NEWSLETTER n.2

CHAGAS DISEASE CLINICAL RESEARCH PLATFORM

PLATAFORMA DE INVESTIGACIÓN CLÍNICA EN ENFERMEDAD DE CHAGAS

RIO DE JANEIRO, DECEMBER 2012

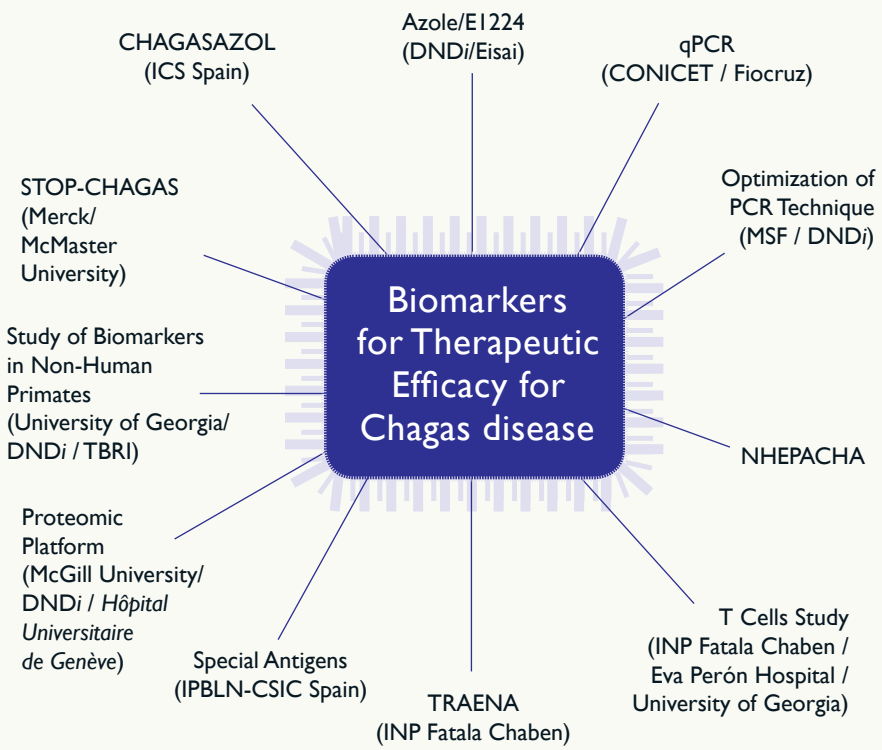
Chagas Disease Clinical Research Platform was inaugurated by DND*i* and partners in 2009, when one hundred years had passed since the discovery of the disease. The Platform's main objective is to provide specific support to the challenges involved in Chagas Research and Development (R&D) through a flexible network oriented to meet the health needs, enable the obtainment and availability of the new drugs for treating the infection caused by *T. cruzi*.

The year 2011 already reflected the birth of a new scenario for the development of new drugs for Chagas, and several clinical trials were started in Latin America and Spain; now, 2012 is a consolidation year, as most of the trials have completed the recruitment of the patients, and entered the follow up phase, thus allowing the thousands of people affected by Chagas disease to keep their expectations.

Since its inauguration, the Platform has committed itself to adapt to the present challenges in the new Chagas R&D scenario, providing tools in support of the studies' application. For this reason, the second edition of the Newsletter intends to go deeper into the challenges to implement those clinical trials, focusing on the need to identify biomarkers for the therapeutic efficacy for Chagas. In this edition we will see the efforts devoted to fill the gap that even today makes it difficult to develop new clinical studies, generate evidence to legitimate therapeutic indications, as well as build trust, so that the carriers of the disease may duly comply with the treatment. Thus, we seek how to map the main initiatives in biomarkers for therapeutic efficacy for Chagas – identifying also the challenges they represent – without pretending, however, an exhaustive presentation. This is just the beginning of the actions to be implemented in order to identify promising findings.

In brief, the second edition of the Platform´s Newsletter provides a new opportunity for all parties concerned about Chagas R&D to work in synergy in the search for answers for those who are affected by the disease.

LANDSCAPE OF THE STUDIES



SUMMARY

Why work on Biomarkers for Chagas? ..... pg. 2

Opening of "Casa Chagas" in Recife ..... pg. 3

Perspectives from the Field ..... pg. 4

Azolic Compounds ..... pg. 6

BENEFIT, Pop-PK and PCR ..... pg. 9

qPCR State of Art pg.10

TRAENA Study ..... pg. 12

T cells response ..... pg. 14

NHEPACHA Network ..... pg. 15

Proteomic Platform ..... pg. 16

Special Antigens ..... pg. 17

Access News ..... pg. 18

Glossary ..... pg. 20

WHY WORK ON BIOMARKERS FOR CHAGAS DISEASE?

Isabela Ribeiro e Eric Chatelain*

Chagas disease is a complex disease with a delicate interplay between the *Trypanosoma cruzi* (*T. cruzi*) parasite and the host immune system to control the infection. It can take a very long time to develop. There is a clear need for new drugs for the treatment of Chagas disease, since currently available drugs have significant compliance issues, including frequent side effects and limited evidence on efficacy in the chronic phase of the disease.

An important hurdle for the development of new drugs for chronic Chagas disease has been the lack of clear and early markers, which correlate with clinical treatment outcome.

If one defines a surrogate marker as a biological marker intended to substitute for a clinical endpoint aiming to predict clinical benefit (on the basis of epidemiological, therapeutic, pathophysiological or other scientific evidence), the traditionally accepted surrogate for Chagas disease –i.e. seroconversion– requires a very long period of follow-up which is not compatible with drug development.

Polymerase chain reaction (PCR) is currently seen as a potential method of choice for detecting parasites in the blood of patients (for the purpose of diagnostics), and a lot of work is being performed based on this method for the detection of *T. cruzi* in blood samples (including a PCR study to optimize the sampling procedure; international RT-PCR method optimization and validation; trials as E1224 Phase II, STOP-CHAGAS, CHAGASAZOL, BENEFIT and TRAENA). Although this technique can be very useful to assess treatment failures in clinical trials, the challenge is to maximize sensitivity of the method and validate it for use as post-treatment follow-up/determination of treatment efficacy.

There is therefore a clear need to explore alternative biomarkers that could be used as surrogate for test of treatment efficacy (on its own or in combination with PCR). Opportunities are arising, but long-term commitment is needed to overcome the existing challenges.

The challenges:

1) Further research on early criteria of therapeutic response
2) Work to further optimize and validate the PCR methods for evaluation of therapeutic efficacy
3) Identification of other biomarkers to be used as surrogate for test of treatment efficacy that is more effective and quicker than seroconversion. Studies are being started using a combination of serological (lytic antibodies, anti-Tc24 antibodies, multiplex), ELISAs for specific markers (ApoAI, BNP, Pro-thrombotic factor), gene expression profiling, and proteomics methods

3.1) Integrate results and preparation of Phase III in collaboration with partners (PAHO, Chagas Disease Clinical Research Platform, NHEPACHA, manufacturers)

3.2) Encourage new partners to be open to new ideas/technologies, break dogma, and define a Target Product Profile for biomarkers for Chagas disease

3.3) Define a development strategy including regulatory aspects

Drugs for Neglected Diseases initiative

DNDi AMÉRICA LATINA
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OPENING OF *CASA DE CHAGAS* IN RECIFE
INTERVIEW WITH DR. WILSON OLIVEIRA JR*


Dr. Wilson de Oliveira Jr.*

The Pernambuco Home for Chagas Disease and Heart Failure was officially opened at the beginning of 2012, but its long history started in fact in the 1980s. This home, a pioneer in this area, is a multiprofessional outpatient center and seat of the Association of Carriers of Chagas Disease, whose members provide voluntary support for the home.

Cardiologist Wilson de Oliveira Jr., who presently coordinates the outpatient center, spoke with us about the history of Casa de Chagas and how it became renowned. Moreover, and thanks to his wide experience in the clinical attention of patients who are carriers of the disease, Dr. Wilson also discusses the difficulties caused by the lack of biomarkers for therapeutic efficacy of Chagas disease.

— Can you tell us the history of the Pernambuco Home for Chagas Disease and Heart Failure?

— By the mid-1980s, the University Hospital Oswaldo Cruz (HUOC), which is associated with the University of Pernambuco (UPE), was a cardiology reference hospital for the state, and as such it received a number of patients suffering Chagas disease, both for diagnosis purposes as well as for the treatment of Chagas-related heart disease. At that time, we coordinated the cardiomyopathy and heart disease infirmary and treated Chagas patients every day, especially those undergoing the more serious phase of the disease. Gradually, the requests of patients made us consider the need for creating an outpatient center to address biopsychosocial aspects and, above all, to have greater access to monitor them. At the same time, we created the Association of Carriers of Chagas Disease, a leading institution in these activities. In August 1987, we started our associative life and care actions. Many barriers have been overcome since then. Sometimes, in the face of so many confrontations, we thought we would be unable to continue. But the team continued to grow, new partners joined us, and we started receiving support. Then, in 2009, thanks to the contribution of UPE Rector Office, and PROCAPE Board, we received the resources to create the Pernambuco Home for Chagas Disease and Heart Failure, which provides a multiprofessional outpatient center and is also the seat of the Association.**

— The Home, which was opened at the beginning of this year, is exclusively devoted to the care of Chagas disease carriers. Can you tell us about the daily routine?

— At Casa de Chagas, as it is commonly called, the outpatient center, which includes healthcare providers, doctors, nurses, psychologists, nutritionists, and biomedical engineers, provides multiprofessional care to Chagas disease carriers and heart failure patients – with or without Chagas etiology. There is also a sector for evaluating patients with pacemakers and defibrillators. Casa de Chagas is also the seat of the Association, including the fundraising bazaar, and a specific area where patients interact and develop education and cultural activities. Since the clinical center is associated with the University of Pernambuco, we receive graduate and research students, who work in different professional areas.

— Today, when providing direct care to Chagas disease carriers, what are the main challenges you face in the assessment of therapeutic efficacy of the disease?

— We, as clinical doctors, look forward to the possibility of having biomarkers that enable us to be more accurate about the best treatment for a specific patient. There are no reliable tools available at the present time that ensure the efficacy of drugs used for curing patients, in particular for those in the chronic phase of the disease. Lack of cure markers has turned clinical follow-up difficult, as well as prognosis stratification and efficacy studies of new drugs.

In consequence, all the community has paid special interest to the studies, in the belief that in the near future we may have biomarkers to evaluate cure criteria. In addition to technical aspects such as sensitivity, specificity, and validation, such biomarkers will have to be accessible in terms of cost and operational feasibility, as we are dealing with a neglected disease.

— In 2010, the International Federation of People Affected by Chagas Disease (FINDECHAGAS) was created. What is the importance of the Federation for the activities of patient associations?

— We believe that FINDECHAGAS actions will strengthen patient associations, by making them more visible and bringing patients to leading roles in the fight for humanized and dignified care.

Through FINDECHAGAS, patients may have a louder voice that may be heard worldwide. The Federation will enable them to raise public awareness in civil society, as well as in the scientific community over priorities and needs of Chagas disease carriers, since Associations, when working together, enhance their negotiation position. •

** University of Pernambuco and Chagas Disease and Heart Failure Outpatient Center*

*** The outpatient center has received affective and effective aid from Professor João Carlos Pinto Dias from the start.*

voices from the field

NO TEST OF CURE: PERSPECTIVES FROM THE FIELD

Julien Potet*

There is a significant hole in the Chagas disease toolbox: a test of cure. Its absence has had a detrimental effect on R&D, as it explains why it takes so long to determine precisely the efficacy of new drugs. It also means that doctors may lack confidence in treating patients, while patients are often unwilling to embark on a two-month course of treatment without knowing the clinical outcome. At *Médecins Sans Frontières*/Doctors Without Borders (MSF), we come up against this obstacle on a daily basis.

The success of anti-parasitic treatment for patients with chronic Chagas disease is currently determined by the disappearance of antibodies, using the same serological tests that are used for primary diagnosis. The time taken to reach 'seronegativisation' can vary widely, ranging from a couple of years in children infected by the *T.cruzi I* strain, up to several decades in some patients with the *T.cruzi II* strain. The rate of antibody clearance is influenced by the patient's age, duration of infection, initial parasite load, and strain of parasite.

In practice, the only option is often to ask patients to undergo serological tests in a hospital-based lab on a regular basis – ideally once a year – until the test shows negative. It is a tedious and uncertain process, and, despite intensive counseling by community health workers, many patients treated by MSF become lost to follow-up in the first few years after treatment.

The existence of a practical test of cure would also make a big difference in collecting data on the outcomes of current treatments. There is strong evidence to recommend treatment of all chronic indeterminate cases. But at field level, knowing more precisely the efficacy rate of benznidazole – in different age groups and in different epidemiological settings – would definitely help convince patients, who quite legitimately want to be told precisely their individual chances of being cured, to start treatment.

Developing a practical test of cure for chronic Chagas disease is therefore a priority if we are to meet patients' needs. This test should confirm or exclude parasitological cure within a period of two years after treatment, be valid for all *T. cruzi* strains, and be available in regional hospitals.

While a number of different initiatives to identify new biomarkers of parasitological cure exist, coordination is lacking to share progress and findings. More incentives would help. When it comes to translating basic research into prototype development, more public funding will be critical. New, innovative R&D incentives – such as an innovation inducement prize that would reward a major scientific breakthrough in Chagas biomarkers research – could attract more potential solvers.

Research would also be enhanced if there were more banks with blood samples of Chagas patients, and if existing serum banks were accessible for research purposes.

More fundamentally, an international R&D convention – as recommended by the World Health Organization's Consultative Expert Working Group on R&D: Financing and Coordination – would secure a sustainable basis for innovation in neglected areas. Under this treaty, countries would agree to adequate and predictable financing to deliver affordable and accessible products focused on the priority health needs of developing countries – a category that would definitely include a Chagas test of cure.

Developing such a test requires good science, but also political will. To make it happen, academics, patients, and civil society organizations will have to work together. It is an effort worth making, because for millions of people living with Chagas disease, the existence of a test of cure will be life-changing. •

** Médecins Sans Frontières Access Campaign*

Miriam Quispe Brito, a Bolivian mother of three, says: "I was treated for Chagas disease in 2004, after my first child was born, and was followed up with controls for two years. But when I was pregnant for the second time, I was still very worried, as I did not know whether I was actually cured and if there was still a risk to my baby."

Miriam gave birth to twins who were later found not to be infected with Chagas disease.

AZOLIC COMPOUNDS

INTEGRATION OF THE EVIDENCE OF THE PROOF OF CONCEPT

At present the approved treatment options against Chagas disease are Benznidazole (BNZ) and Nifurtimox. Registered cases show that the treatment efficacy in the acute phase of the disease is between 65 and 80%, practically reaching 100% in cases of congenital transmission treated during the early years of life. In the cases of chronic infection, the degree of evidence is considerably lower, with serum response rates between 15 and 40%, but with few data on parasitological response collected in a systematic, prospective way. This, combined with a high rate of adverse effects that in 20% of cases forces the definitive suspension of treatment results in a therapeutic scenario that is poor in terms of efficacy and safety against Chagas disease. For this reason there is a pressing need to develop new treatments.

In the search to respond to such need, a new scenario started in 2011 with the development of new drugs, stemming from the several clinical trials in Latin America and Spain. Anti-fungal triazole derivatives are likely prospects of the new treatments and widely regarded as having a potential to inhibit ergosterol biosynthesis in the *T. cruzi*, as it is an essential component for parasite growth and survival. Consensus in this direction has led to the design of two studies with the Posaconazole triazole –STOP-CHAGAS and CHAGASAZOL– and one study with a Ravuconazole pro-drug, E1224. All three studies are currently in the implementation phase, with a scope of investigational assessment, proof of concept (PoC), to ascertain the activity of such components in Chagas disease.

The design of these clinical trials is the result of extensive exchange among the investigators (Alejandro Hasslocher Moreno, Alejandro Luquetti, Anis Rassi Jr., Carlos Morillo, Faustino Torrico, Felipe Guhl, Héctor Freilij, Israel Molina, Jaime Altcheh, João Carlos Pinto Dias, Joaquim Gascón, Jose Rodrigues Coura, Laurence Flevaud, Michel Vaillant, Nines Lima, Pedro Albajar, Sergio Sosa Estani, Tom Ellman, and the participants of the meetings organized by WHO/TDR, DND*i* and Merck in the last years) and thus they have certain similarities, which should facilitate integration and meta-analysis of the results (*vide* description details below). A workshop has been scheduled, at the next Chagas Disease Clinical Research Platform meeting, to review and prepare an integrated analysis protocol involving the different studies.

PROOF OF CONCEPT-POSACONAZOLE STUDIES

STOP-CHAGAS (Study of Oral Posaconazole in the Treatment of Asymptomatic Chronic Chagas Disease) is a randomized, placebo-controlled, blind study for Posaconazole (POS) aimed at exploring POS efficacy as compared to BNZ among patients in the indeterminate phase of Chagas disease without evidence of cardiac compromise. STOP-CHAGAS is conducted in four countries (Argentina, Colombia, Mexico and Venezuela) and is coordinated by Dr. Carlos Morillo, Population Health Institute, McMaster University, Hamilton, Canada.

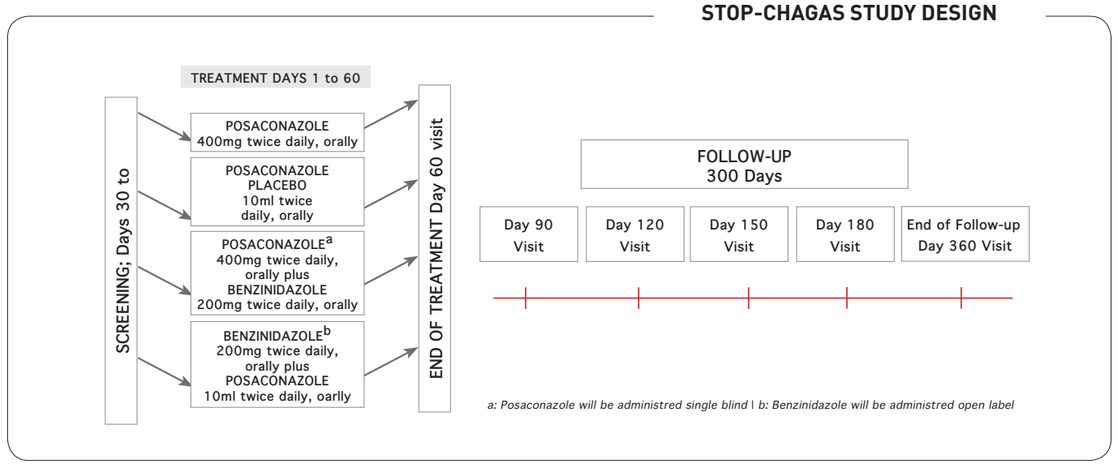
CHAGASAZOL is an open, non-masked, randomized, "futility" assessment study to evaluate POS safety and efficacy to induce a maintained negativity of parasite load in the chronic phase of Chagas disease. The study is conducted in three centers in Barcelona and is coordinated by Dr. Israel Molina, *Hospital Universitario Vall d'Hebron*, Barcelona, Spain.

PROOF OF CONCEPT-RAVUCONAZOLE, PRO-DRUG

DND*i*-CH-E1224-001 is a placebo-controlled, active (BNZ), randomized, prospective, blind to evaluator, blind as regards E1224 and placebo, comparative study that evaluates safety and efficacy of different doses of E1224 in parasitemia negativity at the end of treatment among individuals undergoing the indeterminate chronic phase of Chagas disease. Other evaluations are performed with up to 12-months therapeutic response follow-up by PCR, as well as the evaluation of different likely biomarkers. The study is conducted by Drugs for Neglected Diseases *initiative* (DND*i*), coordinated by Dr. Isabela Ribeiro in association with the Japanese pharmaceutical company Eisai, at two centers in Cochabamba and Tarija, in Bolivia. Principal Investigators are Dr. Joaquim Gascón and Dr. Faustino Torrico.

STOP-CHAGAS STUDY DESIGN

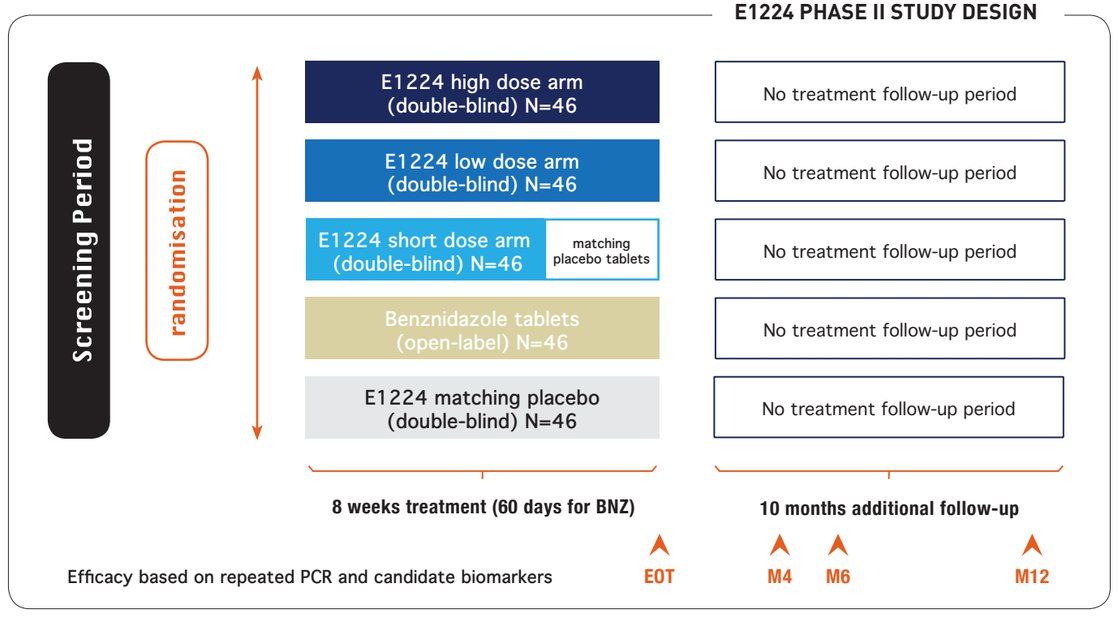
- Group 1: POS 400 mg (10 mL)/12h (n=40)
- Group 2: POS PO placebo 400 mg/12h (n=40)
- Group 3: POS PO 400 mg/12h and BNZ PO 200 mg/12h (n=40)
- Group 4: POS PO placebo 400 mg/12h and BNZ PO 200 mg/12h (n=40)



E1224 STUDY DESIGN

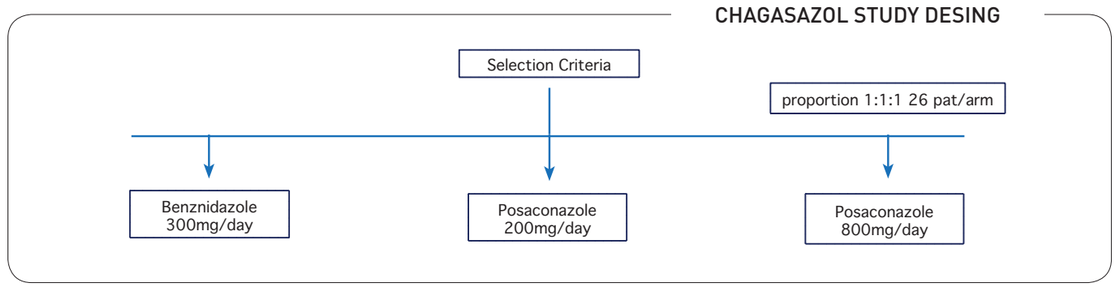
A total of 230 patients were included and divided into five parallel groups (46 in each group):

- Three groups received one of the different E1224 oral doses (high dose for 8 weeks; low dose for 8 weeks; short dose for 4 weeks, matched with placebo until the 8 weeks).
- One group received placebo as negative control.
- One group received BNZ as positive control.



CHAGASAZOL STUDY DESIGN

A total of 78 patients were included, 26 in each arm, having received randomized Benznidazole or Posaconazole in two dosages. One considered the maximum dose authorized in human beings, 800mg/day and another one of 200mg/day. Also a pharmacokinetic study has been conducted in arms receiving Posaconazole.



Visits for blood sampling

	EOT (2 months)	4 months	5 months	6 months	12 months
E1224	x	x	-	x	x
STOP-CHAGAS	x	x	-	x	x
CHAGASAZOL	x	-	-	x	x

Arms of the study

E1224	STOP-CHAGAS	CHAGASAZOL
BZN	BZN	BZN
E1224 (high dose)	POS 400mg	POS (high dose 800mg)
E1224 (low dose)	POS / Placebo	POS (low dose 200mg)
E1224 / Placebo (short doses)	POS 400mg + BZN 200mg BZN 200mg + POS 100mg	
Placebo	Placebo	-

THERAPEUTICAL RESPONSE EVALUATION

In all three studies the primary endpoint will be the parasitemia elimination in PCR-real time (RT-PCR or qPCR) with qualitative evaluation. In the E1224 study, primary response is evaluated at the end of treatment with serial PCR sampling (3 PCR negative results). CHAGASAZOL and STOP-CHAGAS studies evaluate therapeutic response at the end of 12-month follow-up with a single PCR sample in each point. Sampling time(s) in the different studies will allow integrated data analysis (particularly the evaluation of sustained parasite response after 12 months).

PRESENT STATUS OF STUDIES

In the STOP-CHAGAS study recruiting is in progress. The first patient was included in Argentina in November 2011 and patient follow-up should end by early 2013, with results expected by the first quarter of 2014. In turn, recruiting for E1224 study ended in June 2012, with a total of 231 patients. Treatment phase was also completed and the primary endpoint of efficacy evaluation will be performed in the last quarter of 2012. Follow-up of last patients will be performed by late June 2013. CHAGASAZOL study ended its inclusion phase in August 2011 and patient follow-up will be completed in September 2012. Results are expected by September/October 2012.

THERAPEUTIC EFFICACY BIOMARKERS

Lack of early therapeutic effectiveness markers is among the causes of controversy in the treatment of Chagas disease, which has slowed down research and development of new drugs. In the search for a response to this need, E1224 and CHAGASAZOL studies will identify therapeutic effectiveness markers. In the specific case of E1224, the evaluation of parasite response sustained for one year, by negative series of PCR qualitative results, and the evaluation of changes in the levels of different biomarkers (brain natriuretic peptide, troponine, prothrombotic markers, apolipoprotein A1) will be generated as secondary results.

UPDATE ON OTHER STUDIES

BENEFIT

BENEFIT study (BENznidazole Evaluation For Interrupting Trypanosomiasis) is the largest multinational, multicenter, randomized clinical trial ever conducted on *T. cruzi*–infected patients with evidence of early Chagas cardiomyopathy. BENEFIT is evaluating the effect of etiologic treatment with benznidazole in reducing death, cardiac resuscitation, sustained ventricular tachycardia, pacemaker or cardioverter defibrillator implantation, congestive heart failure (CHF), ictus or systemic embolism, and heart transplantation.

The primary objective of the BENEFIT pilot study is to establish the efficacy of benznidazole 5 mg/kg administered for 60 days to reduce parasite load, detected through real-time PCR, in patients with Chagas cardiomyopathy who are PCR-positive at the time of randomization. The study will also look at outcomes in terms of mortality and frequency of clinically relevant cardiovascular events.

Secondary study objectives are to determine if etiologic treatment reverts or slows heart-failure progression based on impaired left ventricular function, emergence of new electrocardiographic alterations, or reduced CHF symptoms, as well as lower parasite load.

BENEFIT is being conducted in five countries (Argentina, Bolivia, Brazil, Colombia, El Salvador) at 50 participating centers.

The first patient was enrolled in Brazil in November 2004, and the last one in October 2011. Follow-up will end in November 2014, and results of the study are expected by the first quarter of 2015. A total of 2,856 patients were randomized. Breakdown on a per-country basis is shown in the table.

COUNTRY	CENTERS	TOTAL RANDOMIZED PATIENTS
Argentina	19	559
Bolivia	1	357
Brazil	24	1360
Colombia	5	502
El Salvador	1	778
TOTAL	50	2856

Central coordination is being conducted by the Population Health Research Institute of McMaster University (Hamilton, Canada), with regional coordination by Dante Pazzanese Hospital (São Paulo, Brazil). BENEFIT is funded by the Canadian Institute of Health Research, the World Trade Organization-TDR, *Hospital das Clínicas de Ribeirão Preto* (Brazil), and the Ministry of Health and Bunge & Born Foundation (Argentina).

PCR TECHNIQUE OPTIMIZATION

Polymerase chain reaction (PCR) is a technique recognized for being able to identify the therapeutic failure in Chagas disease, but also for its potential to become a post-treatment stage tool as an evaluation indicator of the therapeutic efficacy. In this case, *Médecins Sans Frontières* (MSF), DND*i* and *Universidad de San Simón* work together in the implementation of the study "Optimization of the PCR technique sampling procedure to assess parasitological response in patients undergoing the chronic phase of Chagas disease who are treated with Benznidazole, in Aiquile, Bolivia". The "PCR study" seeks to optimize and validate the technique by investigating the ideal volume and the number of samples to improve sensitivity.

The study started in March 2011 and completed the recruitment of 220 patients by December 2011. The study was carried out at a single center located in Aiquile, Bolivia, but patients were recruited from 16 adjacent communities. Patients' age required for the admission was between 18 and 60 years old.

Monitoring visits by DND*i* still continue and MSF is currently performing the 6 and 12 monthly visits to the communities. The data analysis process is conducted through an association of DND*i* with the Oswaldo Cruz Institution (Fiocruz) in Brazil, which began in April 2012. The final study results are expected in March 2013.

POP-PK STUDY

Due to the absolute lack of information about pharmacokinetics (PK) of Benznidazole (BNZ) in the pediatric population and its relationship with the safety and the efficacy of the treatment, there was an emerging need for a Phase IV study to describe the population PK parameters of BNZ in its two formulations (100 mg and 12.5 mg) among children –between 0 and 12 years old – with acute or early chronic indeterminate Chagas disease.

For this purpose, the POP-PK study began on May 31, 2011. A total of 80 patients with Chagas disease were recruited by late April 2012, including the congenital cases, children suffering from the early chronic indeterminate disease and the vector acute cases. The whole duration of the study is 15 months, from the baseline report until the blood collection of the last recruited patient. Follow up of the patients is now underway, and a Statistical Analysis Plan (SAP) of the study was initiated. Next step is to analyze the last samples to prepare a final report.

The trial is performed at five centers in Argentina: *Hospital de Niños Ricardo Gutiérrez* and Instituto *Nacional de Parasitología Dr. Mario Fatala Chabén*, both located in Buenos Aires; *Hospital de Niños* in the Province of Jujuy; *Centro de Chagas y Patología Regional*, in the Province of Santiago del Estero and *Hospital Público Materno Infantil* in the Province of Salta.

STATE OF THE ART QPCR

APPLICATION IN THE FOLLOW-UP OF CHAGAS DISEASE THERAPEUTIC RESPONSE

The two following articles describe the state of the art in the assessment of PCR techniques. In his article, Dr. Schijman describes the process of simultaneous assessment of different PCR strategies, among which is the methodology described in the article by Dr. Constança Britto, following international recommendations for the estimation of precision, sensitivity, specificity and the capability to be repeated for the use in the therapeutic monitoring of patients with chronic infection by *T. cruzi.*

Constança Carvalho Britto*

Because of the dull perspectives for the treatment with present drugs and the absence of immune intervention against infection with *Trypanosoma cruzi*, Chagas Disease (CD) continues posing a serious public health problem. Proof of treatment efficacy in CD shows limitations as regards the absence of the consistent healing criteria as well as low sensitivity of the conventional parasite practices. Studies have pointed out a higher sensitivity of PCR molecular trials in determining parasite persistence in chronic individuals who had been treated and were controlled for long periods after the treatment. However, it has not yet been established if a lower parasite load would result in improved clinical symptoms among people with CD in its chronic phase and myocardiopathy, who have been treated with anti-*T. cruzi* chemotherapy. Evaluation of methodologies to estimate absolute levels of circulating *T. Cruzi* in infected individuals would enable us to describe a possible correlation between parasite load and the progress of the disease, in addition to being very useful in the molecular characterization of the parasite population, prognosis evaluation and the therapeutic efficacy.

This project aims at evaluating markers in the *T. cruzi* genome and their application in the development of a high-sensitivity, reproducible molecular method to estimate the parasite load in CD. With this purpose, SYBR Green and PCR TaqMan® systems shall be compared in Real Time quantitative (qPCR). The one showing the best performance shall be validated with clinical samples related to the BENEFIT (Benznidazole Evaluation for Interrupting Trypanosomiasis) study, an international multi-center, randomized, double-blind, placebo-controlled study designed to evaluate the efficacy of treatment with Benznidazole among patients with chronic Chagas myocardiopathy. Efforts will be made to find out if in the absence of a parasite healing, the drug shall be capable of reducing parasitemia among these patients, and if in the long run a reduced parasite load would result in improved clinical symptoms in humans. The project is being conducted at the Molecular Biology Laboratory and at the Institute of Endemic Diseases, Oswaldo Cruz Foundation (Fiocruz), in cooperation with the Molecular Biology Institute of Paraná (*Instituto Carlos Chagas*/Fiocruz). We hope to achieve the following results: (1) One or more methodologies to estimate the parasite load of *T. cruzi* among the infected individuals, (2) Technology transfer to the health services; (3) The strengthening of the institutional relationships between Fiocruz and the Brazilian Ministry of Health.

The study was designed in 2009, within the framework of the Fiocruz Chagas Disease Integrated Program (PIDC, in Portuguese) – and is expected to end in 2013/2014. Funding is being provided by the universal bid – CNPq No.14/2011 and FAPERJ No.03/2012 – Support for the Study of Neglected and Re-emerging Diseases and by the Program for the Technological Development of Health Supplies (PDTIS/Fiocruz in Portuguese). The project is at an advanced stage, where qPCR methodology is available with the use of SYBR Green for the BENEFIT study; 150 patients (pre-treatment) have been screened in the pilot study. Right now we are close to ending the standardization of a Multiplex proof with TaqMan system (one target is *T.cruzi* and another, a human constitution gene) so as to introduce this study, more robust, for the quantification of BENEFIT patients. As regards to Brazil, 1111 patients will be quantitatively evaluated in two stages: pre-treatment and last visit (2 or 3 years after treatment). Upon the ending of this project we will be able to provide a methodology to be used in molecular diagnosis that will allow us to estimate parasite load among the patients of Chagas disease. •

*Molecular Biology Laboratory and Endemic Diseases, Oswaldo Cruz Institute (Fiocruz).

Schijman, Alejandro Gabriel**

Real-time PCR (PCRq) strategies have been proposed as tools to quantify parasite load in clinical samples of patients with *Trypanosoma cruzi* infection who are receiving etiological treatment, with the purpose of providing a subrogated therapeutic response marker.

Strenuous efforts have been devoted to the standardization and validation of these methodologies, especially considering Chagas disease's complex stages, which feature very different parasite-load ranges, the genetic diversity of natural parasite populations that involve sequence polymorphisms, and the gene dosage of molecular targets used for the essays, all of which determines that the same molecular method may show different diagnostic sensitivities for every clinical and epidemiological scenario.

Within this framework, the World Health Organization (WHO), the Pan American Health Organization (PAHO) and other international entities, such as the Drugs for Neglected Diseases *initiative* (DND*i*) and *Médecins Sans Frontières*/Doctors Without Borders (MSF), have joined efforts to support investigations designed to find standardized operative procedures for obtaining and amplifying nucleic acids in peripheral blood samples of patients with Chagas disease.

For this purpose, between November 2007 and December 2011, coordinated international activities were performed where representatives of molecular diagnosis laboratories were engaged from a number of endemic and non-endemic countries, including Latin America, USA, and Europe. These activities allowed for the simultaneous assessment of different real-time PCR strategies and the comparison of results in sample panels of individuals from the participa-ting countries. The evolution of these investigations resulted in the validation of two multiplex-type real-time PCR strategies, using TaqMan probes of highly repetitive parasite DNA sequences (such as the minicircle conserved region sequence and the satellite nuclear sequence before an extrinsic heterologous control, as an internal amplification control). These methods were based on international recommendations and show adequate sensitivity and specificity levels for the monitoring of chronically infected patients.

One of these methods, based on a DNA satellite sequence, is being or has been applied in clinical studies such as in the Adult Treatment (TRAENA) study (see pg. 12-13); the MSF/DND*i* study "Optimization of sampling procedure for PCR technique to assess parasitological response in patients undergoing the Chronic phase of Chagas disease who are treated with benznidazole in Aiquile, Bolivia" (see pg. 9); E1224 study (see pg. 6-8); and the pharmacokinetic study of benznidazole in children (DND*i*, LAFEPE, Ricardo Gutierrez Hospital, Buenos Aires, Argentina).

Based on these ongoing studies, 2013 is expected to be a highly revealing year, not only about the usefulness of these tools but also about the therapeutic efficacy of the drugs under study. •

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TRAENA STUDY
EVALUATION OF POTENTIAL BIOMARKERS OF THERAPEUTIC EFFICACY

Adelina Riarte*, Elsa Velázquez[1], Nilda Prado*, Alejandro G. Schijman,** Juan C Ramírez**
Ana María De Rissio*, Yolanda Hernández*, Mónica Esteva*, Concepción Luna*, Angel Sinagra*, Andres M Ruiz[1]

TRAENA study (Treatment in adult patients - ***TRA**tamiento **EN** pacientes **A**dultos*, in Spanish) is a clinical randomized, double-blind, Phase III study conducted at *Instituto Nacional de Parasitología Dr. Mario Fatala Chaben* – (INP, in Spanish), the purpose of which is to determine if a parasiticide drug such as Benznidazole (BNZ) is capable of changing natural evolution of chronic Chagas disease in adult patients. Patients will be screened, from the urban population, in the capital city of Buenos Aires and in the province of Buenos Aires; screening breakdown followed by the natural distribution of Chagas disease in adult patients.

The sample was estimated in 750 patients who were randomized to BNZ or Placebo and the administration dose was 5mg/kg/d for 60 days. Post-treatment (p.t.) evolution was performed by ELISA F29, ELISA conventional (ELISAc) and polymerase chain reaction in real time (qPCR).

Under TRAENA, patients are followed-up during a p.t. period of 7-11 years. Serum and parasitology data refer to the total patient population, irrespective of assignment to BNZ or Placebo, since at present the study remains blind. Evaluation of recombinant antigen F29 of *T. cruzi* developed at INP has proved its value as serum indicator of therapeutic efficacy in the study conducted among children with *T. cruzi* chronic infection[1]. TRAENA antibody curve showed at least two populations; one of them with a stable serum profile and the other with a decrease in antibody values until persistent negativity (23.6%) by ELISA F29, which suggests the predictive value of antigen rF29 of *T. cruzi* and the potential therapeutic effect by the action of BNZ in the adult population with chronic Chagas disease evaluated by this biomarker (Fig. 1). An interesting result, ELISAc performed with in-house antigens of the Tulahuen strain showed serum negativity in 22.2% of patients, which seems to indicate that certain *T. cruzi* antigens in conventional serology and obtained through certain procedures might equally be predictors of therapeutic efficacy.

As for parasitemia quantification, measured by qPCR, it was positive in 84.30% of patients in the early period of the study. Table 1 shows variations in the evaluation of samples by qPCR during follow-up, positive in 44.03% after 60 days p.t., in 37.41% between 8 and 16 months p.t. and in 27.19% at the end of the follow-up period 9-11 years p.t., which suggests that one year p.t. would be a potential time of therapeutic effect by qPCR and would stress the value of qPCR as the therapeutic efficacy biomarker in the adult population with Chagas disease treated with BNZ •

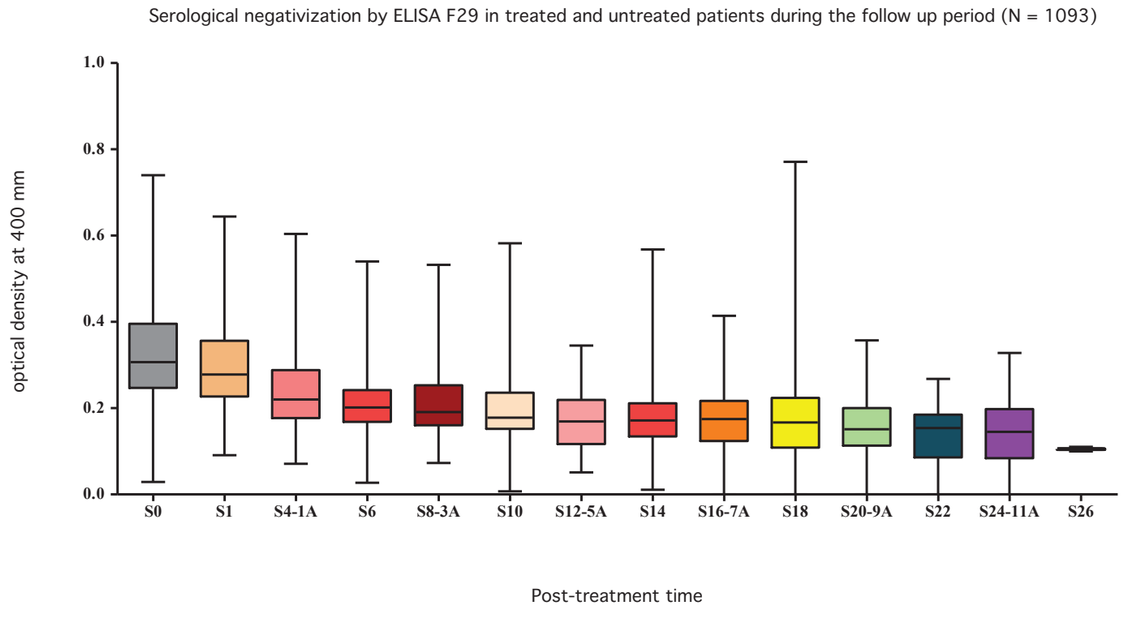

Fig. 1. Serum profile on anti *T. cruzi* antibodies during the follow-up, spotted by ELISA rF29 (cut point 0.175), in the total population irrespective of the treatment assigned. The decrease in antibody value was progressively different since year 1 p.t. until the end of the follow-up as compared to the initial time SO. One-way Anova test - P<0.0001. Dunnet test of multiple comparison.

1 Sosa Estani S, Segura EL, Ruiz AM, Velazquez E, Porcel B, Yampotis C. Chemotherapy with benznidazole in children in indeterminate phase of Chagas Disease. Am J Trop Med Hyg 1998; 59:526-529.

Note: This study was funded by six subsidies from the national agencies of Argentina and international agencies, including PAHO/WHO and DND*i*.

Table 1.
Real-time PCR blind data among treated and non-treated patients. (Benznidazole vs placebo) during treatment.

PCRs	Time 0	60 days p.t.	8-16 months p.t.	9-11 years p.t.
Total (N)	896	285	286	228
Positive (N)	755	211	107	62
Non-Detectable (N)	141	74	181	156
Positive PCR %	84,30	74,03	37,41	27,19
Negative PCR %	15,70	25,96	63,29	68,42

Table shows qPCR values in the general population of the study during the follow-up, irrespective of the assignment to the treatment. High qPCR values are observed at 0-time and variations with a decreasing trend in the follow-up continuity.

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1 CONICET (National Council for Scientific and Technical Research, in Spanish). Ministry of Science, Technology and Productive Innovation, Argentina.

T CELL RESPONSE:
BIOMARKER OF TREATMENT EFFICACY IN CHRONIC CHAGAS DISEASE

Susana Laucella *

Certain immune markers of T cell responses regulated by antigen load hold promise as potential treatment biomarkers for chronic infections (1, 2). After the clearance of an acute infection, T cells shape a pool of stable, highly polyfunctional pathogen-specific memory T cells that can persist long term via self-renewal in the absence of antigen. However, T cells generated during chronic infections are subjected to negative regulation, lose polyfunctionality, and become antigen dependent, rather than developing the ability to persist long term via antigen-independent self-renewal (3,4). A hierarchical loss of different T cell functions, known as immune exhaustion, has been proposed, with the production of IL-2 being the first function compromised followed by the ability to make tumor necrosis factor alpha (TNF-α), while interferon-gamma (IFN-γ) production was most resistant to functional exhaustion (5).

Immune exhaustion also appears to occur in human chronic infection with *T. cruzi*. Chronically *T. cruzi*-infected subjects with no or mild clinical disease have a significantly higher frequency of IFN-γ producing T cells specific for *T. cruzi* antigens than do individuals with more severe disease (6), with IFN-γ single-producing T cells as the predominant functional profile with very low le-vels of dual functional IFN-γ$^+$/IL-2$^+$ T cells (7). The phenotype of these IFN-γ-producing T cells also revealed that the parasite specific T cell compartment is mainly composed by effector/effector memory T cells, supporting the notion that T cell maintenance in the chronic phase of *T. cruzi* infection depends on parasite persistence. Therefore, it can be hypothesized that a decrease in parasite load would result in a reduction of parasite-specific IFN-γ-producing T cells.

Towards this aim, the effect of treatment with benznidazole on the frequency and function of parasite-specific T cells was monitored in chronically infec-ted subjects in a 48- to 60-month follow-up study. Within 12 months post-treatment, the frequency of IFN-γ-producing T cells specific for *T. cruzi* significantly decreased in the treated group compared to untreated subjects and was below the level of detection in 47% out of the total treated subjects evaluated at 36 months post-treatment (8). The shift to negative IFN-γ T cell responses was highly associated with an early (i.e. between 2 and 6 months post-treatment) increase in IFN-γ-producing T cells following treatment with benznidazole, as well as decreases in serological response over time, as determined using a multiplex assay or conventional serologic testing. However, a rebound in parasite-specific T cells enriched in polyfunctional IFN-γ$^+$/IL-2$^+$-producing T cells was observed between 36 and 72 months following treatment (Pérez-Mazliah, personal communication), while serological titers remained low. When exami-ning baseline parasite-specific T cell responses, those subjects with rebound responses or with significant decreases in IFN-γ-producing T cells following treatment with benznidazole had polyfunctional responses prior to treatment. Conversely, those patients in which T cell responses did not vary following treatment with benznidazole displayed mainly a profile of IFN-γ single-producing T cells prior to treatment. Untreated patients did not show any change in parasite-specific T cell responses during the follow-up period.

In summation, monitoring of parasite-specific T cell responses during etiological treatment might be useful as: 1) an early treatment response marker to give an early indication of response to chemotherapy, through the initial increase followed by a decay in IFN-γ-producing T cells; 2) a late treatment marker to denote at least a decrease in parasite load or even sterilizing cure versus parasite persistence, through alterations in the frequency of polyfunctional T cells (i.e. increased specific polyfunctional IFN-γ$^+$/IL-2$^+$/TNF-α$^+$ T cells along with reduced IFN-γ single-positive and TNF-α single-positive T cells have been associated with successful responses to chemotherapy in other chronic infections); and 3) a potential predictor of treatment response according to the functional status of parasite-specific T cell responses prior to treatment. •

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**INP Dr. Mario Fatala Chaben / National Council for Scientific and Technical Research*

NHEPACHA NETWORK: COLLABORATING WITH THE ASSESSMENT OF THERAPEUTIC EFFICACY BIOMARKERS

Joaquim Gascon*

Despite the success of vector control programs in most endemic areas, 8-10 million people are still infected by *T. cruzi*. Significant progress has been seen in recent years in Chagas disease diagnosis, and new serologic techniques have been developed that improve sensitivity and specificity of diagnostic tests; moreover, advances have been made in the development and validation of molecular techniques for detecting *T. cruzi*.

However, much work still needs to be done about the management and treatment of patients affected by Chagas disease. The only available drugs continue to be benznidazole and nifurtimox, both of which must be administered for long periods of time, have toxicity issues and unknown actual efficacy in chronic patients. Only just recently clinical studies were started on posaconazole and pro-ravuconazole. In the next few years, and depending on the results of these first studies, the landscape of anti-parasite treatment may see changes for Chagas disease patients, with improved expectations, reduced mortality, and a better quality of life.

One of the problems in assessing people treated with available drugs, as well as for evaluating the efficacy of treatment in clinical essays of new drugs, is the lack of early biomarkers for therapeutic efficacy. The slow reduction of titers obtained through conventional serology make this technique unproductive for the evaluation of treatment, especially in potential re-infection environments. The lack of early biomarkers for therapeutic efficacy poses a bottleneck for treating individual patients, clinical assays, and research and development for Chagas disease.

The Ibero-American network NHEPACHA (New tools for diagnosis and assessment of Patients with Chagas disease), created in 2011, was organized with the purpose of collaborating, sharing, and promoting research on the subject of biomarkers for therapeutic efficacy. In its first working year, the network's main activities were the creation of joint databases, serum banks, and long-term follow-up biomarker projects, based on systematic review of those biomarkers with more potential.

Chagas disease, as a neglected disease, requires increased attention from academic research groups, to become more visible in the international agenda. Through the Chagas Disease Clinical Research Platform, of which the NHEPACHA network is a part, we want to contribute to coherent research efforts, as well as bring more visibility to people affected by Chagas disease – both carriers and their families. •

** Coordinator, NHEPACHA Network*

PROTEOMIC PLATFORM

ASSESSING THE SUCCESS OF PARASITOLOGY TREATMENT FOR CHAGAS DISEASE

Momar Ndao*

The majority of chronically-infected individuals with blood-borne protozoan parasites (i.e. *Plasmodium, Leishmania, Babesia, Trypanosoma brucei*, and *T. cruzi*) are asymptomatic. Therefore, many become blood donors, despite being infected, because they are unaware of their disease status. The real dilemma of *T. cruzi*, the causative agent of Chagas disease, is the fact that most infected people are asymptomatic for decades despite intermittent parasitemia. Despite decades of effort, there is still no gold standard test for the diagnosis of Chagas disease, and several international organizations have recently emphasized the need for improved serodiagnostic tests. To this end, many companies and research groups have developed a range of parasitologic, serologic, and nucleic acid-based assays. The most common methods used include the indirect immunofluorescence assay (IFA), indirect haemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA), and the radioimmunoprecipitation assay (RIPA).

Therapeutic availability represents yet another important challenge to overcome. Only two drugs are available. Both benznidazole and nifurtimox have trypanocidal activity, in the acute phase of the disease. However, these compounds have been shown to be poorly tolerated, and their efficacy remains controversial. The treatment of the chronic form of the disease, mainly in adult patients, remains a topic of controversy. Due to the delay in the diagnosis of Chagas disease, there is also a lack of evidence supporting the curative effects of late treatment.

As outlined above, these standard diagnostic approaches have both strengths and limitations, but neither can realistically be expected to address the most pressing diagnostic questions in Chagas disease. Presently, there is a lack of markers to assess the parasitologic success or failure of the treatment in clinical practice. Our group is currently working to fill these gaps by looking for defined biomarkers in both animal models and humans infected with *T. cruzi*. We anticipate that we will identify biomarkers of 'cure' following therapy in asymptomatic chagasic subjects.

Towards a potential test of cure for Chagas disease, we aim to assess the use of proteomic signatures and novel biomarkers in serum samples in a cohort of patients treated with nifurtimox at Geneva University Hospitals (Geneva, Switzerland). The samples are from a 2008 study conducted by Jackson *et al*[1]. In 2011, new samples from 53 patients who participated in the 2008 study were obtained. The same number of control samples from uninfected individuals, of same age, was also obtained. Our main objective is to compare their profiles based on 1) serology assay (ELISA); 2) nucleic acid-based assay (PCR); and 3) mass spectrometry platform (SELDI-TOF and MALDI TOF). Our novel proteomic-based assay for latent Chagas disease has proven to be highly sensitive and specific. Therefore, we believe this assay will be the core of the diagnostic tools to be used. Our overall approach is well described in Ndao *et al*[2]. We anticipate that the truncated host-origin biomarkers will disappear after treatment; moreover, cured patients may display new biomarkers following therapy.

This ongoing study is a collaboration among Dr. Eric Chatelain (Drugs for Neglected Diseases *initiative*); Dr. Yves Jackson and Dr. François Chappuis (*Service de Médecine Internationale et Humanitaire, Département de Médecine Communautaire et de 1er Recours, Hôpital Universitaire de Genève*); and Momar Ndao (National Reference Center for Parasitology, Department of Medicine, Division of Infectious Diseases, Research Institute of the McGill University Health Centre). •

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Left to Right: Fabio Vasquez Camargo, Cynthia Santamaria, Alessandra Ricciardi, Momar Ndao, Axel Eluio Renteria Flores, Carlos Melendez-Peña

SPECIAL ANTIGENS

BIOMARKERS OF THERAPEUTIC EFFICACY FOR CHAGAS DISEASE

M Carmen Thomas e Manuel Carlos López *

Chagas disease has an acute phase which often takes the course of a subclinical form. Without treatment, the disease becomes chronic, initially in an asymptomatic stage (indeterminate phase) usually lasting for a long time (15-25 years), characterized by a balance between the host's immune response and parasite replication. This balance is a highly fragile one and may result (in approximately 30-40% of cases) in a proliferation of the parasite in tissue, causing severe pathologies of the disease (symptomatic chronic phase, especially cardiac and/or digestive symptoms). Thus, the etiology base of the chronic phase of Chagas disease is governed by the damage caused by persistence of *Trypanosoma cruzi* in tissue.

Diagnosis of Chagas disease is chiefly made through conventional serological techniques that have high sensitivity and specificity. However, these diagnostic methods are not effective in determining clinical status of patients in their chronic phase, nor in assessing their evolution after treatment, thus not being effective to determine its efficacy. Although PCR has proved useful to spot therapeutic failures, it has the serious disadvantage of showing a high rate of false negatives.

A recent investigation has allowed the identification and evaluation of specific antigens of the *T. cruzi* parasite as biomarkers of Chagas pathology and also to review the efficacy of pharmacologic treatment of the disease. It is a non-conventional serum technique, easy to perform and practical to apply. The biomarker system is based on the independent and simultaneous determination of existing antibody levels in serum from Chagas patients in the presence of three recombinant proteins (KMP11, PFR2 and HSP70) and a synthetic peptide (3973).

The investigators of this study have shown how both patients with chronic Chagas in indeterminate phase and those with cardiac or digestive symptoms have a statistically significant decrease in the level of specific antibodies in the presence of said biomarkers after 6 and 9 months of treatment with benznidazole[1]. Two years after treatment, the antibody levels in the presence of these biomarkers further decrease in a large number of Chagas patients (34-67% of patients, depending on the biomarker). Serum antibody levels in the presence of peptide 3973 are significantly higher in chronic Chagas patients with cardiac and/or digestive symptoms than in patients in the indeterminate phase of disease[2]. Interestingly, peptide 3973 is not observed in the serum of Chagas-negative patients with similar cardiac and/or digestive alterations.

Thus, a biomarker system with high sensitivity and specificity is described, which allows the determination of antibody levels against *T. cruzi* parasite-specific antigens, which differs depending on the level of pathology of the infected person, and –as the case may be – on treatment efficacy.

These results show the establishment of a useful tool to distinguish between different degrees of Chagas pathology severity in its chronic phase, as well as to assess therapeutic efficacy. This knowledge may help with more personalized applications of treatment that may offer better expected outcomes for patients and enable adequate follow-up of their disease status after treatment. •

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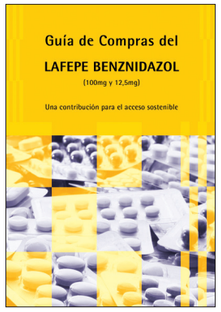
NEW PAEDIATRIC DOSAGE FORM OF BENZNIDAZOLE IS NOW AVAILABLE

The new paediatric dosage form of benznidazole (12.5 mg tablets) for Chagas disease – manufactured by LAFEPE (Pharmaceutical Laboratory of the State of Pernambuco) in association with DND*i* – is already available in the market. The first commercial batch of 240,000 tablets, valid for two years, was released in May 2012. The new drug can be administered to children less than two years of age.



The drug was registered in Brazil by ANVISA (National Health Vigilance Agency), Brazil's drug regulatory authority, and purchases can be made directly with LAFEPE or via the PAHO (Pan American Health Organization) Strategic Fund.

For information about ordering benznidazole from LAFEPE, see the procurement guide: Procurement Guide for LAFEPE Benznidazol (100 mg and 12.5 mg).

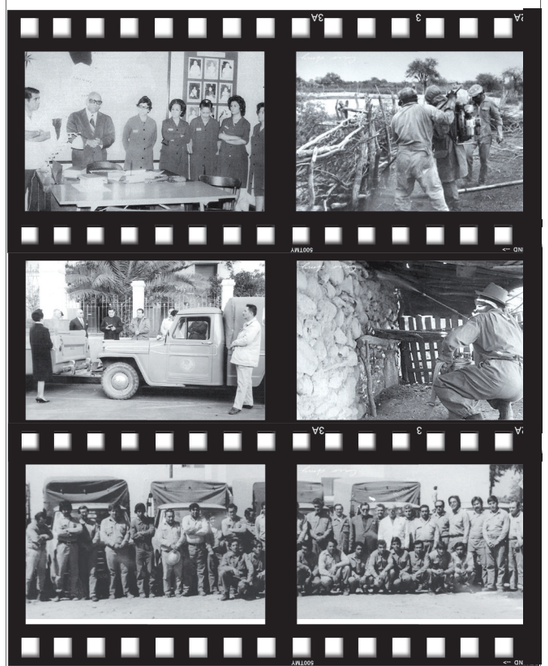
http://www.lafepe.pe.gov.br/LAFEPE/noticias/noticiario/guia-benznidazol.htm (available in Portuguese, Spanish, and English).

ABARAX – BENZNIDAZOL 50 E 100 MG PRODUCED IN ARGENTINA

In March 2012, the Argentinean laboratory ELEA launched 50- and 100-mg tablets of benznidazole. This development is part of an initiative by Argentina's Ministry of Health with Mundo Sano Foundation, ELEA, Pharmochemical MAPRIMED, and research teams involved in the treatment of Chagas disease.

CELEBRATING 50 YEARS OF THE NATIONAL CHAGAS PROGRAM– INP FATALA CHABEN

In Argentina, the research and fight against Chagas disease took institutional shape with the creation, in 1950, of the National Prophylaxis Service and Fight against Chagas Disease. The National Chagas Control Program, which started in 1962, and the present National Parasitology Institute "Dr. Mario Fatala Chaben" (INP, in Spanish) are two fundamental pillars in the history of Chagas disease control through prevention of vector and non-vector transmission, diagnosis, and treatment in Argentina, both of which have made great progress at different times of their history.



In August 2012, Argentina celebrated the 50th Anniversary of the National Chagas Program–INP Fatala Chaben. This was a good time to remember and give testimony of the things done for the benefit of patients who suffer Chagas disease. It was an opportunity to think about and assess the results obtained, as well as to approach new challenges facing the future. From the Chagas Disease Clinical Research Platform, we wanted to share this important commemoration and to join the efforts and dedicated work of all who are engaged in the fight for the people affected by Chagas disease.

LATIN AMERICA COUNTRIES MEET IN BRASILIA TO ESTIMATE DEMAND FOR CHAGAS DRUGS

PAHO and Brazil's Ministry of Health, in collaboration with DND*i* and MSF, invited the heads of the national Chagas control programs of several Latin American countries to a workshop held in July to estimate the needs for drugs to treat Chagas disease. The 13 attending country directors - of Argentina, Brazil, Bolivia, Chile, Ecuador, Peru, Paraguay, Guatemala, Honduras, El Salvador, Panama, Nicaragua and Mexico - received training on the use of a tool designed to estimate drug supply needs. The objective of the meeting was to have a picture of the needs for Chagas drugs and contribute to improve planning for future drug production, as well as to assess drug demand at regional levels based on a progressive scenario of providing Chagas diagnosis and treatment in the countries. The meeting included the participation of the manufacturers of benznidazole in Argentina and Brazil.

CHAGAS PLATFORM MEETING, DECEMBER 2011

"With the beginning of several clinical studies in Latin America and Spain along 2011, we can state that this is an unprecedented historical moment in the development process of Chagas disease control". This was the spirit of the Chagas Disease Clinical Research Platform Meeting on November 30-December 1, 2011 in Rio de Janeiro, Brazil.

During the meeting, investigators updated all Platform members about the clinical studies that were planned for 2012. This was a space to reflect on the challenges posed by access to new and existing technologies and the need for biomarkers to measure therapeutic efficacy for Chagas disease.

Glossary

Biomarker or biological marker: a characteristic objectively measured and evaluated as an indicator of the normal biological processes, the pathogenic processes or the pharmacological responses to a therapeutic intervention.

Pre-clinical Trials: involve both the biological and the chemical characterization of a compound. These studies may be developed *in vitro*, on animal models, isolated tissues or cells and define pharmacology, toxicology, metabolism and pharmacokinetics of the compound. Pre-clinical trials determine if there is a sufficient evidence of appropriate safety and the potential efficacy to justify the risk of using the compound in human beings.

Phase I Clinical Trial: establishes the initial safety of a chemical compound in healthy human beings. Phase I studies usually start with one single dose of the study compound and progress to multiple or higher doses, as soon as the previous dose administration proves to be safe. These trials require a constant monitoring of the subjects involved in the investigation. The pharmacokinetic profile of the compound when used in the human beings is defined in this phase. Other key data are also obtained, for instance the maximum dose tolerated by the human beings and a preliminary profile of the potential toxicity of the compounds. This phase may include a test of concept studies in order to verify that the secondary objective used as the efficacy marker in the pre-clinical studies is also observed in the human beings.

Phase II Clinical Trial: establishes the safety of using certain chemical compounds in the human beings. Phase II studies are generally controlled, employing multiple doses of the compound under investigation in order to identify the appropriate dose to achieve the desired therapeutic effect, acceptably balanced between therapeutic benefits and the risks, evidenced by the adverse effects and the other safety measures.

Proof of Concept (PoC): is a method that seeks to prove its viability/applicability. It aims to verify that certain concepts or theories have the potential to be applied in the real world. The PoC on drug development refers to early clinical development since a study of PoC is intended to demonstrate the clinical efficacy among a small number of patients. It can be defined, therefore, as the earliest stage in the drug development process when decision criteria for the continuity of the study are based on the safety, efficacy, tolerability, bioavailability/ pharmacokinetics and pharmacodynamics. It can be done in both Phase I and Phase II of a clinical study.

Phase III Clinical Trial: establishes the safety and the efficacy of a chemical compound in human patients. Generally, the dose employed in these large multi-centre studies that recruit hundreds and thousands of patients is the optimum dose identified in the Phase II trials. Phase III trials nearly always use placebo or other active compound arms, and the results obtained are used by the regulatory authorities to determine if the safety and the efficacy of a certain drug are appropriate for using it in humans.

Phase IV Clinical Trials: are investigations performed after the product has been marketed. Such investigations are based on the characteristics that the drug had when it was authorized. Typically, these are the post-marketing surveillance studies, whose purpose is to establish the therapeutic value of the drug, the appearance of new adverse reactions and/or the confirmation of the frequency of already known reactions, and the strategies for their treatment. For Phase IV investigations, it is necessary to follow the same ethic and the scientific rules applicable to the previous phases.

Pharmacokinetics: It is a branch of pharmacology that studies absorption, distribution, metabolism and the excretion of the drugs in the living organisms. Pharmacokinetics involves studies, with a restricted number of volunteers that require a large number of samples per patient. Such studies, however, contain little information about co-variables (e.g. age, gender, weight, etc.), provide limited data about variability in the population and have a narrow predictive power.

Polymerase Chain Reaction (PCR): is a method to amplify (create multiple copies of) DNA (deoxyribonucleic acid) with no need to use a living organism, for instance, *Escherichia coli* (bacterium) or yeasts. PCR is mainly applied where available quantities of DNA are limited. In theory, any DNA can be amplified. PCR can be used to identify pathogens existing in samples. The Polymerase Chain Reaction (PCR) is a very sensitive analysis method, it involves great care to avoid the contamination that may turn a result not viable or erroneous. PCR results are analyzed using agarose or polyacrylamide gel electrophoresis, and later interpreted with the help of a competent professional.

Serology Titles: the quantification of antibodies through serology.

Contributions, comments or questions are always welcome. Please feel free to write us to the Platform´s Web Forum:
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