Review

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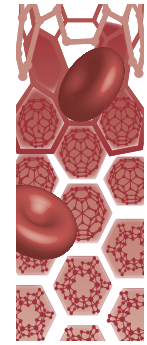
Nanomedicine



Nanomedicines against Chagas disease: an update on therapeutics, prophylaxis and diagnosis

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Chagas disease is a neglected parasitic infection caused by the protozoan *Trypanosoma cruzi*. After a mostly clinically silent acute phase, the disease becomes a lifelong chronic condition that can lead to chronic heart failure and thromboembolic phenomena followed by sudden death. Antichagasic treatment is only effective in the acute phase but fails to eradicate the intracellular form of parasites and causes severe toxicity in adults. Although conventional oral benznidazol is not a safe and efficient drug to cure chronic adult patients, current preclinical data is insufficient to envisage if conventional antichagasic treatment could be realistically improved by a nanomedical approach. This review will discuss how nanomedicines could help to improve the performance of therapeutics, vaccines and diagnosis of Chagas disease.

Keywords: AmBisome • archaeosomes • benznidazole • chronic Chagas cardiomyopathy • microfuidics • nanocapsules • nanomedicine • POC • *Trypanosoma cruzi*

Chagas disease

The American Trypanosomiasis or Chagas disease is a neglected tropical disease [1], considered as highly disabling parasitic infection [2,3]. Nearly 10 million people live with Chagas disease worldwide, a prevalence exceeded only by hookworm and other soil-transmitted helminth infections (Box 1) [1]. Chagas disease itself is a serious opportunistic infection of people living with HIV/AIDS, leading to meningoencephalitis, cerebral lesions and high mortality [4]. Recently impressive similarities between people living with Chagas disease and people living with HIV/AIDS, particularly for those who contracted the disease in the first two decades of the HIV/AIDS epidemic, have been highlighted [5].

Chagas disease is a chronic parasitosis caused by the Kinetoplastid *Trypanosoma cruzi,* which is transmitted by hematophagous *Reduviid* insects. The parasite can also be transmitted by transfusion of contaminated blood, organ transplantation and congenitally from infected mothers to newborns (more than one quarter of the world's new Chagas disease cases). These nonvector modes of transmission together with intense international migrations of the last 15 years have shifted the disease to nonendemic areas [6]. Remarkably, although most of the world's cases occur in Latin America, the infection is becoming increasingly prevalent in Europe and the USA [7].

In all cases, infective but nonproliferative trypomastigotes (metacyclic forms from infected vectors or blood stream forms in transfusional and congenital transmission) enter the bloodstream and invade a variety of cell types, including those of the reticuloendothelial system, muscle and nerve cells of the heart and the gastrointestinal tract. After invasion, the parasites escape the phagolysosomal vacuoles of the host cell and differentiate into replicating amastigotes, which proliferate in the cytoplasm and eventually redifferentiate to trypomastigotes that normally destroy the host cell and reach the bloodstream. Bloodstream trypomastigotes invade other tissues and can also be taken with the blood meal of an appropriate insect vector.

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Box 1. Chagas disease in numbers.

- Endemic in 21 countries in Latin America.
- Approximately 8 million cases worldwide.
- 12,000 deaths annually.
- 430,000 disability-adjusted life years.
- Up to 30% of chronically infected people develop cardiac alterations and up to 10% develop digestive, neurological or mixed alterations which may require specific treatment.
- North America: 1–6 million estimated cases in Mexico and 1 million estimated cases in the USA; 40,000 pregnant women, results in 2000 congenital cases.
- Europe: 59,000–108,000 estimated cases.
- Economic burden of Chagas disease (US$7.19 billion per year) is similar to or exceeds those of other prominent diseases globally (e.g., rotavirus US$2.0 billion and cervical cancer US$4.7 billion).

Tissue damage in the mammalian host results from both direct parasite action and the inflammatory process that ensues. This initial acute phase (that extends by nearly 2 months post-infection) has low mortality (<10%) and generally mild and unspecific symptoms; macrophages, IFN-γ, $CD4^+$ Th1 and $CD8^+$ lymphocytes are the key elements controlling parasite replication [8–10]. The clinical symptoms that lead to chemotherapeutic treatment are manifested in nearly 1% of those suffering the acute phase. The acute phase is followed by a lifelong chronic condition, where the cellular immune response limits the parasite's proliferation but is unable to eradicate the infection. This leads to a sustained inflammatory response that underlies the development of one or more of the symptomatic chronic forms of the disease in 30–40% of patients, including chronic Chagas cardiomyopathy (CCC) and digestive problems [8,11–12]. The most severe of these manifestations is CCC, which typically appears decades after the initial infection, and may result in cardiac arrhythmias, ventricular aneurysm, congestive heart failure, thromboembolism and sudden cardiac death [13,14].

Current treatment & clinical trials

It is currently accepted that parasite persistence is a necessary and sufficient condition to generate and sustain a Th1-biased inflammatory response in infected tissues, which includes some autoimmune phenomena [12,15–18]. The acute cases (all ages) and children up to 14 years old are treated primarily as an infectious, not autoimmune condition [13,19–21]. Specific antiparasite treatment of all chronic phase *T. cruzi*-infected individuals is also recommended by WHO [22].

Only two drugs discovered in the late 1960s and early 1970s, the 5-nitrofuran nifurtimox (NFX; Lampit™, Bayer HealthCare AG, Leverkusen, Germany) and the 2-nitroimidazole benznidazole (BNZ; now produced by LAFEPE as Benznidazol LAFEPE® in Brazil and by Maprimed/ELEA in Argentina as Abarax®) are available for specific antiparasitic treatment. Neither BNZ nor NFX are approved by the US FDA and both of them are contraindicated in pregnancy [5,23]. Both have significant activity in the acute phase (up to 80% of parasitological cures, defined as negativization of parasitological and serological tests, see Table 1), with efficacy varying according to the geographical area. BNZ reduces the severity of the associated inflammatory processes of chronic patients, probably by reducing the parasite loads in infected tissues [24]. Instead, NFX displays potent and specific toxicity on heart and pancreas of rats while BNZ is devoid of such activities, suggesting that the latter may pose a lesser risk to Chagas disease patients with cardiac compromise [25]. Two randomized trials (TRAENA and BENEFIT) are in the process of comparing BNZ to placebo in chronic patients and will provide evidence regarding the evolution of mild or advanced heart disease, respectively. Its results could contribute to modify the actual scenario, where most physicians only prescribe palliative treatment for adult Chagas patients with dilated cardiomyopathy [26]. The reasons why physicians do not prescribe BNZ to chronic adult patients is mainly owed to the lack of a randomized clinical trial showing benefit for patients with this disease, except for children and partly due to the toxic effects caused by BNZ in adults (Figure 1).

The nonprofit organization Drug for Neglected Disease initiative (DNDi), has recently launched a drug research and development platform for Chagas disease [27]. Aiming to reduce cost and enabling translation, a medium term strategy with oral formulations of active principles already approved for another uses (therapeutic switching) was proposed to replace the toxic BNZ. The new triazoles that inhibit the ergosterol biosynthesis (by potent and selective inhibiton of fungal and protozoan cytochrome P-450-dependent C14αsterol demethylase (CYP51) were the most promising candidates, due to their excellent preclinical anticha-gasic activity [28]. However, recent clinical trials have shown higher therapeutic failure and lower efficacy for posaconazole (CHAGASAZOL; SCH 56592; Nox-

afil®, Schering-Plough Research Institute, registered as antifungal against invasive fungal infections) and ravuconazole prodrug (E1224, DNDi in partnership with the pharmaceutical company Eisai Co. [29]) than BNZ [Sosa-Estani s, Pers. Comm.].

These recent clinical failures highlight the need for counting on better, more predictable preclinical models of the cardiac disease. Dogs are proposed as excellent animal models, because present a disease progression much more similar to that of humans than mice [30,31], and because are the main domestic reservoir of *T. cruzi*. Facing the challenge of developing new drugs, a long-term objective proposed by the DNDi, is mandatory in this scenario of counting with one poorly efficient specific treatment against nearly 99% of the chagasic patients. According to the DNDi guidelines, new drugs have to be intended for oral route, in a dosing regimen comparable to systemic antifungals, ideally once daily for 30 days, with a safety superior to BNZ and efficacy not inferior or ideally superior to BNZ; ideally against chronic and acute reactivation caused by all parasite strains. Nonetheless, drugs in development such as cysteine protease (Cruzipain) inhibitors, inhibitors of trypanothione synthesis and metabolism and inhibitors of purine salvage extensively discussed in [32] are still in preclinical tests. This meaning the medium time remaining to reach the market is 10 years at best [33]. In view of this, it is reasonable to search for alternatives others than the classical high throughput screenings and lead optimization approaches used in medicinal chemistry, which could speed the improvement of the antichagasic treatment. Since BNZ activity on humans has not been surpassed by any other antichagasic drug (personal communication), besides of screening for antichagasic activity of already approved active principles, searching for new pharmaceutical forms for BNZ would in principle be a wise decision. However, eradicating intracellular amastigotes and decreasing BNZ's toxicity are not dependent on increasing bioavailability or modifying BNZ's pharmacokinetics. Instead, more radical changes to the ADMET (absorption, distribution, metabolization, excretion, toxicity) of BNZ are necessary and those could be provided by a nanotechnological approach. The first FDA approved (1995) nanomedicine (nano-object [such as liposomes, polymeric or lipid nanoparticles, nanocapsules, micelles] + active principle [API]), is an antitumoral having a nanostructure (pegylated liposome of ~80 nm hydrodynamic diameter) that is responsible for modifying pharmacokinetics and biodistribution of the API (>95% doxorubicin clorhydrate crystalline form) within its inner aqueous space, as compared with the conventional API formulation [34]. In the following 20 years more than 20 nanomedicines – mainly liposomes applied to the oncologic field – were approved by the FDA [35]. Nanomedicines are currently classified as 'nonbiological complex drugs' (NBCDs), that is, drugs showing inherent complexity that determines their pharmacologic activity and ADMET profile, but being of nonbiological, that is, synthetic, origin. Remarkably,

Table 1. Diagnostic test for Chagas disease.

Disease phase	Test	Problems
Acute or reactivation by immunosuppression	Parasitological test: blood wet smear, blood concentration technique such as microhaematocrit or Strout technique	Symptoms are nonspecific or absent. Useful in a short window of time
Chronic	Serologic tests: anti-*Trypanosoma cruzi* antibodies detected by conventional or recombinant ELISA, IHA, IFA or immunochromatographic assay. PCR (parasitologic) test may be recommended, depending on the situation and guideline considered	Needs settings of high throughput, skilled laboratory staff and adequate equipment. Not appropriate for implementation at the prevalent areas (rural and urban primary health care centers). Cross-reaction with antibodies from patients infected with *Leishmania sp.* or *T. rangeli*
Treatment response monitoring	Treatment success is measured by the absence or reduction of antibody titers. Therapeutic failure is defined by the persistence of the parasite, detected using different methods. Standardized qualitative PCR for the assessment of the impact of parasitic load on the overall treatment response is available	Reduction in *T. cruzi*-specific antibody titers often takes many years; the assessment of treatment success becomes insensitive and lengthy. New end points need detected shortly after treatment, such as: anti-*T cruzi* IFN-γ-producing cells; *T. cruzi* antibody titers detected using nonconventional serology (multiplex); and seroreactivity against specific recombinant antigens (complement regulatory protein, recombinant *trans*-sialidase, or kinetoplastid antigen)

IFA: Indirect immunofluorescence assay; IHA: Indirect hemagglutination assay.

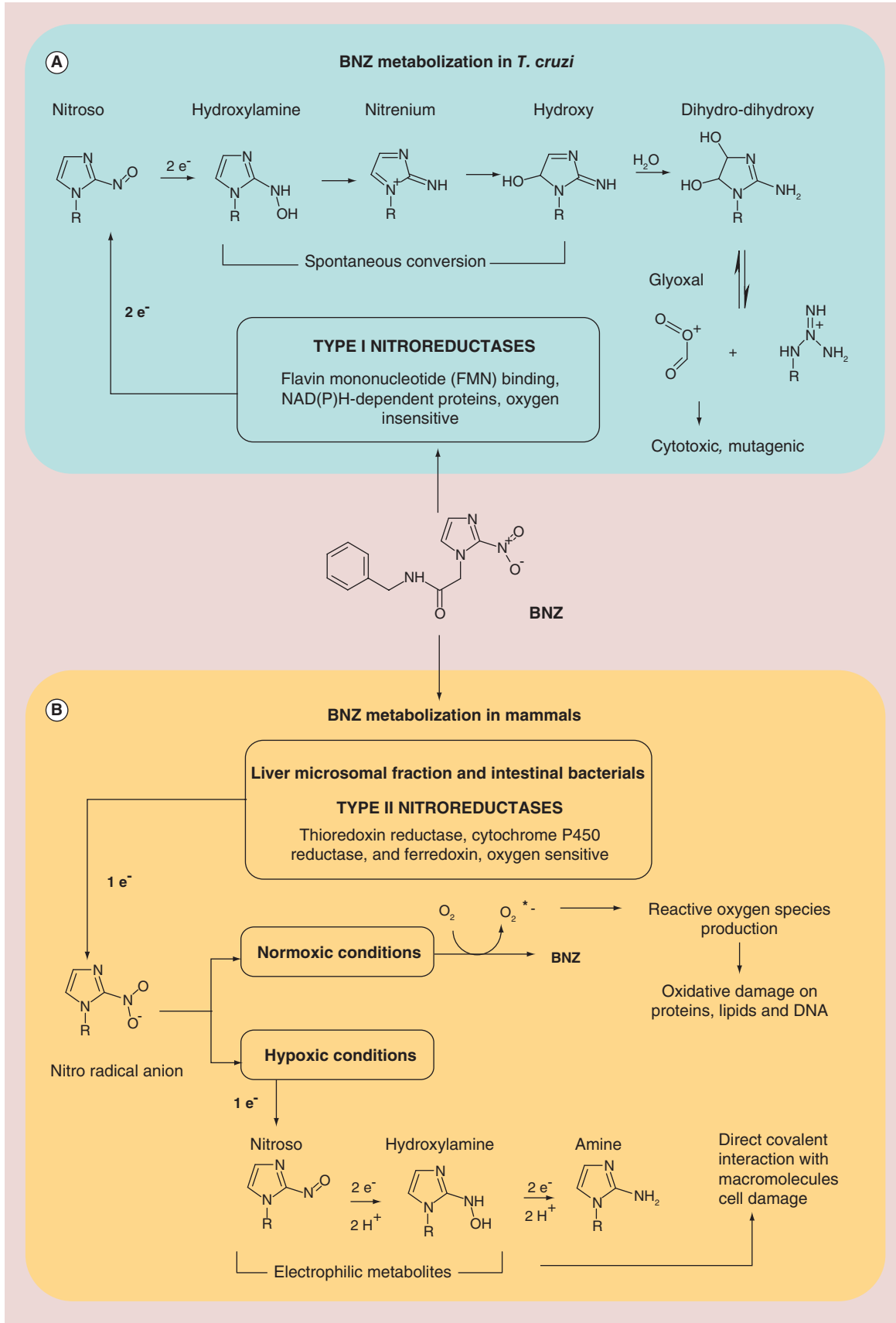
A
BNZ metabolization in T. cruzi
Nitroso
Hydroxylamine
Nitrenium
Hydroxy
Dihydro-dihydroxy
2 e-
H2O
Spontaneous conversion
2 e-
Glyoxal
TYPE I NITROREDUCTASES
Flavin mononucleotide (FMN) binding, NAD(P)H-dependent proteins, oxygen insensitive
Cytotoxic, mutagenic
BNZ
B
BNZ metabolization in mammals
Liver microsomal fraction and intestinal bacterials
TYPE II NITROREDUCTASES
Thioredoxin reductase, cytochrome P450 reductase, and ferredoxin, oxygen sensitive
1 e-
Normoxic conditions
BNZ
Reactive oxygen species production
Oxidative damage on proteins, lipids and DNA
Nitro radical anion
Hypoxic conditions
1 e-
Nitroso
Hydroxylamine
Amine
2 e-
2 H+
Direct covalent interaction with macromolecules cell damage
Electrophilic metabolites

Figure 1. Benznidazole metabolization in *Trypanosoma cruzi* and mammals (see facing page). (A) In *T. cruzi*, BNZ is metabolized by the trypanosomal NADH-dependent type I nitroreductase absent in humans. The reduction of BNZ leads to the formation of derivatives that ends with the cytotoxic and mutagenic agent glyoxal. R corresponds to the benzylacetamide component of BNZ. **(B)** In mammals, the nitro group is reduced to an amino group by type II nitroreductases, with formation of the intermediate nitro radical anion, which is sensitive to oxygen. In the presence of oxygen (normotic conditions), the nitro radical anion is oxidized to BNZ with the formation of reactive oxygen species, such as superoxide and hydrogen peroxide. The resultant futile cycle can potentially cause oxidative cell damage. Under hypoxic conditions, the nitro radical anion is converted to the nitroso form. The nitroso molecule is highly reactive and can lead to cellular damage directly or indirectly through the formation a hydroxylamine derivative. These electrophilic metabolites are metabolized and excreted by glutathione-dependent process (hepatic and intestinal glutathione S-transferases) that reduces their toxicity. Both routes lead to the reported adverse effects of BNZ in humans: (i) hypersensitivity: dermatitis with cutaneous eruptions (days 7–10 of treatment), myalgias, arthralgias and lymphadenopathy; (ii) polyneuropathy, paresthesias and polyneuritis (during the 4th week of treatment); (iii) bone marrow disorders, such as thrombopenic purpura and agranulocytosis (after 2nd week of treatment); (iv) genotoxicity, precludes treatment during pregnancy. New borns and young children show lower nitroreductases activity and fewer side effects than adults.
BNZ: Benznidazole.

the pharmacologic activity of NBCDs is governed by the complexity of their structures (comprising not only nano-objects but also complex mixtures of macromolecules and even small molecules that cannot be fully characterized) [36]. In other words, changes in pharmacokinetics, biodistribution and intracellular traffic of API (as being part of NBCDs) is achieved without modifying API's chemical structure but rationally designing the nano-object's structure [37]. In this approach, the performance of the same API can be radically altered with no need of chemical/enzymatic synthesis, and could speed the search for therapeutic alternatives against Chagas disease. In the following sections we will discuss if in the practice nanomedicines would become a viable alternative to the problem of designing new therapeutics, vaccines and diagnosis methods for Chagas disease.

The therapeutics of Chagas disease: new pharmaceutical forms for benznidazole?

The hand fractionation of the 100 mg BNZ tablets may result in improper dosages and further risks of developing side effects in newborns and children. To solve this, a new formulation of BNZ available as 12.5 mg dispersible tablets, which are easy to disintegrate, has recently been registered in Brazil by LAFEPE with the financial and technical support from DNDi [38]. Moreover, Abarax is available as a pediatric double-scored 50-mg orally disintegrating tablet that can be split into four pieces of 12.5 mg each, and as double-scored 100-mg orally disintegrating tablet for administration to children and adults exceeding 40 kg in weight [39]. Clearly, the problem of a pediatric formulation for BNZ can be successfully solved with no help of nanomedicines. Instead, the important toxicity of BNZ arising from its metabolization and the incapacity to completely eliminate the intracellular parasites – failure to cure the chronic phase – remains as unsolved challenges of the adult's treatment. In spite of its extensive oral bioavailability (>90%) [40], BNZ exerts a relatively poor *in vivo* antichagasic activity. Effectively, if well over 40% of the drug is bound to plasma proteins exhibiting a half-life of 12–15 h during which acts against the circulating forms (trypomastigotes), its apparent volume of distribution (Vap) is 0.56 l/kg. Such low Vap value indicates a poor tendency to penetrate the body tissues and to remain in the vascular compartment [41]. Moreover, since BNZ is a class III (high solubility and low permeability) drug [42], its low permeability would explain its low antiparasitic activity in the chronic phase, which is the most prevalent presentation [43,44]. To overcome its poor permeation across the plasma membrane, a huge concentration gradient is required for BNZ to gain access the cytoplasmic amastigotes [45]. Both low Vap and permeability, together with an extensive intracellular metabolization resulting in toxic metabolites for the host, are the major drawbacks of BNZ (Figure 1).

A recent clinical study showed that the ordinary dosage (5 mg/kg/day in two intakes) of BNZ in chronically infected adults generates sustained trypanocidal plasma concentrations (6–8 μg/ml) [46]. The maximum concentration noted just a few hours after intake was 12.5 μg/ml, almost half of the minimal concentration above which healthy patients manifest toxicity (20 μg/ml) [47]. Even though, 53 out of 54 patients experienced adverse effects and 11 abandoned the treatment. The authors concluded that adverse effects occurred at relatively low and safe levels of plasma BNZ, and suggested that the source of toxicity are the intracellular metabolites of BNZ, that were not quantified. Increasing the BNZ solubility and bioavailability by conventional pharmaceutical techniques such as metal complexation [48], microcrystals [49], co-solvent system [50] or cyclodextrins complexation [51], has been repeatedly achieved in preclinical settings (Table 2). However, increasing the BNZ bioavailability would lead, at best, to increased plasma concentrations with

lower oral dosages. And if well the low permeability of BNZ could be counterbalanced by increasing its plasma concentration, unavoidably the adverse effects of BNZ would remain present as previously shown [46] (in none of the studies shown in Table 2 the generation of toxic metabolites was determined). In other words, if well a higher plasma concentration could increase the intracytoplasmic delivery of BNZ, this would occur at expenses of a potential toxicity. A depot formulation on the other hand, would replace a peak (shown to be 12.5 µg/ml a few hours after oral intake [46]), by a sustained plasma concentration. The adherence to the treatment would certainly be improved if twice daily intakes are replaced by one or less along the 2-months' treatment. Though no data on depot formulations for BNZ have been published yet, the problem of the low permeability of BNZ would not be solved either, and therefore neither accounted to increase its efficacy.

A nanomedicine based in BNZ: harder than expected

Clearly a new pharmaceutical form to increase bioavailability providing a sustained plasma concentration of BNZ, would not overcome the problems posed by toxicity and low permeability of BNZ. In this section we will highlight the main characteristics and discuss pharmacological properties required for NBCDs (nanomedicine) based in the hydrophobic poorly permeable BNZ. Remarkably, the therapeutic outcome of nanomedicines is critically bound to their route of administration. Nanomedicines exert their activity by accumulating within the target tissues, where they are taken up by, or their cargo is released on the close surroundings of, the target cells. Unless vascular permeability is increased, because of their size (50–200/300 nm diameter) the nanomedicines neither extensively cross endothelial (from blood to peripheral tissues), nor epithelial mucosal barriers (from skin surface/gastrointestinal/respiratory tract to blood). The right choice of the administration route of nanomedicines ensures their accessibility to diseased tissues and avoidance of chemical or mechanical destruction through their pathway. In second place, to overcome the BNZ low permeability and extensive metabolization in liver and intestines, a BNZ based nanomedicine would enable its selective delivery to the cytoplasm of infected cells. Challenges like these were successfully solved for paclitaxel (a toxic, class IV low solubility and low permeability API), by different nanomedical formulation (NBCD) such as Abraxane® (developed by Abraxis BioScience acquired by Celgene Corporation, NJ, US; paclitaxel-containing albumin nanoparticles) or Cynviloq™ (Sorrento Therapeutics, CA, US; a micellar formulation of paclitaxel). Once in the bloodstream, paclitaxel released from these formulations binds to albumin that is subsequently taken up

Table 2. Classical strategies used to increase solubility, bioavailability and effectivity of benznidazole.

Formulation	Solubility/dissolution	Bioavailability	Effectivity	Ref.
Complex of ruthenium *trans*-[Ru(BNZ)$(NH_3)_4SO_2$]$(CF_3SO_3)_2$	Increase in BNZ aqueous solubility	Nd	Acute model. Complex at a thousand-fold smaller concentration than BNZ (100 mg/kg/day) protected all infected mice, eliminating the amastigotes in hearts and skeletal muscles observed in micrographics	[48]
BNZ microcrystals prepared by solvent change precipitation	BNZ microcrystals tablets (hydro xyethylcellulose and PEG 400) dissolved 85% in 10 min vs 60% in 60 min of BNZ commercial tablets	Nd	Swiss mice (500 trypomastigotes Y strain), received orally 100, 50 and 25 mg/kg BNZ (crushed and suspended with 4% arabic gum) starting 4 dpi for 7 days. BNZ micronized suppressed parasitemia at 100 and 50 mg/kg; at 25 mg/kg suppressed 33% of the parasitemia. Conventional BNZ only suppress parasitemia at 100 mg/kg. Micronized BNZ achieved suppression for nearly 22 dpi vs 10 dpi for conventional BNZ, and had superior effects on mortality	[49]
Co-solvent system	BNZ in 30% water and 70% PEG400 pH 2.5 (BNZ-PEG400) increase solubility from 0.4 mg/ml up to 10 mg/ml	Nd	C57BL/6 mice (150 trypomastigotes of Tulahuén strain), received orally 60, 40 and 20 mg/kg starting 7 dpi for 14 days. BNZ-carboxymethylcellulose suspension and BNZ-PEG400 showed the same efficacy; mortality at 25 dpi was 0% for both groups vs 100% for placebo group	[50]
Cyclodextrin complexes	Improve dissolution rate by 4.3-fold	Increased 3.7-fold AUC_{0-5} values and 2.5-fold Cmax	Nd	[51]

AUC: Area under the curve; BNZ: Benznidazole; Cmax: Maximum plasma concentration; dpi: Days post-infection; Nd: Not determined.

by tumor cells over expressing the albumin receptor. Remarkably to selectively target infected and inflamed peripheral tissues, nanomedicines have to be administered by intravenous (iv.) route. A blood circulating nanomedicine based in BNZ would be endocytosed by infected cells after extravasation at the inflamed sites, independently of the BNZ plasma concentration. Unfortunately preclinical studies employing iv. nonsterically stabilized liposomes loaded with BNZ, showed that in spite of being extensively taken up by the liver macrophages, the parasitemia of the acute phase was not decreased in mice infected by the reticulotropic RA strain of *T. cruzi* [52]. This was probably owed to the intracellular traffic followed by the liposomes that failed to deliver BNZ to the cytoplasm, to end up within endolysosomes. Instead, by replacing BNZ by a more hydrophilic but less trypanocidal API, and conventional by pH-sensitive liposomes, the API was delivered to the cytoplasm, and a substantial reduction of parasitemia was achieved [53]. The simple multilamellar phosphatidylcholine liposomes therefore were discarded as suitable nano-objects for BNZ delivery. Instead, Kupffer cells targeted liposomes [54], could decrease BNZ toxicity by avoiding its delivery (and subsequent metabolization) to the hepatocytes. The efficacy of such formulation would depend on its quantitative and selective targeting to infected cardiomiocytes, and on the existence of site specific inflammation and deserves further experiments.

The questions posed by the problematic treatments of neglected diseases has also been addressed by the collaborative project BERENICE launched in 2012. BERENICE is aimed to design a more effective, better tolerated and cheaper formulation of antichagasic nanomedicines to be administered by nonparenteral route [55]. This will be achieved by developing highly performing galenic formulations based on the use of sublingually delivered nanomedicines. BERENICE is focused in developing solid lipid nanoparticles (SLNs), to achieve a sustained release of BNZ and small unilamellar vesicles (SUVs) with tailored size, morphology, supramolecular structure and response to external stimuli, in order to improve the pharmacological properties of BNZ. The website, however, does not describe in which realistic manner the SLN and SUV would be rationally designed so as to respond to external stimuli and therefore improve the pharmacological properties of BNZ. Because of their size in the order of tens to hundreds of nanometers, neither SLN nor SUV enter the systemic circulation if applied by the sublingual route. Instead, SLN and SUV would act as drug depots, being stacked and degraded within the first cell layers of the mucosal epithelium, few micrometers below the surface. In the sublingual route, the API released from mucoadhesive formulations enters the vascular bed of the oral mucosa, avoiding the hepatic first-pass effect. A mucoadhesive formulation could probably decrease the toxicity arising from the reductive metabolism of BNZ. Up to this review date, advances of the project have not been published. Challenges encountered to develop mucoadhesive formulations for sublingual administration are several: achievement of prolonged drug retention, uniform drug content, desirable drug release profile, adequate drug permeation and efficient drug delivery. In spite of this, a number of conventional pharmaceutical technology products, such as tablets with a Carbomer 934P mucoadhesive coating for buccal delivery; sucrose/liquid glucose molds and rapidly disintegrating buccal tablets, have already reached the market. As discussed in the section 'Conclusion and future perspective', nanomedicines must efficiently address the unmet needs of the current treatments. Replacing a pre-existent technology by a more sophisticated one to achieve similar results, would surely lead to increase the healthcare costs.

Nanomedicine based in the macrolide amphotericin B

Recently, the antichagasic activity of the antifungal AmBisome (liposomes made of hydrogenated soya phosphatidylcholine, distearoylphosphatidylgycerol and cholesterol carring the polyene amphotericin B, AmB) was tested. AmB is an antifungal that binds to the sterol component of cell membrane, leading to alterations in cell permeability and cell death. While AmB has higest affinity for the ergosterol component of the fungal cell membrane, it can also bind to the cholesterol component of the mammalian cell. AmBisome has lower renal toxicity than free AmB since in AmBisome the AmB binding to high-density lipoprotein (HDL) instead of low-density lipoprotein (LDL) is favored, leading to a decreased uptake by renal LDL receptors to be preferentially targeted to the liver [56]. The trypanosomatids have ergosterol as component of their membranes [57], and AmBisome is first-line treatment for visceral leishmaniasis [58]. In 1999 it was described the effect of AmBisome in mice acutely infected with 3.5 x 10^4 trypomastigotes of the Tulahuén strain of *T. cruzi*. It was showed, on the bases of blood microscopic observations, that a single dose of 25 mg/kg (or 5 mg/kg when infecting with half of the trypomastigotes) of AmBisome was sufficient to suppressed the acute infection [59]. Twelve years later, the efficacy of AmBisome as antichagasic agent was tested again, in a different setting where immunosupression with cyclophosphamide (4 intraperitoneal [ip.] injections of 200 mg/kg on alternate days), was induced to assess the cure rate and the efficacy of the treatment in chronically infected animals.

In a first study, 6 weeks old BALB/cJ mice infected by ip. injection of 1000 blood trypomastigotes of the Tulahuén strain of *T. cruzi* (genotype TcVI) received six ip. injections of AmBisome at 25 mg/kg [60]. The treatments were given on alternate days starting either on the first day post-infection (dpi 1), during the acute phase (dpi 10), the chronic phase (dpi 45) or both phases of infection (dpi 10 and 45). It was found that AmBisome prevented mice from fatal issue in the acute phase of infection, contributed to drastically reduce parasite loads in heart, liver, spleen, skeletal muscle and adipose tissues in acute and in chronic infection, but failed to completely cure animals. Importantly, the treatment given in chronic phase only had also a significant beneficial effect in reducing the parasitic loads in cardiac tissue. However, treatment repeated in acute and chronic phases did not present a significant advantage over the chronic treatment.

Remarkably, the parasite DNA quantified by qPCR in the blood of chronic phase remained similar in non treated and treated mice. This was ascribed to release of *T. cruzi* DNA into blood circulation, subsequent to an intratissue lysis of parasites by AmBisome. Besides, the low levels of parasite DNA found in the acute phase was ascribed to the serum amyloid P protein, known to capture DNA to be rapidly eliminated in liver and produced in response to *T. cruzi* infection. This suggests that the employment of qPCR to determine the parasitic DNA in blood did not reflect the actual parasitic load in other tissues and was not sufficient enough to appreciate the effect of a treatment with AmBisome.

The lack of complete curative activity of AmBisome was ascribed to the preferential tropism of the used parasite strain for muscle tissues. The authors predict a beneficial effect of AmBisome treatment in *T. cruzi* congenital infection, in which parasites are preferentially targeted to the liver by the fetal circulation, since the early treatment was able to reduce drastically parasite loads in liver and spleen, in addition to allowing survival of all infected animals [60]. However, AmBisome is preferentially taken up by liver, spleen and lungs as long as it is iv. administered, but not if ip. injected.

More recently, 7 weeks old BALB/cJ mice were acutely or chronically infected with 1000 blood trypomastigotes of either a BNZ-susceptible (Tulahuén) or a BNZ-partially-resistant (Y) strain of *T. cruzi* were treated with sub optimal doses of Ambisome (short duration, noncurative, 5 ip. administrations at 25 mg/kg every day in chronic and alternate days in acute phase) in combination with BNZ (100 mg/kg for 20 days in acute and 10 days in chronic phase; suboptimal received half of the doses). It was found that the combination did not cure acutely or chronically infected mice [61]. In spite of these discouraging results, the therapeutic failure of the two approaches using Ambisome was probably owed to the nonoptimal route of administration, since the ip. instead of the iv. route was chosen.

Nanomedicine based in the natural product lychnopholide

Recently, the antichagasic activity of sesquiterpen lychnopholide (LYC) loaded into polymeric nanocapsules (NCs) was tested. LYC is a natural product with *in vitro* anti-*T. cruzi* activity related to the presence of alquilant groups, that has limited therapeutic applications by its poor aqueous solubility, high lipophilicity (log p = 5.03) and potential chemical instability in alkaline media that hamper its oral administration. Polymeric NCs consisting of an oil droplet surrounded by a polymeric membrane stabilized by surfactants, have been successfully used to increase the dispersibility of poorly water soluble drugs, to protect drugs against inactivation, to reduce drug toxicity, to control drug release and to prolong blood circulation time after iv. administration. LYC has been loaded in NCs, to be readily dispersed in water, becoming suitable for administration by oral and parenteral routes. In this work, NCs made of poly-ε-caprolactone (PCL) or poly(lactic acid)-co-polyethylene glycol (PLA-PEG) of around 180 and 105 nm mean diameters, respectively containing LYC, were tested on an acute model of murine infection [62]. Swiss mice aged 28–30 days, ip. infected with 1.0 x 10^4 blood trypomastigotes of CL or Y strains received 10 (CL) or 20 (Y) consecutive doses 24 h or 7 dpi (prepatent period) of 2 mg/kg/day of free LYC (dissolved in dimethylacethamide: PEG 300 mixture at 40:60 (v/v) and further diluted in 5% (w/v) glucose), LYC-PCL NCs, LYC-PLA-PEG NCs or 50 mg/kg/day of BNZ solution by iv. route. Cure criterion was based on negativization of parasitological (fresh blood examination, hemoculture and PCR on peripheral blood) and serological tests. Remarkably, animal infected with the Y strain and treated with LYC-PLA-PEG NCs in the prepatent period for 20 days, achieved 100% of cure, whereas those treated with BNZ and LYC-PCL NCs achieved lower of 75% and 62.5% of cure, respectively. Free LYC reduced the parasitemia and improved mice survival, but no mice were cured. The efficacy of LYC PLA-PEG NCs was attributed to the ability of the NCs to maintain the LYC release for longer times in biological media. On the other hand, since the PEG chains retard the rapid removal of NCs from the bloodstream by macrophages, the drug associated plasma half-life is consequently prolonged [63]. However, the drug would only be bioavailable if diffuses out of the NCs. The PLA-

PEG NCs have the chance to extravasate to infected tissues, to be taken up by infected macrophages at the inflammatory sites [64]. According to the authors, the PLA-PEG NCs would exert at the same time a sustained and a passive targeted delivery. However, the sustained delivery could also be achieved by a patch or subcutaneous (sc) depot, avoiding the problem of the pegilated formulation that could cause complement activation related pseudo allergy effect (CARPA effect) once iv. injected (only revealed if pig is used as preclinical model). On the other hand, this study indicated the treatment was more effective when the highest number of blood trypomastigotes is exposed to drug, and immediately after the rupture of highest number of pseudocists (in the prepatent period), at the longest period of treatment (20 days). Hence, the role of a passive targeted delivery was likely negligible.

Nanoparticles proposed as therapeutic nanomedicines

The nano-objects listed in Table 3 have recently been proposed as antichagasic agents although none of them (immunostimulating complexes [65], thiol capped CdTe quantum dots [66,67], a dendrimer produg [68]) has been already tested *in vivo*. The table includes the only report on stem cell therapy applied on Chagas's cardiomyopathy that made use of diagnosis fluorescent nanoparticles as supporting technology [69].

Vaccines for Chagas disease

A preventive Chagas vaccine would be costeffective even where transmission to humans and prevalence of infection are low and even for a vaccine of moderate protective efficacy [70]. A therapeutic vaccine administered alone or in combination with BNZ or NFX 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 and could potential prevent congenital Chagas disease if used in pregnancy [71].

In spite of the immune response elicited by the host [72], *T. cruzi* uses several mechanisms to escape from immunity like T-cell suppression and polyclonal lymphocyte activation. A vaccine 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. In addition, a very stable formulation is required for a vaccine to be efficiently distributed in remote rural areas.

A wide range of prophylactic and therapeutic vaccines have been evaluated in mice, from the use of whole parasites, to purified or recombinant proteins, to viral vectors and DNA vaccines including heterologous prime-boost vaccination strategies, are summarized in recent reviews [73,74]. Few of these vaccine candidates have been recently evaluated in dogs [75–77] (Table 4). The Sabin Vaccine Institute and the Texas Children's Hospital Center for Vaccine Development (Washington DC, USA) are developing a bivalent vaccine for the treatment of chronic Chagas disease comprised of TSA-1 (surface transialidase) and Tc24 (24 kDa antigen) recombinant proteins formulated on alum (insoluble aluminum phosphate or hydroxide salts) and coadministered with the TLR-4 agonist E6020 [71].

Nanovaccines

A limitation of recombinant antigens is their poor immunogenicity that requires of adjuvants to enhance the immune responses. Besides alum-containing vaccines, no other adjuvanted vaccines received approval by FDA until 2009, when 3-*O*-desacyl-4′-monophosphoryl lipid A (AS04, Cervarix vaccine, GlaxoSmithKline, ON, Canada) was approved. AS04 was also approved in Europe, where other adjuvants have gained regulatory approval, including MF59 (submicron oil-in-water emulsion of a squalene, Tween™ 80 and sorbitan trioleate), AS03 (a squalene-based adjuvant) and liposomes. Adjuvants such as alum, MF59, and monophosphoryl lipid A enhance antibody production but are limited in their ability to induce T-cell immunity.

Nanoparticulate antigen delivery systems can enhance and/or facilitate the uptake of antigens by antigen-presenting cells (APCs, dendritic cells or macrophages); may serve as a depot for controlled release of antigen; can protect the integrity of antigens against degradation; and can potentially cross-present antigen (mechanism by which exogenously acquired-antigens are processed into MHC class I pathway) in order to generate cytotoxic T lymphocytes against intracellular pathogens [78].

Phosphatidylserine proteoliposomes

Since phosphatidylserine (PS) containing OVA-carrying proteoliposomes (reconstituted membrane proteins into liposomes) promotes IFN-γ production [79], PS proteoliposomes carrying trypomastigote and amastigote proteins were used to immunize BALB/c mice [80]. Multiple parasite proteins solubilized in SDS were incorporated into 200 nm proteoliposomes (dipalmitoylphosphatidylcholine, dipalmitoyl-PS and cholesterol, 5:2:15 w:w) by co-solubilization. BALB/c mice immunized with one ip. injection of 20 μg total protein generated antibodies and intraperitoneal macrophages that arrested the intracellular replication of trypomastigotes of the Y strain. Four weeks after immunization

Table 3. *In silico*/*in vitro* performance of antichagasic nanomedicines and reporter nanoparticles.

Nano object	Aims and results	Ref.
ISCOMS	ISCOMS containing actinomycin D and a cholesterol-vinyl sulfone compound bound to Staphylococcal protein A covalently and coated with anti-*Trypanosoma cruzi* IgG with an average size of 37 nm. 100% inhibition of epimastigote of Maracay strain of *T. cruzi* grouth at 25 × 10^{-2} ng/ml of actinomycin D, 200-times lower than that required when given in free form	[65]
Thiol capped CdTe quantum dots	Dose-dependent increase in DNA fragmentation, plasma membrane blebbing and mitochondrial swelling of *T. cruzi* epimastigotes induced by incubation with 2–200 μM quantum dots	[66,67]
Dendrimer produg	Theoric dendrimer prodrug development made of myo-inositol (core), a linker molecule (L-malic acid) and three potentially antichagasic agents: 3-hydroxyflavone, quercetin and hydroxymethylnitrofurazone. Results suggest that the carbonyl group next to the myo-inositol is the most promising ester breaking point	[68]
Stem cell therapy with BMMC	Fluorescent nanoparticles (XSight nanospheres) labeled BMMCs were used to follow migration of iv. injected cells in mice infected with trypomastigotes of Brazil strain of *T. cruzi*. Mice treated with BMMCs 1 month after infection showed a small, but significant, number of cells migrated to hearts compared with control animals, whereas the vast majority of labeled BMMCs migrated to liver, lungs and spleen. Therapy reduced right ventricular dilation	[69]

BMMC: Bone marrow mesenchymal cell; CdTe: Cadmium telluride; ISCOMS: Immunostimulating complexes; iv.: Intravenously.

mice were ip. challenged with 300 trypomastigotes of the Y strain. At 10 dpi, control mice presented higher parasitemia than immunized animals. However, between 13 and 30 dpi, the number of circulating trypomastigotes was similar in all groups. While 67% and 33.3% animals immunized with SDS-solubilized proteins and buffer, respectively, succumbed to *T. cruzi* infection before 23 dpi, 100% of mice in the PS proteoliposomes group were alive at that time. However, at 30 dpi the survival rates were not different between groups. These results show that PS proteoliposomes do not induce complete protection to death in mice by a virulent strain of *T. cruzi*, but are able to significantly delay their death.

Archaeosomes

Our group has recently studied the immune response and efficacy of archaeosomes loaded with soluble *T. cruzi* proteins [81]. Archaeosomes are vesicles enclosed by one or more bilayers prepared with total polar lipids (TPL) extracted from microorganisms that belong to the Archaea domain. These lipids are structurally very different from lipids of organisms from Eukarya and bacteria domain: the glycerol backbone is ether linked to saturated isoprenoid chains, mainly phytanyls and diphytanils, in an *sn*-2,3 enantiomeric configuration [82]. Archaeosomes are more avidly internalized by macrophages and APC than liposomes, both *in vitro* and *in vivo* [83,84]. They also differ from liposomes in that the inclusion of immunomodulators such as lipopolysaccharides, their synthetic derivatives, or CpG motifs within lipid bilayers is not needed to improve the adjuvancy. After sc administration in mice, archaeosomes are potent adjuvants for the induction of Th1, Th2 and $CD8^+$ T cell responses to the entrapped soluble antigens [85]. Our group has described that archaeosomes from the hyperhalophile *Halorubrum tebenquichense* induced antigen specific humoral and memory responses upon sc administration in mice [86]. Three administrations of archaeosomes containing 12.5 μg of soluble *T. cruzi* proteins (days 0, 14 and 21) in C3H/HeN mice, generated higher levels of circulating antibodies than those measured in the sera from animals receiving the antigen alone, with a dominant IgG2a isotype, associated with Th1-type immunity. Four weeks after immunization mice were ip. challenged with 150 trypomastigotes of Tulahuén strain. Immunized mice displayed reduced parasitemia during early infection and were protected against the lethal challenge (100% survival of animals immunized with archaeosomes at 30 dpi vs 100% mortality of control groups at 25 dpi).

Archaeosomes have been characterized as poor inducers of innate immunity via toll-like or CD1 receptors [87,88]. However, the presence of glyco-portions of archaetydil phosphate groups glycosidically linked to short oligosaccharides [89,90] seems to be important to the adjuvanting process. Particularly for *H. tebenquichense*-derived archaeosomes, the presence of mannose-containing archaeolipids [91], which could interact with specific receptors on APCs, probably contributed to the enhanced immunogenicity of the archaeosomes.

The potential harmfulness, complexity, expensiveness and difficulties to scale up some promising vaccine approaches can spoil further attempts of industrial production and acceptation by regulatory organisms. In this regard, archaeosomes can be produced by scalable techniques and from sustainable sources. Aditionally, archaeosomes are highly stable, the isoprenoid chains are resistant to peroxidation, the ether linkages are resistant to hydrolysis in a wide range of pH, whereas the *sn-2,3* stereoisomery grants resistance to hydrolysis mediated by stereospecific phospholipases. Remarkably, archaeosomes have displayed low toxicity upon parenteral administration in rodents [92].

Micro-nanoparticles as vaccine adjuvants

The group of the Sabin Vaccine Institute has identified a pH-sensitive biodegradable polymer (hydrophobic complexes of the double-stranded RNA analog poly(inosinic acid)–poly(cytidylic acid), a TLR3 agonist, ion-paired to cetyltrimethylammonium bromide (CTAB), encapsulated in pH-sensitive polyketal microparticles of 1–3 μm) that could be used in the Chagas vaccine project [93]. If well preliminary results using OVA showed that murine splenic dendritic cells induced high percentage of IFNγ-producing $CD8^+$ T cells and also induced TNF-α and IL-2 production in $CD8^+$ T cells, concerns exist about CTAB toxicity.

Diagnosis of Chagas disease

The diagnosis of Chagas disease can be paraitological or serological depending on the stage of the infection (Table 1). The main challenge is counting on detection systems type point-of-care (POC), by which screening campaigns could be effectively implemented. A POC device needs to be practical, costeffective and portable with high sensitivity (ideally greater than 98%) and specificity (not below 95%). Nanotechnology holds the promise to reach the sensitivity and specificity to lower the cost and to enable portable microfluidic platforms suitable for resource-constrained settings. At the moment there are few developments, the most advanced at the level of prototypes.

Nanodiagnosis

The most advanced in development diagnosis nanodevice is an electrochemical immunosensing platform named Nanopoc®, developed by a public–private partnership in Argentina (Instituto Nacional de Tecnologia Industrial and Universidad Nacional de San Martín; Buenos Aires) [94]. The system consists of four recombinant proteins immobilized on silica-coated superparamagentic iron oxide nanoparticles. Once the antigen-antibody complex is formed in solution, the nanoparticles are attracted to the electrode by means of a magnetic field where the final amperometric measurement takes place. This strategy is more efficient and faster with respect to direct electrode immobilization; as a consequence, the incubation time has been reduced to a few minutes, while an analog ELISA usually would require 30–60 min incubation. This strategy also allows the repeated reused of the electrodes. Currently the device is being validated, and there is no public information available in order to assess its comparative performance.

Recently the development of a microfluidic immunosensor with screen printed carbon electrode modified by electrodeposition of gold nanoparticles and functionalized with *T. cruzi* proteins from epimastigote for the quantification of anti-*T. cruzi* antibodies present in serum samples, has been reported [95]. Antibodies in serum samples were quantified through the addition of horseradish peroxidase enzyme-labeled secondary antibodies specific to human IgG, using 4-tert-butylcatechol (4-TBC) as enzymatic mediator. HRP in the presence of H_2O_2 catalyzes the oxidation of 4-TBC whose back electrochemical reduction was detected on the modified electrode at -100 mV. A linear relation was observed between i and the IgG concentrations (11–205 ng/ml) with good correlation coefficient. The detection limit was 3.065 ng/ml (far below of classical ELISA test ~480 ng/ml). The assay showed good precision (coefficient of variation within-assay <4.12% and between-assay <6.95% for 100 ng/ml). The total assay time was 26 min. The authors claim that the device offers the possibility of obtaining miniaturized, integrated and portable systems for on-site analysis. If well only 20 positive and five negative human serum samples were analyzed, such sample number is too small so as to assess its relative sensitivity and specificity. No published data on the cost is available so as to evaluate the feasibility of implementation.

Finally, an immunosensor was constructed on a modified conducting glass substrate (ITO-indium doped tin oxide film) by layer by layer method intercalating silver nanoparticles incorporated into a silane matrix (SiPy+Cl-) and polyvinyl sulfonate (PVS) to form a bilayer [96]. The *T. cruzi* Ag were attached into the sensor by electrostatical interaction. The biosensor obtained with four bilayers (PVS/AgNP/SiPy+Cl)4 presented more sensitivity than a commercial ELISA test, with a titer of at least 1/40, besides was not quantitative.

Other important point is monitoring the treatment of the patients. Standardized qualitative PCR for the assessment of the impact of parasitic load on the over-

Table 4. Inmunization in dogs.

Vaccination strategy	Results	Ref.
Preventive DNA encoding TcSSP4 (an amastigote specific glycoprotein), 2 intramuscular administrations	Shorter period of parasitaemia and lower parasite load in the immunized/infected dogs (Ninoa strain isolated from a patient in Mexico intraperitoneally 15 days after the last immunization), than in nonimmunized dogs. Increased the levels of IFN-γ and IL-10 and stimulated the spleen cell proliferation. Prevents splenic but not cardiac tissue damage. However, the lesions in heart did not reach myocardial or subendocardial tissues, covered only subepicardial tissue lesions	[75,76]
Therapeutic DNA encoding TSA-1 (surface transialidase) and Tc24 (24 kDa antigen), 2 intramuscular administrations during the acute phase	Reduces the density of amastigote nests, cardiac arrhythmias and mortality	[77]

all treatment response is now available and is being used in ongoing preclinical and clinical studies. In this respect nothing was done for development of PCR for POC for *T. cruzi*.

Conclusion & future perspective

Today there is insufficient data to envisage whether nanomedicine will offer improved antichagasic treatments or not. Whenever the conventional pharmaceutical technology has already developed efficient treatments – as in the acute cases of young people – the true need for nanomedicines is arguable. Besides, to be worth effective, any new therapeutics would have to accomplish with most of the long-term objectives proposed by the DNDi. However, antichagasic nanomedicines will have to be administered by the iv. route (preferably in a single dose). The expected benefits have to be high enough to counterbalance the potential toxicity due to acute infusion reaction such as the CARPA effect if dealing with pegilated nanomedicines.

The current preclinical data have not clearly shown yet the succeeding elimination of the intracellular parasites in chronic asymptomatic cases, or successfully immunized suitable (nonrodents) animal models. However, the findings from [60,61] suggest that AmBisome, if iv. injected, could be a suitable shortcut toward a better antichagasic treatment deserving to be deeper investigated. Although the intracoronary injection of autologous bone marrow mesenchymal cells was recently shown not to improve left ventricular function or quality of life in patients suffering CCC [97], extracellular matrices made of bionanomaterials and nanoparticles could be used to favor and track the differentiation process of future, improved stem cell-based therapeutics [69]. However, such approach is far from having the chance of being realistically applied on patients from endemic countries. Accordingly, once counting on adequate end points markers (detected shortly after treatment), the development of POC nanosensors would enable an easier detection and follow up of the presence and course of the infection both in clinics and preclinics. However – and differing from oncology – the advances on the diagnosis field need of a concomitant improvement of the current antichagasic therapy. In the absence of improved treatment, the sole development of better nanosensors will be worthless. Finally, Mexico, Brazil and Argentine are the endemic countries with higher research and development development in nanotechnology in Latin America [98]. However, the emergence of antichagasic nanomedical therapeutics would likely not emerge from these countries, but from public/private partnerships involving academics, industry and government stakeholders of the developed nations. The Chagas disease is a doubly neglected disease, not only for pharmaceutical companies but for public politics of the endemic countries. Opposing, developed countries having a clear vision of the strategic needs in the short and long term provide confidence to high risk economical investments. Such sociopolitical context is a prerequisite to promote alliances between academia, industry and government. Those are the reasons why Spain (a nonendemic developed country with reported cases of Chagas) has promptly generated projects such as BERENICE (Spain, France, Brazil and Argentine). Similarly, the multinational SABIN consortium (USA, Mexico, Germany, Japan) aimed to develop therapeutic antichagasic vaccines, was readily organized.

Nanomedicines are said to reduce treatment (better efficiency, lower side effects) and personnel (reduction inpatient care day) costs caused by the four major healthcare costs causing diseases: cancer, cardiovascular, neurodegeneratives, musculoskeletal disorders. For a developed country's perspective, in such diseases, the technology-dependent costs account for a maximum of 20% of the total costs [99]. In Latin

America on the other hand, counting on a new (conventional) drug treatment for Chagas disease is predicted to be a very costeffective (less than 1 per capita gross domestic product) [100] intervention, that could save many millions of life years, avoiding morbidity and mortality. Nanomedicines pose the risk of increasing healthcare costs, as long as they are aimed at diseases of minor costs relevance such as infections, or if they come as add-on technology with an unfavorable cost-benefit ratio [101]. The CCC, however, is a cardiovascular disease that could fit within the scope of diseases deserving nanomedical treatments. Up to date there are no studies evaluating the impact of future antichagasic nanomedicines on the healthcare costs of endemic or developed countries. Overall, because of such uncertainties, a strong preclinical assessment of high efficacy antichagasic nanomedicines will be critical to truly overcome the effectiveness and efficiency of the current treatment based on the oral BNZ.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Chagas in numbers

- Chagas disease is endemic in 21 countries in Latin America.
- Chagas disease afects approximately 8 million cases.
- Chagas disease causes 12,000 annually deaths.
- Chagas disease results in 430,000 disability-adjusted life years. Decades after the acute phase up to 30% of chronically infected people develop chronic Chagas cardiomyopathy (cardiac arrhythmias, ventricular aneurysm, congestive heart failure, thromboembolism and sudden cardiac death), digestive problems or neuropathies.

Therapeutics

- Together with the nitrofuran nifurtimox, the less cardiotoxic 2-nitroimidazole benznidazol are the only available drugs against Chagas disease.
- None of the available drugs are approved in the USA and cannot be used in pregnant women.
- The toxicity and low permeability of benznidazole, with subsequent impaired plasma membrane penetration to access the intracellular amastigotes, are the main drawbacks associated to the treatment of seropositive adults.
- Intraperitoneally administered AmBisome (unilamellar liposomes loaded with Amphotericin B) alone or in combination with benznidazole, failed to exert any curative effect.
- Intravenously administered sterically-stabilized nanocapsules (made of an oily core enclosed within a polymeric coat loaded with a hydrophobic natural product) induced 100% cure in a murine model of acute Chagas disease.
- Aiming to develop solid lipids nanoparticles and small unilamellar vesicles loaded with benznidazole and triazoles intended for sublingual administration, a public–private multinational partnership has recently been launched (BERENICE).

Vaccines

- A single subcutaneous dose of proteoliposomes (phosphatidylserine-containing phospholipid vesicles loaded with *Trypanosoma cruzi* Y strain trypomastigotes and amastigotes proteins) failed to protect mice against a lethal challenge.
- Three subcutaneous doses of archaeosomes (vesicles made of total polar lipids extracted from the archaea *Halorubrum tebenquichense*, loaded with *T. cruzi* Tulahuén strain trypomastigotes proteins) protected mice against lethal challenge.
- Neither therapeutic or prophylactic strategy have evolved beyond preclinics nor induced complete cure of animals in a suitable model of chronic disease or eliciting immune responses similar to humans.

Diagnosis

- Parasitological or serological depending on the stage of the infection. The main challenge is counting on a point-of-care detection systems.
- An electrochemical immunosensing platform (Nanopoc®) based on recombinant proteins immobilized on silica-coated superparamagentic iron oxide nanoparticles is being validated.
- Microfluidic immunosensor with screen printed carbon electrode modified by electrodeposition of gold nanoparticles is under development.

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