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Review

Chagas Disease: Current View of an Ancient and Global Chemotherapy Challenge

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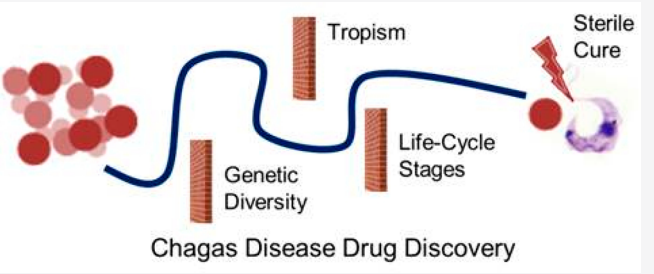
ABSTRACT: Chagas disease is a neglected tropical disease and a global public health issue. In terms of treatment, no progress has been made since the 1960s, when benznidazole and nifurtimox, two obsolete drugs still prescribed, were used to treat this disease. Hence, currently, there are no effective treatments available to tackle Chagas disease. Over the past 20 years, there has been an increasing interest in the disease. However, parasite genetic diversity, drug resistance, tropism, and complex life cycle, along with the limited understanding of the disease and inadequate methodologies and strategies, have resulted in the absence of new insights in drugs development and disappointing outcomes in clinical trials so far. In summary, new drugs are urgently needed. This Review considers the relevant aspects related to the lack of drugs for Chagas disease, resumes the advances in tools for drug discovery, and discusses the main features to be taken into account to develop new effective drugs.



KEYWORDS: *American trypanosomiasis, Chagas disease, chemotherapy, drug discovery, neglected tropical disease, screening cascade, target product profile, Trypanosoma cruzi*

Chagas disease (CD) or American trypanosomiasis is a life-long and life-threatening tropical infection caused by the protozoan parasite *Trypanosoma cruzi*. *T. cruzi* is naturally transmitted through faeces of blood-sucking triatomine bugs, although congenital transmission, blood transfusion, transplantation, parasite-contaminated food or drink, and laboratory accidents are other important means of transmission.[1]

After its discovery, CD was limited for many decades to Latin America as a silent, rural and invisible disease.[2,3] The classical endemic region comprises 21 countries, ranging from the southern USA to the northern Argentina and Chile.[3,4] However, CD has recently spread to other nonendemic regions as a result of constant mobility and migratory flows,[5] with recorded outbreaks in Canada, USA, Japan, and several European and Oceanian countries.[1,6] Therefore, CD has become a global health problem that affects 6−8 million people[2,5,7] and causes 12−14 thousand deaths annually,[4,8] and it is hypothesized that 70−100 million people are living at risk of infection worldwide, most of them in Latin America.[2,4,8] Figure 1 shows the estimated distribution of CD worldwide in 2018.

According to the World Health Organization (WHO), CD is classified as the most prevalent of the poverty-caused and poverty-promoting neglected tropical disease (NTD). Moreover, it is the most important parasitic disease and the third most spread infectious disease in Latin America.[2,10] Nevertheless, fewer than 10% of infected people are diagnosed, less than 1% of those infected are treated, and drugs frequently fail.[4,10,11]

In 1909, CD was discovered by Brazilian doctor Carlos Chagas,[12] and more than 110 years later, there is no effective treatment for CD yet. Nowadays, CD is considered a major public health and socioeconomic problem by several international and national organizations. Most pharmaceutical companies were not interested in developing new drugs for CD because of its low financial return,[13,14] although there has been a change in recent years in collaboration with international organizations, such as the Drug for Neglected Diseases Initiative (DNDi). Nevertheless, there is a lot to be done to address the challenges.[14,15] In this context, pharmaceutical companies, donors, endemic countries, and nongovernment organizations included CD as one of the NTDs in the London Declaration of 2012,[16] and WHO officially declared April 14th as "World Chagas Disease Day" during the 72nd World Health Assembly in 2019. This day was celebrated for the first time in 2020, and it will make CD more visible since the disease is termed as a "silent and silenced

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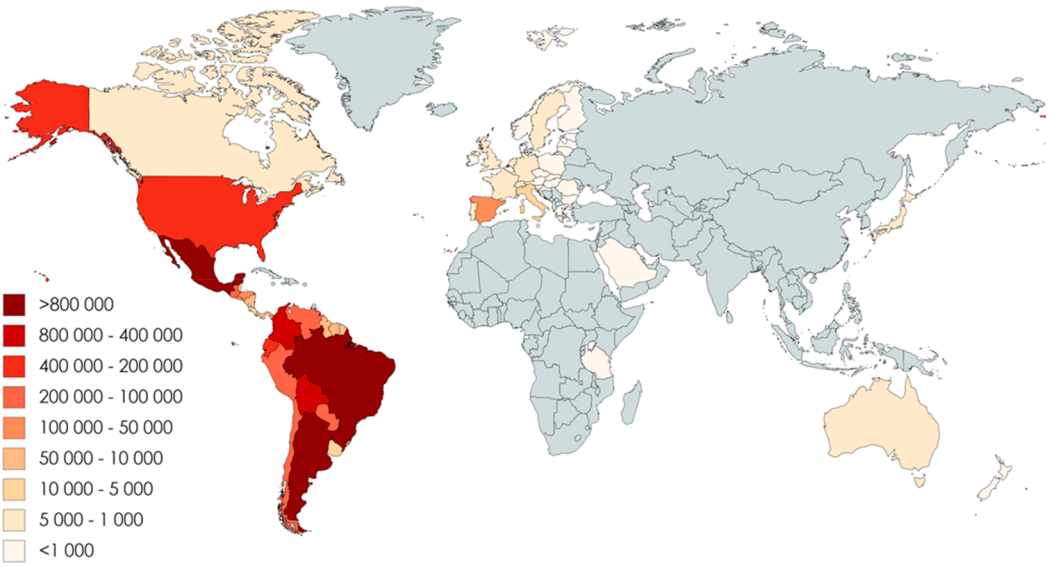

Figure 1. Estimated global distribution of cases of Chagas disease in 2018. Gray map, no data available.[6,9]

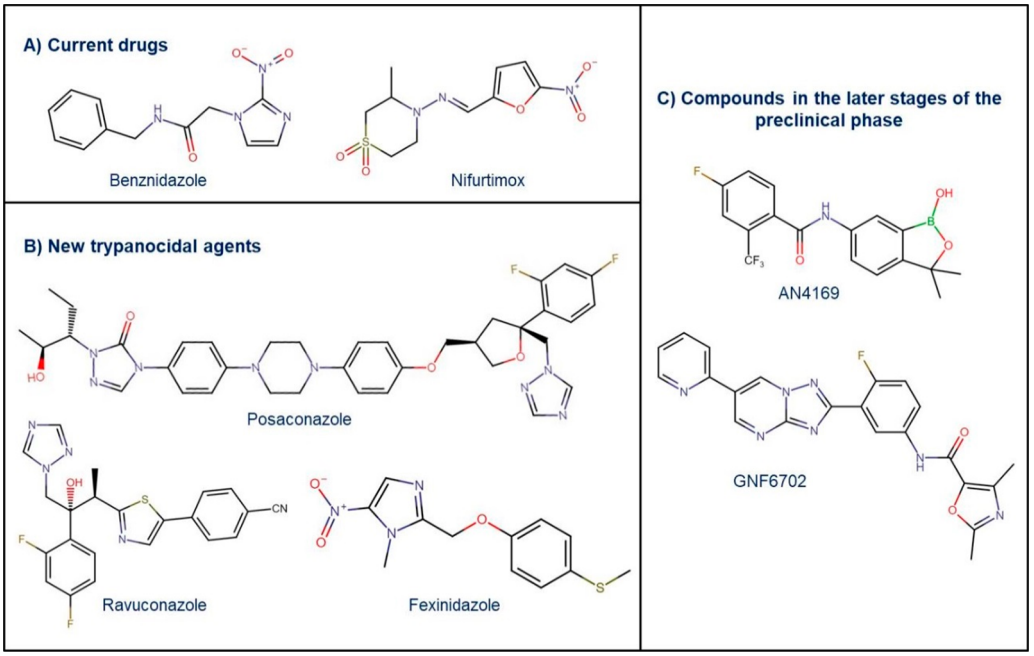

Figure 2. Structures of (A) current drugs, (B) new trypanocidal agents, and (C) potential compounds for the treatment of Chagas disease.

disease", not only because of its slow and often asymptomatic clinical course but also because it mainly affects poor people without political voice or access to health services.[17]

Despite all research efforts, gaps in the knowledge of *T. cruzi* biology and the available methodologies have been major factors in limiting progress. In short, there is an urgent need for tolerable, safe, accessible, and effective new drugs for CD.[4] In this Review, the current status and challenges related to CD drug discovery are discussed.

CURRENT TREATMENTS

Despite many efforts done over the past decades, the infection biology, the complex pathology, the long-term nature of CD, and the controversy about the autoimmune etiology have resulted in a lack of available vaccines.[5,18] Currently approved treatments are limited to two obsolete nitroheterocyclic drugs, benznidazole (BZN), and nifurtimox (NFX) (Figure 2A). These drugs were developed more than 50 years ago and lead to serious drawbacks, such as toxic side-effects, long treatment periods, and frequent treatment failures.[11]

Both drugs are highly effective in curing CD if given soon after infection (onset of the acute phase). However, they require extended treatment and their efficacy gets reduced the longer a patient has been infected, showing low efficacy in the chronic phase, as well as other limitations.[19] BZN has the best safety, tolerance and efficacy profile, and therefore it is often used for first-line treatment.[20] Table 1 shows treatment recommendations, limitations, effectiveness and guidelines for different patient profiles. It is important to note that cure rate and its confirmation depend on several factors, such as disease phase, patient age, and immune response, along with the susceptibility of the parasite genotype to the drugs used.[9]

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Table 1. Recommendations, Effectiveness, and Treatment Guidelines for Current Drugs (Benznidazole and Nifurtimox) for Chagas Disease (CD)[5,9,10,21−26]

patient	recommendation[a]	objective	curative efficacy	dose of treatment (mg/kg/day)	length of treatment
neonates	strongly recommended	sterile cure	nearly 100%	5[d] for BZN 10[e] for NFX	60 days
children and young people			up to 80−90%	5−10[d] for BZN 15−20[f] for NFX	60 days
adults aged 19−50 years with acute infection			65−100%. In a timely manner[c]	5−7[d] for BZN 8−10[f] for NFX	
asymptomatic adults aged 19−50 years Adults aged 19−50 years with early chronic infection	recommended	sterile cure	50−75%. In a timely manner[c]	5−7[d] for BZN	60 days
adults aged 19−50 years with late chronic infection adults suffering from reactivation		to prevent or delay disease progression	30%	8−10[f] for NFX	
adults older than 50 years old	optional[b]	to prevent or delay disease progression	30%	5−7[d] for BZN 8−10[f] for NFX	60 days
adults with advanced disease pregnant women, and people with kidney/liver insufficiency or neurological/psychiatric disorders	contraindicated				

[a]In general, nifurtimox (NFX) is the better-tolerated option in children, and benznidazole (BZN) is used in adults. [b]Benefit not proven: potential benefit should be weighed against possible adverse reactions, occurring in up to 40% of patients. [c]More effective in curing CD the sooner drugs are administered after infection. [d]Divided in two doses. [e]Divided in three doses. [f]Divided in four doses.

RECENT ANTICHAGASIC AGENTS IN CLINICAL TRIALS

Although no safe and effective drugs are available for the treatment of CD, the Chagas research and development (R and D) field has not been very active in the discovery of new chemical entities (NCEs) (Table 2). This research is mainly based on old and far-from-ideal drugs, some of them repurposed from other areas, and on modifying current treatments (adjustment dose of BZN) to increase their trypanocidal efficacy or reduce their toxicity.[27]

In summary, these trials evaluate the efficacy of current drugs (BZN and NFX) and new trypanocidal agents (posaconazole (POS), ravuconazole (RAV), and fexinidazole (FEX)) in chronic CD (Figure 2B). Unfortunately, none of the compounds evaluated have shown more effectiveness than BZN in chronic CD, except for FEX. In 2017, a new phase II proof-of-concept study using shorter and lower-dose treatment regimens of FEX is in the follow-up phase and its results will be available in 2020. If this study shows that FEX is effective, this will be the first new drug developed to treat CD in more than 50 years.[46] It is noteworthy that although FEX is a new drug for CD treatment, it belongs to the same class as BZN and NFX, and it has been repositioned from human African trypanosomiasis.[48]

TARGET PRODUCT PROFILE (TPP)

To lead the development of new treatments, either improved schedules of current drugs or new trypanocidal agents, it is essential to define a clear TPP. In this context, DNDi together with other partners launched the Chagas Clinical Research Platform (CCRP) in 2009 (the 100th anniversary of the discovery of CD). This platform convened a multidisciplinary group of experts in Brazil (2010) to provide a TPP for CD, as well as support for the evaluation and development of new drugs, the standardization of methodology to assess drug efficacy, and the revision of alternatives (guidelines, doses, and combination) for using current approved drugs.[4] In 2019, another TPP has been published by several experts in the field.[24] Both TPPs are summarized in Table 3.

Overall, both profiles are very similar. As far as drug is concerned, it must be safe and highly effective in both the acute and chronic phases of CD to be used for the widest range of population. Besides, it must be highly stable in tropical conditions, allow oral administration and a simple treatment regimen, that is, short-course practical therapies because of the environment in which most infected people are found.

A new topic to be taken into account, and included in the last TPP proposed, is that of sterile parasitological cure, that is, total clearance of the *T. cruzi* infection. New drugs must eradicate all parasites in both blood and tissues to avoid any relapse after treatment. In contrast to the human African trypanosomiasis, where blood−brain barrier (BBB) penetration is frequent, *Trypanosoma cruzi* can spread to the brain—probably in infected white blood cells—and form nests in astrocytes in acute Chagas disease in children younger than 2 years old and immunocompromised patients.[50] Permeation into the cerebrospinal fluid (CSF) is a critical point since this BBB blocks the passage of most small molecules. Therefore, the requirement for active compounds to cross the BBB and achieve sterile parasitological cure is a major issue in drug design, which also increases the risk for adverse effects.

POS, a CD drug candidate that has previously shown effectivity in animal models, has failed in clinical trials because of the difficulties in achieving sterile cure.[39] It should be noted that assessment of sterile cure in CD is controversial due to the absence of irrefutable tests to ensure parasite elimination. PCR techniques are mainly used to ascertain the failure of clinical treatments—even consistently negative results using blood are not sufficient to confirm parasite elimination.[51] Currently, assessment of cure is based on the seroconversion (the disappearance of anti-*T. cruzi* antibodies) serological tests. However, antibodies may take up to 5 years to disappear. Accordingly, positive serology does not mean active infection, whereas negative serology indicates cure (except for immunocompromised individuals).[5]

DRUG DISCOVERY PIPELINE

Drug discovery is a time-consuming and high-investment process used to identify new drugs for different disciplines.

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Table 2. Main Clinical Trials in the Last 20 Years for the Treatment of Chagas Disease (CD)

clinical trial	NTC number	sponsor	period	country	phase	treatment	objective[a]	main outcome	reference
TRAENA	NCT02386358	Instituto Nacional de Parasitologia Dr. Mario Fatala Chaben	1999−2013	Argentina	III	BZN	evaluation of the clinical progression	no clinical improvements	28−30
BENEFIT	NCT00123916	Population Health Research Institute	2004−2015	Argentina, Bolivia, Brazil, Colombia and El Salvador	III	BZN	evaluation of the clinical progression in chronic cardiac CD	no clinical improvements for the most severe forms of cardiomyopathy	25,28,31,32
CHAGASAZOL	NCT01162967	Hospital Universitari Vall d'Hebron Research Institute	2010−2013	Spain	II	POS	effectiveness evaluation, and safety	80−90% treatment failure during patient follow-up	28,33−35
		Merck Sharp and Dohme Corp.	2011−2013	Argentina					
proof-of-concept study E1224	NCT01489228	DNDi and Eisai, Co.	2011−2013	Bolivia	II	RAV	effectiveness evaluation, and safety	treatment failure: transient, suppressive effect on parasite clearance	36−38
STOP CHAGAS	NCT01377480	Merck Sharp and Dohme Corp.	2011−2015	Argentina, Colombia, México and Venezuela	II	POS and BZN	effectiveness evaluation in asymptomatic CD after oral treatment	long-term ineffectiveness of POS in asymptomatic CD; BZN monotherapy ≈ BZN/POS combination therapy	39−41
Pop PK Chagas	NCT01549236	DNDi	2012	Argentina	IV	BZN	study of pharmacokinetic parameters in children	treatment gap closed for children with CD: new pediatric formulation for children	31,42
CHICAMOCHA-3	NCT02369978	Universidad Autónoma de Bucaramanga	2015−present	Colombia and Argentina	II−III	NFX	effectiveness evaluation, and safety	in progress	43
proof-of-concept study FEX	NCT02498782	DNDi	2014−2016	Bolivia	II	FEX	effectiveness evaluation, and safety	high efficacy findings at the lowest dose tested	31,44
	NCT03587766		2017−present	Spain			determination of the minimal efficacious and safe dose	in progress	31,45,46
BENDITA	NCT03378661	DNDi	2016−2018	Bolivia	II	BZN and RAV	effectiveness evaluation of different BZN regiments and in combination with RAV	BZN monotherapy ≈ BZN/RAV combination therapy; > 80% efficacy in all tested groups	31,47

[a]In adults with chronic Chagas disesase (CD), unless specified; TRAENA, treatment with benznidazole (BZN) in adults with chronic CD; BENEFIT, the BZN evaluation for interrupting trypanosomiasis; CHAGASAZOL, clinical trial for the treatment of chronic CD with posaconazole (POS) and BZN; STOP CHAGAS, a study of the use of oral POS in the treatment of asymptomatic chronic CD; Pop PK Chagas, population pharmacokinetics study of BZN in children with CD; CHICAMOCHA-3, equivalence of usual interventions for trypanosomiasis (EQUITY); BENDITA, BZN new doses improved treatment and associations; DNDi, Drugs for Neglected Diseases Initiative; RAV, ravuconazole; NFX, nifurtimox; FEX, fexinidazole.

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Table 3. Target Product Profile (TPP) for Chagas disease (CD)

	Drugs for Neglected Diseases Initiative (DNDi), 2010[49]		Rao et al., 2019[24]
	acceptable	ideal	proposed
target population	chronic	chronic and acute	acute, chronic, and reinfections
geographic distribution/DTUs	all regions	all regions	active against all DTUs
efficacy	≥BZN standard[a] dose in all regions (parasitological)	>BZN standard dose to different phases of disease (acute and chronic) (parasitological)	eliminates all parasites, including in blood and tissue
safety	>BZN[a] in the frequency of definitive treatment discontinuations for medical indication (clinical and laboratory)[b]	>BZN[a] in the frequency of definitive treatment discontinuations for medical indication (clinical and laboratory)[b]	safe and well tolerated
contraindications	pregnancy	no contraindications	no contraindications; use at all ages and during pregnancy and lactation with no monitoring required
precautions/side effects	no genotoxicity[b]; no pro-arrythmic potential	no genotoxicity; no teratogenicity; no pro-arrythmic potential	no side effects
interactions	no clinically significant interaction with anti-arrythmic and anticoagulants drugs	no clinically significant interactions	not affected by pharmacogenomic factors
presentation	oral, parenteral (short POC)[c]Age-adapted	oral age-adapted	oral
stability	3 years, climatic zone IV	5 years, climatic zone IV	>2 years under tropical conditions
dosing regimen	oral−any duration parenteral − <7 days	<30 days	simple treatment regimen, amenable for use in a setting of weak health systems/infrastructure, accessible and affordable
cost	current treatments	lowest possible	

[a]As per World Health Organisation (WHO) recommendation. [b]No genotoxicity is a condition only for novel chemical entities (NCEs). [c]Need for parenteral treatment for severe disease; DTUs, discrete typing units; BZN, benznidazole.

Table 4. Proposed Screening Cascade in Preclinical Studies by Different Institutions and Experts

		in vitro models		in vivo models
institution	report	trypanocidal activity	toxicity	efficacy/safety/other considerations
Fiocruz[a]	Romanha et al., 2010[67]	>BZN in trypomastigote and amastigote forms	SI ≥ 50	1. Maximum tolerated dose (MTD) 2. Efficacy only in acute CD (3 phases): a. >BZN b. parasitological cure c. parasitological cure in BZN-resistant strains
TDR, WHO	Nwaka et al., 2011[68]	IC_{50} < 1 μg/mL in amastigote forms	SI > 50	
DNDi	Don and Ioset, 2014[69]	IC_{50} ≤ 10 μM in amastigote forms	SI > 10	1. 80% parasitaemia reduction or no parasites detected 2. Not overtly toxic at the efficacious dose
GHIT	Katsuno et al., 2015[70]	IC_{50} < 10 μM in amastigote forms	SI > 10	1. 80% parasite burden reduction in acute CD or no parasites detected 2. no acute toxicity in efficacy studies 3. oral efficacy and bioavailability
DNDi	Chatelain, 2015[15]	1. IC_{50} < 5 μM in amastigote forms	SI > 10 (SI > 100 for a lead compound)	1. ADMET profile
DND*i*	Chatelain and Ioset, 2018[61]	2. max. activity >90% 3. activity against all genotypes		2. parasitological cure in both the acute and chronic CD

[a]Fiocruz Program for Research and Technological Development on Chagas disease (CD); TDR, Tropical Diseases Research, Special Programme for Research and Training in Tropical Diseases; WHO, World Health Organisation; DNDi, Drugs for Neglected Diseases Initiative; GHIT, Global Health Innovative Technology; BZN, benznidazole; IC_{50}, inhibitory concentration 50; SI, selectivity index; ADMET, absorption, distribution, metabolism, excretion, and tolerability/toxicology.

According to the European Federation of Pharmaceutical Industries and Associations (EFPIA), it usually takes about 15 years to develop a new drug, and its success rate is only 0.02%.[52] As the Food and Drug Administration (FDA) demonstrated, the number of drugs approved has been reduced since 1995, but the investment has been increasing.[53,54]

Scientists involved in CD drug discovery face multiple specific challenges linked to a wide range of unsolved questions, ranging from the disease itself and parasite−host interactions to the development of screening cascades and the establishment of progression criteria for compounds used during early drug discovery.[55] It is well-known that the clinical event cascades in CD are induced by the presence of parasites, and evidence suggests that eradicating parasites as soon as possible after infection can prevent serious disease.[24] Sterile cure is a new and important topic in CD as it has been shown to be essential to prevent parasite reproliferation, relapse after treatment, and long-term pathogenicity.[24]

Unfortunately, current treatments—BZN and NFX—for CD are meager and inefficient, and the latest candidates—POS and RAV—have failed in clinical trials. At the moment FEX is the only candidate in clinical phase for the treatment of CD.[45,46] In addition, the number of compounds in later stages of the preclinical phase is low as only a few compounds have shown to reach high cure rates in animal models: proteasome

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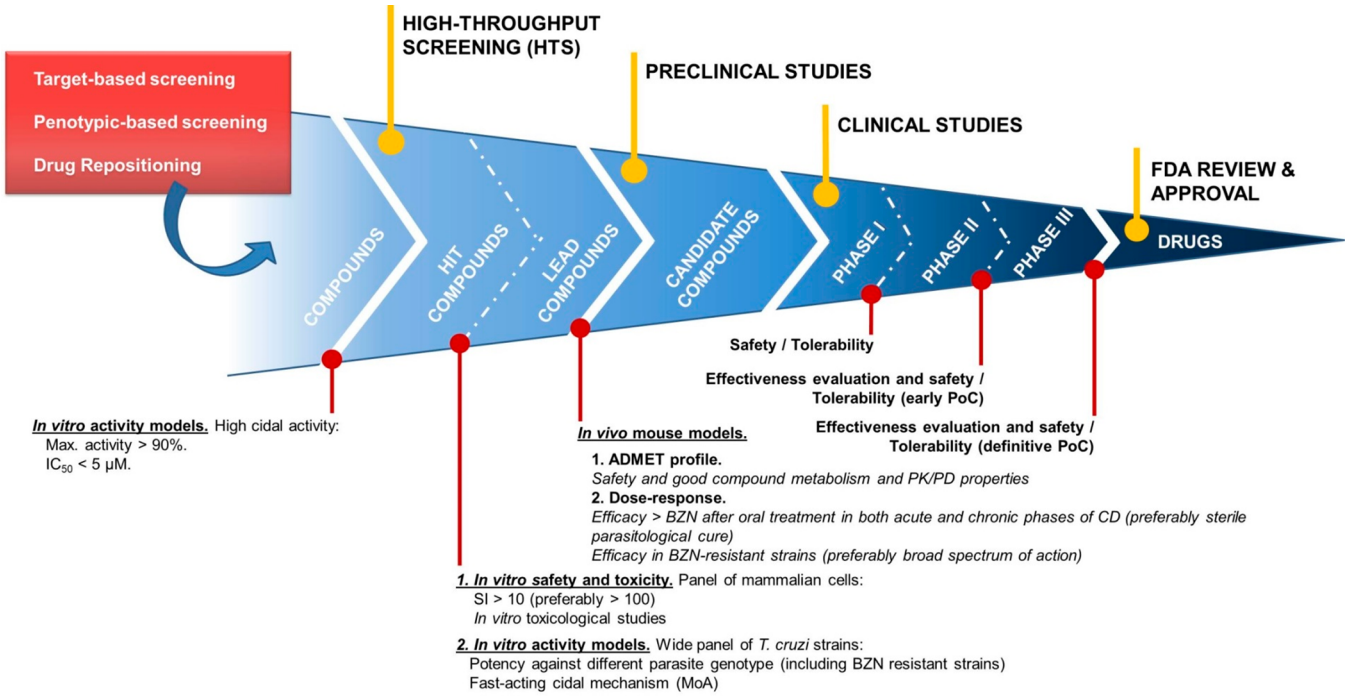

Figure 3. Chagas disease drug discovery pipeline in preclinical trials. IC_{50}, inhibitory concentration 50; SI, selectivity index; MoA, mode of action; ADMET, absorption, distribution, metabolism, excretion, and tolerability/toxicology; PK/PD, pharmacokinetic/pharmacodynamic; BZN, benznidazole; CD, Chagas disease; PoC, proof-of-concept; FDA, Food and Drug Administration.

inhibitors (e.g., GNF6702), benzoxaboroles (e.g., AN4169/SCYX6759) (Figure 2C), and nitroimidazoles (e.g., FEX).[56−58]

The first step in drug discovery process is the identification of hit compounds via screening assays using either target-based or phenotypic-based approaches. Target-based are rare due to very few confirmed targets for CD drug discovery.[59] Phenotypic-based approaches are the main focus,[60] several trials have been reported,[61] and many compounds have been identified.[62] However, very few compounds have advanced through lead optimization into preclinical and clinical research. Drug repositioning is considered a fruitful approach for the development of new treatments, as it is advantageous in terms of costs and time-consuming processes required versus drug discovery.[63] Several drugs have already been tested but no encouraging results have been obtained yet.[64−66]

An important aspect in drug discovery pipeline is the adoption of balanced selection criteria for the optimization process in preclinical studies. In this regard, a multidisciplinary group of experts have discussed this point, but no single criterion has been adopted. Table 4 summarizes the proposed screening cascade by different experts for in vitro cell assays and in vivo animal models (mice).

In 2010, a group of experts from the Fiocruz Program for Research and Technological Development on CD published the first requirements to be followed in drug discovery pipeline. In addition to publishing in vitro criteria against *T. cruzi* (activity) and mammalian cells (toxicity), they included a critical topic for in vivo assays, which refers to the concept of sterile parasitological cure, even using different *T. cruzi* strains. However, the group only included trials in acute models as they considered that chronic models did not contribute to the identification of trypanocidal drugs.[67] In 2015, another critical topic included by the Global Health Innovative Technology (GHIT) experts concerned the route of drug administration at the lead stage: any trypanocidal compound needs to demonstrate good bioavailability and high efficacy after oral treatment;[59,70] it will reduce the cost of the treatments and would be no longer considered a topic only for ideal compounds (TPP−DNDi).[4]

Finally, Chatelain reported updated screening criteria by the DNDi. After an interesting revision on the data obtained and the methodologies used in previous studies, Chatelain proposed a new screening cascade in CD drug discovery process. When considering in vitro models, Chatelain underlined that potential compounds must show high cidal activity (>90%), efficacy against all parasite genotypes, and exhibit a fast mode of action (MoA). He also made an exhaustive statement of different factors associated with the in vivo models: route of parasite inoculation, route of drug administration, treatment dose, and immunosuppression schedule. He emphasized the importance of evaluation in both the acute and chronic phases of CD, eradicating *T. cruzi* parasites. Special attention is also focused on searching for a potential clinical candidate; Chatelain (2014)[15] highlighted the importance of safety and good drug metabolism and pharmacokinetic properties, that is, the ADMET profile (abbreviation in pharmacokinetics and pharmacology for absorption, distribution, metabolism, excretion, and tolerability/toxicology).[15,61] This scheme is the most predictive of clinical outcomes, at least for the reference compounds tested so far.[61]

All these considerations were included in the TPP proposed by Rao et al.[24]—some of them already included in the TPP for ideal compounds by the DNDi (2010),[4] as shown in Table 3. A possible strategic outline of the current CD drug discovery pipeline in preclinical trials is shown in Figure 3. Comprehensive in vitro assays against a panel of different *T. cruzi* strains and toxicological tests must be conducted where fast-acting and cidal compounds with a broad spectrum of

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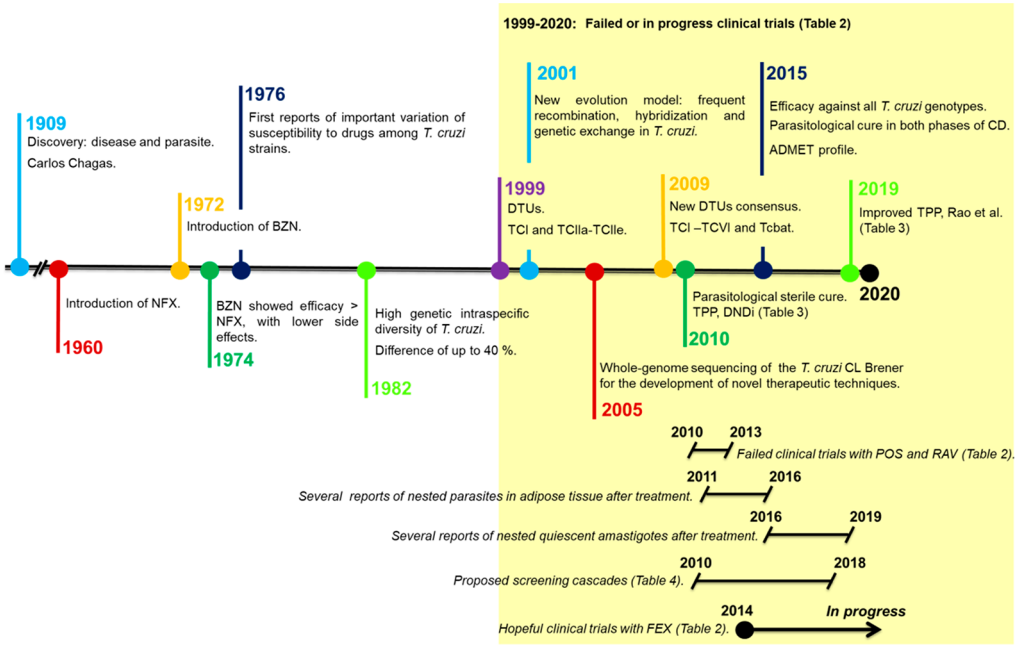

Figure 4. Important events in the Chagas disease drug discovery.[66,71−90] NFX, nifurtimox; BZN, benznidazole; DTU, discrete typing unit; TC, *Trypanosoma cruzi*; TPP, target product profile; DNDi, Drugs for Neglected Diseases Initiative; POS, posaconazole; RAV, ravuconazole; FEX, fexinidazole; ADMET, absorption, distribution, metabolism, excretion, and tolerability/toxicology.

action (potency against different *T. cruzi* strains) would be examined. For in vivo trials performed in mouse models, toxicological and pharmacokinetics/pharmacodynamics (PK/PD) tests must be completed first, and then lead compounds must be active after oral treatment in both the acute and chronic phases of CD.

CHALLENGES IN DRUG DISCOVERY AND TREATMENT

CD faces many particular challenges, especially after recent failed clinical trials evaluating POS and RAV (Table 2). These disappointing results are mainly linked to the variety of factors regarding both the disease biology and the parasite genetic background. As new findings have been made, challenges have increased and both the drug discovery strategy and the TPP needed to be modified. Figure 4 shows the timeline with these events. Because of all these factors, discovering a new drug effective against all mammalian forms and the DTUs of the parasite is quite a difficult task.[61]

***T. cruzi* Genetic Diversity and Drug Efficacy.** *T. cruzi* belongs to a highly diverse species consisting of a pool of strains and isolates that circulate among vectors and mammalian hosts.[10] The high genetic intraspecific diversity of *T. cruzi* was already reported in 1982,[89] and since 2001, several authors have reported recombination, hybridization, and genetic exchange that challenged the traditional paradigm of the clonal evolution model of *T. cruzi*.[56,72−76,90,91] Currently, the evidence indicates that these processes have contributed to the evolution of multiple genotype subgroups reported so far. After years of vigorous debate, an expert committee has classified *T. cruzi* into six discrete typing units (DTUs) designated TcI−TcVI and Tcbat.[9,29,78,92,93]

This genetic diversity is a major challenge for the clinic since there is variability among strains and DTUs in terms of infectivity, progression, tissue tropism, and drug susceptibility.[94−97] Alternatively, polyparasitism or mixed/concurrent infections occur very frequently and contribute to the high degree of variability.[94] The increasing availability of genomic technologies should make it possible to determine the extent to which parasite genetics contributes to disease outcome.

There are multiple reports of wide divergence in the drug susceptibility of different *T. cruzi* strains and isolates in both in vitro and in vivo trials, even no correlation with parasite lineage or biological origin has been observed.[95,98−100] Moreover, BZN and NFX susceptibility was independent of the mitochondrial nitroreductase (TcNTR) sequence. In summary, intra-DTU differences seem to be as extensive as those between lineages. This fact implies that this susceptibility must be associated with additional factors, which must be studied to avoid drug resistance and treatment failures.[57,95,100,101]

Thus, the target validation becomes very difficult. To overcome this challenge, a representative and wide panel of strains is essential to evaluate the efficacy of new compounds against *T. cruzi*, and must be considered an integral step in the drug discovery pipeline (Figure 3).[29,61,102−104]

***T. cruzi* Tropism and Therapeutic Efficacy.** Nowadays, it is widely known that the *T. cruzi* infection is also dependent on the genetic composition of the infecting strain: *T. cruzi* is able to infect a large variety of cells and its tissue homing ability is strain-specific.[94,97] Parasites can be present in a number of organs during chronic CD, such as heart, muscle, esophagus, stomach, intestine, kidney, liver, or adipose tissue, among others.[83,105−107] In addition, parasites can become widely disseminated after reactivation of CD in immunocompromised patients. Given the obvious difficulties in knowing the infection dynamic and parasite tropism during chronic CD in humans, experimental models are at the forefront of the research. Several trials using bioluminescence[108] or PCR[56,109] demonstrate the parasite dissemination in animal models, even crossing the blood−brain barrier and infecting the brain.[109,110]

The inability of current treatments to reliably cure chronic CD has led to speculation that the parasite may survive in

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organs/tissues where access to drugs is limited. It has been proposed that inadequate PK/PD between drugs and tissue localization of nested parasites is related to treatment failure.[111] Consistent with this, a detailed study of PK and tissue distribution of BZN has revealed that inadequate biodistribution is unlikely to be responsible for therapeutic failure in murine models after oral administration.[112]

However, adipose tissue, among others, has been identified as a possible reservoir of parasites and one of the sites responsible for relapse after treatment: parasites can egress from this tissue and repopulate the blood. Both animal model studies[113,114] and clinical trials[83] report that current potential candidates for the treatment of CD (such as POS) show nested parasites in this tissue after treatment. Less drug accessibility for hydrophilic drugs (most of them) or low parasite susceptibility in a lipid rich environment could be the reasons for the ineffectiveness of the treatments.[80,81] Hence, this tissue has been proposed as a reservoir of parasites, such as African trypanosomiasis,[81,115] and further research is needed to draw the situation in *T. cruzi*.

***T. cruzi* Life-Cycle Stages and Drug Susceptibility.** In addition to the high genetic diversity, this parasite presents a complex life-cycle. Recent research has revealed that the classical description of *T. cruzi* life-cycle is rather superficial and that the process in mammalian host cells is certainly more complex. One of the findings is that some amastigote forms may become metabolically quiescent—called quiescent or dormant amastigotes—do not replicate and could reside long-term in tissues.[84,86]

These quiescent amastigote forms pose challenges as they have been shown to be uniquely resistant to extended drug treatment using highly effective trypanocidal candidates: they have reduced metabolism compared to actively dividing parasites, are less susceptible to drugs, and ultimately can convert into trypomastigote forms and establish infections in new host cells.[86] These findings explain for the failure of highly trypanocidal compounds to completely resolve the infection. Although ergosterol biosynthesis inhibitors, such as POS and RAV, have low IC_{50} values against a panel of different strains in vitro, they are unable to completely eradicate parasites from patients.[28,33−35,39−41] In 2015, studies in murine model have shown that POS does not completely eliminate the infection through bioluminescence methods, even when the dose is increased or the treatment period is extended.[116] In 2016 several wash-out assays demonstrated that these drugs maintain residual infected cells after treatment, but viable and infective.[82,85] Finally, in 2018, it was reported that these residual infected cells were infected with quiescent amastigotes, which appear soon and spontaneously after infection, arrest replication, have a reduced requirement for ergosterol biosynthesis and resume replication weeks after entering a dormant state.[86]

These findings bring into question current methods for identifying new effective and curative drugs: simply identifying more compounds that efficiently kill metabolically active parasites is not likely on its own to be the solution to achieving the desired sterile parasitological cure. This dormant state is the key to the failure of the current drugs for CD. While other factors—genetic diversity, drug efficacy, tissue tropism, and drug biodistribution—surely also contribute to treatment outcomes, only quiescence has been definitely linked.[86]

NEW TOOLS FOR DRUG DISCOVERY

Given the wide variety of unanswered questions about the *T. cruzi* biology and the lack of validated drug targets, most approaches to CD drug discovery are based on phenotype. Nevertheless, new approaches have been widely used to overcome the challenges of treating this infection.[117] Nowadays, computational approaches, in vitro high-throughput screening (HTS), in vitro fluorescence imaging, and in vivo bioluminescence imaging are highly used methods in drug development.[118,119] These tools have become the main pillar of Chagas R and D.

HTS is used to screen large number of compounds from appropriate chemical libraries using established selection criteria. Moreover, HTS is really useful to screen compounds against a wide panel of *T. cruzi* strains in vitro.[118,119] The largest HTS campaigns against *T. cruzi* have been prosecuted by GSK[120,121] and Norvatis,[122] screening nearly 2 million compounds and over 300 000 molecules, respectively. Alternatively, several studies use computational approaches to identify potential inhibitors of key molecular targets, such as virtual screening based on quantitative structure−activity relationships (QSAR) models.[62,123,124]

Since tests must be designed to only identify cidal compounds, fluorescent in vitro imaging techniques are subsequently used to allow the identification of drugs that kill the parasite from those that only block growth and division in culture mammalian cells. It is very important to assess the antiparasitic effect of compounds, and this imaging method is compatible with cidality, time-kill, and wash-out assays which further enhance the confidence of hits (compounds capable of achieving sterile cure in vitro) to be progressed into in vivo trials.[61,85]

Finally, transgenic parasites expressing luciferase and in vivo bioluminescence imaging techniques are used to evaluate the efficacy of potent compounds in murine model: these assays facilitate research in terms of monitoring the course and the dynamic of the infection in real time, reducing the number of animal and generating data with superior accuracy. This method highlighted the spatiotemporal dynamic distribution of the parasite during chronic CD, with foci that appear/disappear over the course of even a single day.[108] Hence, this new finding questions the validity of in vivo trials that use the PCR method to evaluate the effectiveness of a treatment. Currently, imaging methods are pivotal assays for effective CD drug discovery, along with cyclophosphamide-induced immunosuppressions. Immunosuppression is the formula employed to prove cure because seemingly cured immunocompromised mice present parasitaemia reactivation, and any remaining parasites are responsible for this reactivation.[125] This is an important matter because apparently cured immunocompromised individuals and cured patients submitted to transplantation (diagnosed with AIDS or treated with anticancer chemotherapy) present clinically aggressive parasitaemia reactivation.[126]

However, bioluminescence imaging technology is not sensitive enough to allow the microscopic detection of individual infected cells and visualization of host-parasite interactions. To address this problem, Taylor et al.[127] have developed a transgenic parasite that expresses dual bioluminescence: fluorescence reporters. This new transgenic parasite allows the visualization of intracellular amastigotes by

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fluorescence microscopy after locating, excising, and sectioning infected foci.

CURRENT SCENARIO FOR DRUGS DEVELOPMENT

Most of the current candidates for treating CD are not potent enough to render a sterile parasitological cure. According to the WHO, the goal is to eradicate the parasite from infected individuals, thereby decreasing the likelihood of both symptomatic CD and the spread of the disease.[128] In this regard, all current drug discovery programs are aimed at this goal. Overall, there is an urgent need for drugs to enable safe and effective therapies for CD.[24]

Since 2010, different clinical trials have evaluated the efficacy of POS and RAV, candidate compounds that actively inhibits the synthesis of ergosterol (Table 2). These compounds previously showed excellent activity in both in vitro and in vivo models of CD, but they failed to disclose the same efficacy in humans.[34] As stated above, further studies using new methodologies—in vivo bioluminescence imaging[116] and in vitro wash-out assays[82,86]—confirmed the low efficacy of these ergosterol biosynthesis inhibitors. This failure is linked to the MoA of these compounds and thus to the nonreplicative and drug-insensitive quiescent amastigotes. In addition, BZN and NFX both require metabolic activation,[129] the activity of which is likely to be reduced in these metabolically reduced quiescent amastigotes.

From here, the prerequisite of cidality has been notably supported. POS and RAV are ergosterol biosynthesis inhibitors, which means that inhibit the main sterol in the cell membranes and the crucial component for the proliferation of the replicative stages of *T. cruzi*: these compounds inhibit cell division (static activity) and are time-dependent (slow-acting), so quiescent amastigotes have reduced susceptibility to them.[130,131] The time and period of action are also important features, without forgetting potency against a wide panel of *T. cruzi* strains.[15] Future work is needed to understand the development of quiescent amastigotes and to find novel inhibitors capable of overcoming this stage.[86] Shortly, new fast-acting, potent, and cidal candidates, capable of killing quiescent amastigotes, are needed to avoid treatment failures (see Drug Discovery Pipeline and Figure 3).

Alternatively, it seems that the time that amastigotes stay quiescent is finite. On the basis of this, the use of current drugs over longer periods (months, beyond the dormant stage potential of the parasite) at less intensive dosing routines (to keep overall dose down) should also be further explored.[132] Barret et al.[87] reported that nondividing quiescent amastigotes respond to signals that trigger conversion to trypomastigotes. This offers the possibility of finding molecules that might activate quiescent parasites and sensitize them to existing drugs.

New drugs and treatment schedules to find adequate PK/PD between them and tissue localization of nested parasites is also a major challenge—it is one of the main obstacles in CD drug discovery[133]—as it is the improvement and systematic validation of higher-order mammal models that more closely mimic the human CD.[104,133] Lastly, the identification of new molecular targets can also have a strong impact in drug discovery.[55]

A synergistic treatment represents another alternative that is also being explored to fight CD:[66] a combination of different compounds, preferably with different MoA, generally improves trypanocidal activity and could increase the efficacy until a sterile parasitological cure is achieved. Additionally, this treatment reduces the drug toxicity and the possibility of developing resistance.[134]

CONCLUSIONS

The aim objective is to find a specific treatment that achieve a sterile parasitological cure, that is, the eradication of the parasite and, hence, the elimination of the signs and symptoms of CD. In view of recent challenges facing the treatment of CD thanks to the new technology developed, it appears that many gaps must be filled and the likelihood of new optimal treatments arising in the coming years remains uncertain. Nevertheless, it is clear that research has increased, an innovative line of research with an unconventional strategy is currently observed, and promising results are being obtained in drug discovery projects (new methodologies, treatment guidelines, chemical series, molecular targets, and complementary approaches).

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Notes

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ABBREVIATIONS

ADMET, absorption, distribution, metabolism, excretion, and tolerability/toxicology; BZN, benznidazole; CCRP, Chagas Clinical Research Platform; CD, Chagas disease; DND*i*, Drug for Neglected Diseases Initiative; DTU, discrete typing unit; ERG, Eastern Research Group; FDA, Food and Drug Administration; FEX, fexinidazole; GHIT, Global Health Innovative Technology; HTS, high-throughput screening; IC_{50}, inhibitory concentration 50; MoA, mode of action; NCEs, novel chemical entities; NFX, nifurtimox; NTD, neglected tropical disease; PD, pharmacodynamics; PK, pharmacokinetics; PoC, proof-of-concept; POS, posaconazole; QSAR, quantitative structure−activity relationship; R&D, research and development; RAV, ravuconazole; SI, selectivity

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index; TPP, target product profile; WHO, World Health Organization

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