Advances and challenges toward a vaccine against Chagas disease

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Chagas disease is major public health problem, affecting nearly 10 million people, characterized by cardiac alterations leading to congestive heart failure and death of 20–40% of the patients infected with *Trypanosoma cruzi*, the protozoan parasite responsible for the disease. A vaccine would be key to improve disease control and we review here the recent advances and challenges of a *T. cruzi* vaccine. There is a growing consensus that a protective immune response requires the activation of a Th1 immune profile, with the stimulation of CD8⁺ T cells. Several vaccine types, including recombinant proteins, DNA and viral vectors, as well as heterologous prime-boost combinations, have been found immunogenic and protective in mouse models, providing proof-of-concept data on the feasibility of a preventive or therapeutic vaccine to control a *T. cruzi* infection. However, several challenges such as better end-points, safety issues and trial design need to be addressed for further vaccine development to proceed.

Introduction

Chagas disease or American Trypanosomiasis is major public health problem, affecting nearly 10 million people, mostly in Latin America where it is endemic, but international migrations are making the disease increasingly frequent in non-endemic countries as well. It is one of the most neglected tropical diseases, frequently associated with poverty and margination in rural areas, although it is also expanding in richer suburban and urban areas in many countries. The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas disease, and it is transmitted mainly by hematophagous vectors from the triatominae family, commonly known as kissing bugs. Secondary routes of transmission include blood transfusion, organ transplant and materno-fetal transmission, and oral outbreaks have also been described.

The disease initiates by an acute phase lasting a few weeks and characterized by an elevated parasitemia associated with fever, headache, nausea, that is rarely lethal. This phase is followed by a chronic phase, which remains asymptomatic for many years. The parasites are then difficult to observe in the blood,

and diagnosis relies principally on serologic tests. The majority of patients remain in this indeterminate stage for life, while 20–40% start presenting a symptomatic chronic phase characterized by a progressive and debilitating Chagasic cardiomyopathy and sometimes megasyndromes. Arrhythmias of increasing severity lead to congestive cardiac failure and death of the patients.¹

Drug treatments of infected patients are based on Nifurtimox and Benznidazol since the 1960s, and while their efficacy is fairly well established during the acute phase of infection and in children, their usefulness in chronic phase and adult patients is still debated and a large clinical trial is currently underway to clarify this point.^{2,3} Both drugs require prolonged treatments and can have severe adverse effects.^{4,5} Patients also require palliative care for the cardiomyopathy, ranging from drug therapy to pacemakers and cardiac transplants.⁶ Thus treatment can cost up to over 1,000 USD per year and per patient.⁷

Prevention currently only relies on vector control, which has considerably reduced disease incidence and prevalence in most Latin America.⁸ Nonetheless, a number of triatomine species have proven difficult to control and the most likely scenario is that low level vectorial transmission to humans will be maintained in many regions and countries in spite of vector control interventions.⁹

In this context, a vaccine would be key to improve the control of Chagas disease, and a recent study highlighted the large cost-effectiveness that a preventive vaccine for humans would have in a variety of scenarios. Thus, a vaccine would be cost-effective even where transmission to humans and prevalence of infection are low (i.e., after vector control or in regions of low endemicity), and even for a vaccine of moderate protective efficacy.¹⁰ Alternatively, a therapeutic vaccine administered alone or in combination with current drug treatment may greatly improve the prognosis for Chagasic patients by increasing treatment efficacy, reducing its duration and cost, or at least delaying disease progression to advanced stages and heart failure. Such a therapeutic vaccine is also likely to be very cost effective. In addition, in some regions, domestic animals such as dogs, have been found to play an important role as reservoir of the parasite and its transmission to human.^{11–14} Control of infection in these animals has thus been proposed as an important complementary strategy to vector control, for example using insecticide-impregnated collars.¹⁵ However, a veterinary vaccine, either preventive or therapeutic, would thus also greatly contribute to Chagas disease control by reducing infectiousness of the main domestic reservoir

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of the parasite, as well as improving the care of companion animals that are increasingly being detected as infected by *T. cruzi*.¹⁶ We review here some of the recent advances and challenges in *T. cruzi* vaccine development, and an analysis of earlier work can be found in previous reviews in reference 17–19.

Correlates for Immune Protection against *T. cruzi*

A good understanding of the interactions between *T. cruzi* and the immune system is key to the rational design and improvement of vaccines, and important advances have been made in recent years. However, it is important to recall that *T. cruzi* vaccine research has been hindered by the fear of exacerbating myocardial lesions through an activation of a severe autoimmune response, based on the hypothesis that Chagas disease was actually an autoimmune disease.^{20,21} Nonetheless, parasite persistence rather than an imbalanced immune response, has been shown to be associated with disease progression,²² strengthening the rationale for a vaccine (see also below).

Recognition of *T. cruzi* by the immune system relies on the activation of the innate and adaptive immunity. Recognition of pathogen-associated molecular patterns (PAMP) by Toll-like receptors (TLR) such as TLR-2 and TLR-9, lead to the activation of T and B cells, making TLRs an important link between innate and adaptive immunity. The absence of TLR signaling reduces the activation of CD4⁺ cells against *T. cruzi*, while CD8⁺ responses may be maintained.²³ Indeed, *T. cruzi* is able to downregulate the immune system through the release of several molecules.^{23,24} *T. cruzi* can promote IL-10 production by dendritic cells allowing for its persistence, while in a model of dendritic cell-based immunization, IL-10-deficient dendritic cells pulsed with *T. cruzi* antigens conferred protection against infection to recipient mice.²⁵ Specific parasite proteins such as cruzipain and Tc52 can directly increase IL-10 expression, leading to a non-protective immune response.²⁶ Thus, aiming at both early killing of parasites and neutralizing suppressive mechanisms may be necessary for effective therapies and vaccines.

IFN γ plays a key role during *T. cruzi* infection, increasing the production of nitric oxide by macrophages, which inhibits the development of the intracellular form of *T. cruzi*.²⁷ CD4⁺ Th1 lymphocytes that produce the cytokines interleukin-2 (IL-2) and IFN γ and can stimulate the expansion of cytotoxic CD8⁺ T lymphocytes, appear central for systemic protection against *T. cruzi* infection.^{28,29} Recent studies have confirmed that CD8⁺ T cells act both indirectly by secreting IFN γ to activate macrophages and directly through the production of perforin and their concomitant cytotoxic activity.³⁰ IFN γ producing CD4⁺ and CD8⁺ T cells also play a key role in the liver for the clearance of blood trypomastigotes.³¹ These cells are stimulated following oral infection, which is supposed to be a common route of infection for several animal reservoir species.³² Thus, there is now a growing consensus that a protective immune response against *T. cruzi* requires the activation of a Th1 immune profile, with the stimulation of CD8⁺ T cells, while antibodies may play a rather secondary role.

Current Status of Vaccine Research

A very wide range of vaccine formulations have been evaluated over the years, ranging from whole parasites, to purified or recombinant proteins, viral vectors and DNA vaccines (Table 1). Initial attempts at using killed *T. cruzi* parasites resulted in modest but encouraging protection against infection,³³ possibly because of a rather low immunogenicity. Live attenuated parasites on the other hand are much more immunogenic and early studies indicated that they were able to confer significant protection against infection.³⁴ Similarly, immunization with live *T. rangeli*, a related parasite species non-pathogenic in humans, can also provide some cross-reactive immunity and partial protection, possibly due to homologous antigens.^{35–37} However, these approaches raise the issues commonly associated with live vaccines, i.e., safety and the challenges associated with their large-scale production and distribution. Nonetheless, there is a renewed interest in a live attenuated vaccine approach and recent studies have described the generation of *T. cruzi* mutants for specific genes such as LYTI or ECH1 and 2, which can provide good protection against infection in mice, including through oral administration.^{32,38}

Early attempts at immunizing mice with more defined preparations were based on proteins purified from parasite culture, including cruzipain, the paraflagellar rod protein or trypomastigote excretory-secretory antigens.^{39–41} As expected, the outcome of such vaccines is critically dependent on the formulation used, and the orientation of the immune response that is induced. For example, immunization with cruzipain administered with alum or Freund adjuvants, which bias the immune response toward a Th2 type and high antibody levels, is not protective. On the other hand, the same antigen administered with CpG oligonucleotides induces a Th1 type immune response with elevated IFN γ production, which protects mice against infection by reducing parasitemia and increasing survival.⁴² More recent studies are based on the use of recombinant proteins, which have now been extensively evaluated. Some of the most promising antigens tested include cruzipain, GP82, and several proteins from the trans-sialidase superfamily such as ASP-2 and TS, and all can provide significant protection in a variety of mouse models when administered with CpG oligonucleotides as adjuvants (Table 1). This again, highlights the need to induce a strong cellular immune response with CD8⁺ cell activation and cytotoxic activity for the control of *T. cruzi* infection, rather than an antibody response.

Accordingly, several of these antigens have been formulated as recombinant viral vaccine vectors as well as DNA vaccines since these formulations may be more effective than recombinant proteins at inducing a Th1 type immune response with cytotoxic CD8⁺ T cells.^{43,44} Sendai, vaccinia and adenovirus vectors are among those that have been evaluated, expressing several of the antigens mentioned above, including ASP-2 and TS proteins from the trans-sialidase superfamily (Table 1). As expected such vaccines were able to induce strong IFN γ responses as well as the activation of CD8⁺ cytotoxic T cells, which led to a strong protection against infection as indicated by a decreased parasitemia and increased survival of mice. Such protection was often better than that achieved by the corresponding recombinant

Table 1. Preventive vaccines against *T. cruzi* evaluated in mice

Formulation	Antigens	Immune response	Parasitemia	Heart Parasite Burden	Heart Histopathology	Survival	Reference
Whole organism	Live attenuated <i>Trypanosoma cruzi</i>	NR	Decreased	Decreased*	NR	NR	32
	Live <i>Trypanosoma rangeli</i>	NR	Decreased	Decreased	No change	Increased	37
Recombinant protein	rASP-2 + Alum or CpG ODN	Increased IFN γ	Decreased	NR	NR	Increased	94, 95
	rTS (trans-sialidase) + CpG ODN	IgA, IgG and IFN γ	NR	Decreased	NR	Increased	96, 97
	rCruzipain + CpG ODN	Humoral and cellular response, IFN γ and lytic activity	Decreased	NR	Decreased inflammation**	NR	98
	rGP82 + CpG ODN	IFN γ	NR	NR	NR	Variable according to challenge route	99
Viral vector	adenovirus or vaccinia expressing TSSA CD8 ⁺ epitope	IFN γ	Decreased	NR	NR	Increased	47
	adenovirus expressing TS and ASP-2	Antibodies and cytolytic activity	Decreased	NR	NR	Increased	100
	Sendai virus expressing ASP-2	IFN γ and CD8 ⁺	Decreased	NR	NR	Increased	101
DNA	TSA-1, ASP-1 and ASP-2 with IL-12 and GM-CSF	Antibodies and cytolytic activity	Decreased	NR	Decreased inflammation	Increased	102
	ASP-2 or UB-ASP-2	IFN γ , CD8 ⁺ , cytolytic activity	Decreased	NR	NR	Increased	94, 103
	TS + IL15	IFN γ and CD8 ⁺	NR	NR	NR	Increased	104
	TSA-1 and Tc24	NR	Decreased	NR	Decreased inflammation	NR	60
Heterologous prime-boost	TcVac2 (TcG1, TcG2, TcG4) + IL-12 + GM-CSF DNA and recombinant protein boost + saponin	Humoral and cellular response, IFN γ and CD8 ⁺	Decreased	Decreased	Increased	NR	53
	DNA + adenovirus expressing TS and ASP-2 clone 9 (Prime-Boost)	IFN γ , CD8 ⁺ , cytolytic activity	Decreased	NR	NR	Increased	105

NR, not reported. *Parasite burden was actually measured in skeletal muscle rather than heart. **No alterations were observed in the heart, but inflammation was reduced in skeletal muscle.

protein, strengthening the idea that vaccine type is key to achieve the desired immune response. Furthermore, heterologous prime-boost immunization strategies, based on a combination of DNA immunization followed by recombinant protein or viral vectors, have also been evaluated and may further enhance the protective immunity induced. However, such complicated immunization protocols may be difficult to translate into a field vaccine. New recombinant virus vectors have also recently been described, such as Yellow fever virus expressing ASP-2⁴⁵ and influenza virus

expressing a single dominant CD8⁺ epitope,⁴⁶ which may provide additional alternatives for immunization. Indeed, immunization with a vaccinia and adenovirus expressing this epitope was previously found to be sufficient to induce protection, highlighting the central role of this T cell population in parasite control.⁴⁷ In fact, highly immunodominant CD8⁺ epitopes have been identified in the trans-sialidase protein family, resulting in a restricted immune response during *T. cruzi* infection, as observed in both mice and humans.^{48,49} This explains why most vaccine studies have focused

Table 2. Therapeutic vaccines against *T. cruzi* infection evaluated in mice

Formulation	Antigens	Immune response	Parasitemia	Heart Parasite Burden	Survival	Heart Histopathology	Reference
Recombinant protein	rSA85-1.1	Increased antibody levels	NR	NR	NR	No exacerbation	58
DNA	TSA-1, Tc24, and Tc52	Increased IFN γ and CD8 $^{+}$	Decreased	Decreased	Increased	Decreased inflammation	57, 59, 106, 60
	ASP-2 like clone 9 and TS	NR	No effect	No effect	No effect	No effect	106

NR, not reported.

on antigens from the trans-sialidase family, but while such epitope-specific CD8 $^{+}$ T cells are important for *T. cruzi* control, they only provide strain-specific protection.⁵⁰ Importantly, mice tolerated for these dominant epitopes are still able to control acute *T. cruzi* infection through the activation of CD8 $^{+}$ T cells specific for unknown alternate epitopes.⁵¹ Identifying these epitopes may thus lead to additional vaccine antigens that could complement trans-sialidase proteins.

Indeed, as noted before,¹⁹ a very limited variety of *T. cruzi* antigens have been evaluated so far, and some studies have focused on the identification of novel antigens. Thus for example, the antigens TcG1, TcG2 and TcG4, corresponding to a putative ribosomal protein and hypothetical proteins, respectively, have been recently identified and immunization with DNA vaccines encoding all three antigens followed by recombinant protein boosts (TcVac2) was found able to induce potent antibody, CD8 $^{+}$ T cell and cytokine responses and protected mice from acute parasitemia and chronic cardiac tissue damage.^{52,53} Alternatively, immunization with a trypomastigote cDNA library and the sequential selection of protective pools has also been used to identify a series of novel antigens able to confer increased survival following infectious challenge.⁵⁴ Similarly, a novel family of surface proteins has been recently identified that seems to be specific for *T. cruzi*.⁵⁵ Taken together, these studies may thus lead to some promising antigens with important potential as novel vaccine candidates.

While the studies mentioned above support the feasibility of a preventive vaccine against *T. cruzi*, it is also noteworthy that therapeutic vaccines can control an ongoing *T. cruzi* infection (Table 2). Indeed, the administration of DNA vaccines encoding a trans-sialidase antigen (TSA-1) or the secreted antigen Tc24⁵⁶ following a *T. cruzi* infection was found able to reduce the parasitemia and cardiac inflammatory reaction, while increasing survival of treated mice.⁵⁷ Similarly, the immunotherapy with recombinant SA85-1.1 from the trans-sialidase superfamily, allowed to reduce parasitemia and cardiac tissue damage.⁵⁸ It is also important to stress that no exacerbation of disease has been observed in these studies, in spite of the significant activation of the immune response following vaccine administration. Indeed, in agreement with the data presented above on preventive vaccine, the efficacy of such therapeutic vaccine appears to rely on a reorientation of the immune response toward a Th1 type, with the production of IFN γ and the activation of CD8 $^{+}$ T cells, and the lack of CD8 $^{+}$ completely abrogate the therapeutic vaccine

effect.^{59,60} It is thus of key interest that the same antigens and vaccine formulations may be used for both the prevention of Chagas disease as well as for the therapy of an ongoing infection, making these a very flexible tool for disease control.

Given these promising results in mouse models, a few of these vaccine candidates have been evaluated in dogs. These are considered an excellent animal model, which presents a disease progression much more similar to that of humans than mice.⁶¹⁻⁶³ In addition, as mentioned above, a veterinary vaccine for the control of canine Chagas disease would be very helpful for the control of this important parasite reservoir, as well as for the care of pets which are increasingly found to be infected by *T. cruzi*.¹⁶ Thus, immunization with a live attenuated *T. cruzi* strain was found to significantly reduce natural *T. cruzi* infection in dogs in a large field study, as assessed by xenodiagnosis.⁶⁴ Similarly, as observed in mice, immunization of dogs with *T. rangeli* was able to reduce their infectiveness to triatomine vectors following experimental infection, although all dogs developed parasitemia and it is unclear if they were protected from further disease development.⁶⁵ More recently, immunization with four doses of TcVac1, based on a combination of DNA vaccines encoding TcG1, TcG2 and TcG4, dog IL-12 and GM-CSF was evaluated.⁶⁶ Immunization induced an increase in antibody levels, together with some increase in IFN γ and CD8 $^{+}$ T cells, confirming the immunogenicity of the vaccine. However, immunized dogs presented only a small decrease in infectivity to triatomines, as well as in cardiac arrhythmias, and the inflammatory reaction in the heart appeared unchanged compared with control dogs. Importantly, the density of necrosis, inflammatory foci and amastigote nests (parasite burden) were increased in vaccinated dogs, as well as their mortality, suggesting a limited efficacy of this vaccine.⁶⁶

As in mice, the therapeutic administration of two doses of DNA vaccines encoding TSA-1 and Tc24 during the acute phase has also been tested in dogs and EKG recording indicated a decrease in the severity of cardiac arrhythmias⁶⁷ (Quijano-Hernandez et al. unpublished data). Importantly, this DNA vaccine can also reduce the density of amastigote nests, cardiac arrhythmias and mortality when used as a preventive vaccine in dogs, emphasizing the potential of this formulation (Quijano-Hernandez et al. unpublished data). Taken together, these encouraging studies set the basis for further evaluation of both preventive and therapeutic vaccines in this animal model, which will be key to further our understanding of *T. cruzi* control in non-murine models.

Perspectives and Challenges to *T. cruzi* Vaccine Development

While many of the studies highlighted above provide proof-of-concept data on the potential of a preventive or therapeutic vaccine to control a *T. cruzi* infection by at least decreasing parasite burden, cardiac tissue inflammation and damage or increasing survival, a number of important challenges and key questions remain to be answered for further vaccine development to proceed.

First of all, some authors have argued against the rationale presented above about the actual need for a vaccine.^{68,69} Indeed, as transmission of the disease has been effectively controlled by vector control interventions in large parts of Latin America, the population at risk of infection has been dramatically reduced. Thus, there seem to be no population left to vaccinate. However, as we briefly mentioned, it is important to stress that domiciliated *Triatoma infestans* and *Rhodnius prolixus* are the only vector species which have been effectively controlled (although partially as insecticide resistance has now been detected in *T. infestans*), and that many autochthonous triatomine species are much more difficult to control and will remain responsible for significant *T. cruzi* transmission to humans, although at lower levels. Furthermore, the cost-effectiveness of a vaccine has been demonstrated even when there is a low prevalence of *T. cruzi* infection (about 1%¹⁰), which is what can be expected to remain after such vector control efforts. A vaccine would thus definitively be an asset for populations still at risk, or for infected patients in the case of a therapeutic vaccine. In addition, a veterinary vaccine targeting domestic reservoirs would be an attractive complement to vector control, to help mitigate the rising problem of insecticide resistance in some vector species.

Second, a major concern with Chagas disease has been the fear of exacerbating disease by stimulating autoimmunity through vaccination instead of providing protection,^{20,21,70} which has considerably slowed down vaccine research. Importantly, this situation is very similar to that of several other pathogens for which there is also concern about vaccine-increased susceptibility to infection.^{71,72} A typical example is dengue virus, in which case antibody-dependent enhancement (ADE) caused by weakly neutralizing but cross-reactive antibodies is believed to favor a secondary infection by a heterologous serotype of the virus. While the mechanisms of these processes are still not understood in detail, these aspects are carefully taken into consideration in vaccine development to avoid such adverse effects.^{73,74} In the case of *T. cruzi*, there is now a large body of evidence indicating that disease progression is due to parasite persistence, and autoimmune reactions are rather a consequence of cardiac tissue damage than the main mechanism driving disease progression.⁷⁵⁻⁷⁸ Also, several independent pre-clinical vaccine studies such as those highlighted above have repeatedly shown that the induction of a strong cellular immune response allows for a reduction in parasite burden as well as in cardiac tissue inflammation, rather than exacerbation of disease (Tables 1 and 2). Taken together, these data clearly suggest that both preventive of therapeutic vaccination against *T. cruzi* are safe and do not lead to autoimmune reactions. However, it is also clear that more extensive evaluations are needed. Thus, severe adverse effects including autoimmunity

will need to be closely monitored in future vaccine trials, but do not justify further delaying vaccine development.

Another major issue is the design and feasibility of clinical trials. Indeed, chronic Chagas disease takes years to develop, leading some authors to argue that trials would need to last at least 20 y to see any significant effect.⁶⁸ In addition, given the low incidence of disease in most countries where intensive vector control has been implemented, randomized controlled trials would need to include a very large number of volunteers to allow for the detection of a modest vaccine efficacy. Again, these issues are not specific to *T. cruzi* vaccines and have been raised for other chronic diseases including TB or HIV-AIDS.^{79,80} It is clear that they certainly complicate trial design, but it does not mean that such trials are not feasible. A therapeutic vaccine trial may be easier to design than a preventive vaccine trial, although as in the case of HIV trials it would be necessary to administer the vaccine together with conventional drug treatment, which would make it more difficult to observe a moderate vaccine effect. In any case, novel trial design such as adaptive trial designs may help to reduce duration and number of volunteers needed, while ensuring the scientific quality and integrity of such trials.⁸¹ A correlate problem to trial design is that of the lack of widely accepted biomarkers to evaluate vaccine efficacy. Thus, neither the end-points to be used in a clinical trial, nor the expected target product profile are easily defined. As illustrated above, commonly used markers in animal studies, which include cardiac tissue damage, parasite burden in the heart, disease reactivation following immunosuppression or survival, are too invasive and/or unethical to be used in clinical trials. Also, there is some debate about the need for a vaccine to be able to provide sterile immunity, leading some authors to discuss the usefulness of a vaccine unable to reach this end-point. However, it seems that while desirable, sterile immunity may not be a necessary target. Indeed, disease progression to Chagasic congestive cardiomyopathy and cardiac failure is what is causing the major morbidity of Chagas disease and its economic cost. Thus, preventing or at least delaying the onset of congestive cardiomyopathy may confer sufficient value to a *T. cruzi* vaccine. Thus, clinical indicators such as EKG or echocardiography may provide valuable end-points to evaluate progressive cardiac damage and vaccine efficacy.^{82,83} Also, such clinical indicators may be able to detect difference in disease progression rate within a reasonable time, as suggested by studies in dogs^{66,67,84} and in patient follow-up evaluations.^{82,83,85} In that respect, much can be learned from the current “BENEFIT” trial, which is evaluating the efficacy of drug treatment in chronic-stage patients. Nonetheless, ongoing efforts for the identification of additional biomarkers are underway, including electrocardiographic, immunologic and biochemical markers, which may greatly improve and facilitate the monitoring of Chagas disease progression and thus vaccine evaluation.⁸⁶⁻⁹²

As indicated above, a successful vaccine will need to induce a strong cellular immune response, with the activation of CD8⁺ cytotoxic T cells in order to effectively control *T. cruzi* parasites. This requirement places some constraints on the formulations and types of vaccines that may be used, since such a response is harder to induce than a humoral response. In addition, for a

vaccine to be efficiently distributed in remote rural areas with limited health infrastructure, a very stable formulation would be a key advantage.

A therapeutic vaccine targeting infected adult patients in either the indeterminate or symptomatic chronic phase could be administered through health centers, alone or in combination with conventional drug therapy, to prevent or delay disease progression. Such a vaccine would be of interest in most Latin American countries where the disease is endemic, as well as in non-endemic countries with large populations of migrants from this region. Alternatively, a preventive vaccine could be aimed at both children and adult populations living in endemic areas, even where vectorial transmission has been considerably reduced, to further reduce the incidence of new cases of Chagasic cardiomyopathy. Such a vaccine could eventually be integrated into established vaccination programs.

In conclusion, after years of neglect, it appears that vaccine development against *T. cruzi* is finally coming of age. Abundant

mice studies provide strong proof-of-concept data on the feasibility of controlling *T. cruzi* infection with both preventive and therapeutic vaccines, and it is clear that such vaccines would be extremely helpful for Chagas disease control. Importantly, the emergence of novel international partnerships bringing together academics and manufacturers is expected to provide a renewed momentum to vaccine development against neglected diseases.⁹³ While many challenges remain to be addressed, including technical, ethical and logistical issues, advances in our understanding of Chagas disease pathogenesis and in vaccine development against other diseases are providing some clues about how to address these. Encouraging results from dog trials suggest that a veterinary vaccine could be developed in the short-term, which would provide further support for the development of a human vaccine.

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