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Drug Discovery for Kinetoplastid Diseases: Future Directions

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ABSTRACT: Kinetoplastid parasites have caused human disease for millennia. Significant progress has been made toward developing new treatments for leishmaniasis (particularly on the Indian subcontinent) and for human African trypanosomiasis (HAT) in Africa. The sustained decrease in the incidence of HAT has made the prospect of elimination a tantalizing reality. Despite the gains, no new chemical or biological entities to treat kinetoplastid diseases have been registered in more than three decades, and more work is needed to discover safe and effective therapies for patients with Chagas disease and leishmaniasis. Advances in tools for drug discovery and novel insights into the biology of the host−parasite interaction may provide opportunities for accelerated progress. Here, we summarize the output from a gathering of scientists and physicians who met to discuss the current status and future directions in drug discovery for kinetoplastid diseases.

Nearly a billion people are at risk from the group of vector-borne kinetoplastid diseases composed of Chagas disease, leishmaniasis, and human African trypanosomiasis (HAT, also known as sleeping sickness). These ancient parasitic illnesses have burdened humans for thousands of years, as evidenced by *Trypanosoma* DNA sequences found in South American mummies.[1] In the current era, kinetoplastid diseases cause an estimated 30 000 deaths annually and induce crippling morbidities in millions more.

There is reason to be optimistic about trends concerning HAT. Public and private partners have jointly tackled the disease in recent decades, with the World Health Organization (WHO) coordinating public health activities and the Drugs for Neglected Diseases Initiative (DNDi) directing global efforts for new therapies. The introduction of nifurtimox-eflornithine therapy in 2009 was a pivotal milestone, which was followed recently by the demonstrated efficacy of oral fexinidazole[2] for late-stage disease. (Approval for use is now pending assessment by medicine regulatory agencies.) Fewer than 1500 new cases were reported to WHO in 2017, making disease elimination a tangible goal.

Successes in combating HAT are encouraging and contrast with slower progress in containing other kinetoplastid diseases. Most of the current kinetoplastid drugs are repurposed and are often not potent enough to render a sterile cure (i.e., to eliminate all parasites). Safe, effective, short-course practical therapies are urgently needed for Chagas disease and leishmaniasis yet remain elusive.

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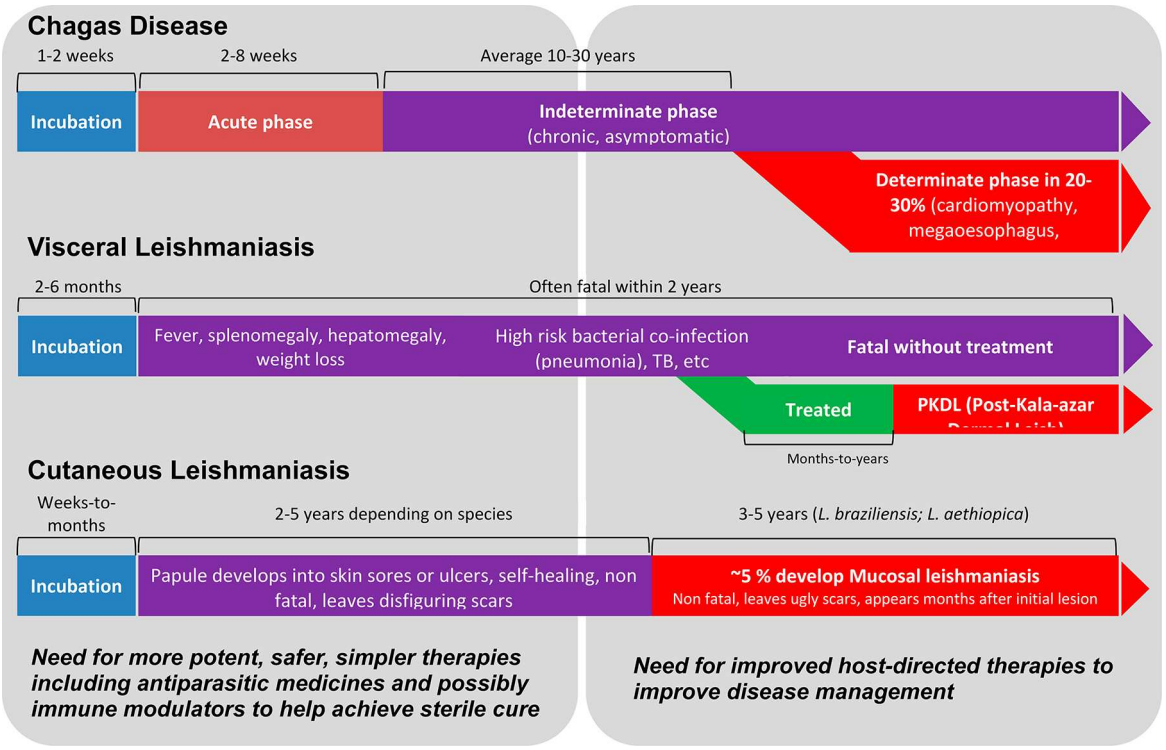

Figure 1. Clinical manifestations of kinetoplastid diseases and opportunities for intervention.

To understand the present-day challenges and opportunities related to new medicines for Chagas disease, visceral leishmaniasis (VL), and cutaneous leishmaniasis (CL), the Novartis Institute for Tropical Diseases convened a multidisciplinary group of scientific and medical specialists in parasitology, immunology, and drug discovery in June 2018. The scope of the discussion encompassed unmet medical needs, the global pipeline of preclinical drug candidates, parasite biology, assays, models, and the potential use of novel immunomodulatory and adjunct therapies to target disease sequelae. This Viewpoint summarizes key workshop information.

CURRENT DRUGS: OLD, TOXIC, AND OFTEN INEFFECTIVE

Chagas disease, which is endemic in the Americas, is caused by *Trypanosoma cruzi*. Inoculation typically occurs through infected feces from the triatomine bug, which is scratched or rubbed into the skin or mucosa. Transmission also takes place through blood transfusion and congenital and oral routes. The pathogenesis of Chagas disease is not fully understood, and it is currently impossible to predict which fraction of the patient population (approximately 30%) will develop the serious cardiac or gastrointestinal sequelae that appear years after the initial infection (Figure 1). Sudden death from chronic Chagas cardiomyopathy is an all too common outcome. Two drugs, nitroheterocyclic agents benznidazole and nifurtimox, are used for the treatment of Chagas disease; both were developed decades ago, are contraindicated during pregnancy, and can have serious adverse effects that substantially restrict their use. Benznidazole is the better-tolerated option in adults, although 15−30% patients are unable to finish the standard 60-day course, mainly because of skin and nervous system complications. In children, nifurtimox is better tolerated than benznidazole.

In both acute and chronic *T. cruzi* infection, treatment reduces the parasite load and can yield clearance from blood using available assays (e.g., PCR). Even so, in some cases, parasites presumably may persist intracellularly, and it is unclear in adults how the reductions in the parasite load modulate the severity of chronic disease in the absence of complete parasite clearance. Current drugs are inadequate because they fail too often and are not dependable. However, WHO recommends off-label use of benznidazole for the treatment of chronically infected patients, even though its efficacy in later stages of the disease is debatable. A large study of patients with chronic Chagas cardiomyopathy (the BENEFIT trial) demonstrated that benznidazole treatment reduced the parasite burden but did not significantly reduce disease progression. However, most had New York Heart Association class I (largely asymptomatic) heart disease, which may have confounded the findings.[3] Some nonrandomized, unblinded studies using benznidazole in indeterminate patients without heart failure showed reduced disease progression, emphasizing the need for controlled randomized studies for indeterminate patients.[4]

Similarly, the approved therapeutic arsenal for leishmaniasis has important limitations. Leishmaniasis is distributed across the tropics and subtropics, though a majority of VL cases are reported in only seven countries: Brazil, Ethiopia, Kenya, Somalia, Sudan, South Sudan, and India. Patients succumb to VL gradually over a period of 2 years. Anorexia and pancytopenia give rise to wasting and increased susceptibility to bacterial superinfections. VL is fatal without treatment, and even those who undergo therapy remain at risk of a disfiguring dermal form of relapsing disease called postkala-azar dermal leishmaniasis (PKDL) that may also contribute to continued disease transmission (Figure 1). CL has a higher global burden than VL, with the greatest prevalence in Africa, the Mediterranean, and South America. It does not cause systemic

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morbidities or death but can result in grievous disfiguration and stigma. Drugs targeting *Leishmania* parasites have generally been repurposed from other indications. Antimonials, amphotericin B, paromomycin sulfate, and miltefosine have variable efficacy against the more than 20 *Leishmania* species that cause disease. While WHO-recommended treatment regimens for VL on the Indian subcontinent include liposomal amphotericin B or oral miltefosine, these medicines are poorly effective in patients in other global regions. Treatment courses are generally long, require hospitalization, and have significant toxicities that mandate frequent monitoring. Differences in the treatment protocol by region, high costs, and low availability of some drugs understandably stretch the limits of under-resourced health systems in countries where these diseases are endemic.

THE ANTIPARASITIC PIPELINE IS FILLING, BUT THERE ARE GAPS

Antiparasitics are the cornerstone of therapy for kinetoplastid diseases. It is well accepted that the clinical event cascades in Chagas disease and leishmaniasis are induced by the presence of parasites, and evidence suggests that eliminating parasites as early as possible after infection could mitigate severe disease. Unfortunately, the current preclinical pipeline for Chagas disease treatments is meager. Only three classes of compounds have been shown to achieve high cure rates in stringent mouse models of infection: nitroimidazoles (e.g., fexinidazole, currently in phase II), oxaboroles (e.g., DNDi-6148, active against both leishmaniasis and Chagas), and proteasome inhibitors (e.g., GNF6702[5]). The future is brighter in drug discovery for leishmaniasis, where there are at least six candidates in preclinical or clinical phases that have five distinct mechanisms of action.[6]

Proposed target product profiles for new drugs are listed in Box 1. For Chagas disease, medicines should achieve cures that prevent the development of chronic disease. Any treatment to be given beyond the acute stage must be simple to administer and safe because patients in the indeterminate phase typically feel healthy and are unlikely to comply with a complex or poorly tolerated drug regimen. In leishmaniasis, a short-course therapy that achieves a relapse-free cure with no or minor adverse effects would be ideal, but even a medicine with efficacy similar to that of current drugs and improved safety would be a step forward.

Whether a sterile cure (i.e., the elimination of all parasites) is essential is a topic of debate. Some parasitologists advocate strongly that a sterile cure must be achieved in Chagas disease to prevent the reproliferation of parasites and enduring pathogenicity. A sterile cure may not be critical for VL and CL. Reducing the parasite load in these infections could be sufficient if the host immune system can complete the job of parasite control or clearance. A sterile cure is more likely needed for PKDL (which appears to result from latent parasites) and for VL in individuals with HIV coinfection or other immunodeficiency syndromes. A condition known as leishmaniasis recidivans in CL may also result from the recrudescence of latent parasites that survive therapy.

NEW INSIGHTS INTO DISEASE BIOLOGY WILL INDICATE THE NEED FOR NEW MODELS

Given how little we know about the biology of *T. cruzi* and *Leishmania* species and the lack of validated drug targets, it is not surprising that most current pipeline compounds originated from phenotypic screens. Assays are available to test the growth inhibition of amastigotes for *T. cruzi* (intracellular) and *Leishmania* (intracellular and extracellular), and these are compatible with high-throughput screening. It may be important to evaluate the antiparasitic effect of compounds by using intracellular parasites grown in disease-relevant tissues. Cidality, time-to-kill kinetics, and washout assays may be used to further enhance the confidence of hits and to assist prioritization.

Highly sensitive in vivo imaging with bioluminescent *T. cruzi* that enables the monitoring of the mouse parasite burden in real time has highlighted how the parasite load varies by tissue type over time.[7] Furthermore, this model has predictive power. It demonstrated the limited efficacy of posaconazole, a Chagas disease drug candidate that had previously shown potency in animal models but has failed to consistently eliminate parasitemia in patients. By comparison, benznidazole was shown to be efficacious in both mice and humans.[8] Similarly, novel murine and hamster models for VL and CL using bioluminescent parasites have improved our understanding of disease progression. New chemical entities should be tested in mouse models with specific questions in mind, such as how the treatment duration and curative exposures could translate from mice to humans.

An important unknown for Chagas disease is the role played by amastigotes that spontaneously adopt a "persister" phenotype. These nonreplicative and phenotypically drug-resistant forms of the parasite are later able to differentiate to

Box 1. Proposed Target Product Profile (TPP) for Chagas Disease and Leishmaniasis

Proposed TPP for Chagas

- Eliminates all parasites, including in blood and tissue
- Active against all distinct typing units (DTUs)
- Oral, safe, and well tolerated for use at all ages and during pregnancy and lactation with no monitoring required
- Simple treatment regimen, amenable for use in a setting of weak health systems/infrastructure, accessible and affordable
- Potency and safety not affected by pharmacogenomic factors
- Can be used repeatedly (e.g., in the case of reinfections)
- No significant drug−drug interaction
- Low probability of resistance
- Shelf life >2 years under tropical conditions

Proposed TPP for Leishmaniasis

- Effective against all VL and CL parasites from varying geographic regions
- Potency and safety not affected by pharmacogenomic factors
- Potency/efficacy, >95% parasite clearance for VL, 99.9% parasite clearance from periphery, 99% from seclusion sites for CL
- Short treatment regimen (as short as 1 week for both VL and CL, 14 day maximum for VL, 21 day maximum for CL)
- Amenable for use in a setting of weak health systems/infrastructure, accessible and affordable treatment regimen
- Oral, safe, and well tolerated for use at all ages and during pregnancy with no monitoring required
- Effective in immune-deficient individuals (e.g., HIV-VL) and against PKDL
- Avoids risk of resistance

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trypomastigotes and reinfect new host cells.[8] Future work is needed to understand how the development of persistent forms is triggered, if they are metabolically active, whether they can be forced out of dormancy, and what their role is in disease progression. In the meantime, screening against persistent parasites to find novel inhibitors would be beneficial.[9]

Persistence in *Leishmania* may also be a concern. Persistent *L. mexicana* and *L. major* parasites have been reported in mouse models,[10,11] although similar forms have not yet been sought in animals for *L. donovani* or *L. infantum*. However, nonreplicating *L. donovani* were identified in a macrophage model, and these could represent persister-type cells, the existence of which is implicated through the recrudescence that can occur following VL treatment being manifest as PKDL.[12]

IMMUNE MODULATION HAS PROMISE IN ANTIPARASITIC THERAPY

Kinetoplastid infections provoke robust innate and adaptive immune reactions, which can be protective or disease-promoting. This provides a rationale for investigating host-directed strategies such as immune modulators as an add-on to antiparasitic therapy. Lessons from immuno-oncology may offer a roadmap. Indeed, there are similarities in the dynamics of host−tumor and host−parasite interactions. Both tumor cells and cells harboring intracellular parasites are perceived by the immune system as foreign, both retain features of normal cells that could trigger an immune tolerance, and both can create a microenvironment that facilitates immune escape and promotes disease progression. In certain types of cancer, adding immunotherapy to cytotoxic chemotherapy improves survival. For example, a monoclonal antibody that binds to T cells and blocks their inhibition by tumor cells is now part of the first-line treatment for some lung cancers.[13] An analogous approach could conceivably help antiparasitic medicines to work more quickly, more effectively, or with less variability.

Harnessing the immune system to treat kinetoplastid diseases is not a new idea. Beginning in the early 1990s, interferon-gamma was tested in VL and CL patients (usually in combination with antimony), with mixed results. Interleukin (IL)-10 has been studied extensively in VL. It appears to promote parasite growth, and experimental models suggest that the IL-10 blockade can reduce disease progression.[14,15] A clinical trial with a humanized anti-IL-10 antibody was planned but later withdrawn due to problems in securing quality drug for the study (NCT01437020). IL-10 neutralization may also provide a benefit in CL.[16] Additionally, TLR9 agonist CpG D35 is currently undergoing preclinical development in preparation for clinical trials for CL.[6]

In Chagas disease, the association of several pro- and anti-inflammatory cytokines has been observed with cardiac and indeterminate forms of the disease, respectively.[17] Some immunomodulation strategies postulated for Chagas disease include limiting regulatory T cells and increasing IL-17,[18] although overall there is less evidence to support immune modulation in Chagas disease compared with leishmaniasis.

Naturally, there are challenges to testing and deploying immunotherapies. Success or failure in experimental models is not necessarily predictive of outcomes in humans. Patients with leishmaniasis are at high risk of coinfection with bacteria; therefore, modulating immunity in these populations will require careful safety monitoring. Even if immunotherapy is successful in enhancing the response to treatment in acute disease, there are no tools to definitively assess latent infection. Finally, the immune system is dynamic and changes with age, pregnancy, coinfections, and other conditions, which would need to be considered.

ADJUNCT THERAPIES ARE ALSO NECESSARY IN THE CLINICAL ARMORY

Adjunct therapies that improve outcomes including quality of life should be pursued in parallel with work to discover parasite-specific agents. These include medicines to improve wound healing in CL, to address nutrition or coinfection complications in VL, and to better manage cardiovascular and gastrointestinal complications in Chagas disease. This also applies to preventative or therapeutic vaccines, which are in various phases of development.

Progress toward adjunct therapies is hampered in part by our limited understanding of many features of disease biology. There are, however, illustrative examples. In CL, the disease is in large part mediated by the inflammatory immune response. For example, lesions from *L. braziliensis* patients have few parasites but severe ulceration, and experimental studies indicate that a blockade of IL-1β or the NLRP3 inflammasome may ameliorate the disease in these patients.[19] There is evidence that wound treatment with pharmaceutical sodium chlorite 0.045% or radio-frequency-induced heat therapy has clinical benefits for CL.[20] In chronic Chagas cardiomyopathy, patients generally suffer worse outcomes than those with heart failure from other causes, despite the fact that Chagas disease patients are usually younger and have fewer comorbidities.[21] Recently, angiotensin receptor−neprilysin inhibition was found to reduce mortality and hospitalization in a large group of heart failure patients with a reduced ejection fraction, including a subgroup of patients with Chagas disease.[22] Future work will be needed to determine the specific implications for Chagas disease patients.

SUMMARY

While there have been substantial advances in recent years to address kinetoplastid diseases, on the whole these conditions remain severely neglected across the domains of health policy, advocacy, funding, and research. For HAT, more work is needed to ensure that the gains realized are not lost. With respect to finding safe and effective new therapies for Chagas disease and leishmaniasis, we highlight several key priorities. To start, the fundamental pathobiology of these diseases must be further demystified to pave the way for targeted treatments; the discovery of persister parasites is a sobering reminder that we have much to learn before definitive medicines can be generated. Novel tools will be needed to successfully validate clinical candidates in patients, including biomarkers capable of measuring intracellular parasite clearance and predicting the clinical benefit without the need for extended follow-up. The potential benefits in kinetoplastid diseases for immune modulation and adjunct therapies need to be carefully evaluated. Finally, we advocate continued and even greater multidisciplinary collaboration. In the face of limited resources, with an all too small scientific and medical community focused on these complex diseases, harmonized research and development strategies will be essential to accelerating progress toward the common good of transformative new therapies for patients.

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