REVIEW OF PHARMACOLOGICAL OPTIONS FOR THE TREATMENT OF CHAGAS DISEASE

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Abstract

Introduction: Chagas disease (CD) is a worldwide problem, with over 8 million people infected in both rural and urban areas. CD was first described over a century ago, but only two drugs are currently available for CD treatment, benznidazole (BZN) and nifurtimox (NF). Treating CD infected patients, especially children and women of reproductive age, is vital in order to prevent long term sequelae such as heart and gastrointestinal disfunction, but this aim is still far from being accomplished. Currently, the strongest data to support benefit-risk considerations come from trials in children. Finally, treatment response biomarkers need further development as serology is being questioned as the best method to assess treatment response. Areas covered: This article is a narrative review on the pharmacology of drugs for CD, particularly BZN and NF. Data on drug biopharmaceutical characteristics, safety and efficacy of both drugs are summarized from a clinical perspective. Current data on alternative compounds under evaluation for CD treatment, and new possible treatment response biomarkers are also discussed. Conclusion: Early diagnosis and treatment of CD, especially in pediatric patients, is vital for an effective and safe use of the available drugs (i.e. BZN and NF). New biomarkers for CD are urgently needed for the diagnosis and evaluation of treatment efficacy, and to guide efforts from academia and pharmaceutical companies to accelerate the process of new drugs development.

Introduction

Chagas disease (CD) is a zoonosis caused by infection with *Trypanosoma cruzi* , a protozoan parasite. Humans can acquire this infection by contact with insect vectors (hematophagous triatomine or Reduviidae bugs), by ingestion of contaminated food[1], congenital transmission, blood transfusions or organ or tissue transplants from infected donors. In the past, CD was believed to exclusively affect rural populations in Latin America, but movement of people from rural to urban areas (as well as expanding screening strategies to urban dwellers), has revealed the infection as a worldwide problem[2,3]. Congenital transmission in particular has become an important route of infection and the main reason for acute CD in non-endemic countries, such as North America and Europe.

The World Health Organization (WHO) estimates that over 8 million people worldwide are infected with *T cruzi* , and that an excess of 10,000 deaths occur every year due to CD [2]. Under-diagnosis of CD cases is suspected to be as high as 90%, and even higher in cases of congenital CD which is alarming considering that estimated *T. cruzi* prevalence among pregnant women ranges from 2% to 40% depending on geographical area [4,5].

CD has a clinical course characterized by an acute phase, commonly asymptomatic, that resolves spontaneously in most cases but which can sometimes (i.e. less than 5% of cases) be severe, leading to serious sequelae and even death. Following the acute phase, a chronic stage ensues, with patients usually remaining asymptomatic for many decades. However, approximately 30% of infected patients eventually develop progressive and irreversible target organ damage, mainly in the heart and/or esophagus and colon. The

'silent' asymptomatic phase between acute and chronic phases is referred to as 'indeterminate stage' by some authors.[6,7]

The decision to implement CD treatment was historically based on age, due to limited evidence of efficacy, and an increasing frequency and severity of side effects in relation to patient age. [8]Currently there is agreement in international clinical guidelines that anti-parasitic treatment is effective and therefore should be offered at least to 1) patients with acute CD, 2) all children with congenital or acquired acute CD 3) immunosuppressed hosts with acute or reactivation of chronic disease 4) women of childbearing age in order to prevent congenital transmissions [9–12].

Treatment effectiveness in chronic CD continues to be highly debated[13–15]; for adults over 50 years old, trypanocidal therapy is still considered optional due to an unclear risk-benefit balance. On the one hand there is a general agreement that parasitic persistence increases the risk for development or progression of cardiac lesions in chronically infected patients and therefore parasite eradication may be necessary in the early stages of the disease[16,17]. On the other hand, advanced CD seems to involve irreversible cardiac damage, and therefore parasiticidal treatment of affected older patients may be futile[13]. However, the evidence for either position is still limited[18].

Unfortunately, anti-parasitic therapy has not been widely implemented, even for those age groups that can clearly benefit from it (e.g. pediatric patients, early chronic infections, etc.) in spite of existing national and international guidelines that support treatment. This failure to treat may possibly be explained by many obstacles, including health care providers' low awareness of CD and its treatment options, overblown concerns about side effects, low access to healthcare for many patients with CD, lack of an optimal straightforward test of CD cure, widespread drug shortages and irregular supplies, and regulatory barriers[19]. Even though WHO 2020 Goals for CD included access to treatment and/or care of all infected/ill patients, and The London Declaration on Neglected Tropical Diseases [20] announced plans for the elimination or control of Chagas disease by 2020, current estimates indicate that less than 1% of CD infected patients are treated and those lofty aims are far away from becoming a reality.[21] Sub-optimal CD treatment implementation continues in many countries in spite the fact that failing to treat a CD patient could be considered medical negligence in many jurisdictions[22].

In South America, CD causes the loss of over 750,000 working days because of premature deaths and $1.2 billion in productivity loss every year [23]. The calculated annual global burden of disease is over $600 million dollars per year in health-care costs and 10% of this burden affects non-endemic countries[24]. According to a study conducted in Mexico that evaluated the impact and economic outcomes (costs, cost-effectiveness, cost-benefit) of identifying and treating different percentages of CD patients in the acute and indeterminate phases, identifying and treating CD cases earlier was always economically dominant compared to no treatment[21]. Authorities in charge of health policies should acknowledge that this would result not only in reduced transmission rates and better health outcomes but also in huge cost-savings, besides being a medical duty, and human rights issue.

Despite the fact that CD was first described over a century ago[25], only two drugs are currently available for treatment, benznidazole (BZN) and nifurtimox (NF), which were developed over 40 years ago. Both drugs require prolonged treatments (30 to 60 days) and are associated with adverse events that increase in severity and prevalence with age. Prompt diagnosis and treatment, especially in pediatric patients, are vital for an effective use of these medications.

Pharmacological treatment of CD

Both NF and BZN are nitroheteroerocyclic drugs developed over four decades ago by Bayer and Roche, respectively. Their mechanism of action is believed to rely on intracellular activation, that generates intermediates affecting the parasite's vital biological functions [26,27]. Both drugs are highly liposoluble, with very low water solubility. The parasite's mechanisms against these drugs relies on detoxifying molecules such as

trypanothione [28] a vital part of the free radical scavenging cycle that is recycled by the enzyme trypanothione disulfide reductase.

Treatment with BZN and NF is contraindicated during pregnancy in most guidelines, due to limited evidence on safety, yet there is evidence of low concentration of BZN or NF in breastmilk with no risk to infants during lactation [29–31].

These drugs are usually not recommended in patients with renal or hepatic impairment, mostly on the basis of lack of safety data. However, given that there are almost completely metabolized, renal elimination only plays a marginal role in their clearance and use in kidney failure would be possible with appropriate monitoring for adverse events. Similarly, in cases requiring emergency treatment (e.g. CD meningoencephalitis), hepatic impairment should not be an obstacle for treatment assuming that strict monitoring can be implemented[32].

Although BZN is more commonly used than NF, both drugs seem to have similar efficacy and safety profiles. Reported treatment responses in the chronic indeterminate phase in children (mostly based on measuring serologic titers) are near 90% after NF treatment [33]and 94% for BNZ [34,35]. In adults NF has treatment response rate of 7-8% [36,37] and BZN between 2 and 40% [13,38], with more studies carried for BZN than NF in this area (see **table 1**). There are no current data formally comparing both drugs, but some clinical studies are currently ongoing attempting to address this issue, such as TESEO (NCT03981523) and CHICAMOCHA 3[39].

Unfortunately, given the natural history of CD and heterogeneity of response follow-up techniques, it is logistically challenging to treat this disease during the earlier asymptomatic chronic phase and follow that patient cohort to determine clinical outcomes, which can take decades to appear, with sufficient statistical power to differentiate potential effects in treated versus control patients. About 30 years ago two controlled placebo clinical trials assessed the efficacy of treatment in CD chronic phase in pediatric patients with good results[35,40], and other studies followed those, leaving no doubt that the earlier children are treated, the better the response achieved[8,40,41]. Women in fertile age should also be treated to prevent congenital CD transmission[9,40–42].

Unlike treatment for children or women of reproductive age, controversies regarding treatment of adult patients still abound; in 2016 the first prospective multi-centric and randomized CD cohort study in older adults with advanced CD, the 'Benznidazole Evaluation for Interrupting Trypanosomiasis' (BENEFIT), was published, describing the outcomes of 2854 patients with established Chagas heart disease that received BZN or placebo and where followed for 5.4 years[13]. This study concluded that no significant morbidity or mortality reduction was achieved with anti-parasitic treatment in patients with advanced cardiac stage. On the other side, the evidence from cohort and historical controlled trials has supported treating most chronic patients at early stages, with the available drugs[16,43–47].

Monitoring treatment is recommended for either drug, with complete blood counts, hepatic, and renal function testing. Frequency varies through different guidelines between every two and five weeks, always with a pre-treatment laboratory evaluation to compare later findings[34,48].

Benznidazole

Brief Recent History

Benznidazole (N- phenylmethyl-2-nitro-1H-imidazole-1-acetamide; CAS Number 22994-85- 0) is the most commonly used drug for treatment of CD. It was developed by Roche (Ro 07-1051)[49] and there have been three producers of BZN so far: Roche, Lafepe (public pharmaceutical company of Brazil), and Chemo (formerly Elea, an Argentine pharmaceutical company). Roche manufactured and distributed the drug (as Radanil© or Rochagan©) from 1967 until the early 2000s, when production was discontinued due to economic reasons[49]. Later, encouraged by pressure from scientific and medical organizations, Roche eventually transferred BZN production technology and remaining stocks to Lafepe, which committed to re-establish supply. Lafepe developed a pediatric formulation for children weighing < 20 kg (12.5 mg tablet) that was tested in clinical pediatric study in Argentina (sponsored by Drugs for Neglected Diseases initiative)[8] and

this formulation was registered in Brazil in 2011 and was included on the WHO's Essential Medicines List for children in 2013.

BNZ was the first drug approved by the United States Food and Drug Administration (FDA) in 2017 for children ages two to twelve years with CD[50,51] and in April 2018, a pediatric formulation of BZN was approved in Argentina to treat children under the age of 2 year. BZN may is also prescribed off-label for adolescents, adults, and children under 2 in countries where the drug has not been registered specifically for these age groups.

BNZ Pharmacology

Benznidazole is an oral, broad spectrum nitroimidazole antimicrobial that has activity against bacteria and several parasites. It has demonstrated efficacy against in vitro *T. cruzi* strains in several in vivo animal models [52–56]. According to the Biopharmaceutical Classification System (BCS)[57,58] BNZ belongs to Class IV drugs (reduced solubility and permeability); it is a liposoluble drug with very low solubility in water, and a weak base at physiological pH range. BNZ solubility in distilled water or simulated gastric and enteric fluids is reported between 0.2 mg/ml and 0.4 mg/ml. According to this, BZN is classified as a low-permeability drug with a log P of 1.64[58].

BZN is considered a prodrug, requiring activation by parasite nitroreductase enzymes that reduce BZN, initiating a cascade of reactions leading to the formation of highly reactive drug metabolites[27]. The main parasite enzyme involved in BZN activation is believed to be a type 1 nitroreductase. The resulting BZN metabolites, such as dialdehyde glyoxal, bind to parasite macromolecules disrupting *T. cruzi* metabolism and other vital functions, and leading to parasite cell death[27]. However, using a metabolomics approach to asses BZN mechanism of action, Trochine et al. proposed that the covalent binding of BZN with low molecular weight thiols as well as with protein thiols is a primary cause of the drug's toxicity against *T. cruzi,* instead of glyoxal generation as formerly stated[59]. This suggests that BZN acts in a complex manner and there are still some remaining uncertainties about its mechanisms of action: metabolomic studies are a promising frontier in this research area.

T cruzi resistance to BZN is not well described in literature. Some in-vitro studies have reported that some parasite strains have a 'natural' in-vitro resistance to BZN associated with overexpression of ABCG transporter[60], but this evidence has been questioned, as in-vitro results do not correlate with therapeutic outcomes in humans[61]. Other studies with in vitro data suggest that susceptibility of different *T.cruzi* strains to BZN fluctuates, but the 50% inhibitory concentration (IC50) values remain [?]19.5 μg/mL (75 μM) and can vary 10-fold within the same assay. Activity against different forms of the parasite (epimastigotes, trypomastigotes, or amastigotes) also appears to vary within a relatively small range[62,63], and it should be considered that many studies are performed on the epimastigote stage, which is easier to culture but not the human stage of the parasite. Additionally, time-kill studies indicate that BZN trypanocidal effect is both time and concentration dependent [62–64]. Using multiple *T.cruzi* strains and a high-throughput screening platform, a rapid trypanocidal effect was demonstrated with 100% parasite clearance against multiple divergent *T. cruzi* genotypes, a rate superior to that for ergosterol biosynthesis inhibitors[63].

After oral administration, BZN is quickly absorbed from the human intestine (Ka = 1.14/h), with a plasmatic peak within 2–4 hours after drug intake[8,65]. The impact of food on absorption has not been systematically investigated. Some evidence points to first step elimination by hepatic biotransformation and entero-hepatic recirculation, possibly with some degree of enteric metabolism as well, but little research has been conducted in this area. Absolute bioavailability in humans has never been formally estimated due to the absence of an intravenous formulation apt for human use, though a mean relative oral bioavailability of 91.7% in three healthy adults when comparing liquid to solid oral formulations was reported[66]. Steady-state plasma concentrations are reached within 3 days of initiation of a twice-daily dosing regimen[8,66]. BZN distributes widely into tissues, including the central nervous system (CNS)[67,68], with higher volume of distribution in children compared to adults[8]. The drug reaches CNS concentrations close to 70% of those observed in plasma, which has allowed successful treatment of Chagas CNS infections (e.g. meningoencephalitis) in

immunosuppressed patients[69–72]. Plasma protein binding of BZN is approximately 50% and is thus not expected to lead to significant interactions with other drugs [54].

Clearance of BZN is mainly by biotransformation (>80%)[68,73], believed to take place mostly in the liver, probably by members of the cytochrome P450 (CYP) family and/or tissue nitro-reductases. However, few studies to date have explored the details of the metabolic pathways responsible for BZN elimination. Approximately 6–20% of the drug can be found unchanged in urine, with differences depending on age of the patient (e.g., children seem to eliminate more unchanged drug in urine compared to adults); and the rest of the drug has been observed as reduced and conjugated.[74]

Mean BZN half-life is 13 hours in adults[66] and significantly shorter in children (3 to 6 hours for 2 to 7 year-old patients and 9 to 10 hours in children 7 to 12 years) as observed in two prospective clinical trials[8]. This difference in clearance and half-life between different age groups implies average steady-state concentrations of BZN lower in children than in adults. Interestingly, this difference does not seem to affect the efficacy of BNZ since in a prospective clinical trial, all treated children showed good response to treatment despite lower plasma concentrations of the drug[8]. When comparing the data obtained in this study with previously reported adult results[66], a progressive decrease in the clearance rate of BZN with increasing age was observed (i.e. the older the patient, the slower the drug was eliminated). The specific mechanisms for drug elimination in children and adults remain undiscovered. Research in the area is actively testing different hypotheses such as slower drug metabolism in adults and impaired drug absorption in younger children. BNZ pharmacokinetics and treatment response in teenagers and young adults have never been studied, so the assumption that it would be in between children and adults is so far unsupported by actual evidence.

The most commonly used BNZ dosing regimen, reported in the majority of the evidence published to date (see **table 1**) uses doses ranging from 5 to 8 mg/kg/day orally, in two daily doses for 30 to 60 days. BZN can also be administered in three daily doses, with a clear tendency in international guides for recommending 5 rather than 8 mg/kg/day and twice daily rather than thrice.[48]

Duration of treatment in children and adults is currently under review and some expert guides are already recommending shorter treatments[8,75], supported by the fact that treatment in children is proven to be effective despite differences in PK with adults leading to lower concentrations and shorter half-lives, without detectable drop in effectiveness and with less adverse reactions[8,75]. A few trials enrolling children who received 30 days of treatment have showed good results[42,76], and recent evidence points towards possible efficacy of lower BNZ doses or less frequent dosing for adults and teenagers too[65,77,78]. Lower treatment duration has also proven to be effective in adults in preliminary results of an unpublished clinical trial (NCT03378661) that showed 89.3% of therapeutic response (measured as a negative PCR) after a 4 week treatment compared to 82.8% after 8 week-treatment. There are currently other ongoing trials addressing BZN dairy doses and duration as well such as BETTY trial[79] and MULTIBENZ (NCT03191162), that may change treatment regimen in the next few years, assuming that sustained long term responses are demonstrated.

The most commonly observed adverse drug reactions (ADRs) associated with BZN use includes rash and pruritus (usually after 7 to 12 days of treatment), headache, myalgia, and gastrointestinal discomfort (in the first days of the treatment). Drug-associated hepatitis, leucopenia, peripheral neuropathy, and severe drug hypersensitivity (Stevens-Johnson syndrome and other reactions with systemic symptoms) are less frequent. The median proportion of severe side effects is 2.7%[45] and trough BZN serum concentrations did not appear to be related to the appearance of serious ADRs in a small study in adults[80], evidence for a concentration-adverse event relationship has been observed in pediatric studies[8,34]. A recent prospective study in 99 participants reported some previously unreported ADRs; ten subjects presented psychiatric symptoms (anxiety, panic attacks, emotional lability and persecutory delusions), four patients reported sexual alterations (erectile dysfunction or delay in menstrual cycle with no alternative explanations) and one patient had a bronchospasm. The results of this study were in other aspects similar to previously published literature about BZN adverse reactions.[81]The safety profile of BZN in children is well described

in the literature; data are consistent and do not suggest any signals of clinical concern[8,34].

The incidence of ADRs between children and adults has not been compared directly (i.e. in a study enrolling both age groups), but ADRs seem rare and almost universally mild in younger children, and appear to increase gradually after 7 years of age in both frequency and severity. It is very infrequent to observe ADRs in newborns and children under 1 year old, and rates of treatment discontinuation due to ADRs are significantly low in children[34,47,76,82–85] while these ranges between 11 and 45% in adult studies[16,45,82,86].

The underlying biological mechanisms for the observed ADRs have not been studied in depth, but the immune system seems to play an important role, particularly in the case of cutaneous rashes and hypersensitivity reactions. This assumption is based on the timing for the moderate cutaneous reactions (7–12 days after onset of treatment) that mimics the time course of similar reactions associated to other unrelated medications known to cause rash (e.g. lamotrigine), and the observation of rare severe adverse reactions such as Stevens-Johnson syndrome and drug reactions with eosinophilia and systemic symptoms[83,84]. A common immunological trigger for these reactions and possibly a pharmacogenetic predisposition could explain these similarities, but studies of potential pharmacogenomic markers are lacking. Some authors had formerly proposed to associate BZN with thioctic acid in order to prevent ADRs, based on this compound to increase hepatic elimination of BZN, but this has proven not to be effective when evaluated in-vivo.[87]

BZN has never been formally studied during pregnancy, but it is not recommended for pregnant women due to the lack of safety data; there is insufficient information about reproductive safety of this drug, other than the fact that there have been no reports of malformations or any other pregnancy complications. However, it should be considered that it is likely that an unknown number of women were exposed to BZN in the first trimester by accident and the lack of reports on safety data might be a good sign so far. Also, there are some reports of treatment during late-stage pregnancy in emergency situations that did not result in any complications for the baby and may have saved the mother's life[69]. The main recommendation therefore remains to avoid BZN during the first trimester of pregnancy and throughout pregnancy whenever possible until further information becomes available, though in case of an emergency or a life-threatening situation caused by CD, we recommend not delaying treatment because of an unproven teratogenic risk[69].

BNZ has been classically contraindicated during lactation, but recent prospective studies and pharmacokinetic evaluations suggest that the risk of exposure to BZN from breastmilk for a breastfed baby is negligible, and lactation should not be considered a contraindication for CD treatment in those circumstances when treatment cannot be postponed. [29]

Nifurtimox

Brief Recent History

NF was manufactured in the 1970s by Bayer -as Lampit®- but its development started earlier, in the 1960s as Bayer-2502. Similar to what happened with BZN, Bayer discontinued production in the 1990s due to low demand and almost null profitability, but reconsidered and restarted production later. Since then, country-level access to the drug has depended on individual states' agreements and negotiations with WHO and Bayer, and local bureaucratic and political decisions. Availability currently seems erratic in many South American countries, but it is expected to improve in the near future, after a clinical trial performed in children -CHICO study (NCT02625974) - that supported FDA approval of NF formulations for CD in the pediatric population.

Nifurtimox Clinical Pharmacology

Many aspects of NF clinical pharmacology are similar to those of BZN. There is also considerable lack of knowledge on many aspects of its PK, effectiveness, and metabolism. However, NF is currently undergoing extensive redevelopment, with some clinical trials already completed and others in process (NCT04274101) (see **table 1**).

NF mechanism of action is believed to be the generation of nitro-anion radicals, after activation by parasite

nitroreductases in the presence of oxygen. This leads to production of free radicals that damage vital *T. cruzi* cell components, block DNA synthesis and accelerate DNA and RNA degradation[88,89].

Similarly to BZN, NF is hydrophobic, highly liposoluble and distributes widely to tissues, including the central nervous system[90], a useful property for the treatment of T. cruzi CNS infections. It has a rapid absorption from gut (Ka 0.77/h), but undergoes extensive first-pass elimination (much higher than BZN), leading to only a small fraction of orally administered NF reaching systemic circulation[91,92]. NF is administered orally and reaches peak plasma concentrations after 2 to 4 hours[32,90,93] with a relatively short half-life (approximately 3 hours in adults, and similar in children based on very limited data)[94,95]. Liver elimination accounts for virtually all NF clearance (i.e., unchanged NF elimination in urine is less than 1% of the administered dose).[96]

According to a recent trial that reported biopharmaceutical characteristics after oral administration of 30 mg and 120 mg tablets[96], total systemic exposure to NF was approximately 71% greater after food than in a fasted state. Mean (%CV) NF AUC estimates ranged between 1676-2670 μg[?]h/L (19–32%) and Cmax estimates ranged between 425-568 μg/L (26–50%) following administration of single dose 120 mg NF with food in adult CD patients. The median time to reach maximum concentration (Tmax) of NF under fed conditions was 4 hours (range: 2 to 8 hours). Interestingly, in this study Cmax increased 68%, AUC increased 71%, and Tmax increased by 1 hour after a high-fat meal compared to fasted conditions.[96]

Animal liver experiments of NF metabolism have suggested a number of metabolites[97], but this aspect has only been studied in a limited number of humans, with preliminary confirmation of the metabolites observed in animal experiments and further observation of a range of minor metabolites[98]. Data from animal studies also suggests that CYP enzymes are responsible for NF metabolism, but no human data is publicly available identifying specific CYP isoforms, or associated enzymes, responsible for biotransformation[98,99]. NF plasma protein binding is approximately 50% and not expected to play a significant role in drug-drug interactions[100]. This drug is a substrate for the ABCG2 transporter, commonly known as Breast Cancer Resistance Protein (BCRP), which has been shown to influence NF transport across the blood-brain barrier, as well as its excretion into breastmilk[30,31,101].

NF apparent volume of distribution is high (V/F = 760 L), suggesting both an extensive distribution into tissues and also a significant pre-systemic elimination (i.e., a limited bioavailability), such as that observed in animal studies [91].

Neither NF optimal dose nor the optimal treatment duration for CD is well defined. Initially, treatments tended to be long (90–120 days)[102] but were subsequently reduced to mimic BZN treatment spans (approximately 60 days)[103,104]. The recent trial CHICO study (NCT02625974) studied alternative dosing (30 versus 60 days) observing similar serological and parasitological treatment responses for children under 2 years; but in order to apply these conclusions to all pediatric patients, long term follow-up would be crucial. Commonly used dose ranges from 8 to 15 mg/kg/day divided in three daily administrations, but optimal daily dose frequency has never been duly studied either, and was defined only on the basis of NF half-life.

The most commonly observed ADRs are anorexia and weight loss, irritability, sleepiness, and other nervous system signs and symptoms[83,103,105]. NF use is also associated with rash, pruritus, and drug-associated hepatitis but less frequently than BZN. Depression, peripheral neuropathy, and psychiatric symptoms have also been reported, less commonly. Similar to BZN, NF-associated ADRs seem much more common and severe in adults and are usually mild in children, including neonates[96,106,107]. Notably, there is some evidence that suggests that patients who develop a severe drug reaction to BZN may still be treated safely with NF [108].

Similar to BZN, NF is considered contraindicated during pregnancy and lactation: virtually no data is available on the safety of this drug during the first trimester of pregnancy, and therefore it is still advisable to avoid its use at this stage. About lactation, recently published and ongoing studies on NF transfer into breastmilk strongly suggest that the drug is safe during breastfeeding, and treatment of a lactating mother should not be discouraged if needed[30,31].

assessment of treatment Efficacy: Existing biomarkers of treatment response and new advances

The appropriate markers of CD cure (i.e. a patient being free from CD and not at risk of developing target organ involvement such as cardiomyopathy, cardiac failure, mega-esophagus or mega-colon, etc.) have been subject to intense debate for decades, in part due to prolonged persistence of *T.cruzi* specific antibodies, lack of sensitivity of parasitological tests, and need for long-term follow-up (generally years or decades) to observe negative seroconversion of conventional serological tests, as well as a general lack of understanding of the parasite biology in the human and the kinetics of drug response. Serology (and, in particular, negative seroconversion) has been heralded for many years as the gold standard for treatment response, largely guided by the successful results observed after treating acute infections or early chronic infections in children[8,109] (see **table 1**). However, treatment of older patients, or even children over 7 years of age, does not lead to negative seroconversion for decades (if ever)[8,40,42,109], even if a drop in antibody titers is observed early after pharmacological treatment. This fact is easy to understand, if one considers that persistent immune system stimulation (e.g. as would be the case in chronic CD due to persistent antigen shedding by deep-tissue *T cruzi* nests) is bound to generate immune responses that would last for a long time even after complete parasite clearance by NF or BZN.

Negative seroconversion continues to be the (somehow arbitrarily) chosen method to ascertain a treatment response, both in general practice and research. Reported serologic response rates are as high as 96% for congenitally infected infants [8,109–111], 76% for acute infections[112], 63% [40,113] to 90% [113] for chronically infected children, and 37% for chronically infected adults [114]. These rates have marked variability among different published studies due to different serologic techniques employed, with sometimes poorly evaluated, different sensitivities and specificities, used to determine treatment response as the primary outcome of clinical trials[115,116].

It would be reasonable to consider that more sensitive serological techniques would under-estimate time and rates of cure (i.e. would yield positive antibody results with lower titers), with no correlation with clinical outcomes such as organ impact, but this still requires more research to confirm. In order to study the correlation between serologic response and organ damage, a recent study of a pediatric cohort performed long-term follow up of treated children with electrocardiograms (ECG), 24 hours ECG (Holter) and Speckle-tracking strain echocardiography and observed no CD untoward impact on heart function in this population years after treatment, supporting the low correlation between serological tests and clinical response[117]. Also, *T. cruzi* detection tests currently in use in some countries for long term follow up of patients such as polymerase chain reaction against *T.cruzi*- DNA (PCR) or different serology techniques, were initially developed for diagnostic purposes. Furthermore, many of the methods used have been repeatedly changed across the years, and comparison of results from recent clinical studies to older studies involves a degree of uncertainty even if comparing tests that are nominally the same (e.g. RT-PCR done in recent years would have used primers and protocols very different to those used 10 years ago) [41,46]. In this context, new markers of cure are needed. Alternative early markers of cure have been suggested, such as decrease of total anti-*T.cruzi* antibody titers (i.e. instead of negative seroconversion) or use of non-conventional serological techniques [118,119] such as specific lytic anti-α-Gal antibodies known as anti-F2/3 antibodies[120]. Other CD biomarkers suggested by scientific literature so far have been reviewed by different authors too, but the general impression is that they all still require more research, and validation. **Table 2** summarizes the biomarkers studied.[46,121,122]

PCR has been proposed as a sensitive and specific method to detect *T.cruzi* parasitemia in newborns[41,123,124] and has also shown good results for the assessment of treatment failure, as a persistently positive result after treatment clearly is evidence of failure to eliminate the parasite[125]. However, while PCR may be more sensitive than current methods in some cases, the lack of standardization of the method across centers is a still unresolved issue. Furthermore, actual rate of false positives is still under debate, and may vary among testing laboratories (and different techniques used). Other issues such as cost and instrument availability and technical skills, conspire to limit the use of this method at the moment, but considering its good results so far and its feasibility of being easily applied in clinical settings, the investment in improving PCR methodologies

is worthwhile. The CD community must focus on suitable strategies for parasite DNA extraction in lower sample volumes, the equivalence between blood and tissue parasitemia; the reduction of false negatives, as well as the validation and standardization of PCR assays; and the correlation of PCR readouts with negative seroconversion.[109,126–128]

Considering all available evidence, we could conclude that despite the need of trials in this area, a negative PCR -associated to a persistent decrease of *T.cruzi* antibodies titers- should be the chosen criteria used to assess treatment response and to follow-up after treatment in our time.

Pharmacological Treatment: New treatment strategies and Alternative drugs

As mentioned before, there are few recent advances in BZN and NF pharmacology, which is disappointing considering their longevity. Some improvements in drug formulation have been proposed (e.g. application of nanotechnologies such as nanocrystals, polymeric nanoparticles, and lipid nanostructures) as an attractive approach to improve solubility and dissolution of BNZ and NFX, hopefully leading to dose reductions and, perhaps, novel treatment schemes, but virtually no clinical research has been undertaken with this proposed formulations[129,130].

New potentially effective drugs have been proposed on the basis multiple targets in the parasite cell. Ergosterol biosynthesis enzymes in particular have been well studied, and CYP51 (sterol 14-Demethylase) was proposed as an interesting target, both due to its importance in parasite survival, and the availability of multiple medications already in the market (i.e. azole antifungal drugs) that could be easily repositioned for clinical trials in CD [104,131–134]. This repositioning approach is advantageous in view of the cost and time-consuming process required compared to the development of new medicines, especially in neglected diseases, since repositioned drugs already have their toxicological and pharmacokinetic profile assessed when used on their previous therapeutic target [135]. Unfortunately, only allopurinol and a few azoles have been studied in clinical trials, observational studies, and case reports - there is an ongoing randomized double-blind, placebo controlled trial being carried (NCT03193749) comparing Amiodarone hydrochloride with placebo but there are no preliminary results disclosed so far. Despite allopurinol has shown to be useful in combination with NF or Benznidazole in small trials, evidence is still insufficient[136–138]. From azoles, posaconazole was compared in high and low doses versus placebo and research results concluded it has an acceptable antitrypanosomal activity, but also a significant increase in treatment failure compared with BZN group [139]. Another randomized placebo-controlled trial in adults tested E1224 (a ravuconazole pro-drug in different dosing regimens) and BZN versus placebo, and found that E1224 + BZN group displayed a transient, suppressive effect on parasite clearance, whereas BZN showed early and sustained efficacy until 12 months of follow-up. This transitory effect was shown only in high dose sub-group while parasite levels in the low-dose and short-dose E1224 groups gradually returned to placebo levels [140]. In summary, from azole's research, some former promising repositionable drugs such as monotherapy with ketoconazole, ravuconazole or posaconazole has not proven to be efficacious for the treatment of chronic T. cruzi infection [139–141] and the combination of posaconazole and BZN did not provide any further efficacy or safety advantages over BZN monotherapy [142,143].

Similarly, pre-clinical studies have identified interesting targets for drug action including cruzipain (parasite lysosomal cysteine), B citocrome, trypanothione reductase system, cyclophilins, N-myristoylome, carbonic anhydrases and NMDA glutamate receptor.[143,144] However, none of these targets have drugs in clinical trials yet, and the ever-mounting costs of drug development and human clinical trials make it difficult to believe that many new molecules for CD would be coming into the market in the foreseeable future.

Fexinidazole is a drug previously repositioned for *Trypanosoma brucei gambiense* infection (African trypanosomiasis) after demonstrating effectiveness in a randomized controlled trial[145]. Also, fexinidazole's safety and pharmacokinetics had been properly studied in humans, proving that oral administration is safe and well tolerated [132,133,146]. Considering this drug is effective in clearing *T.cruzi* as well in pre-clinical studies, an ongoing randomized, double-blind, placebo controlled trial is being carried out in Argentina, Bolivia and Spain to assess its efficacy in CD (NCT02498782).

Interestingly, some natural compounds and dietary supplements such as microalgae extracts[147], wasp venom[148], coumarins[149], South American Vernonieae[150], curcumin[151]and Resveratrol[152] have been also studied for anti-tripanosomal activity, but more research is required to draw conclusions, and there is still close to no human clinical data. The use of natural compounds to treat known diseases might lead to effective benefit-cost resources, considering that many of these compounds are not subject to patent restrictions and may be widely available. However, formal clinical testing should be performed before any of these compounds is used in patients.[104]

In spite of a relative abundance of preclinical molecular candidates and potential repositionable drugs, there are currently no new classes of drugs in the clinical development pipeline for CD and BZN and NF remain the only two available drugs for treatment with relatively solid clinical data to support their use.

Conclusion

CD is a highly neglected tropical disease, and it has increasingly become a worldwide problem. There are an alarming number of undiagnosed and untreated patients, and an urgent need for researchers and providers to change this fact. The choice for treatment remains between two drugs, created a century ago. The strongest data to support benefit-risk considerations come from trials in children (see **Table 1**).

Scientific and economic effort should be urgently aimed to supply early diagnose and treatment in this population, in addition to more research in this area. New biomarkers for CD are strongly needed for the diagnosis and detection of treatment efficacy and efforts from academia and pharmaceutical companies to accelerate the process of new drugs development are necessary. Also, an extra effort to standardize a predictive Chagas disease *in vivo* model should be done and validated in order to improve its predictability and to ease its comparison and reproducibility.

Early diagnosis and treatment of Chagas diseases, especially in pediatric patients, are vital for an effective and safe use of the available drugs (BZN and NF) medications.

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Conflicts of interest: Dr Facundo Garcia Bournissen has consulted for Chemo for projects not related to benznidazole, and has participated in nifurtimox clinical trials sponsored by Bayer.

Dr Jaime Altcheh has consulted for Bayer, and for Chemo. Dr Fernanda Lascano has no potential conflicts of interest to disclose.

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