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Chapter

The saga of Selenium Treatment Investigation in Chagas Disease Cardiopathy: Translational Research in a Neglected Tropical Disease in Brazil

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Abstract

This chapter describes the steps from basic research to the definition of a putative public health recommendation in the clinical protocols and therapeutic guidelines for selenium (Se) supplementation for patients with Chagas disease. From 1998 to 2018, we conducted a translational research project to test the concept that chronic Chagas disease cardiopathy (CCC) severity could be associated with low levels of blood selenium (Se), and if oral Se supplementation could help to sustain the asymptomatic cardiac stage and reduce disease severity. Pre-clinical studies in mice and a clinical trial conducted in the early asymptomatic cardiac stage of CCC patients (B stage) were performed, identified as "Selenium Treatment of Chagasic Cardiopathy (STCC)" trial. The roadmap of the selenium project was/is a real saga, with important obstacles that tested team resilience and revealed Brazilian conditions of science development. We discuss the main possible mechanisms involved in the physiopathology of CCC and the lessons learned in this process. In this chapter, we also organized the timeline of the translational project and described the crucial moments of the journey, as well as the next steps driving the research teams and their international and health industry connections.

Keywords: neglected tropical diseases, myocardiopathy, *Trypanosoma cruzi*, selenium, pathogenesis, poverty, translational research, trace elements, clinical trial

1. Introduction

From 1998 to 2018, we conducted a translational research project to test the concept that chronic Chagas disease cardiopathy (CCC) severity could be associated with low levels of blood selenium (Se), and if supplementation with Se could help to sustain the asymptomatic cardiac stage and reduce disease severity. Pre-clinical studies in mice [1–3], clinical studies in affected people [4], and a clinical trial [5] with patients in the early stages of CCC were performed. Here we revisit and build the narrative of this process, from the very first ideas, through all the difficulties that we faced until the present days and the perspectives to introduce a new treatment for a neglected disease.

2. Research in context

Chagas disease (CD) is caused by infection with the protozoa *Trypanosoma cruzi* and affects 6–7 million people, leading to 12,000 deaths annually in more than 21 countries, mainly in Latin America, where Brazil, Bolivia, and Argentina are the most affected nations [6]. CD is considered by the WHO as a neglected tropical disease, due to several factors such as (i) the global disinterest of the pharmaceutical industry related to the low-profit perspective in the development of new drugs and vaccines for a vulnerable population that is affected by the CD; (ii) related to social determinants of the affected population, including poverty, malnutrition, poor housing/sanitation, and low education levels [7]. As a consequence of the low investment in drug development for CD, only first-generation drugs dated from the seventies are currently in use for trypanocidal treatment—benznidazole (BZN) and nifurtimox (Nif). Nevertheless, it is estimated that only 1% of the infected persons are etiologically treated, due to the second dimension of negligence that is related to the negligence of public health policies to identify, diagnose, treat, and monitor chronically affected CD people.

To enhance the visibility to the real dimension of CD, WHO instituted a *World Chagas disease Day* in 2020 (https://www.who.int/campaigns/world-chagas-disease-day). The real dimension of CD worldwide is still only estimated, based on data that is more than 10 years old. In Brazil, national guidelines for therapeutics and clinical protocols (PCDT-Chagas) were adopted only in 2018 [8]. Treatment is recommended to prevent or reduce CD progression in both the acute and early chronic phases by using BZN or Nif [6]. About 20–30% of the infected persons will develop chronic complications related to cardiovascular and digestive systems, and CCC is the most relevant infectious heart condition in Latin America [6, 9, 10]. We recently reviewed the complex physiopathology of CCC and the possible interactions where Se could act to reduce oxidative damage that is caused by multiple determinants [11, 12]. **Figure 1** shows the main strategies that we could mobilize to face the complex physiopathogenesis of CD, which involves anti-parasite drugs (AP), modulators of inflammation (MI), immune stimulators (IS), regulators of pathology (RP), and the combination of different therapeutic strategies (CT).

The idea of using Se as a complementary treatment rises from its role in 25 human selenoproteins, some of them acting as antioxidants and others in endocrine and immune pathways [13, 14]. Therefore, the effect of Se therapy in CD development needed proof of concept related to experimental infection and in patients. Our group and other researchers are adding evidence in this direction.

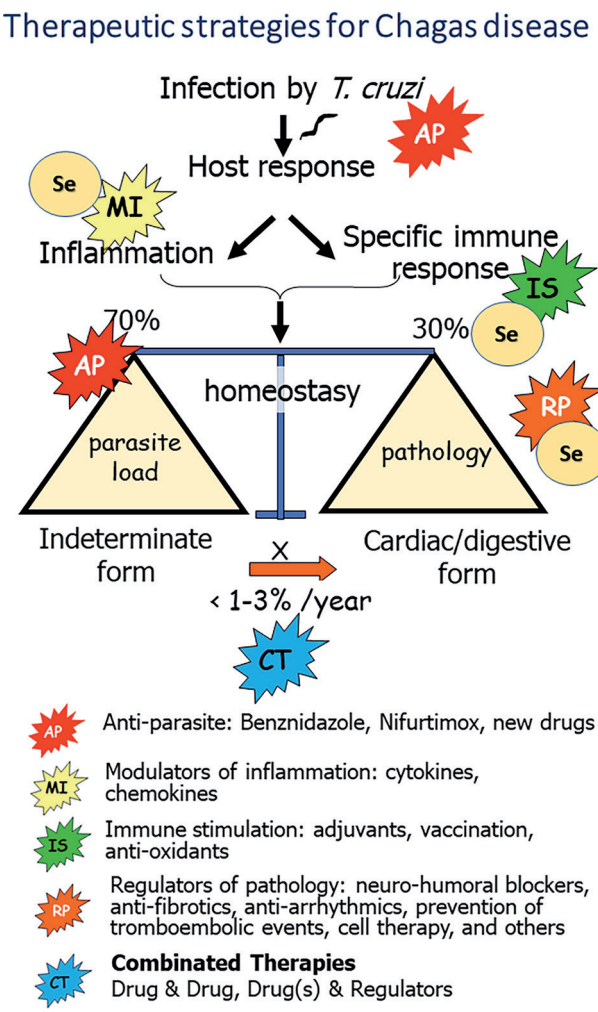

Figure 1.
Five different therapeutic strategies that may be applied for Chagas disease treatment in the complexity of its multifactorial causality. Anti-parasite (AP) drugs such as benznidazole and nifurtimox, as well as others that are currently been studied, may reduce the parasite load in the early acute phase and in chronic infection when the parasite still evading the immune system. Infection in the hosts/patients triggers both local and systemic inflammation and a strong specific immune response. An adequate balance of these two mechanisms regulates the control of parasite growth and the regulation of physiopathology. Different strategies are under study in basic research for intervention in these four arms—Modulators of inflammation (MI), as cytokines, chemokines and their antagonists, immune stimulators (IS) as adjuvants, vaccines, and antioxidants, such as Se, regulators of pathology (RP) as neuro-humoral blockers, anti-fibrotic and anti-arrhythmic agents, prevention of thromboembolic events, cell therapy and others. The combination of different therapeutic strategies (CT) should help to face this complexity. However, the translation from basic to clinical studies and to patient access is still far.

However, combined therapies are scarcely being tested in basic science [15, 16], and its translation to humans must also be investigated, mainly related to more clinical trials, as we performed for Se [5], opening more new doors than solving questions. As shown in **Figure 1**, the strategies of using combined trypanocidal chemotherapy and other agents to mitigate inflammatory disbalance, in which cytokine networks assume pathological roles, as well as increased fibrinogenic and neurodegenerative mechanisms, became co-protagonists in CD physiopathology [9, 10, 17].

3. Good beers and good ideas: the selenium hypothesis

In 1996, we were introduced to Se effects in cardiopathies through contact with Belgian colleagues that worked with *Médicins sans Frontières* in China and who studied experimental CD with us during a postdoctoral stage [18]. Good Belgian beers composed the scene for this open and free discussion. These colleagues were testing the use of Se to improve the treatment of Kashin-Beck osteoarthropathy [19], and they

Figure 2.
Main landmarks of the STCC clinical trial. Starting from the first ideas in 1996, three decades will lead to evidence to support public policies. Financial supports are shown in blue and main publications are shown in red; yellow dots at left indicate the critical points that threatened or delayed the project. The orange gradient background indicates the three main phases of the translational roadmap—(a) ideas, conceptualization, and basic preclinical studies, from 1996 to 2015, including the three PhD thesis, (b) pretrial activities conditioning the start of recruitment phase, from 2004 to 2014, and (c) the first clinical trial (STCC) from 2013 to 2020, with results published in 2021. This first trial used a short follow-up and showed a significant effect only in patients of the B2 stage of chronic Chagas cardiopathy, leaving some questions that implicated the design of new clinical trials to elucidate points that remained open, thus opening a fourth phase (white background) that deserves planning, financial funding, and implementation.

were aware of Se involvement in an endemic cardiopathy, the Keshan disease [20]. Later, Keshan disease was linked to Se scarcity in the soil of some Chinese regions. Important projects were also conducted testing Se in an experimental virus disease and the possibility of pathology reversion after Se treatment [21]. Se deficiency in food turns benign viral strains into more virulent and pathogenic ones. The original idea was planted (a good idea)—does Se have any relationship with Chagas' disease cardiomyopathy?

We then asked Prof. Alejandro Luquetti, from the Federal University of Goiás, to give us some serum samples from CD patients at different stages of cardiac disease and measured Se levels. In the five pilot samples tested, two from non-infected and three from CD-infected people, we found that those with the lowest level of Se were the ones with the more advanced cardiac disease. It would be worth persisting in testing the hypothesis, thus starting the timeline of this long project. **Figure 2** shows this 30-year timeline, writing in black the yearly landmarks, in blue the application and approval of financial support projects, and in red the main publications.

4. Timeline of selenium treatment in Chagas Cardiopathy (STCC) clinical trial, the strength of a scientific network and the social arm of the project

The next step, in 1999, was to enhance the number of patients studied to confirm whether Se levels would be lower in patients with more severe cardiac disease. The first partnership was made with Dr. Alejandro M. Hasslocher-Moreno, who was the physician responsible for the clinic management and follow-up of CD patients at the Evandro Chagas National Institute of Infectious Diseases (INI), Fiocruz, Rio de Janeiro city. Se levels were measured and a very large variation was observed. We then decided that patients with different nutritional habits from another state in Brazil should also be analyzed. It implicated a new partnership with colleagues from Belo Horizonte city and CD patients from Dr. Manoel Otávio Rocha, enrolling a total of 273 patients in the study [4]. We observed that Se levels in patients with moderate and severe cardiopathy were lower than in patients with mild cardiopathy and indeterminate form, as well as in uninfected individuals [4].

In parallel, we performed some proof-of-concept studies, showing in experimentally *T. cruzi*-infected mice—(i) that Se diet deficiency turn mice much more susceptible to acute-phase mortality [1]; (ii) the parasite survival and replication was not affected by Se [2]; (ii) that mice parasitemia did not vary in situations of nutritional deficiency or Se supplementation [2]; (iii) that in the acute [2] and in the chronic [3] phases in mice, treatment with Se was able to reduce cardiac inflammation, regulate arrhythmias, and prevent cardiomegaly; (iv) digestive mega disease was prevented by Se supplementation [22]. A second idea was planted—could Se treatment or supplementation prevent the progression of chronic forms of CCC?

In 2004, we started to move on to the clinical trials stage. We designed a placebo-controlled, double-blind trial, recruiting patients with mild heart disease (stages B1 and B2 defined by the Brazilian Consensus on CD) followed at the Chagas clinic at INI-Fiocruz, for treatment of 100 mcg/day Se for 1 year. Two outcomes were defined—50% reduction in progression of heart disease and a significant reduction in left ventricular ejection fraction (LVEF) value in 5 years of follow-up. However, we did not previously contact a Se supplier. Our first impediment was to think that it should be easy to find a Se source in the Brazilian pharmaceutical market. The saga was in the early beginning. **Figure 2** represent the

historical saga of the Se clinical trial, including the first step (1996–2004 period) of developing ideas, hypothesis, and pre-clinical studies.

1. In 2005, the Brazilian National Health Surveillance Agency (ANVISA), the drug regulatory agency, changed the criteria for daily supplementation dose of Se for 34.5 mcg. Therefore, the predicted dose of 100 mcg/day would be considered as treatment and not supplementation, implying the need to change the clinical trial regulatory licenses. Then, in June 2006, a second version of the trial was approved by the Ethics Committee.

2. In 2005, we found no Se supplier in Brazil under good manufacturing practices (GMP) conditions for clinical trials, either organic or inorganic. In sequence and based on the opportunity that Fiocruz has an industry sector for drugs and medicines production (FarManguinhos/Fiocruz), we tried a partnership for Se production, but it was not possible due to technical restrictions.

3. In 2006, the production was staggered at other Fiocruz industry sectors (Bio-Manguinhos/Fiocruz) and we initially succeeded. One year later we signed a contract and the first batch of liquid sodium selenite in ampoules was manufactured by BioManguinhos.

4. In June 2009, we submitted the essay proposal to ANVISA, which approved it in 5 months, after a single meeting for clarification and adjustments.

5. In early 2010, we received the bad news that BioManguinhos would have to interrupt the GMP production of Se due to a priority of the factory's response to the shortage of yellow fever vaccine.

6. We then started a journey to find a private industry partner. In 2011, based on the experience of the recently organized Fiocruz Clinical Research Platform (http://www.ppt.fiocruz.br/fiochagas/2021/09/27/quem-somos/), we found the *Relthy Co*., a national company as a partner that was interested in manufacturing Se and placebo under the same conditions. In 2011, a contract with *Relthy* was signed and we started the studies with the first batch of softgel capsules containing placebo or 100 mcg sodium selenite.

7. In 2013, the team that worked in the pre-clinical studies and that was initially trained for the clinical trial, was partially disarticulated with the departure of the project manager and other technicians.

8. In 2013, we reorganized the STCC team, with the completion of the database preparation and training of the new group in Good Clinical Research Practices (GCP). The database was structured with 389 fields to be filled in the Research Electronic Data Capture (REDCap), a web-based application to capture data for clinical research and create databases, divided into (i) Tracking and inclusion, (ii) Baseline visit for cardiac and clinical data (history and physical examination); (iii) Clinical follow-up, electrocardiogram (ECG) and echocardiogram (ECO); (iv) polymerase chain reaction for *T. cruzi*; (v) Se measurement; (vi) Thyroid hormones; (vii) Pregnancy test; (viii) Laboratory biomarkers; (ix) Clinical analyzes (hematology blood count and biochemistry);

(x) Immunological analyzes (humoral and cellular); (xi) Nutritional assessment; (xii) Follow-up ECG and ECO; (xiii) Follow-up echocardiogram. A challenge was the complexity of dealing with so many fields, multiplied by 135 expected participating patients multiplied by 14 visits each, with 10 only in the first year. It resulted in 1890 visits, with all the variables and biomarkers collected in most of them. In 2014, we had overcome the main obstacles to crossing the "valley of death," as shown in Declan Butler's publication in *Nature* in 2008 [23], and we the first patient was enrolled, enabling the publication of the STCC trial protocol in 2014 [24]. But then...

9. In 2015, the manufacturer *Relthy Co*. was purchased by a multinational company, Catalent, and we needed to re-discuss the clinical trial project Se supply and to renew the contract. Fortunately, the new partner agreed to maintain the project follow-up in 2015 and even prepared a second GMP batch.

10. On April 6th, 2016, a meeting of the external data safety monitoring board [24] was called to analyze some adverse effects recorded and to discuss the possible need for interrupting the clinical trial. Without the need of breaking the STCC blinding, this analysis concluded that a possibly intercurrent dengue virus endemic infection could have caused the noted effects (leukopenia/neutropenia) and the continuity of the trial was recommended. After ending the first year of STCC follow-up, the results published clearly confirmed the absence of adverse effects related to Se treatment [5].

11. During this long time, it occurred many advances in the knowledge about the effect of Se treatment in cancer and heart disease. It became clear that perhaps the dose provided in our clinical trial (100 mcg/day) could not be sufficient. From 2013 to 2018, four articles showed that 200 mcg was ideal for cardiovascular protection [25–28], and Swedish studies in elderly people proposed the benefits of the association of Se with the co-factor coenzyme Q10 for the expected outcomes. There was then a risk that STCC could end with no significant results, due to the lower concentration of Se chosen in 2004, based on cardiovascular literature related to Se treatment available at that time.

12. At the end of 2014, after 1 year of patients recruitment, we noted that the rate of recruitment was very slow (**Figure 3**), compromising the ability to reach the 163 volunteers needed for STCC protocol. It was 2015 when the third idea was planted—how to think about strategies to bring people with Chagas disease closer together and interest them in participating in the study to test/develop new medication and vaccines? Would it be possible to make them partners in the study or in actions to engage more patients in the knowledge of their own disease?

At this point, we decided to develop what we call the social arm of the clinical project.

April 14, 2015, we organized a meeting to make a public launch of the project, and to propose workshops of playful activities to "Talk about Chagas with Science and Art," inspired by the work of the Argentine group "Hablamos de Chagas" [29]. These initiatives quickly produced results—(i) we organized five successive editions of the course "We talk about Chagas with Science and Art" from 2015 to 2019, for people with CD, their family members, and health professionals; (ii) we created a

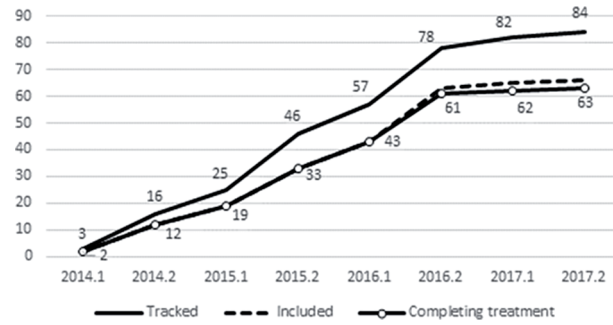

Figure 3.
Time sequence of inclusion of participants on the STCC clinical trial. Black solid line shows the putative participants tracked, dashed line shows the participants included after signing the project voluntary consent form, and solid line with white dots shows the number of patients that completed the one-year treatment. Note that after 2015 the slope angle of the inclusion curve rise, denoting a success in the recruiting phase.

"Rio Chagas Collective" on social media, which gave rise to the (iii) "Rio Chagas Association," founded on April 8, 2016, in which we participate in the Scientific Council; and (iv) the Rio Chagas Association participants inspired us to conceive the "Chagas Express XXI" social technology [30].

13. In 2018, we created a *YouTube* channel called "We talk about Chagas" (https://www.youtube.com/c/FalamosdeChagas) to provide videos about Chagas disease, and in 2019, we created the social technology for those affected by the disease named "Expresso Chagas XXI" to talk about Chagas in endemic areas, bringing science, culture, and art to those most in need of information for health promotion, prevention of Chagas disease and access to diagnosis and treatment in the Unified Health System (SUS – Sistema Único de Saúde). In 2019, we carried out a pilot expedition in four cities in an endemic area for CD in northern Minas Gerais. In addition, in 2020, we organized solidarity strategies for coping with the COVID-19 pandemic together with Rio Chagas Association (virtual WhatsApp meetings called "Coffee with Affection").

Chagas Express XXI was created as an "imaginary train" with around 40 ArtScience workshops, games, laboratory activities, and conversation circles. It was structured with an entry and exit point, followed by six more modules of activities that combined a focus on associations of affected people, on opportunities for the public to rediscover Carlos Chagas' discoveries, on microscopic observations and play, on health education in approach. A One Health approach was adopted, with a focus on home care, environment and reservoirs, and wellness activities. Chagas Express XXI was conceived as a social technology since all processes were co-created by scientists and patients with Chagas disease and worked with local cross-sector partnerships. We observed that 81% of the more than 2000 participants were unaware of the possibility of treating Chagas disease and 52% requested a blood test to diagnose CD. From the 1100 adults tested, 20% were diagnosed as positive for *T. cruzi* infection [30].

The fourth idea would then be: if the clinical trial of Se becomes unfeasible, we will have a social legacy to be able to continue later.

14. However, the problem was not only in the communication and mobilization of patients to participate in STCC, since in INI/Fiocruz cohort the number of patients

in the B1 and B2 stages of CCC was small as compared to patients in other clinical stages (indeterminate, A and C/D). Besides, a new risk for the project was arising: a long time had passed and the patients in the INI/Fiocruz cohort aged, acquiring comorbidities that were predicted as exclusion criteria and entering other studies that were ongoing, almost making the trial unfeasible due to a very slow rate of inclusion, as shown in **Figure 3**, during the years 2016 and 2017. To overcome this problem, the clinicians proposed to increase the age of recruitment from 65 to 75 years and to consider diabetes mellitus as a non-exclusion criterion.

Besides, the statisticians carried out other scenario studies for the feasibility of the STCC. They concluded that by extending the age range to up to 75 years, reducing the time of follow-up to one year, and focusing as a primary outcome only on the reduction of LVEF values (and not on the rate of CCC progression), we would conclude the inclusion of participants and generate some valid results. Then, in 2018, we published an update of the protocol [31] and started working to include 62 patients, divided into the two groups (placebo and Se treatment) for a follow-up of one year. We were then able to include all the expected patients and on August 8th, 2018, we were able to complete the last 12 months' visit of the last participant included.

15. The next step was to include all the laboratory and the nutritional data in the database, to run quality control for it, and to start the statistical analysis, both for descriptions of the clinical findings of participants and for comparisons between the placebo and Se-treated groups, as well as sub-group analyses, whenever possible. The study results were finally published in September 2021 [5]. When comparing the mean values of LVEF recorded in Se treated with the placebo group at baseline and after 1 year of follow-up, we did not find significant differences in the B1/B2 stages patients (overall), nor in the B1 stage patients, but found a significant effect (p = 0.02) in the B2 stage patients (**Figure 4**).

After one year (**Figure 4**, gray bars), all the groups showed a lower mean of LVEF when compared to baseline values (**Figure 4**, black bars). However only those already on the B2 subgroup, with LVEF <45%, showed a high decrease in LVEF in 1-year, which

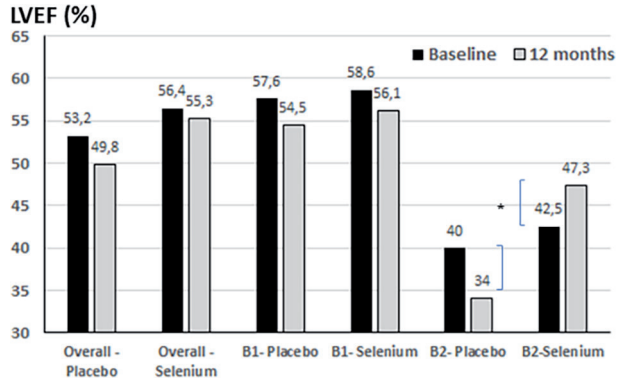

Figure 4.
Mean values for left ventricular ejection fraction recorded for all the patients participating in the STCC clinical trial, at baseline (baseline, black bars) and after 12 months (gray bars). In the X-axis it is shown the comparison of the groups treated with placebo or selenium, considering all the participants in each group and in the subgroups B1 and B2 stages. For details, see reference #5. ***Figure 4*** *Was prepared with data published in the STCC results [5].*

was reversed by Se treatment. The differences in LVEF longitudinal changes between groups were evaluated using linear mixed effect models in intention-to-treat analyses. This type of analysis assesses the rate of change of the outcomes by the time X intervention group interaction term, considering the correlations between repeated measures over time and missing data. In the B2 subgroup, the worsening of cardiac dysfunction was significantly reduced by Se treatment, and while the placebo group had a drop in LVEF, the Se group remained stable. In addition, only in the Se group, we observed cases of LVEF increase of more than 10 absolute points in 5 patients (n = 3) and recovery from stage B2 to B1 (n = 2), as reported [5]. In this short-term follow-up (1 year), the secondary outcomes observed were all related to LVEF as the underlying cause and could not be weighted in the analysis. We also observed that treatment with Se was safe for patients with CCC and that the low percentage of adverse effects detected were similar in the two groups. However, there was no complete shift of patients treated with Se to a safe range of serum Se (>100mcg/L), indicating that the dose of 100 mcg per day may have been insufficient. Further studies are needed to explore higher doses and/or associations of Se at different stages of CCC (B2 and C) at a short follow-up (one year) and at early diseases stages (A and B1), with longer follow-up.

16. The paper with STCC results [5] discussed some possibilities and pointed that "new clinical trials with a longer follow-up are needed to investigate the effects of Se in the mild (stages A and B) or severe (stages C and D) CCC, and in the asymptomatic indeterminate clinical form". The limitations of STCC were reported and the future outspread of the project is depicted in **Figure 2** from 2022 to, at least, 2026. The design of new projects (STCC#2 and STCC#3) is underway, and when the adequate industry partnership(s) will be signed, the proposal is that the new clinical trials will have a following of 3 to 10 years to generate the necessary evidence to support public policies recommendations.

5. Team resilience to face all the difficulties

From basic research to a putative public health recommendation in clinical protocols and therapeutic guidelines, the roadmap of the Se project was a real saga, with many obstacles that tested team resilience and Brazilian conditions of science development.

This resilience derived from the strength of the encounter between a basic research group (in Oswaldo Cruz Institute (IOC), founded by TCAJ) and a clinic research group (INI, founded by AMHM). Both groups were very active in producing new knowledge in CD and were excited with the idea of conceiving and performing the first Brazilian clinical trial [23] with a strategy based on one of the pathological mechanisms implicated in CCC [8, 9]. Overpassing all the difficulties that were listed in **Figure 2** (item 3), one after another, and attaining the conclusion of the trial gave even more strength to this partnership, our self-confidence, and our mutual respect increased.

The progress attained in the social arm of the project was also a source of resilience. More IOC/Fiocruz laboratories are associated with the project, as we could see from the Chagas Express publication [30] in August 2021. A project recently approved is preparing a virtual version of Chagas Express (https://expressochagas.com/), integrating contents related to COVID-19 as well as new expeditions to other endemic regions, planned for 2022.

In addition, this social arm of the project was developed at a very important moment in the struggle for the rights of patients with Chagas' disease. In 2018, the Ministry of Health, through its National Commission of Technology Incorporation, offered Therapeutic Guideline for CD Diagnosis and Treatment (PCDT Chagas) to public consultation, fostering discussions that lead to reformulations and publication in 2019. It was one of the main documents disseminated in the expedition to Minas Gerais by Expresso Chagas XXI [30]. We also participated in the advocacy movement that led to compulsory notification of chronic cases. Together with the traditional surveillance of cases in the acute phase, ordinance No. 1.061, May 18, 2020, introduced notification of chronic cases in Brazilian territory, strengthening the construction of public policies based on scientific evidence.

In a recent review [32], Morel discussed that CD is an example of successful translational research integrating basic and applied science, attaining the control of its transmission by insect vectors in large regions of the Southern Cone countries in the 90s. However, if the successful control of CD transmission by insect vectors is, in fact, a paradigmatic achievement in science translation, in addition to organizing a strong scientific community in Brazil and in Latin America, the translational research to focus on vaccines and treatments suffers extremely negative pressures as the ones we reported above. One important lesson to take home after this saga is that translational science on a neglected disease in *Innovative Developing Country, such* as Brazil, is not simple and needs specific policies to help scientists to overcome the valley of death. Morel also recognized that success in translation derives from "a long process led by incredible people, each one a leader in her or his area of work, who were able to collaborate with equally dedicated partners at the decision-making and political levels." As Morel reminded, thought the words of Lewis Carroll in his book, "Alice through the looking glass": "now, here, you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!"

6. Conclusions

What are the lessons learned and the next steps?

Four lessons were learned during the STCC saga—#1: the harmonious interaction between the clinical and basic research teams is essential to allow translational research to proceed; # 2: even in the pre-clinical phase, it is essential to identify potential suppliers in the market for the formulations that are intended to be used as treatment and/or industry partners interested in leading the product to the market; #3: the inclusion of master's and doctoral students should only be done in pre-clinical studies. In clinical trials, the stable inclusion of trained and mature professionals, economically stable, is necessary to avoid the shortage of human resources during the study; #4: a national and international network of experts is critical to overcoming the numerous doubts that arise in such a long study. In this case, we learned a lot about the clinical use of Se during the 15 years of the clinical trial, and we continue to learn about the physiopathology and clinical management of patients with Chagas disease.

Concerning the next steps, we are at the stage of preparing the reports for the sponsors and for our patients participating in the study. We will have several consequences in the clinical scope:

i. to carry out the cardiological follow-up of the study participants for another 4 to 10 years;

ii. to conduct a clinical trial with Brazil nuts as a natural source of Se;

iii. to identify PCR-*T. cruzi* positive patients at the end of the trial to treat them with benznidazole for 6 months plus Se;

iv. to accomplish the study of immunological biomarkers and gene polymorphism for cytokines and selenoproteins in samples from participants in the published study;

v. to contact suppliers and industry partners to enable further studies with Se in different settings, conditions, and associations and in multicenter and international arrangements.

As a second initiative, we will prepare a dossier for the National Commission of Technology Incorporation in the Health System (Comissão Nacional de Incorporação de Tecnologias no SUS" – CONITEC) to evaluate the recommendation of dietary supplementation for patients with Chagas disease with Se, either by supplementation with one Brazil nut per day (about R $20 per month) or by supplementation with Se and Coenzyme Q10 (about R$80 per month), due to type B levels of evidence for therapeutic studies in the literature and the safety when administering Se to elderly persons [25–27].

Last, but not least, we intend to incorporate Chagas Express XXI as cutting-edge educational technology, since we have already demonstrated its potential as an instrument of field epidemiology. In the next expeditions planned for 2022 in the states of Pernambuco, Goiás, and Minas Gerais, we will include a rapid test for a local screening of positive people and inclusion in the National Health System for diagnostic confirmation and clinical follow-up. We will also include a local digital electrocardiogram to screen for possible abnormalities related to Chagas disease in individuals with mild cardiac form/stage A. The Chagas Express XXI is a potentially useful social technology for health and science education and active search for chronic cases of disease of asymptomatic CD patients, contributing to the notification of chronic cases and their inclusion in the lines of care of PCDT-Chagas. Furthermore, this technology can be adapted to understand and cooperate in other potentially epidemic situations, especially related to other neglected diseases, such as leishmaniasis, tuberculosis, and arboviruses.

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Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study nor in the writing of the manuscript, or in the decision to publish the results.

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