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A strategic discovery roadmap towards high-quality leads and drug development candidates for kinetoplastid diseases. Part 1: setting the stage

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Given the impact of kinetoplastid diseases, the limited therapeutic options and risk of treatment failure, continued research efforts to discover novel drug entities are required. The ambition to deliver drug development candidates has mainly been taken on board by academia and public private partnerships, but remains highly challenging because of the lack of adequate funding and standardized laboratory procedures. Establishing a systematic roadmap of experiments and decision criteria to attain high-quality leads and drug candidates with lower risk profiles remains the logical path to deliver more compelling proof-of-concepts for impactful diseases, such as African trypanosomiasis, Chagas disease and visceral and cutaneous leishmaniasis.

In a three-part series, a structured roadmap from 'hit finding' to 'drug development candidate' is presented with a focus on the minimal essential data package, laboratory experimental models and endpoints. Part 1 introduces the concept of a pragmatic framework with reference to specific preclinical R&D stages: (i) hit finding, (ii) hit profiling, (iii) lead definition and (iv) drug development candidate to support a more focused early development path that remains accessible to engaged stakeholders. The experiment-oriented roadmap is presented in the next parts addressing the discovery and characterization of confirmed hits (Part 2) and the lead discovery phase towards identification of a drug development candidate (Part 3).

Although specifically focusing on kinetoplastid diseases, the principles also apply to small-molecule preclinical R&D against other microbial diseases, evidently with specific adaptation of the primary pharmacology models.

Introduction

Kinetoplastids, including parasites such as *Trypanosoma* spp. and *Leishmania* spp., are mostly transmitted by arthropod vectors and cause Neglected Tropical Diseases (NTDs) that compromise human and animal health and economic development in different parts of the world.

Human African trypanosomiasis (HAT, sleeping sickness) manifests in two clinical forms determined by the subspecies of *Trypanosoma brucei* that are both transmitted by tsetse flies.[1] The acute form caused by *T. brucei rhodesiense* is found in Eastern and Southern Africa, whereas the chronic form caused by *T. brucei gambiense* predominates in Western and Central Africa and accounts for 92% of reported HAT cases. The infection with these extracellular parasites progresses from a haemolymphatic (stage 1) to encephalitic stage where parasites have gained access to the central nervous system (stage 2). The existence of cryptic reservoirs and asymptomatic carriers could compromise the projected WHO 2030 goal of *gambiense* HAT elimination.[2] Other species and subspecies of trypanosomes include *T. b. brucei*, *T. congolense*, *T. vivax*, *T. b. evansi* and *T. b. equiperdum* that are pathogenic to a wide range of animals and cause animal trypanosomiasis (AT). Treatments for HAT include pentamidine and suramin for early-stage disease and NECT or melarsoprol for late-stage disease, although toxicity and logistical barriers persist.[3] Fexinidazole, the first oral drug for HAT, improves accessibility but is less effective in advanced CNS disease.[3,4] Acoziborole, a promising single-dose oral candidate, is in the last phase of the regulatory process.[3] AT is currently managed with diminazene, isometamidium, homidium, quinapyramine, suramin and cymelarsan, although drug resistance is widespread.[5]

Chagas disease is caused by *T. cruzi* parasites that are transmitted by triatomine bugs and alternate between intracellular amastigotes and extracellular trypomastigotes in the mammalian host. It is endemic in Latin American countries affecting an estimated 7 million people and causing ~10 000 deaths each year.[6] Other sources of infection are congenital transmission, contaminated food and drinks, blood transfusion and organ transplants.[7,8] About 30% of patients develop chronic disease involving mainly the cardiovascular and digestive systems.[9,10] Current treatment for *T. cruzi* infection relies on benznidazole and nifurtimox, but both drugs have limited efficacy in late-stage disease and cause frequent adverse effects.[9]

Leishmaniasis, caused by the intracellular *Leishmania* spp. parasites acquired via the bites of phlebotomine sand flies, results in different clinical forms depending on the infecting species,[11] host species and immune status. Visceral leishmaniasis (VL) caused by *L. infantum* and *L. donovani* is fatal if left untreated. In addition to causing human disease, *L. infantum* also causes pathology in dogs known as canine leishmaniasis. Other species, such as *L. major*, *L. tropica* and *L. braziliensis* and many others cause cutaneous leishmaniasis resulting in skin lesions and ulcers. Mucocutaneous leishmaniasis typically causes stigmatizing destruction of the mucous membranes and cartilage of the face.[12] Post-Kala-azar dermal leishmaniasis typically occurs as a complication after an apparent period of recovery from VL.[13] Treatment of leishmaniasis largely depends on the form and region, with pentavalent antimonials still widely used despite toxicity and resistance.[11] Liposomal amphotericin B is the preferred treatment for VL in many settings. Miltefosine is the only oral drug, but suffers from teratogenicity and from treatment failure in some geographical areas.[14] Cutaneous leishmaniasis forms are often treated with topical agents, cryotherapy or systemic drugs depending on severity and species. In dogs (reservoirs for *L. infantum*), treatment with allopurinol and antimonials may reduce symptoms but rarely achieves parasitological cure, implying a risk for ongoing transmission.[11]

Framework overview

Strategic roadmap towards high-quality leads and drug development candidates for kinetoplastid diseases

Although significant progress has been made in drug discovery and understanding the biology of kinetoplastid parasites, there is still a major need for novel drugs for human and veterinary use to overcome limited efficacy, side effects, environmental impact and increasing drug resistance.[15] The drug discovery process faces a high attrition and a strategic approach is imperative to make properly use of the limited budgets that generally available for NTDs. Therapeutic shortcomings generally link to specific discovery failures, such as toxicity, metabolic instability and lack of CNS penetration. While drug repurposing efforts in some cases have proved to be cost effective,[16] exploring novel classes for reduced toxicity and better PK-PD may further improve the efficacy of current medical treatments.[17]

The drug discovery process is long and complicated, requiring the translation from *in vitro* identification to *in vivo* evaluation in an extensive and interactive set of laboratory models. While diverse or focused compound libraries or natural products generally serve as a chemical starting point in disease-oriented screening campaigns, drug discovery is now moving beyond the standard approach (Figure 1) to further embrace virtual screening and artificial intelligence-driven platforms.[18,19] Stringent baseline criteria must be adopted during hit finding, hit profiling, lead definition and selection of a drug development candidate. Any undesirable effect may justify the rejection of a candidate and prioritize back-up de-risked high-quality leads. Besides potency and selectivity, other parameters such as biological and physicochemical properties, metabolic stability, (eco)toxicity, formulation and pharmacokinetics are fundamental in the decision-making process. These relatively expensive and time-consuming methods can be complemented and in some cases be replaced with *in silico* methods that offer the potential to reduce the overall drug discovery cycle time and cost.[20] In addition, target product profiles[21–25] (see Table S1, available as Supplementary data at *JAC* Online), precision design and reporting of experiments remain key in an animal-friendly '3Rs' (replacement, reduction, refinement) approach[26] to achieve high-quality compounds. Some recommendations have already been made to define a more robust 'proof-of-concept' for lead development.[15,27,28]

Here, we provide an overview of pivotal analyses (Figure 2) that are required during the different stages of early drug R&D to ensure a successful transition from a chemical hit compound to a high-quality lead or drug development candidate eligible for clinical development. This work provides a detailed overview of a recommended path to follow when progressing from early 'hit' identification towards 'lead' and 'drug development candidate'

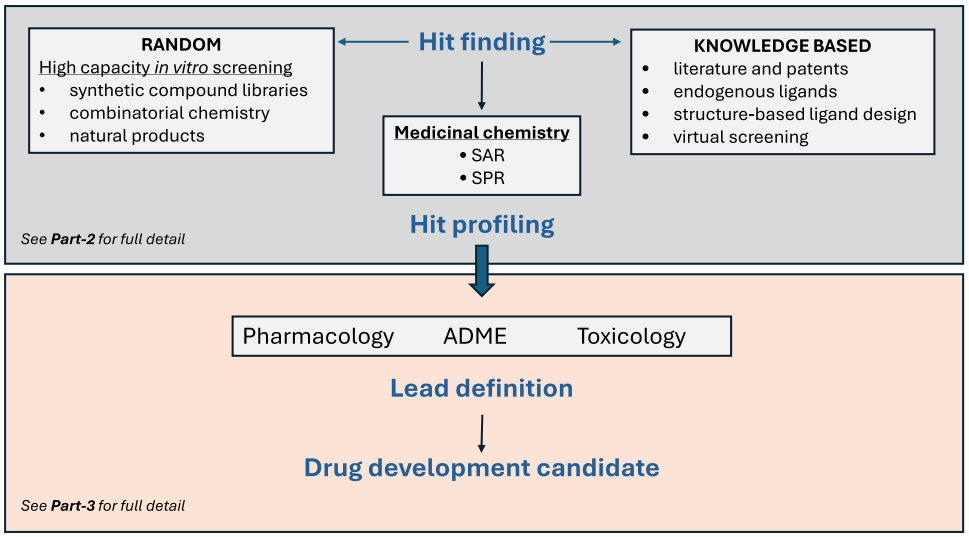

Figure 1. Schematic representation of the standard preclinical R&D workflow from 'hit finding' to selection of 'lead compound' and 'drug development candidate'. SAR: structure-activity relation; SPR: structure-property relation; ADME: absorption, distribution, metabolism, excretion.

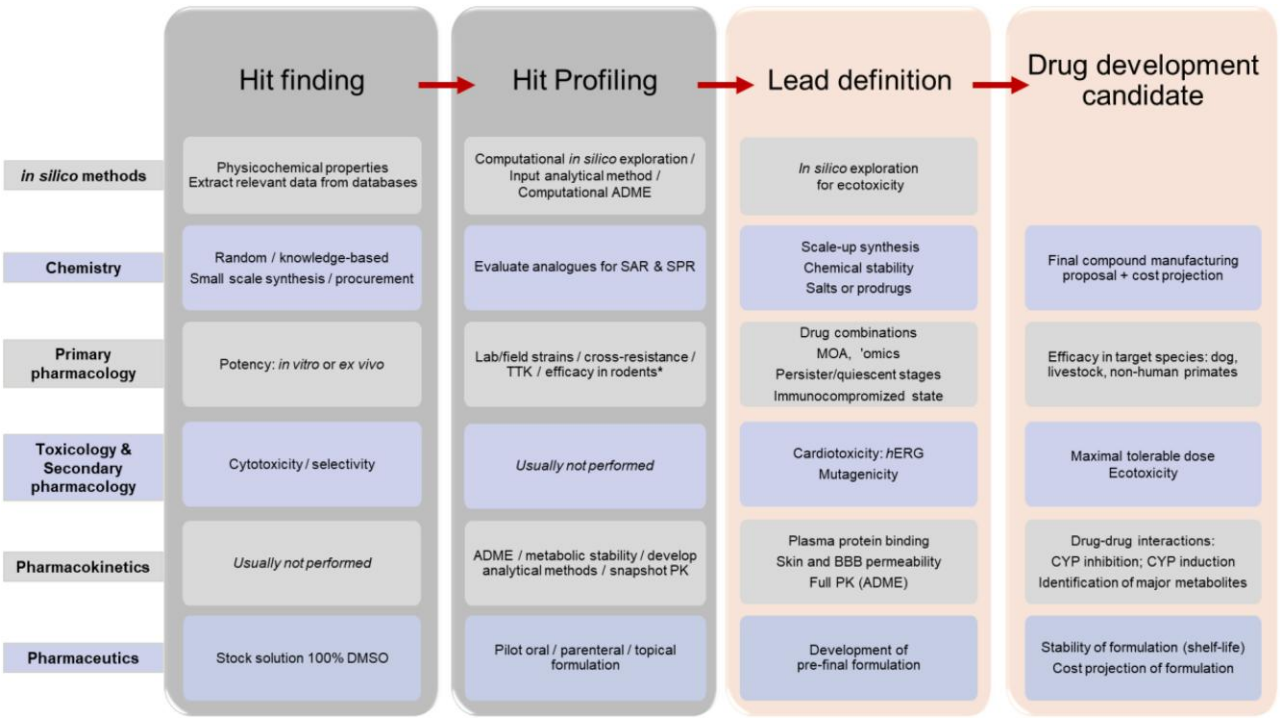

Figure 2. Overview of the 'baseline' preclinical data package required during 'hit finding', 'hit profiling', 'lead definition', and selection of a 'drug development candidate', adopting a vertical (R&D stage) versus horizontal (discipline) tabular design. * Conditional to a proper selectivity and PK profile. PK: Pharmacokinetics; SAR: Structure-Activity Relationship; SPR: Structure-Property Relationship; TTK: Time to Kill; ADME: Absorption, Distribution, Metabolism, Excretion; MOA: Mode of Action; BBB: Blood Brain Barrier; CYP: Cytochrome P450.

against neglected tropical kinetoplastid-induced infections, taking into account the capabilities that are present within a drug R&D network. A variety of disciplines (cell biology, molecular biology, chemistry, primary pharmacology, (eco)toxicology, bio-informatics, pharmacokinetics and pharmaceutics) are discussed in parts 2 and 3 with a strong emphasis on what is practically achievable within 'non-GLP' (academic) research networks.[29,30] In a low- and middle-income countries context, the execution of the pivotal primary pharmacological studies is achievable provided the availability of a functional laboratory infrastructure (with access to a CO_2 incubator, laminar flow, microscope) suitable for aseptic cell culture and the manipulation of pathogenic protozoa under the

appropriate biosafety levels. In addition, an ethically approved animal facility (preferably with housing in individually ventilated cages) is necessary for *in vivo* pharmacodynamic research. To bridge the technological gap, a hybrid model can rely on local sample collection (for example, via dried blood spots or cryopreservation) and external advanced bioanalytical platforms. This ensures that high-quality data can be acquired, even when local resources are limited to fundamental biological assays.

Acknowledging that complete harmonization of the drug discovery process will remain challenging, the proposed comprehensive roadmap specially focuses on the minimal set of experimental data that should cover the essential acceptance criteria required for the successful transition from a new chemical entity to a high-quality drug lead or drug development candidate, with added emphasis on the 3Rs principle of animal use and consideration of the environmental impact. Furthermore, it should enable accelerating and harmonizing discovery pipelines for NTDs through a shared framework adaptable to academic and PPP settings, including in disease-endemic low- and middle-income countries.

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Transparency declarations

None to declare.

Supplementary data

Supplementary Table S1 is available as Supplementary data at *JAC* Online.

References

1 Büscher P, Cecchi G, Jamonneau V *et al*. Human African trypanosomiasis. *Lancet* 2017; **390**: 2397–409. https://doi.org/10.1016/S0140-6736(17)31510-6

2 WHO. *Ending the Neglect to Attain the Sustainable Development Goals: a Road map for Neglected Tropical Diseases 2021–2030*. World Health Organization, 2020.

3 Lejon V, Lindner AK, Franco JR. Human African trypanosomiasis. *Lancet* 2025; **405**: 937–50. https://doi.org/10.1016/S0140-6736(25)00107-2

4 Lindner AK, Lejon V, Barrett MP *et al*. New WHO guidelines for treating rhodesiense human African trypanosomiasis: expanded indications for fexinidazole and pentamidine. *Lancet Infect Dis* 2025; **25**: e77–85. https://doi.org/10.1016/S1473-3099(24)00581-4

5 Ungogo MA, de Koning HP. Drug resistance in animal trypanosomiases: epidemiology, mechanisms and control strategies. *Int J Parasitol Drugs Drug Resist* 2024; **25**: 100533. https://doi.org/10.1016/j.ijpddr.2024.100533

6 Global age-sex-specific fertility, mortality, healthy life expectancy (HALE), and population estimates in 204 countries and territories, 1950-2019: a comprehensive demographic analysis for the Global Burden of Disease Study 2019. *Lancet* 2020; **396**: 1160–203. https://doi.org/10.1016/S0140-6736(20)30977-6

7 Pereira KS, Schmidt FL, Barbosa RL *et al*. Transmission of chagas disease (American trypanosomiasis) by food. *Adv Food Nutr Res* 2010; **59**: 63–85. https://doi.org/10.1016/S1043-4526(10)59003-X

8 Gonzalez-Guzman S, Gonzalez-Cano P, Bagu ET *et al*. Seroprevalence of *Trypanosoma cruzi* in Eight Blood Banks in Mexico. *Arch Med Res* 2022; **53**: 625–33. https://doi.org/10.1016/j.arcmed.2022.08.007

9 Pinazo MJ, Malchiodi E, Ioset J-R *et al*. Challenges and advancements in the development of vaccines and therapies against Chagas disease. *Lancet Microbe* 2024; **5**: 100972. https://doi.org/10.1016/j.lanmic.2024.100972

10 Echavarría NG, Echeverría LE, Stewart M *et al*. Chagas disease: chronic chagas cardiomyopathy. *Curr Probl Cardiol* 2021; **46**: 100507. https://doi.org/10.1016/j.cpcardiol.2019.100507

11 Burza S, Croft SL, Boelaert M. Leishmaniasis. *Lancet* 2018; **392**: 951–70. https://doi.org/10.1016/S0140-6736(18)31204-2

12 Nuwangi H, Agampodi TC, Price HP *et al*. Stigma associated with cutaneous and mucocutaneous leishmaniasis: a systematic review. *PLoS Negl Trop Dis* 2023; **17**: e0011818. https://doi.org/10.1371/journal.pntd.0011818

13 Kumar P, Chatterjee M, Das NK. Post Kala-Azar dermal leishmaniasis: clinical features and differential diagnosis. *Indian J Dermatol* 2021; **66**: 24–33. https://doi.org/10.4103/ijd.IJD_602_20

14 Sundar S, Madhukar P, Kumar R. Anti-leishmanial therapies: overcoming current challenges with emerging therapies. *Expert Rev Anti Infect Ther* 2025; **23**: 159–80. https://doi.org/10.1080/14787210.2024.2438627

15 De Rycker M, Wyllie S, Horn D *et al*. Anti-trypanosomatid drug discovery: progress and challenges. *Nat Rev Microbiol* 2023; **21**: 35–50. https://doi.org/10.1038/s41579-022-00777-y

16 Vijayasurya, Gupta S, Shah S *et al*. Drug repurposing for parasitic protozoan diseases. *Prog Mol Biol Transl Sci* 2024; **207**: 23–58. https://doi.org/10.1016/bs.pmbts.2024.05.001

17 Rao SPS, Barrett MP, Dranoff G *et al*. Drug discovery for kinetoplastid diseases: future directions. *ACS Infect Dis* 2019; **5**: 152–7. https://doi.org/10.1021/acsinfecdis.8b00298

18 Niazi SK. Artificial intelligence in small-molecule drug discovery: a critical review of methods, applications, and real-world outcomes. *Pharmaceuticals* 2025; **18**: 1271. https://doi.org/10.3390/ph18091271

19 Villalta F, Rachakonda G. Advances in preclinical approaches to Chagas disease drug discovery. *Expert Opin Drug Discov* 2019; **14**: 1161–74. https://doi.org/10.1080/17460441.2019.1652593

20 Pun FW, Ozerov IV, Zhavoronkov A. AI-powered therapeutic target discovery. *Trends Pharmacol Sci* 2023; **44**: 561–72. https://doi.org/10.1016/j.tips.2023.06.010

21 DNDi. *Target product profile for sleeping sickness*. https://dndi.org/diseases/sleeping-sickness/target-product-profile/.

22 DNDi. *Target product profile for Chagas disease*. https://dndi.org/diseases/chagas/target-product-profile/.

23 DNDi. *Target product profile for cutaneous leishmaniasis*. https://dndi.org/diseases/cutaneous-leishmaniasis/target-product-profile/.

24 DNDi. *Target product profile for visceral leishmaniasis*. https://dndi.org/diseases/visceral-leishmaniasis/target-product-profile/.

25 GALVmed. *Animal African Trypanosomosis Target Product Profiles downloads*. https://www.galvmed.org/news/animal-african-trypanosomosis-target-product-profiles-downloads/2020).

26 Percie du Sert N, Hurst V, Ahluwalia A *et al*. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol* 2020; **18**: e3000410. https://doi.org/10.1371/journal.pbio.3000410

27 Cos P, Vlietinck AJ, Berghe DV *et al*. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. *J Ethnopharmacol* 2006; **106**: 290–302. https://doi.org/10.1016/j.jep.2006.04.003

28 Soeiro MNC, Sales-Junior PA, Pereira VRA *et al*. Drug screening and development cascade for Chagas disease: an update of in vitro and in vivo experimental models. *Mem Inst Oswaldo Cruz* 2024; **119**: e240057. https://doi.org/10.1590/0074-02760240057

29 Hendrickx S, Ilbeigi K, Thoré ESJ *et al*. A strategic discovery roadmap towards high-quality leads and drug development candidates for kinetoplastid diseases. Part 2: from molecule to confirmed hit. *J Antimicrob Chemother* 2006; https://doi.org/10.1093/jac/dkag110

30 Hendrickx S, Ilbeigi K, Thoré ESJ *et al*. A strategic discovery roadmap towards high-quality leads and drug development candidates for kinetoplastid diseases. Part 3: from lead towards a drug development candidate. *J Antimicrob Chemother* 2026; https://doi.org/10.1093/jac/dkag111