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1 Drug Discovery for Kinetoplastid Diseases: Future Directions

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22 **ABSTRACT:** Kinetoplastid parasites have caused human disease for millennia. Significant progress has been made toward
 23 developing new treatments for leishmaniasis (particularly on the Indian subcontinent) and for human African trypanosomiasis
 24 (HAT) in Africa. The sustained decrease in the incidence of HAT has made the prospect of elimination a tantalizing reality.
 25 Despite the gains, no new chemical or biological entities to treat kinetoplastid diseases have been registered in more than three
 26 decades, and more work is needed to discover safe and effective therapies for patients with Chagas disease and leishmaniasis.
 27 Advances in tools for drug discovery and novel insights into the biology of the host–parasite interaction may provide
 28 opportunities for accelerated progress. Here, we summarize the output from a gathering of scientists and physicians who met to
 29 discuss the current status and future directions in drug discovery for kinetoplastid diseases.
 30
 31

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

41 There is reason to be optimistic about trends concerning
 42 HAT. Public and private partners have jointly tackled the
 43 disease in recent decades, with the World Health Organization
 44 (WHO) coordinating public health activities and the Drugs for
 45 Neglected Diseases Initiative (DNDi) directing global efforts
 46 for new therapies. The introduction of nifurtimox-eflornithine
 47 therapy in 2009 was a pivotal milestone, which was followed

recently by the demonstrated efficacy of oral fexinidazole² 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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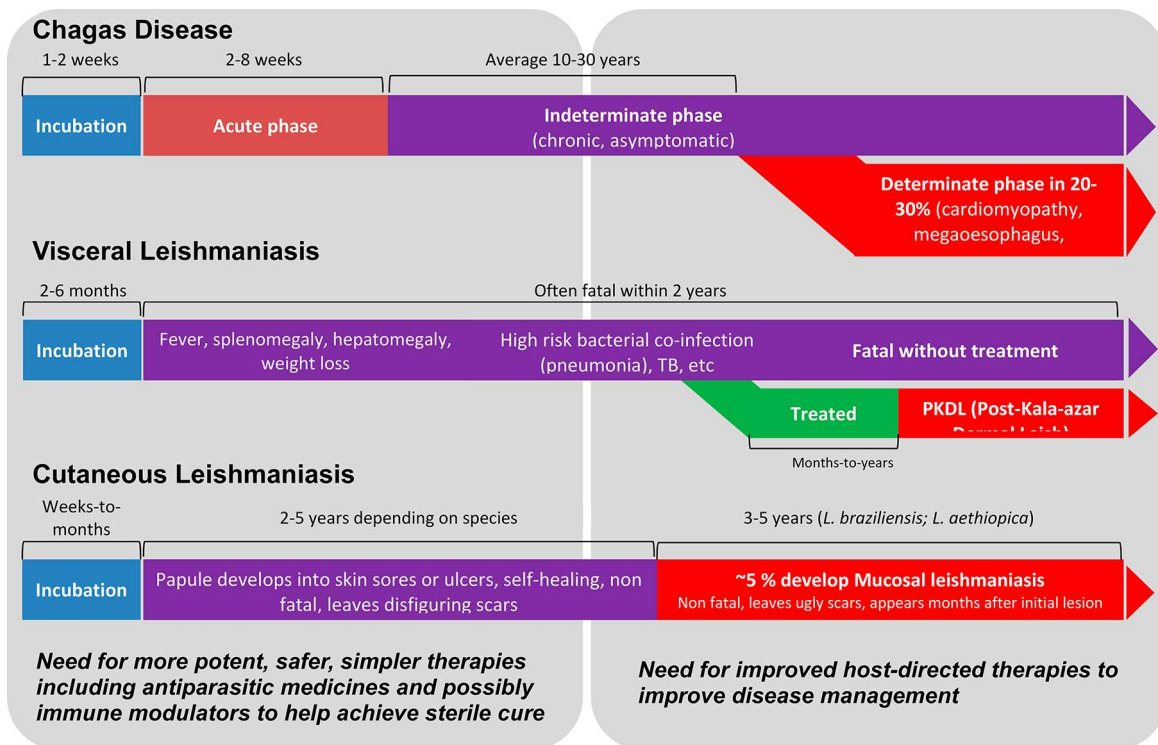


Figure 1. Clinical manifestations of kinetoplastid diseases and opportunities for intervention.

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

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

144 ■ THE ANTIPARASITIC PIPELINE IS FILLING, BUT 145 THERE ARE GAPS

146 Antiparasitics are the cornerstone of therapy for kinetoplastid
147 diseases. It is well accepted that the clinical event cascades in
148 Chagas disease and leishmaniasis are induced by the presence
149 of parasites, and evidence suggests that eliminating parasites as
150 early as possible after infection could mitigate severe disease.
151 Unfortunately, the current preclinical pipeline for Chagas
152 disease treatments is meager. Only three classes of compounds
153 have been shown to achieve high cure rates in stringent mouse
154 models of infection: nitroimidazoles (e.g., fexinidazole,
155 currently in phase II), oxaboroles (e.g., DNDi-6148, active
156 against both leishmaniasis and Chagas), and proteasome
157 inhibitors (e.g., GNF6702⁵). The future is brighter in drug
158 discovery for leishmaniasis, where there are at least six
159 candidates in preclinical or clinical phases that have five
160 distinct mechanisms of action.⁶

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

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

185 ■ NEW INSIGHTS INTO DISEASE BIOLOGY WILL 186 INDICATE THE NEED FOR NEW MODELS

187 Given how little we know about the biology of *T. cruzi* and
188 *Leishmania* species and the lack of validated drug targets, it is

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

not surprising that most current pipeline compounds 189 originated from phenotypic screens. Assays are available to 190 test the growth inhibition of amastigotes for *T. cruzi* 191 (intracellular) and *Leishmania* (intracellular and extracellular), 192 and these are compatible with high-throughput screening. It 193 may be important to evaluate the antiparasitic effect of 194 compounds by using intracellular parasites grown in disease- 195 relevant tissues. Cidalty, time-to-kill kinetics, and washout 196 assays may be used to further enhance the confidence of hits 197 and to assist prioritization. 198

Highly sensitive in vivo imaging with bioluminescent *T. cruzi* 199 that enables the monitoring of the mouse parasite burden in 200 real time has highlighted how the parasite load varies by tissue 201 type over time.⁷ Furthermore, this model has predictive power. 202 It demonstrated the limited efficacy of posaconazole, a Chagas 203 disease drug candidate that had previously shown potency in 204 animal models but has failed to consistently eliminate 205 parasitemia in patients. By comparison, benznidazole was 206 shown to be efficacious in both mice and humans.⁸ Similarly, 207 novel murine and hamster models for VL and CL using 208 bioluminescent parasites have improved our understanding of 209 disease progression. New chemical entities should be tested in 210 mouse models with specific questions in mind, such as how the 211 treatment duration and curative exposures could translate from 212 mice to humans. 213

An important unknown for Chagas disease is the role played 214 by amastigotes that spontaneously adopt a “persister” 215 phenotype. These nonreplicative and phenotypically drug- 216 resistant forms of the parasite are later able to differentiate to 217

218 trypomastigotes and reinfect new host cells.⁸ Future work is
219 needed to understand how the development of persistent
220 forms is triggered, if they are metabolically active, whether they
221 can be forced out of dormancy, and what their role is in disease
222 progression. In the meantime, screening against persistent
223 parasites to find novel inhibitors would be beneficial.⁹
224 Persistence in *Leishmania* may also be a concern. Persistent
225 *L. mexicana* and *L. major* parasites have been reported in
226 mouse models,^{10,11} although similar forms have not yet been
227 sought in animals for *L. donovani* or *L. infantum*. However,
228 nonreplicating *L. donovani* were identified in a macrophage
229 model, and these could represent persister-type cells, the
230 existence of which is implicated through the recrudescence that
231 can occur following VL treatment being manifest as PKDL.¹²

232 ■ IMMUNE MODULATION HAS PROMISE IN 233 ANTIPARASITIC THERAPY

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

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

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

272 Naturally, there are challenges to testing and deploying
273 immunotherapies. Success or failure in experimental models is
274 not necessarily predictive of outcomes in humans. Patients
275 with leishmaniasis are at high risk of coinfection with bacteria;
276 therefore, modulating immunity in these populations will
277 require careful safety monitoring. Even if immunotherapy is
278 successful in enhancing the response to treatment in acute
279 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.

283 ■ ADJUNCT THERAPIES ARE ALSO NECESSARY IN 284 THE CLINICAL ARMORY

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

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

313 ■ SUMMARY

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

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343 Notes

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