Status of vaccine research and development of vaccines for Chagas disease

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1. About the disease and pathogen

Chagas disease (American trypanosomiasis caused by *Trypanosoma cruzi*) is a vector-borne parasitic infection transmitted by triatomines (kissing bugs), today considered one of the most important neglected tropical diseases globally (NTDs) [1].

1.1. Global disease and economic burden

An estimated 7–8 million people are infected worldwide with almost all of the cases occurring in the Americas, predominantly in the poorest countries in the region [2–4]. However, revised estimates taking into consideration underreporting and poor diagnosis suggest that the number of total worldwide Chagas cases is much closer to 9–10 million [5,6]. Further estimates are that Chagas disease is responsible for 10,600 deaths per year in addition to 97,500 years lived with disability (YLDs) [5,7]. In total, combining years of life lost and YLDs, Chagas disease causes approximately 0.6 million disability adjusted life years (DALYs), a measure integrating a disease's morbidity and mortality [4,5,8]. According to the World Health Organization, currently the largest number of people living with Chagas disease are found in focal areas of poverty within Latin America's three wealthiest countries: Argentina (1.5 million), Brazil (1.2 million), and Mexico (0.9 million), while the highest prevalence of the disease (in terms of percentage of the population infected with *T. cruzi*) is seen in Bolivia (6.104 cases per 100 people) [7]. Chagas disease has also become "globalized" with thousands of cases also found in Southern Europe, Australia, and Japan [9]. Also, of particular concern are the large numbers of cases in the southern United States, especially Texas where emerging evidence indicates a significant level of autochthonous transmission [10,11].

Despite its global importance in the most affected Western Hemispheric countries, it is estimated that only a small percentage of Chagas disease patients are diagnosed and receive access to essential medicines and other healthcare [12]. Patients with Chagas disease typically live in extreme poverty and represent a highly vulnerable population. The poor who live in the Western Hemisphere's wealthiest countries, including Argentina, Brazil, Mexico, and the United States are especially neglected, a concept consistent with the tenets of "blue marble health" [13]. Moreover, Chagas also has an important impact on veterinary public health, with canines and sylvatic mammals serving as important reservoir hosts.

In addition to its global health impact, Chagas disease has been demonstrated to be one of the largest causes of why Latin America's poorest population, the "bottom 100 million", remain impoverished ($7 billion annual losses to the economy) [14,15].

1.2. Natural history and clinical disease

Typically, the *T. cruzi* protozoan parasite enters its vertebrate hosts with insect faeces deposited into fresh bite sites, mucosal surfaces or other skin breaks. In its trypomastigote form the parasite then can invade cells such as fibroblasts, macrophages, and epithelial cells, thus largely evading the host's immune system. Within its mammalian host the parasite then transforms into its intracellular amastigote form, and concomitant with the eventual rupture of these host cells, blood-form trypomastigotes are released. The cycle is completed when triatomines take up trypomastigotes during subsequent feedings, and the parasite develops into the epimastigote form in the midgut, which multiplies by binary fission, and back into trypomastigotes in the hindgut [16].

Exposure to the *T. cruzi* parasite results in the development of an acute infection, which is often asymptomatic and lasting for 4–8 weeks, but can also be characterized by a rather telling localized swelling at the site of infection (Chagoma), or a special form of

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conjunctivitis (Romaña's sign). Virtually all of these acutely infected individuals seroconvert to *T. cruzi*, but approximately 60–70% of these individuals do not progress to clinically apparent disease (*indeterminate status*), while the remaining 30–40% move on to develop the chronic form of the disease (*determinate status*), concomitant with the appearance of cardiac and/or gastrointestinal signs and symptoms. Cardiac complications in particular, occurring in 20–30% of patients, cause the most severe morbidity and mortality and are characterized by arrhythmias, aneurysms, and heart failure. Ventricular tachycardia and fibrillation leading to sudden death are connected to two-thirds of the mortalities from Chagas disease, followed closely by heart failure and thromboembolism [17]. Pathological findings in chronic Chagasic gastrointestinal disease include dilatation and muscular hypertrophy of the colon or the oesophagus, as well as injury to the parasympathetic nervous system [18].

While most of Chagas disease occurring in the Americas is transmitted by triatomines, there is now also evidence for increasing maternal-to-child transmission of the disease, resulting in congenital Chagas disease. Hundreds of thousands of pregnant women in the Americas now live with Chagas disease, while maternal-to-child transmission of *T. cruzi* leading to congenital Chagas disease has resulted in 8668 documented neonates infected in Latin America (in 2010), with Mexico having the largest number of cases of congenital Chagas disease [7], though these estimates do not likely represent the true number of cases due to poor detection of the disease [19,20].

1.3. Current approaches to treatment

Current treatment is dependent on two drugs, benznidazole or nifurtimox, both of which are highly effective, provided therapy is initiated at the onset of infection and during the acute phase of the disease. However, very few patients are diagnosed in the acute stages because these stages are often silent, or because the patients do not have access to proper diagnosis and treatment. The efficacy of both drugs for many patients with chronic Chagas disease diminishes the longer a person has been infected. In fact, a recent randomized trial of patients showing evidence of Chagasic cardiomyopathy failed to show any benefit for patients treated with benznidazole versus an untreated control cohort when it came to the progression of cardiac clinical deterioration [21]. Therefore, the benefits of medication in preventing or delaying the development of Chagas disease has to be weighed against the long duration of treatment (up to two months) and relatively high rates of adverse reactions that occur in up to 40% of treated patients. Furthermore, the drugs are contraindicated in pregnancy and for those with kidney or liver failure. Additionally, specific treatment for cardiac or digestive manifestations may be required. Up to 20% of patients cannot tolerate full treatment courses [22,23]. Given this current situation, there is an urgent need for a new treatment strategy is crucially needed for chronic Chagas disease, including a safe and effective vaccine.

2. Overview of current efforts

2.1. Biological feasibility for vaccine development

Currently there are at least two major potential Target Product Profiles (TPPs) for a Chagas disease vaccine (Box 1). The first is a prophylactic vaccine that would prevent acute infection and the second is a therapeutic vaccine for patients that have seroconverted and are at the indeterminate stage. It is widely noted that a therapeutic or preventative *T. cruzi* vaccine would be highly beneficial in controlling Chagas disease [23,24]. A preventative

vaccine if used in highly endemic areas could potentially prevent acute infection (especially in a hyperendemic setting found in some parts of Latin America) but is unlikely to interrupt disease transmission because of the large number of animal reservoirs and zoonotic hosts, typically found in these endemic areas. A therapeutic vaccine administered alone or in combination with chemotherapy would be used to prevent or delay the onset of Chagasic cardiomyopathy in patients who have seroconverted to *T. cruzi* infection and are at the indeterminate or early determinate stage [15]. Hence a third TPP would include the use of a therapeutic vaccine used in conjunction with benznidazole or other newly developed antiparasitic drugs. Some investigators refer to this combination as "vaccine-linked chemotherapy".

Box 1: TPPs for Chagas disease vaccine
Preventive Chagas disease vaccine
Therapeutic Chagas disease vaccine in patients with indeterminate infection
Therapeutic Chagas disease vaccine linked to anti-parasitic chemotherapy ('vaccine linked chemotherapy')

Cost-effectiveness studies have been conducted on both the preventive and therapeutic vaccine TPPs [14,15,25]. While both are considered cost-effective, the therapeutic vaccine is considered more cost-effective and cost-saving and therefore more economically dominant [15]. There is as yet no consensus among the community of Chagas disease researchers on the urgency for developing a prophylactic vaccine. However, there is general agreement on the need for new therapeutic anti-trypanosomal products that go beyond the currently available drugs, benznidazole and nifurtimox.

Both scientific and socioeconomic challenges have hindered the development of vaccines against Chagas disease. Chagas disease is an NTD almost exclusively affecting people in poverty and thus there has been an absence of market incentive for pharmaceutical companies to undertake research and development on a vaccine. In addition, initial safety concerns over exacerbating pre-existing cardiac lesions through activation of an autoimmune reaction, based on earlier assertions that there is an important autoimmune component to Chagas disease pathogenesis. However, most investigators currently believe that it is parasite persistence that is associated with disease progression, and that a strong CD8$^+$ T cell immune response will need to be induced, encompassing either interferon-gamma or cytotoxic activity, or both, in order to control *T. cruzi* infection through vaccination [18,22,23].

Evidence from the testing of a wide range of vaccine formulations over the years that range from whole parasites, to purified or recombinant proteins, viral vectors and DNA vaccines have provided preliminary pre-clinical proof-of-concept for vaccination as a preventive and potentially therapeutic strategy. It has further been observed that the efficacy of *T. cruzi* vaccines in pre-clinical models will be greatly influenced by the vaccine formulations used and their resulting immune response [22,23]. Along these lines, many preclinical models have been employed in the study of Chagas disease, most of which include rodents, dogs, and non-human primates (NHP). The advantages of rodent models, most commonly mice, are lower cost for housing and handling, and a shorter lifespan resulting in quicker results. However, it is often difficult for the murine systems to completely reflect the pathology seen in human Chagas disease. Canine and NHP models often more successfully mimic the human responses and pathologies observed, but, with their longer lifespan, experimental results are often delayed. In addition, ethical concerns that surround their use and the

Table 1
Development status of current vaccine candidates.

Candidate Name/identifier	Developer	Platform/antigens	Preclinical	References
Tc24	UADY, CINVESTAV, Sabin Vaccine Institute-PDP	DNA, recombinant protein	X	[34,35]
TSA-1	UADY, CINVESTAV, Sabin Vaccine Institute-PDP	DNA, recombinant protein	X	[35,36]
Live attenuated *Trypanosoma cruzi*	Universidad Nacional de Salta	Live attenuated parasite	X	[37,38]
Live *T. rangeli*	Universidad Nacional de Córdoba	Live parasite	X	[39,40]
ASP-2	Universidade Federal de São Paulo	Adenoviral vector	X	[41–43]
TS	Universidad Nacional del Litoral	Recombinant protein	X	[44]
Cruzipain	Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad de Buenos Aires	Salmonella vector	X	[45,46]
GP83	University of Alabama at Birmingham	Adenoviral vector	X	[47]
TSSA CD8+ epitope	Universidade de São Paulo	Recombinant protein	X	[48,49]
Tc52	Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad de Buenos Aires	Salmonella vector	X	[45,50]
TcG1, TcG2, TcG4, TcVac2, TcVac4	University of Texas Medical Branch	DNA-recombinant protein prime-boost	X	[51–53]
MASP	University of Texas at El Paso	Peptide	X	[54]

significant increase in cost involved must be considered when planning these studies [26].

2.2. General approaches to vaccine development for this disease for low- and middle-income country markets

As alluded to above, several recent preclinical studies have used recombinant proteins, recombinant viral (adenovirus) vectors, DNA platforms, and heterologous prime-boost vaccination regimens and elicited protection against infection and increased survival in murine models (Table 1).

The success of either a preventative or therapeutic vaccine would depend on its ability to induce a T_H1-mediated immune response suggesting that the same antigens and formulations would be efficacious for both types of vaccine. A therapeutic vaccine though, as a standalone therapy or used together with benznidazole and nifurtimox in a vaccine-linked chemotherapy approach, is likely the currently desired (and probably most feasible) strategy for vaccine development with the potential to reduce the dose and/or the treatment duration, therefore ultimately reducing the toxicity of current and even emerging chemotherapeutics [15].

It is estimated that an effective vaccine could have a significant impact on the prevention of cardiac complications. This would be of paramount importance given the approximately 40,000 new cases of Chagas disease that occur in Latin America every year. Remarkably, it is estimated that a vaccine would further prevent 10,000 deaths or more annually and would save up to 600,000 DALYs, caused by cardiomyopathy and gastrointestinal disease [2,27]. In addition, it is expected that a vaccine could serve to reduce child mortality and improve maternal health and pregnancy outcome in Latin American countries and could simultaneously reduce cost of treatment for this disease, while increasing efficacy in treating disease symptoms [25,28].

3. Technical and regulatory assessment

Currently no clinical trials have begun for a Chagas disease vaccine. However, national regulatory agencies for endemic countries such as the U.S. Food & Drug Administration (FDA), Comisión Federal para la Protección contra Riesgos Sanitarios (COFEPRIS, Mexico), European Medicines Agency (EMA) and Agência Nacional de Vigilância Sanitária (ANVISA, Brazil) could be engaged following submission of an Investigational New Drug application. The critical path for clinical development could likely involve a first test of safety of the vaccine in an NHP model prior to *first-in-human* trials. It has been noted that a significant percentage of NHPs living outdoors in primate centers in Chagas endemic areas of the Western

Hemisphere are actually infected with *T. cruzi* [29]. The parameters used to evaluate proof-of-concept may be similar to those employed for evaluating new small molecule therapeutics including parasitemia [30]. In addition, efforts are underway at PAHO and the WHO to assess the response to anti-parasitic treatment in the chronic phase of the disease, which we expect to be equally beneficial for the evaluation of vaccine efficacy in humans [31].

4. Status of vaccine R&D activities

A variety of different approaches to the development of Chagas vaccines is underway as shown in Table 1. These approaches range from immunization with attenuated parasites of non-pathogenic *Trypanosoma* strains, to DNA vaccines and recombinant protein vaccines. Additionally, the combination of various candidate antigens into a multivalent strategy is also being explored. Currently, all candidates are still in the preclinical phase of testing and not yet in clinical trials.

Pre-clinical testing is currently underway for several therapeutic vaccine candidates in mice, dogs, or non-human primates at academic-based institutions (Table 1, [32]). Many of these candidates are DNA vaccines undergoing testing in mice. The Sabin Vaccine Institute Product Development Partnership (Sabin PDP), in collaboration with the Carlos Slim Foundation, the Universidad Autónoma de Yucatán, the Laboratorios de Biológicos y Reactivos de México, and the Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, is pursuing a therapeutic vaccine comprised of recombinant antigens expressed in yeast or bacteria and formulated with an immunostimulant [24]. In addition, the Sabin PDP collaborates with several other academic groups of investigators to evaluate and test their vaccine candidates.

5. Likelihood for financing

According to G-Finder, from 2007 to 2013, just under US$6 million in funding was awarded for Chagas disease vaccine research and development (R&D, preventative and therapeutic), primarily from major public sector funders including the Argentine Ministry of Science, Technology and Productive Innovation, German Federal Ministry of Education and Research (BMBF), Institut Pasteur, and the US National Institutes of Health (NIH) [33]. Major philanthropic funders such as the Bill & Melinda Gates Foundation and the Wellcome Trust have not yet prioritized vaccine R&D for the disease, though the Carlos Slim Foundation and the Japanese Global Health Innovative Technology (GHIT) Fund have initiated investment strategies for Chagas disease vaccines, together with

other private organizations including the Texas-based Kleberg Foundation, the Southwest Electronic Energy Medical Research Institute, and Texas Children's Hospital. Because Chagas disease is endemic to both wealthy Latin American nations and the United States, and there is evidence of globalization of Chagas disease to Europe, Australia, Japan, and elsewhere, this could potentially incentivise investment from the pharmaceutical industry upon pre-clinical and clinical proof-of-concept trials.

Conflicts of interest

Several of the authors are investigators and patent holders on vaccines against hookworm and other neglected tropical diseases and are supported in part by grants to develop these vaccines.

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