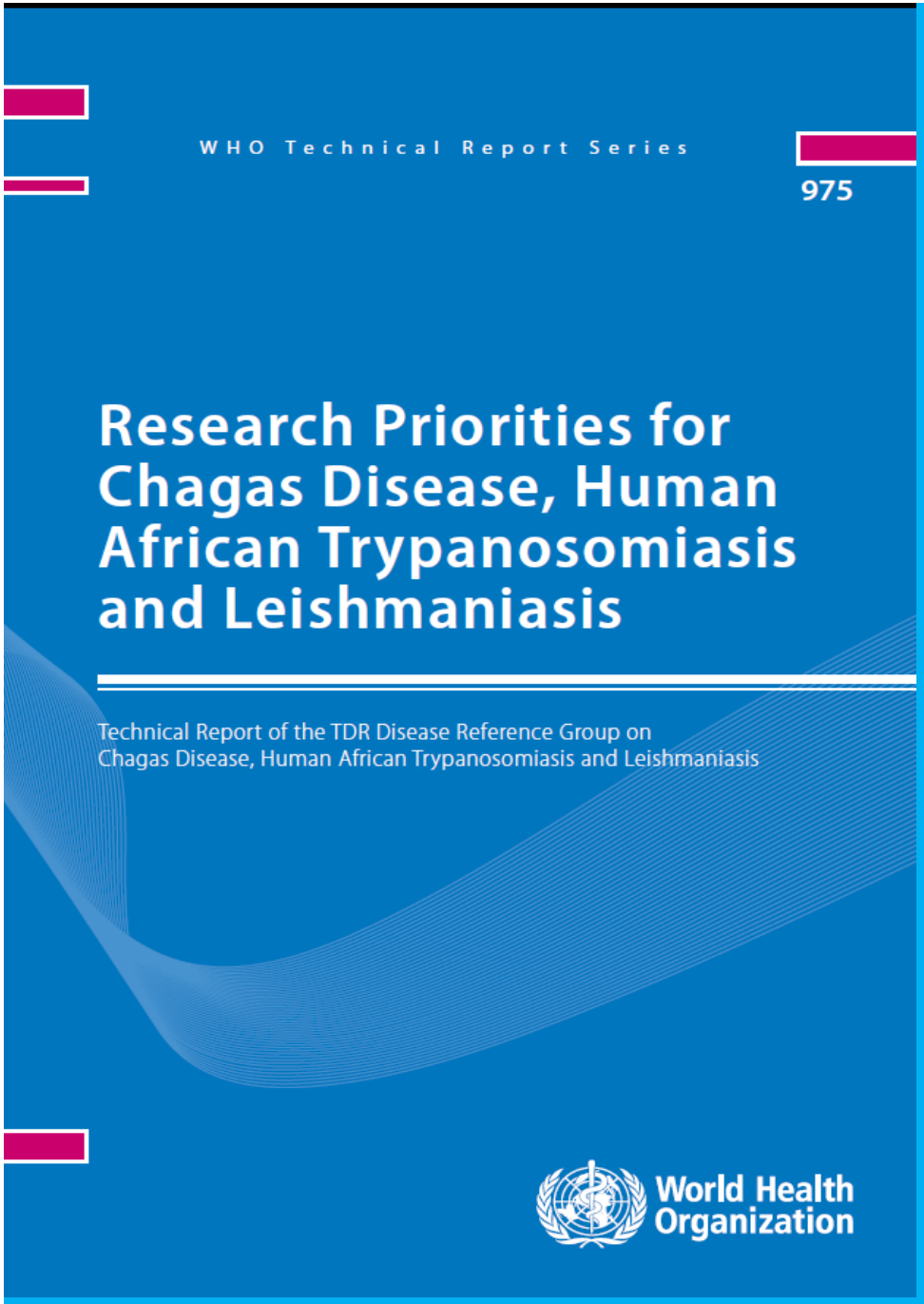
Grupo de Biologia Molecular de La Enfermedad de Chagas

INSTITUTO DE INVESTIGACIONES EN INGENIERIA GENETICA

Y BIOLOGIA MOLECULAR "Dr. Héctor N. Torres"

C O N I C E T

Buenos Aires, Argentina



Top research priorities for Chagas disease, human African trypanosomiasis and leishmaniasis:

Research on new diagnostics for case detection and characterization, including drug resistance and tests of cure.

Research on new therapeutics to avoid drug resistance, including exploring combinations of approved anti-kinetoplastid drugs, repurposing of existing approved drugs, and developing new drugs.

Research on new vector control technologies, including markers of successful vector control.

Research on vector population characteristics, including insecticide resistance.

Operational research on integrated disease and vector control.

Research on vaccines to prevent Leishmania infection and disease, and vaccines to block transmission of Leishmania

Research to assess the importance of asymptomatic infection.

INTERNATIONAL INITIATIVES

WHO Consultation on International Biological Reference Preparations For Chagas Diagnostic Tests,

23-24 April 2007, Buenos Aires, Argentina

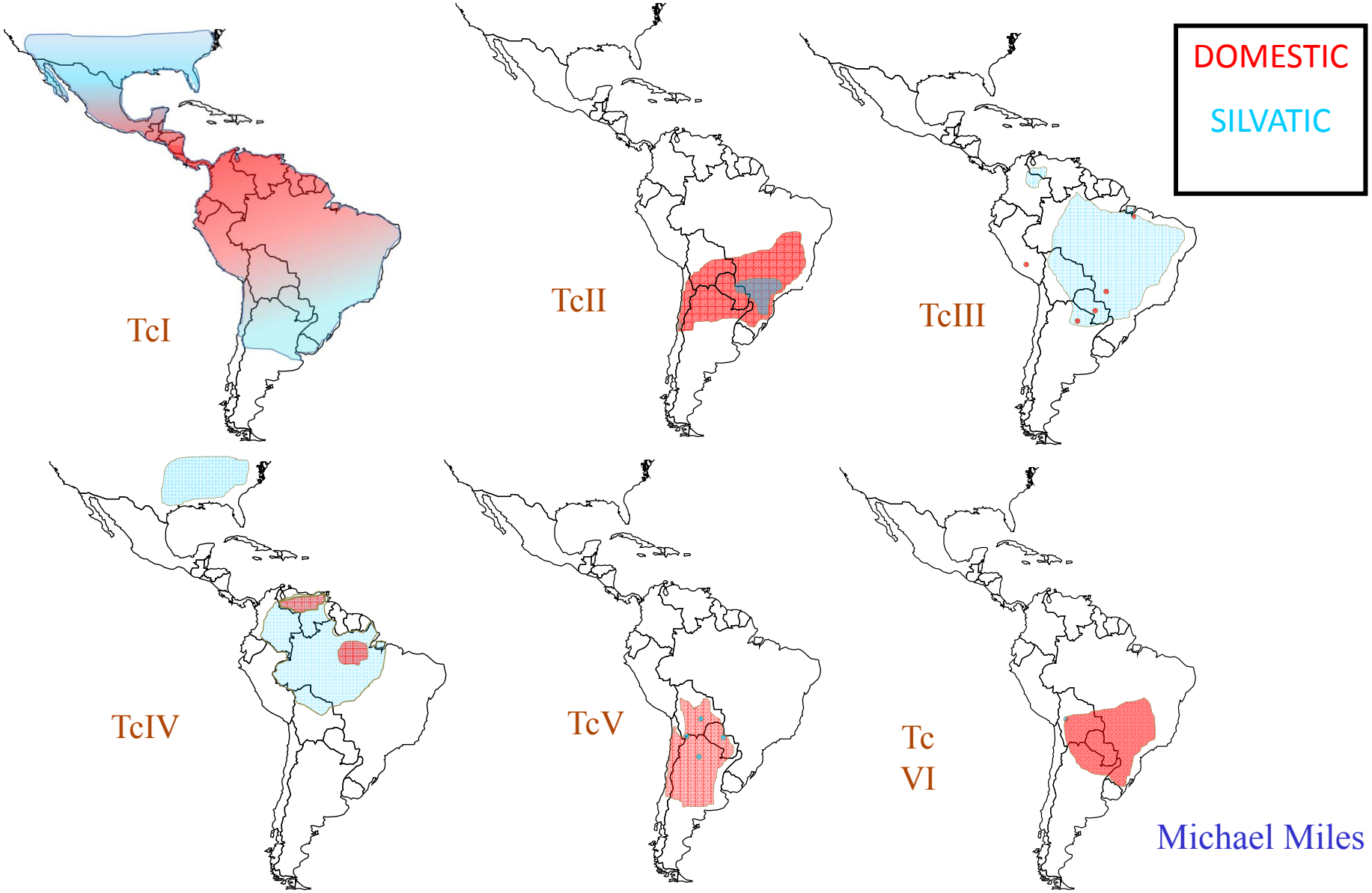
It has been recognized that the application of polymerase chain reaction (PCR) to detect Trypanosoma cruzi directly in blood with high sensitivity and specificity has opened new possibilities for the diagnosis of infection and evaluation of trypanocidal chemotherapy.

Revisiting Chagas disease: From a Latin American Health perspective to a Global Health perspective,

2-3 July 2007, WHO, Geneva, Switzerland;

GENETIC DIVERSITY OF *TRYPANOSOMA CRUZI*

DISCRETE TYPING UNITS



DTUs and Molecular Diagnosis

- Variations in accuracy of PCR in different regions could be due in part to the geographical diversity of DTUs distribution.
- Copy numbers of sequences used as targets for molecular diagnosis differ among different DTUs and strains.
- Therefore, PCR should be validated in this context.

Preparation of control panels and distribution to 29 laboratories

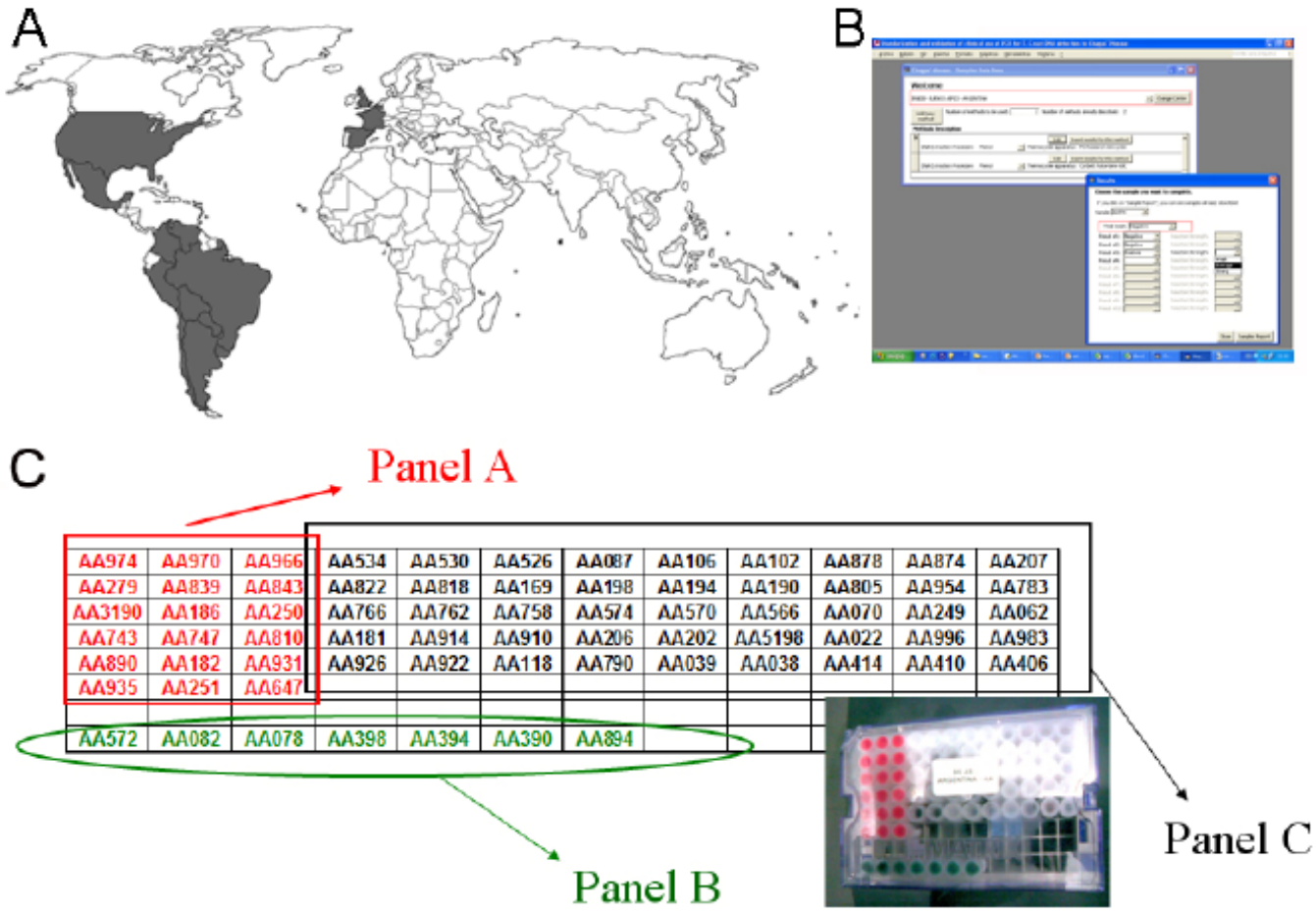


Figura M4. Países participantes en el estudio interlaboratorio de PCR y materiales enviados. A. Países participantes. **B y C.** Materiales enviados a cada laboratorio: Base de datos para informar procedimientos y resultados (B) y paneles de muestras identificados por color (C).

Table 1. PCR tests reported by the participating Laboratories.

Laboratory / Test	Extraction Method	Target	Primer Names	Amplification	Master Mix	Cycles N
A	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	35
B	Solvent extraction (HM)	kDNAv	S35 - S36	C	In-House (HM)	30
C1	Solvent extraction (HM)	kDNAv	S35 - S36	C	In-House (HM)	32
C2	Solvent extraction (HM)	Sat-DNA	tcz1 - tcz2	C	In-House (HM)	40
C3	Solvent extraction (HM)	24s	D71-D71	C	In-House (HM)	40
C4	Solvent extraction (HM)	CO II-DNA	Tcmit 31-40	C	In-House (HM)	48
C5	Solvent extraction (HM)	CO II-DNA	Nested Tcmit 10-21	C	In-House (HM)	48
C6	Solvent extraction (HM)	SL-DNA	Tcc- Tc1-Tc2	C	In-House (HM)	30
D1	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	36
D2	Solvent extraction (HM)	Sat-DNA	TczF-TczR	RT	QiaGen (Kt)	50
D3	Solvent extraction (HM)	Sat-DNA	TczF-TczR	C	In-House (HM)	41
E	Chelex Resine (HM)	kDNAv	121-122	C	In-House (HM)	35
F1	Roche Glass fibers column (Kt)	Sat-DNA	cruzi1-2	RT	Roche (Kt) *	NA
F2	Roche Glass fibers column (Kt)	kDNAc	32f-148r	RT	Roche (Kt) *	NA
G1	Roche Glass fibers column (Kt)	kDNAc	32f-148r	RT	Roche (Kt) *	NA
G2	Roche Glass fibers column (Kt)	kDNAc	32f-148r	RT	Roche (Kt) *	NA
G3	Roche Glass fibers column (Kt)	kDNAc	32f-148r	RT	Roche (Kt) *	NA
G4	Roche Glass fibers column (Kt)	Sat-DNA	cruzi1-2	RT	Roche (Kt) *	NA
H1	Favorgen Glass fibers column (Kt)	kDNAv	121-122	C	GoTaq (Kt)	33
H2	Favorgen Glass fibers column (Kt)	kDNAv	121-122	C	In-House (HM)	33
I1	Favorgen Glass fibers column (Kt)	kDNAv	121-122	C	In-House (HM)	0
I2	Favorgen Glass fibers column (Kt)	kDNAv	S35 - S36	C	In-House (HM)	0
J	Solvent extraction (HM)	Sat-DNA	Tcz1-Tcz2	C	In-House (HM)	40
K1	Silica gel column (Kt)	Sat-DNA	cruzi1-2	RT	In-House (HM)	NA
K2	Silica gel column (Kt)	kDNAv	121-122	C	In-House (HM)	NA
L1	Blood mini Kit (Kt)	Sat-DNA	cruzi1-2	C	In-House (HM)*	40
L2	Blood mini Kit (Kt)	Sat-DNA	Satellite DNA based kit	C	OligoC-T Coris (Kt)*	40
M	Silica gel column (Kt)	kDNAv	TC1-TC2	C	In-House (HM)	1
N1	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	40
N2	Solvent extraction (HM)	Sat-DNA	Tcz1-Tcz2	C	In-House (HM)	35
O	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	40
P1	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	35
P2	CTAB (HM)	kDNAv	121-122	C	In-House (HM)	35
Q	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	37
R	Roche Glass fibers column (Kt)	kDNAv	121-122	C	In-House (HM)	40
S1	Qiagen Silica gel column (Kt)	18s	Tc18s F3-R4	C	AmpliTaq Gold (Kt)	40
S2	Qiagen Silica gel column (Kt)	Sat-DNA	cruzi1-2	RT	Platinum qPCR w/ROX (Kt) *	40
S3	Qiagen Silica gel column (Kt)	18s	Tc18s F1042- R1144	RT	Platinum qPCR w/ROX (Kt) *	40
S4	Qiagen Silica gel column (Kt)	kDNAv	121-122	C	AmpliTaq Gold (Kt)	40
T	ATGEN kit (Kt)	kDNAv	121-122	RT	Invitrogen (Kt)	40
U1	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	40
U2	Solvent extraction (HM)	24s	D71-D72	C	In-House (HM)	32
V1	Silica gel column (Kt)	kDNAv	121-122	C	In-House (HM)	40
V2	Silica gel column (Kt)	Sat-DNA	Tcz1-Tcz2	C	In-House (HM)	30
W	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	40
X	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	35
Y	Solvent extraction (HM)	kDNAv	121-122	C	In-House (HM)	35
Z	Silica gel column (Kt)	Sat-DNA	cruzi1-2	RT	TaqMan Univ (Kt)	45

Laboratory, letter code; PCR strategy, number code; HM, home made; C, commercial; kDNAc, constant region; kDNAv, variable region of minicircle DNA; Sat-DNA, satellite DNA; 24s, 24sa rDNA; 18s: 18s rDNA; SL, Spliced Leader; NA, not available, * Master mix containing Uracyl DNA N-Glycosylase and dUTP to prevent amplicon carry-over contamination.

Table 2 . Performances of PCR tests in Sets A and B

Laboratory / Test	Extraction Method	PCR	Test Target	Set A							Set B		
					T. cruzi I		*T. cruzi* IV		*T. cruzi* VI		*T. cruzi* VI		
				Sp	Co	DL	Co	DL	Co	DL	Sp	Co	DL par/ml
A	HM	C	kDNAv	Y	Y	0.1	N	0.01	N	0.01	Y	N	0.005
B	HM	C	kDNAv	Y	N	0.001	N	0.001	Y	0.1	Y	N	0.005
C1	HM	C	kDNAv	N	N	0.001	N	0.001	Y	0.1	N	Y	ND
C2	HM	C	Sat-DNA	Y	Y	ND	N	1	Y	10	Y	Y	0.00005
C3	HM	C	24s	Y	Y	ND	Y	ND	Y	ND	NA	NA	NA
C4	HM	C	CO II-DNA	Y	Y	ND	Y	ND	Y	ND	NA	NA	NA
C5	HM	C	CO II-DNA	N	Y	1	N	0.1	N	0.001	NA	NA	NA
C6	HM	C	SL-DNA	Y	Y	ND	Y	ND	Y	ND	NA	NA	NA
D1	HM	C	kDNAv	Y	Y	1	Y	10	Y	1	N	N	0.005
D2	HM	RT	Sat-DNA	Y	Y	1	Y	10	Y	1	Y	Y	0.05
D3	HM	C	Sat-DNA	Y	Y	1	Y	10	Y	1	Y	Y	0.05
E	HM	C	kDNAv	Y	Y	1	Y	10	Y	10	Y	Y	0.005
F1	Kt	RT	Sat-DNA	Y	Y	0.1	Y	1	Y	0.01	Y	Y	0.05
F2	Kt	RT	kDNAc	Y	Y	1	N	0.01	Y	1	Y	Y	0.5
G1	Kt	RT	kDNAc	Y	Y	0.1	Y	1	Y	ND	Y	Y	0.05
G2	Kt	RT	kDNAc	Y	Y	0.1	Y	1	Y	1	Y	Y	0.05
G3	Kt	RT	kDNAc	Y	Y	0.1	Y	1	Y	1	Y	Y	0.05
G4	Kt	RT	Sat-DNA	Y	Y	1	Y	1	Y	1	Y	Y	0.5
H1	Kt	C	kDNAv	Y	Y	1	Y	10	N	0.001	Y	N	0.05
H2	Kt	C	kDNAv	Y	Y	1	N	0.1	Y	10	Y	N	0.05
I1	Kt	C	kDNAv	Y	Y	1	N	0.001	Y	10	Y	Y	0.005
I2	Kt	C	kDNAv	Y	Y	1	Y	10	Y	10	Y	Y	0.05
J	HM	C	Sat-DNA	Y	Y	0.01	N	0.001	N	0.001	Y	N	0.5
K1	Kt	RT	Sat-DNA	Y	Y	10	Y	10	Y	10	Y	Y	0.5
K2	Kt	C	kDNAv	Y	Y	1	Y	10	Y	10	Y	Y	5
L1	Kt	C	Sat-DNA	Y	Y	ND	Y	10	Y	1	Y	Y	0.5
L2	Kt	C	Sat-DNA	Y	Y	ND	Y	ND	Y	1	Y	Y	0.5
M	Kt	C	kDNAv	N	Y	0.001	Y	0.001	N	0.001	Y	Y	ND
N1	HM	C	kDNAv	Y	Y	0.1	Y	ND	N	0.1	Y	N	0.005
N2	HM	C	Sat-DNA	Y	Y	1	Y	ND	Y	10	Y	Y	0.5
O	HM	C	kDNAv	Y	Y	10	N	1	Y	ND	Y	Y	0.05
P1	HM	C	kDNAv	Y	Y	0.1	Y	10	Y	1	Y	Y	5
P2	HM	C	kDNAv	Y	Y	0.1	Y	10	Y	1	Y	Y	0.5
Q	HM	C	kDNAv	Y	Y	1	Y	10	Y	1	Y	Y	0.5
R	Kt	C	kDNAv	Y	Y	0.1	Y	1	Y	0.1	Y	N	0.00005
S1	Kt	C	18s	Y	Y	1	Y	ND	Y	ND	Y	Y	ND
S2	Kt	RT	Sat-DNA	Y	Y	1	Y	10	Y	1	Y	Y	0.5
S3	Kt	RT	18s	Y	Y	10	Y	10	Y	ND	Y	Y	ND
S4	Kt	C	kDNAv	Y	Y	1	Y	1	Y	10	N	N	0.00005
T	Kt	RT	kDNAv	N	N	0.001	Y	1	N	0.01	N	N	0.05
U1	HM	C	kDNAv	N	Y	0.001	Y	0.001	Y	0.001	Y	N	0.005
U2	HM	C	24s	N	Y	0.001	Y	0.001	Y	0.001	N	N	0,005
V1	Kt	C	kDNAv	Y	Y	0.1	Y	10	Y	10	Y	Y	0.05
V2	Kt	C	Sat-DNA	Y	Y	0.1	Y	ND	Y	10	Y	Y	0.05
W	HM	C	kDNAv	Y	Y	0.01	Y	1	Y	0.1	Y	Y	0.005
X	HM	C	kDNAv	N	N	0.01	N	0.001	N	0.1	Y	N	0.0005
Y	HM	C	kDNAv	N	Y	1	Y	1	Y	0.1	Y	Y	ND
Z	Kt	RT	Sat-DNA	Y	Y	1	Y	1	Y	0.01	N	N	0.0005

Grey boxes, Good Performing Methods (GPM) in sets A or B, Black boxes, GPM in both sets A and B
Core Lab, Coordinating Lab. C, Conventional PCR, RT, Real Time PCR; K, kDNA; S, Satellite DNA; 24s, 24sα rDNA; 18s: 18s rDNA; SL, Spliced Leader
Sp, 100% of specificity in the three controls without DNA, Co, Coherence in PCR positive reports
DL Detection limit in fg DNA/ul. Y. Affirmative, N. Negative. NA. Not available. ND. Not detectable

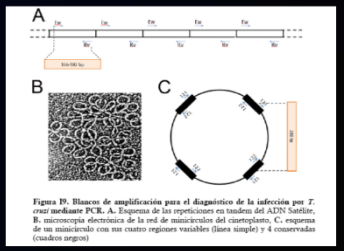


Figura I9. Blancos de amplificación para el diagnóstico de la infección por *T. cruzi* mediante PCR. A. Esquema de las repeticiones en tandem del ADN Satélite, B. microscopía electrónica de la red de minicírculos del cinetoplasto, C. esquema de un minicírculo con sus cuatro regiones variables (línea simple) y 4 conservadas (cuadros negros)

OPEN ACCESS Freely available online

PLoS NEGLECTED TROPICAL DISEASES

International Study to Evaluate PCR Methods for Detection of *Trypanosoma cruzi* DNA in Blood Samples from Chagas Disease Patients

Alejandro G. Schijman[1*], Margarita Bisio[1], Liliana Orellana[2], Mariela Sued[2], Tomás Duffy[1], Ana M. Mejia Jaramillo[3], Carolina Cura[1], Frederic Auter[4], Vincent Veron[5], Yvonne Qvarnstrom[6], Stijn Deborggraeve[7], Gisely Hijar[8], Inés Zulantay[9], Raúl Horacio Lucero[10], Elsa Velazquez[11], Tatiana Tellez[12], Zunilda Sanchez Leon[13], Lucia Galvão[14], Debbie Nolder[15], María Monje Rumi[16], José E. Levi[17], Juan D. Ramirez[18], Pilar Zorrilla[19], María Flores[20], Maria I. Jercic[21], Gladys Crisante[22], Néstor Añez[22], Ana M. De Castro[23], Clara I. Gonzalez[24], Karla Acosta Viana[25], Pedro Yachelini[26], Faustino Torrico[12], Carlos Robello[19], Patricio Diosque[16], Omar Triana Chavez[3], Christine Aznar[5], Graciela Russomando[13], Philippe Büscher[7], Azzedine Assal[4], Felipe Guhl[18], Sergio Sosa Estani[27], Alexandre DaSilva[6], Constança Britto[28], Alejandro Luquetti[29], Janis Ladzins[30]

PCR Strategy						PANEL A					PANEL B			PANEL C	
Extraction	Target		Primers	Master Mix	Visualization	Sp	Co	Detection limit fg/ul x-10	Cl-Br	Can III	Sp	Co	DL Par/ml	Sp	Se
phenol	RT	S	TczF-TczR	QiAgen	Sybr Green	Y	Y	1	1	10	Y	Y	0.05	100,00	66.67
phenol	C	S	TczF-TczR	In-House	Agarose Gel	Y	Y	1	1	10	Y	Y	0.05	100,00	60,00
Silica Gel Col	RT	S	cruzi1-2	Roche	Taq-Man	Y	Y	0.1	0.01	1	Y	Y	0.05	100,00	60,00
Phenol	C	K	121-122	In-House	Agarose Gel	Y	Y	1	1	10	Y	Y	0.5	100,00	60,00

OPEN ACCESS Freely available online

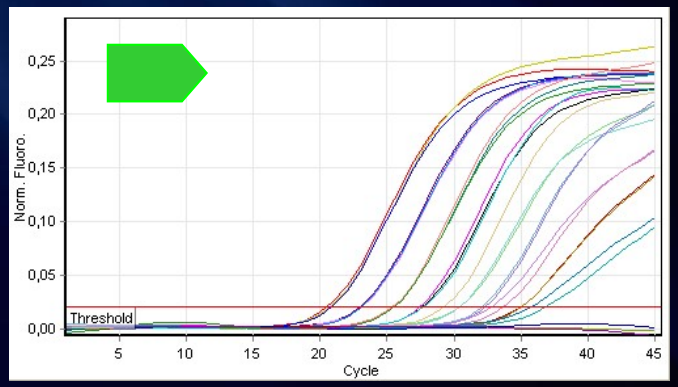
PLOS | NEGLECTED TROPICAL DISEASES

Analytical Performance of a Multiplex Real-Time PCR Assay Using TaqMan Probes for Quantification of *Trypanosoma cruzi* Satellite DNA in Blood Samples

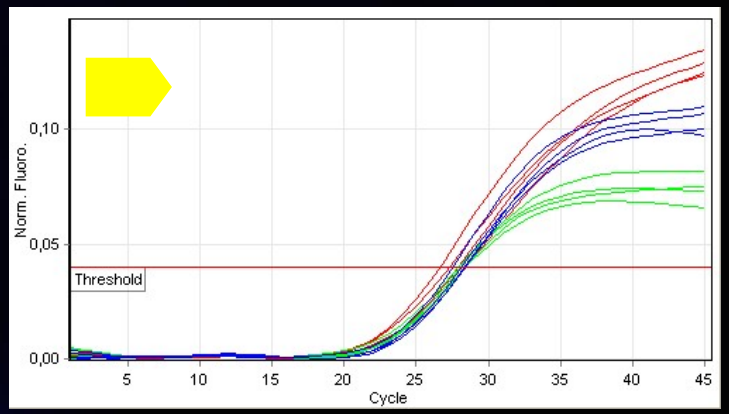
Tomas Duffy[1☯], Carolina I. Cura[1☯], Juan C. Ramirez[1☯], Teresa Abate[2], Nelly M. Cayo[3], Rudy Parrado[4], Zoraida Diaz Bello[2], Elsa Velazquez[5], Arturo Muñoz-Calderon[2], Natalia A. Juiz[1], Joaquín Basile[1], Lineth Garcia[4], Adelina Riarte[5], Julio R. Nasser[6], Susana B. Ocampo[3], Zaida E. Yadon[7], Faustino Torrico[4], Belkisyole Alarcón de Noya[2], Isabela Ribeiro[8], Alejandro G. Schijman[1*]

1 Grupo de Biología Molecular de la Enfermedad de Chagas, Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr. Héctor N. Torres" (INGEBI-CONICET), Buenos Aires, Argentina, **2** Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas, Venezuela, **3** Instituto de Biología de la Altura, Universidad Nacional de Jujuy, Jujuy, Argentina, **4** Universidad San Simón, Cochabamba, Bolivia, **5** Instituto Nacional de Parasitología "Dr. Mario Fatala Chaben", ANLIS, Buenos Aires, Argentina, **6** Laboratorio de Química Biológica, Facultad de Ciencias Naturales, Universidad Nacional de Salta, Salta, Argentina, **7** Pan-American Health Organization, Washington, D.C., United States of America, **8** Drugs and Neglected Diseases Initiative, Genève, Switzerland

T.Cruzi DNA sequence



Internal Amplification Control



WHO-TDR /PAHO / DNDi Initiatives

Drugs for Neglected Diseases Initiative (DNDi)

Chagas Clinical Research Plataform

22-23 March 2010, Buenos Aires.

PCR Technical Group Meeting

PAHO Meeting to organize validation studies of Q PCR

31 Mayo 2011, Buenos Aires.

Setiembre 2011, Bogotá, Colombia.

INTERNATIONAL WORKSHOP FOR ANALYTICAL VALIDATION OF QUANTITATIVE PCR FOR DETERMINING PARASITIC LOADS IN HUMAN BLOOD

DECEMBER 2011, Buenos Aires, INGEBI-CONICET OPS/TDR

All calls for applications
Empowerment training and fellowships
Research activities
- Lead discovery for drugs
- Innovation research
- Vector control interventions
- Drug development for helminths
- Quality assured diagnostics
- Evidence for treatment of TB/HIV
- Antimalarial policy/access
- Visceral leishmaniasis elimination
- Community-based interventions

Application and reporting forms

Home > Grants > All calls for applications > Call for research labs

Standardization and validation of qPCR for *Trypanosoma cruzi* Call for research laboratories

Deadline: 16 October 2011

Background
How to apply

I. Background

PAHO Communicable Diseases Research Program and the Special Programme for Research and Training in Tropical Diseases (TDR) invite research laboratories to joint standardization and validating exercise for quantitative Polymerase Chain Reaction (qPCR) for the quantification of *Trypanosoma cruzi* DNA loads in Chagas patients.

Selected laboratories will be invited to attend a one-week workshop to hosted by the Instituto de *Investigaciones en Ingenieria Genetiva y Biologia Molecular* (INGEBI) in Buenos Aires in December 2011 and provided with an opportunity to evaluate/compare their qPCR techniques against a set of reference samples. The objective is to be able to harmonize procedures that can be applied in the future evaluation of treatment response to new products.

Applications are expected from public and private laboratories with demonstrated experience in processing clinical samples for qPCR for *T. cruzi* DNA and willingness

INTERNATIONAL WORKSHOP - 24 PARTICIPANTS

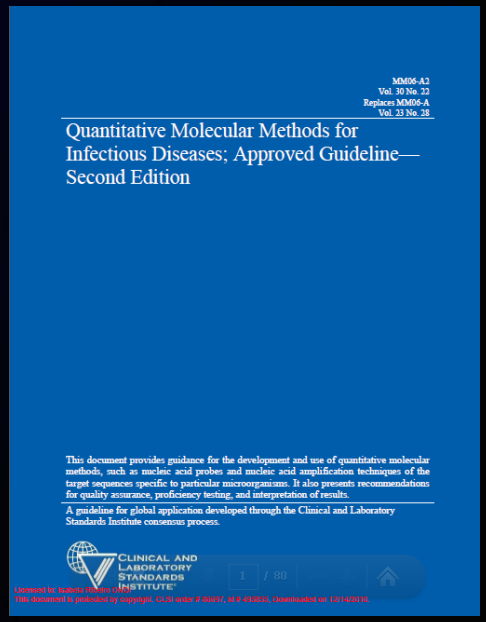
Q-PCR - DUPLEX TaqMan

Satellite DNA – IAC and Kinetoplastid DNA- IAC.

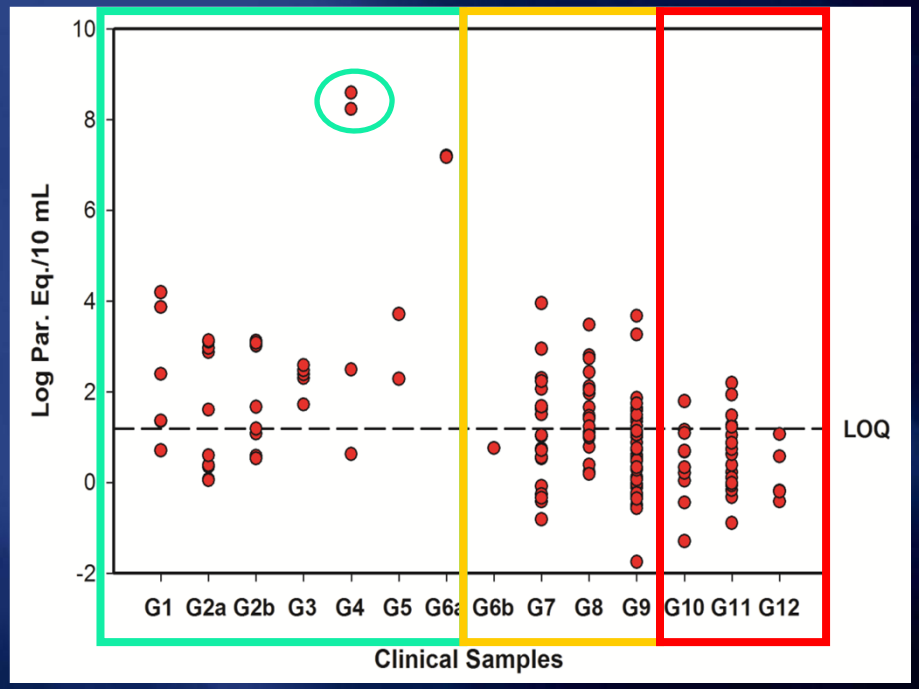
CLINICAL SPECIMENS PROVIDED BY PARTICIPANTS

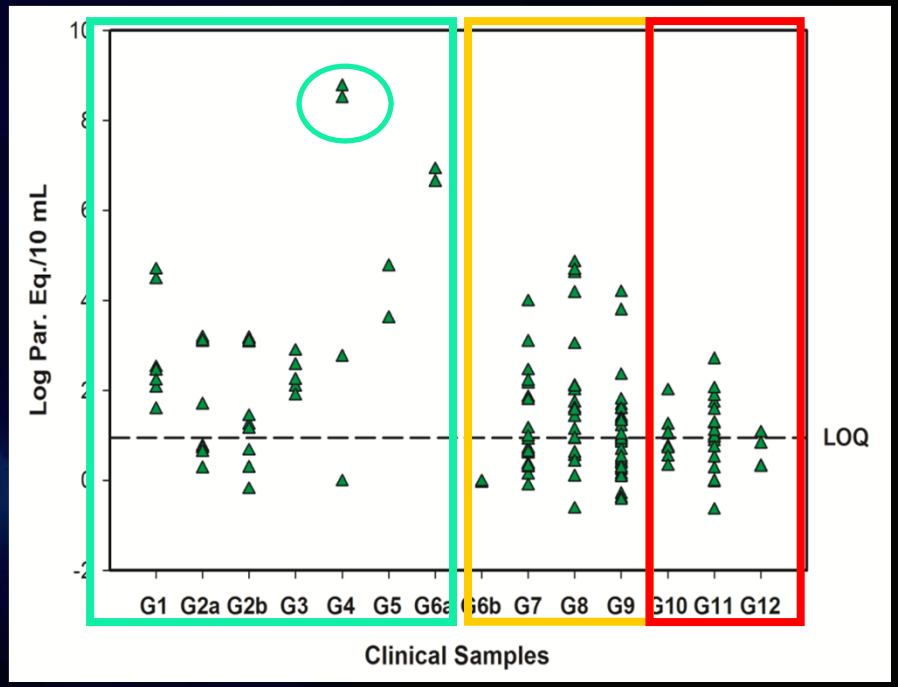
Analytical validation of qPCR following Clinical Laboratory Standard Institute Guidelines

		Sat qPCR	kDNA qPCR
Diagnostic Sensitivity	Acute CD	100 % (11/11)	100 % (11/11)
	Chronic CD	80.69 % (117/145)	84.14 % (122/145)
Diagnostic Specificity		100 % (50/50)	100 % (50/50)
Reportable Range		10^5-0.5 par eq/mL	10^5-0.25 par eq/mL
Limit of Detection (LOD)		0.698 par eq/mL	0.234 par eq/mL
Limit of Quantification (LOQ)		1.531 par eq/mL	0.895 par eq/mL
Precision	0.25 par eq/mL	---	31.98 %
	0.5 par eq/mL	46.60 %	---
	10 par eq/mL	6.00 %	8.79 %
	1000 par eq/mL	1.72 %	2.92 %
Inclusivity	Tc Ia	0.0625 fg/uL	0.0625 fg/uL
	Tc Id	≥ 0.25 fg/uL	0.0625 fg/uL
	Tc Ie	≥ 1 fg/uL	0.0625 fg/uL
	Tc II	0.0625 fg/uL	0.0625 fg/uL
	Tc III	0.0625 fg/uL	0.0625 fg/uL
	Tc IV	≥ 0.25 fg/uL	0.0625 fg/uL
	Tc V	0.0625 fg/uL	0.0625 fg/uL
	Tc VI	0.0625 fg/uL	0.0625 fg/uL
	Tc Bat	0.0625 fg/uL	0.0625 fg/uL
Exclusivity	*T. rangeli*	1 pg/uL	1 fg/uL
	L. major	1000 pg/uL	1000 pg/uL
	L. mexicana	1000 pg/uL	1000 pg/uL
	L. amazonensis	1000 pg/uL	1000 pg/uL



Clinical Samples from participating laboratories





G1	Caracas
G2a	Bogotá
G2b	
G3	Bogotá
G4	México DF.
G5	Cayenne
G6a	La Paz
G6b	
G7	Madrid
G8	Buenos Aires
G9	Salta
G10	Rio de Janeiro
G11	Uberaba
G12	Natal

Satellite qPCR

Tc I

kDNA qPCR

Tc V/ VI

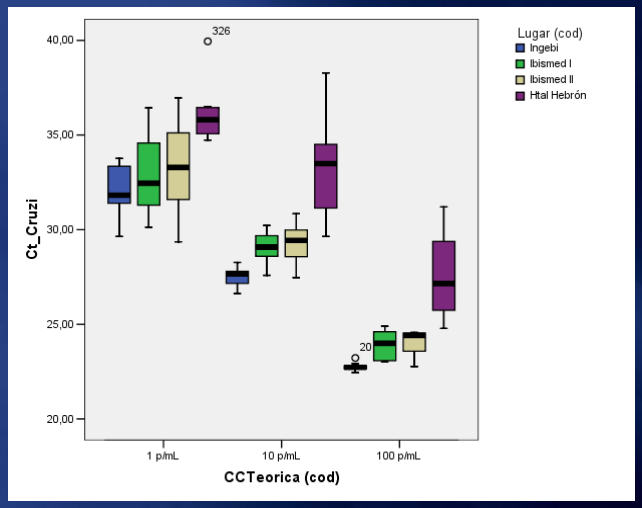
Tc II

External Quality Control Program

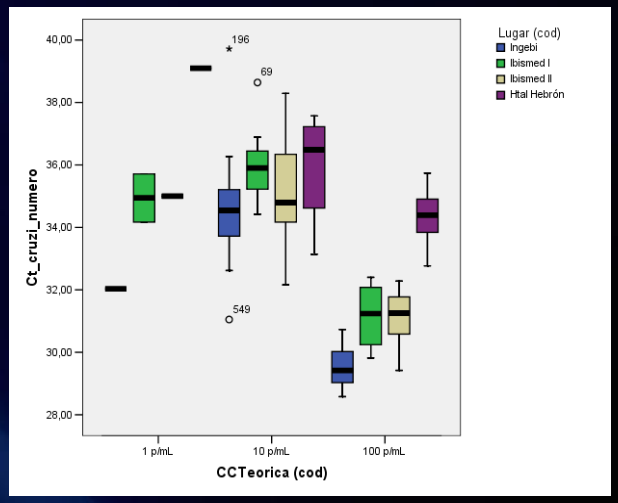
Panels of Guanidine EDTA-blood spiked with T.cruzi cells.

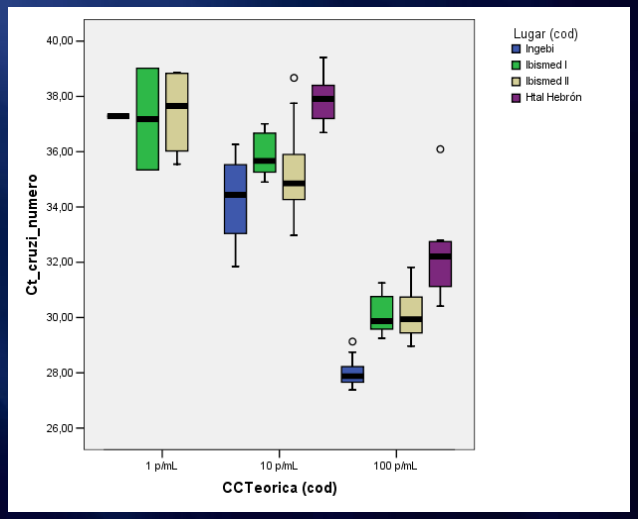
	Concent.	Panel I	Panel II	Panel III	Panel IV
Tc I K 98	C. Negat	CCP 113	CCP 209	CCP 307	CCP 401
	1 p/mL	CCP 114	CCP 210	CCP 306	CCP 403
	10 p/mL	CCP 115	CCP 212	CCP 305	CCP 402
	100 p/mL	CCP 116	CCP 211	CCP 308	CCP 404
Tc I SX10	C. Negat	CCP 109	CCP 216	CCP 301	CCP 406
	1 p/mL	CCP 111	CCP 214	CCP 303	CCP 405
	10 p/mL	CCP 110	CCP 215	CCP 302	CCP 407
	100 p/mL	CCP 112	CCP 213	CCP 304	CCP 408
Tc V	C. Negat	CCP 108	CCP 201	CCP 316	CCP 411
	1 p/mL	CCP 106	CCP 202	CCP 314	CCP 410
	10 p/mL	CCP 107	CCP 203	CCP 315	CCP 409
	100 p/mL	CCP 105	CCP 204	CCP 313	CCP 412
Tc VI ClBr	C. Negat	CCP 101	CCP 207	CCP 309	CCP 413
	1 p/mL	CCP 102	CCP 206	CCP 310	CCP 416
	10 p/mL	CCP 104	CCP 205	CCP 312	CCP 415
	100 p/mL	CCP 103	CCP 208	CCP 311	CCP 414

Tc I K98

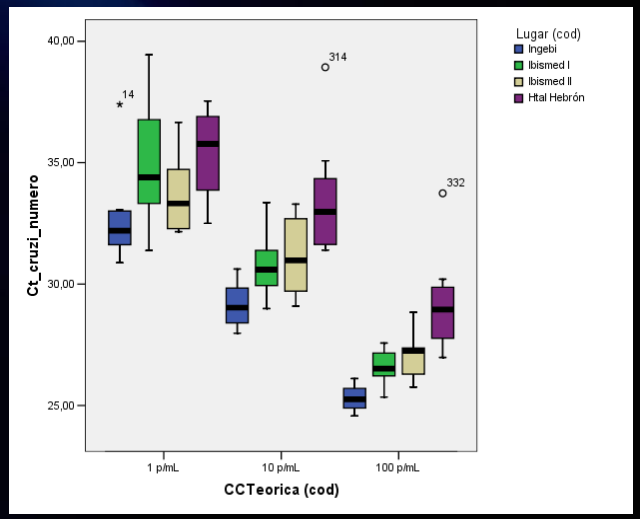


Tc I Silvio X-10





Tc V



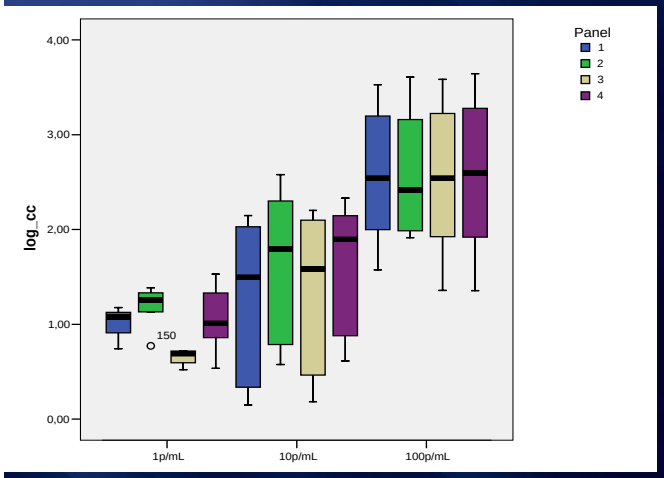
Tc VI

QUANTIFICATION OF PARASITIC LOADS

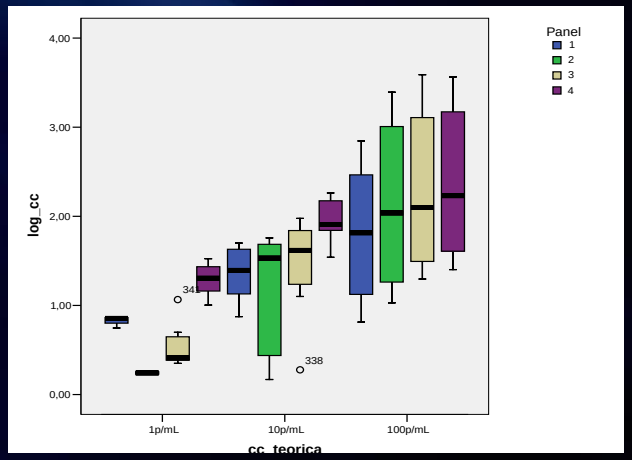
Four Panels, 0, 3, 6, 9 months after preparation and transport

Three concentrations

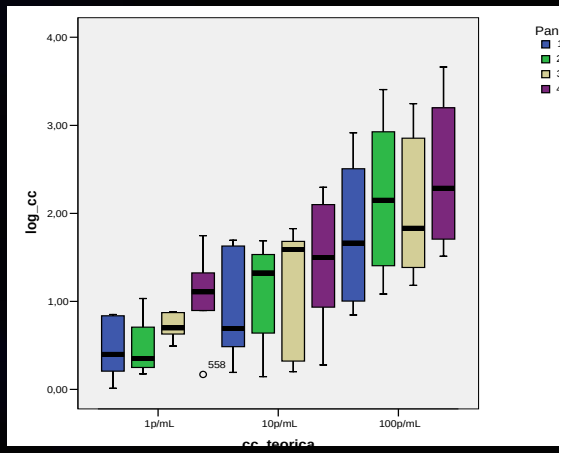
INGEBI Core Laboratory



IBIMED I Lab 1 Operator 1



IBIMED Lab 1 Operator 2



Consultancy Bioq:Marcelo Rodriguez

Team Operativo Gestion de Calidad

Departamento Parasitlogía

Instituto Nacional de Enfermedades Infeccios

ANLIS "CarlosG.Malbran"

Alejandro1

Improvement of Findings after technical modifications

Cepa T. cruzi	Muestra	Conc. Teorica	Smartcycler			C F X		
			Ct1	Ct2	Resultado	Ct1	Ct2	Resultado
Tc Ia K98	CCP 401	C. Negat	ND	ND	Negativo	ND	ND	Negativo
	CCP 402	10 p/mL	31.7	33.4	Positivo	27,64	27,91	Positivo
	CCP 403	1 p/mL	35.3	36.5	Positivo	30,85	31,76	Positivo
	CCP 404	100 p/mL	31.2	27.6	Positivo	23,06	22,83	Positivo
Tc Id Silvio X10 Cl1	CCP 405	10 p/mL	ND	ND	Negativo	37,26	34,35	Positivo
	CCP 406	1 p/mL	ND	ND	Negativo	ND	37,39	Positivo
	CCP 407	C. Negat	ND	ND	Negativo	ND	ND	Negativo
	CCP 408	100 p/mL	33.5	34.2	Positivo	29,23	30,2	Positivo
Tc V LL014-1-R1 Cl1	CCP 409	C. Negat	ND	ND	Negativo	ND	ND	Negativo
	CCP 410	1 p/mL	ND	ND	Negativo	34,55	ND	Positivo
	CCP 411	100 p/mL	32.7	32.1	Positivo	28,99	28,17	Positivo
	CCP 412	10 p/mL	37.2	38.4	Positivo	34,21	34,07	Positivo
Tc VI ClBr Bianca	CCP 413	100 p/mL	29.1	28.8	Positivo	25,49	25,57	Positivo
	CCP 414	1 p/mL	35.6	36.9	Positivo	33,03	32,31	Positivo
	CCP 415	10 p/mL	33.6	31.4	Positivo	28,65	29,14	Positivo
	CCP 416	C. Negat	ND	ND	Negativo	ND	ND	Negativo

Slide 157

Alejandro1 Volumen: 5 uL (ADN) + 15 uL (Mix) = 20 uL (Final) ..... OK

Master Mix: TaqMan Universal PCR Master Mix (Applied Biosystems) ..... OK

PCR Multiplex: T. cruzi Satellite DNA - RNase P Detection Reagent ..... Imagino que te refieres al reactivo del control interno. La referencia correcta sería: Taqman Human RNase P Control reagents kit (Applied Biosystems). El ADN que amplifica es el satélite como bien indicas y está descrito en el artículo de María Piron.

Dime si necesitas alguna cosa más (concentraciones de los primers, condiciones del termociclador, etc) y te lo envío, espero que más rapidamente, ahora que ya estoy de nuevo en Barcelona...

¿Que tal va el paper? ¿Te está dando mucho trabajo?

Gracias por todo y especialmente por la paciencia!!!!!

Besos

Elena

Alejandro, 4/22/2015

APPLICATION IN FIELD STUDIES AND CLINICAL TRIALS

MSF-DNDi PCR Study
Optimization of Sampling

NCT01678599

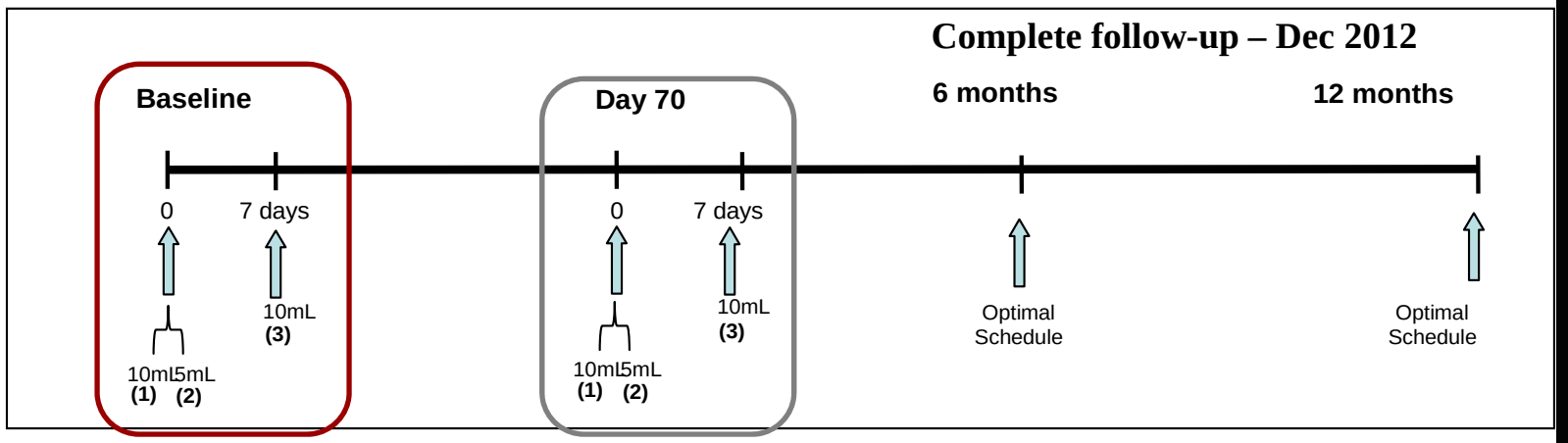
Benznidazole 5mg/kg/d

during 60 days

Target recruitment n=220

Study initiation April 13th (Recruitment Dec '11)

Complete follow-up – Dec 2012



Primary endpoint:

+ or – PCR

in sero+ patients

Secondary endpoint

Definition of optimal sampling

+ or – PCR

in PCR +(10 or 5+10 ml)

Current Strategy = 1 sample - 10 ml

Enhancement Strategy = additional samples

Substitution strategy = SS1: 5 ml; SS2: 5+10 at D7

MSF-DNDi PCR study

NCT01678599

Sample combination (baseline)

Combination of results	Sample 1+2 2 PCR done	Sample 1+2+3 3 PCR done	Sample 1+2+3 At least 2 PCR
True Positives	193	180	202
False Negatives	27	15	18
Missing PCR	0	25	0
Sample size	220	195	220
% true positives	87.73%	92.31%	91.82%

3 samples increase PCR clinical sensitivity in adults

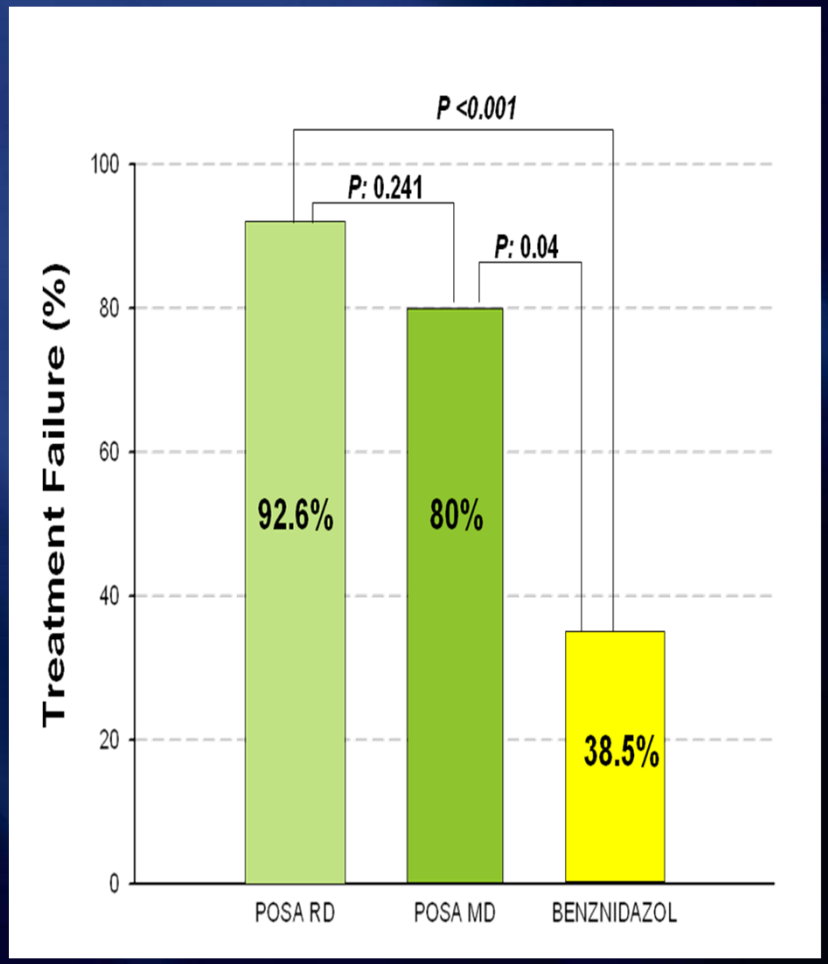
No difference betrween 5 and 10 ml blood

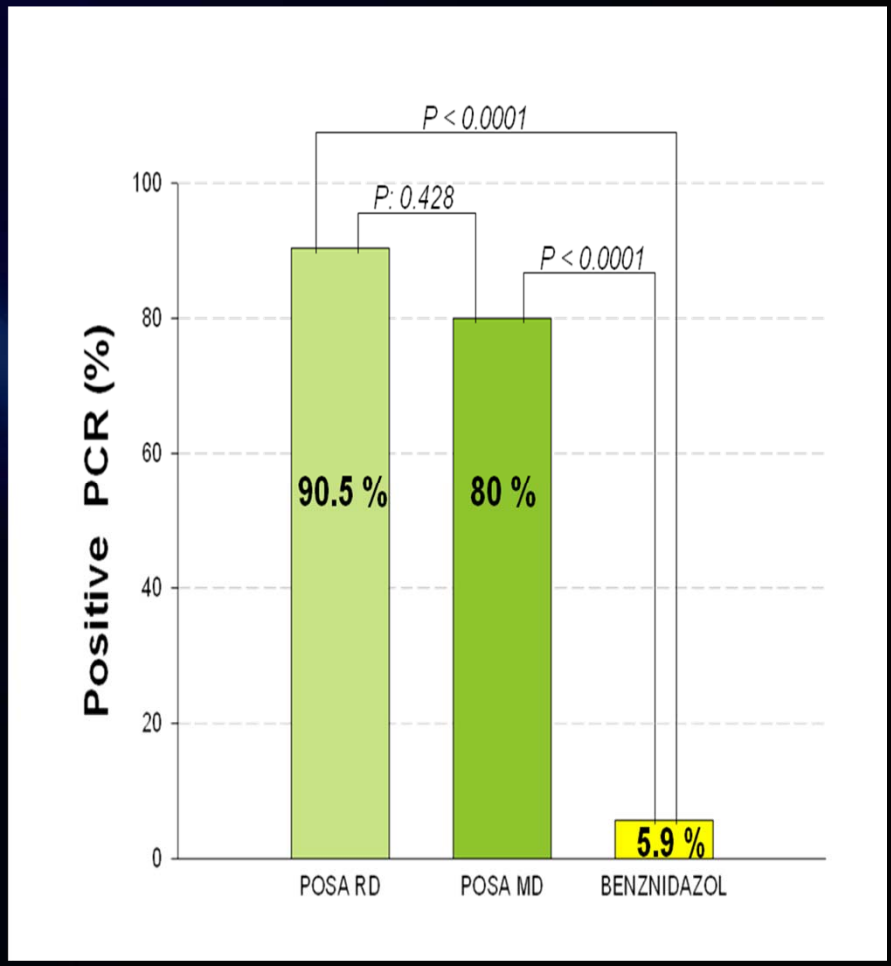
No neccesary to wait 7 days for additional sample

Vall d'Hebron Hospital

Prosics
Institut Català de la Salut
Programa de salut internacional
Barcelona

CHAGASAZOL STUDY





DNDi-CH-E1224-001

NCT01489228

Efficacy Results

Assessment by PCR at D65 and 12 months

Day 65 (EOT)

			Placebo (N=47)	LD (N=48)	SD (N=46)	HD (N=45)	BZN (N=45)	All (N=231)
Parasite clearance at D65		N	**47**	**48**	**46**	**45**	**45**	**231**
		Missing	**0**	**0**	**0**	**0**	**0**	**0**
	No	n (%)	**35 (74.5)**	**5 (10.4)**	**5 (10.9)**	**11 (24.4)**	**4 (8.9)**	**60 (26.0)**
	Yes	n (%)	**12 (25.5)**	**43 (89.6)**	**41 (89.1)**	**34 (75.6)**	**41 (91.1)**	**171 (74.0)**

12 Month Follow-up

			(N=47)	(N=48)	(N=46)	(N=45)	(N=45)	(N=231)
Sustained clearance At 12 months	No	n (%)	43 (91.5)	44 (91.7)	41 (89.1)	32 (71.1)	8 (19.0)	**168 (72.7)**
	Yes	n (%)	4 (8.5)	4 (8.3)	5 (10.9)	13 (28.9)	37 (81.0)	**63 (27.3)**

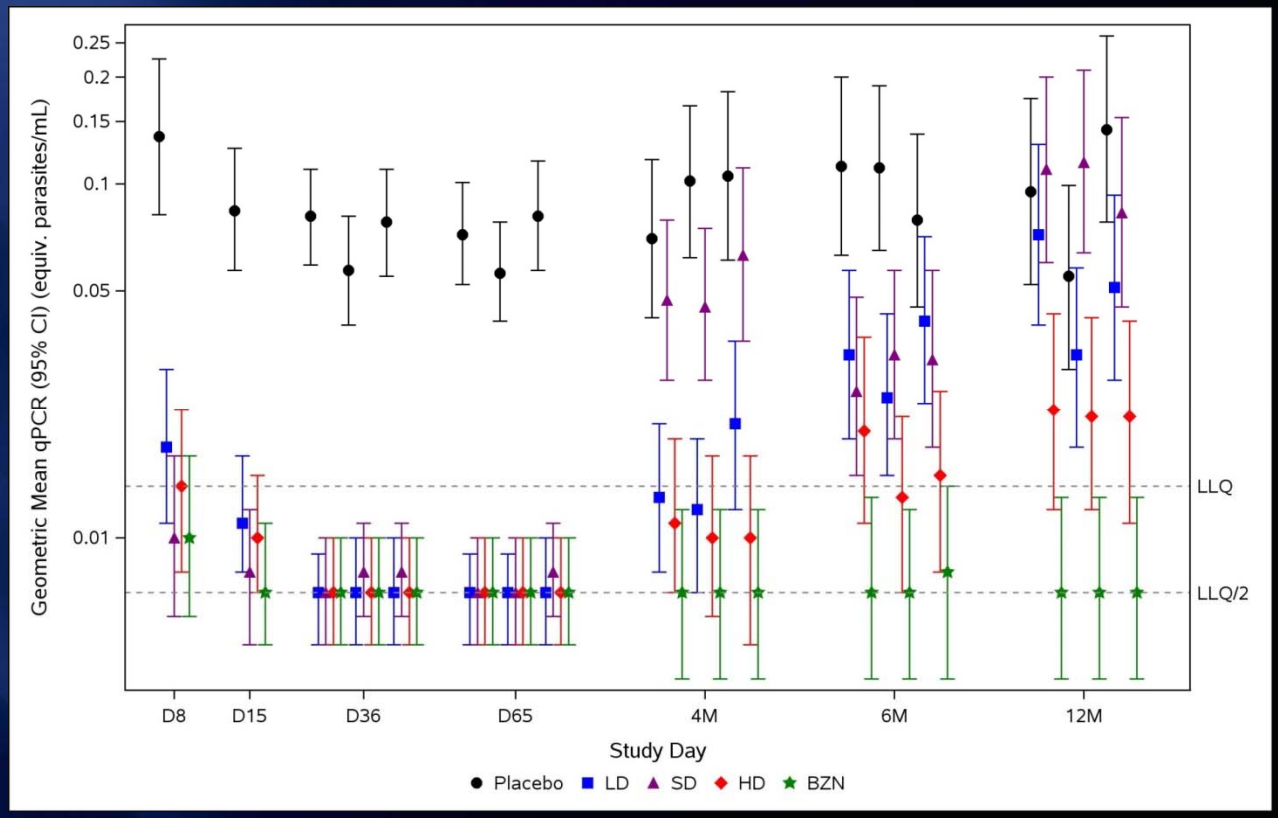
- Significant difference at EOT for all comparisons vs. placebo (<.001)
- Significant difference (one-sided) $p < 0.025$ for the comparison of HD arm vs. placebo and BZN arm vs. placebo for sustained response at 12 months

DNDi-CH-E1224-001

NCT01489228

qPCR Repeated Measure Analysis:

Estimated Values (Population: ITT/Safety)



Stepwise Cox model - time to first relapse from day 8 post .tmt

- Increased hazard of relapse with treatment group (placebo vs. LD and SD) and higher quantitative PCR at baseline (1.10 (1.03, 1.16)
- Decreased hazard of relapse with HD E1224 (0.60 (0.26, 1.37)) and BZN (0.06 (0.02, 0.21))


Gracias
Thank you

Panel Discussion

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Afternoon Panel Discussion

- Trial designs and trial endpoints
  - *What are feasible and acceptable clinical trial designs?*
  - *What primary endpoint(s) would be appropriate for a clinical trial? What are the strengths and weaknesses of clinical outcome endpoints (For example, Is the clinical outcome endpoint well-defined and reliable? When should treatment benefit be assessed? How long would patients need to be followed?)*

Afternoon Panel Discussion

- Trial designs and trial endpoints
  - *What are the strengths and weaknesses of the evidence that change in serology (sero-negative or reduction in titers), negative PCR, or other laboratory test result at a specified time point after treatment are predictive of later clinical outcome? Is accelerated approval a regulatory pathway that could be considered?*

Open Public Comment

Closing Remarks