Chagas Disease Clinical Research Platform was inaugurated by DNDi 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.
**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 diagnosis), 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 outcome.

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

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**OPENING OF CASA DE CHAGAS IN RECIFE**

INTERVIEW WITH DR. WILSON 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.

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*Interview with Dr. Wilson Oliveira Jr.*
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 induction 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.

* 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.

Cure: Perspectives from the Field

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

A 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 Albarja, Sergio Sosa Estani, Tom Ellman, and the participants of the meetings organized by WHO/TDR, DNDI 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–POSA CONA ZOLE 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–RAVUC ONA ZOLE, PRO-DRUG

DNDI-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 (DNDI), 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.

E1224 PHASE II 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.

E1224 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)
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.

**CHAGASAZOL STUDY DESIGN**

**Visit for blood sampling**

<table>
<thead>
<tr>
<th>EOT (2 months)</th>
<th>4 months</th>
<th>5 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1224</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>STOP-CHAGAS</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>CHAGASAZOL</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

**Arms of the study**

<table>
<thead>
<tr>
<th>E1224</th>
<th>STOP-CHAGAS</th>
<th>CHAGASAZOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZN</td>
<td>BZN</td>
<td>BZN</td>
</tr>
<tr>
<td>E1224 (high dose)</td>
<td>POS 400mg</td>
<td>POS (high dose 800mg)</td>
</tr>
<tr>
<td>E1224 (low dose)</td>
<td>POS / Placebo</td>
<td>POS (low dose 200mg)</td>
</tr>
<tr>
<td>E1224 / Placebo (short doses)</td>
<td>POS 400mg + BZN 200mg</td>
<td>BZN 200mg + POS 100mg</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>Placebo</td>
</tr>
</tbody>
</table>

**Therapeutic 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.

**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, MínÖcuenas Sàins Frànteres (MSF), DNDI and Universidade 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 DNDi 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 DNDi with the Oswaldo Cruz Institution (Fiocruz) in Brazil, which began in April 2012. The final study results are expected in March 2013.

**Update on Other Studies**

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 cardiologyopathy. BENEFIT is evaluating the effect of etiology treatment with benznidazole in reducing death, cardiac resuscitation, sustained ventricular tachycardia, pacemaker or cardioverter defibrillator implantation, competitive 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 cardiologyopathy 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 reverses or slows heart-failure progression based on improved 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.

**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 Gutierrez and Instituto Nacional de Parasitología Dr. Mario Falleta Chabén, both located in Buenos Aires; Hospital de Niños in the Province of Jujuy; Centro de Chagas y PatologíaRegional, in the Province of Santiago del Estero and Hospital Público Materno Infantil in the Province of Salta.
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 and 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.

**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 and 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.

Be cause 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 possibility of a parasite healing, the drug shall be capable of reducing parasitemia in 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 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).

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 5 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.

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 (DNDi) 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 participate-timing 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 specificity and sensitivity 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 (TRACEA) study (see pg. 12-13); the MSF/DNDi 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 (DNDi, 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.

**Molecular Biology Laboratory and Endemic Diseases, Oswaldo Cruz Institute (Fiocruz).**
TRAENA study (Treatment in adult patients - TRATamiento EN pacientes Adultos, in Spanish) is a clinical randomized, double-blind, Phase III study conducted at Instituto Nacional de Parasitología Dr. Mario Fata 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 antigens 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. 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.

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

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

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

Susana Laucella *

C ertain 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 polygonal 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 with the production of IL-2 being observed between 36 and 72 months following treatment (Pérez-Mazliah, personal communication), while serological titers remained low. When examining baseline parasit-specific T cell responses, those subjects with re-bound 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 follow up 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 summnation, monitoring of par-aside-specific T cell responses during etiological treatment might be useful as: 1) an early treatment re- sponse marker to give an early indica- tion 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 polyfunc- tional T cells (i.e. increased spe-

cific polyfunctional IFN-γ/IL-2/7 TNF-α’ T cells along with reduced IFN-γ single-positive and TNF-α single-positive T cells have been shown to have been associated with successful responses to chemotherapy in other chronic infections); and 3) a potential pre- dictor of treatment response according to the functional status of parasite-specific T cell responses prior to treatment.

References
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+NHEPACHA Network

**NHEPACHA NETWORK: COLLABORATING WITH THE ASSESSMENT OF THERAPEUTIC EFFICACY BIOMARKERS**

Joacim Gascon*
PROTEOMIC PLATFORM
ASSESSING THE SUCCESS OF PARASITOLOGY TREATMENT FOR CHAGAS DISEASE

Mohar Ndao*1

C hagas 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 benznidazole2. 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 disease2. 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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BIOMARKERS

SPECIAL ANTIGENS
BIOMARKERS OF THERAPEUTIC EFFICACY FOR CHAGAS DISEASE

M Carmen Thomas e Manuel Carlos López *

Left to Right: Fabio Vasquez Camargo, Cynthia Santamarta, Alessandra Ricciardi, Mohar Ndao, Axel Eluio Renteria Flores, Carlos Melendez-Peria

1. Inglese Renteria, Flores, et al. Proteomic Platform for Chagas Disease

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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 DNDi – 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).

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, Pharmaceutical MAPRIMED, and research teams involved in the treatment of Chagas disease.

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.

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.

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

PAHO and Brazil’s Ministry of Health, in collaboration with DNDi 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

“We 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.
**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.