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Review

Challenges in drug discovery targeting TriTryp diseases with an emphasis on leishmaniasis

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ABSTRACT

Tritryps diseases are devastating parasitic neglected infections caused by *Leishmania* spp., *Trypanosoma cruzi* and *Trypanosoma brucei* subspecies. Together, these parasites affect more than 30 million people worldwide and cause high mortality and morbidity. Leishmaniasis comprises a complex group of diseases with clinical manifestation ranging from cutaneous lesions to systemic visceral damage. Antimonials, the first-choice drugs used to treat leishmaniasis, lead to high toxicity and carry significant contraindications limiting its use. Drug-resistant parasite strains are also a matter for increasing concern, especially in areas with very limited resources. The current scenario calls for novel and/or improvement of existing therapeutics as key research priorities in the field. Although several studies have shown advances in drug discovery towards leishmaniasis in recent years, key knowledge gaps in drug discovery pipelines still need to be addressed. In this review we discuss not only scientific and non-scientific bottlenecks in drug development, but also the central role of public-private partnerships for a successful campaign for novel treatment options against this devastating disease.

1. Background

Leishmania spp., *Trypanosoma cruzi* and *Trypanosoma brucei* subspecies are the causative agents of leishmaniasis, American trypanosomiasis (Chagas disease) and Human African trypanosomiasis (sleeping sickness), respectively. Together, these protozoal infections are known as TriTryp diseases. They represent a serious public health problem worldwide, especially in Africa, South America and Asia. TriTryp diseases are responsible for high mortality and morbidity rates in developing countries and impact affected regions economically and socially (Barrett et al., 2003; Hotez et al., 2009; WHO, 2018a). As there are no vaccines available, the treatment of infected people is one of the main strategies to control these diseases. However, drugs in use present major drawbacks, such as high toxicity, relevant contraindications and complicated administration regimens (Table 1) (Nussbaum et al., 2010; Singh et al., 2012).

2. *Leishmania* and leishmaniasis

Leishmaniasis is a complex group of diseases caused by different species of protozoan parasites that are members of the genus *Leishmania*, and impose a serious public health problem worldwide. According to the World Health Organization (WHO), leishmaniasis is endemic in 98 countries affecting around 12 million people. It is estimated that over 1 billion people live in endemic areas at risk of infection. Also, around 1.3 million new cases of the disease are registered annually and death counts 20,000 to 30,000 per year (Alvar et al., 2012; WHO, 2018a).

Leishmania has a digenetic life cycle, involving both invertebrate (phlebotominae sandflies) and vertebrate (mammals, including humans) hosts and presents two very distinct stages: promastigotes (extracellular and flagellated forms found in the insect gut) and amastigotes (intracellular and round forms that multiply within phagocytic immune cells). Mammals are infected by the bite of female sandflies that regurgitate infective promastigotes during a blood meal. Upon host infection, promastigotes are phagocytosed mainly by macrophages, where they differentiate into amastigotes inside phagolysosomal compartments. After successive multiplication, amastigotes are released from macrophages and re-infect new cells, such as macrophages, dendritic cells and fibroblasts. Occasionally, sandflies become infected by ingesting infected cells during next blood meal (Killick-Kendrick, 1990; Sacks and Kamhawi, 2001).

The disease leads to different clinical manifestations determined both by host parameters, such as genetic characteristics and immunological status (Jeronimo et al., 2007; Blackwell et al., 2009; Sakthianandeswaren et al., 2009), and parasite features, including heterogeneity in the virulence of different species/strains (Naderer et al., 2004). Clinical manifestations range from cutaneous lesions

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Table 1
TriTryp diseases.

	Leishmaniasis	American Trypanosomiasis (Chagas disease)	Human African Trypanosomiasis (sleeping sickness)
Causative agent	*Leishmania* species (*Leishmania* and *Viannia* subgenera)	*Trypanosoma cruzi*	*Trypanosoma brucei* subspecies
Endemic region	Mainly in Asia, South America, East Africa, and Mediterranean countries	Mainly in Latin America	Exclusively in Africa
Clinical manifestation	Cutaneous Leishmaniasis (skin lesions and mucous ulcers) Visceral Leishmaniasis (enlarged spleen and liver, fever, pallor)	Acute phase with variable symptoms (fever, headache, enlarged spleen and liver) Chronic infections: cardiac and/or digestive forms (megaesophagus and megacolon)	General manifestations: fever, headaches, neurological manifestation: seizures, poor coordination, somnolence, coma
Current treatments	Pentavalent antimonials, Amphotericin B, miltefosine and paromomycin	Benznidazole and nifurtimox	Suramin, pentamidine, melarsoprol, eflornithine, and nifurtimox-eflornithine combination
Disadvantages of chemotherapy	Toxicity, severe side effects, hospitalization requirement and parasite resistance emergence	Variable response in chronic disease, poor tolerability, severe toxic effect and contraindications	High toxicity and inefficacy against the neurologic phase

(cutaneous leishmaniasis, CL) and mucous ulcers (mucocutaneous leishmaniasis, MCL) to systemic visceral damage (visceral leishmaniasis, VL). VL is the most severe form of the disease and is potentially fatal if untreated (Piscopo and Mallia Azzopardi, 2007). Bangladesh, Brazil, India, Ethiopia, Kenya, Nepal and Sudan concentrate more than 90% of world's VL cases, while CL and MCL are predominantly diagnosed in Afghanistan, Algeria, Colombia, Brazil, Iran and additional African and Latin countries (Alvar et al., 2012; WHO, 2018a).

Currently, chemotherapeutic options show major disadvantages limiting the treatment of infection and clinical success (Table 1). Pentavalent antimonials (Glucantime® and Pentostam®), Amphotericin B (Fungizone® – salt formulation and Ambisome® - liposomal formulation), miltefosine (Impavido™) and paromomycin (Humatin®) are classically used for the treatment of leishmaniasis; however, these drugs present a number of limitations, including high cost, limited efficacy, and disabling side effects due to high toxicity and extended period of treatment. Of all the above drugs, miltefosine is the only one administered orally. Also, the emergence of antimonial-resistant *Leishmania* strains and variable susceptibility regarding distinct species/strains have been reported (Croft et al., 2006a; Barrett and Croft, 2012; Freitas-Junior et al., 2012; Uliana et al., 2017). Collectively, these factors contribute to the therapeutic failure observed in clinical practice.

Given the epidemiologic impact of leishmaniasis as well as the lack of appropriate treatment options, the development of safer, more effective and affordable new drug candidates and/or the improvement of existing therapies remains a priority.

3. Drug discovery criteria regarding leishmaniasis

Despite the advances observed in the anti-*Leishmania* drug discovery field, the innovation cycle is a challenging process that still faces gaps (Fig. 1).

Several approaches have been reported to identify and optimize new candidates against *Leishmania* parasites, including *de novo* drug discovery (Fig. 1), focusing on the identification of new chemical entities by screening both chemical and natural product libraries (Siqueira-Neto et al., 2012; Annang et al., 2015; Khare et al., 2016; Peña et al., 2015; Zulfiqar et al., 2017), and *short-term* strategies, including combinatory therapies, new formulations for drugs in use and drug repurposing (Alirol et al., 2013; Andrews et al., 2014; Hamill, 2013; Trinconi et al., 2014).

Target Product Profile (TPP) - defined as a planning tool for promising therapeutic candidates - has a major role in *de novo* drug discovery. Basically, TPP takes into account factors, such as compounds'

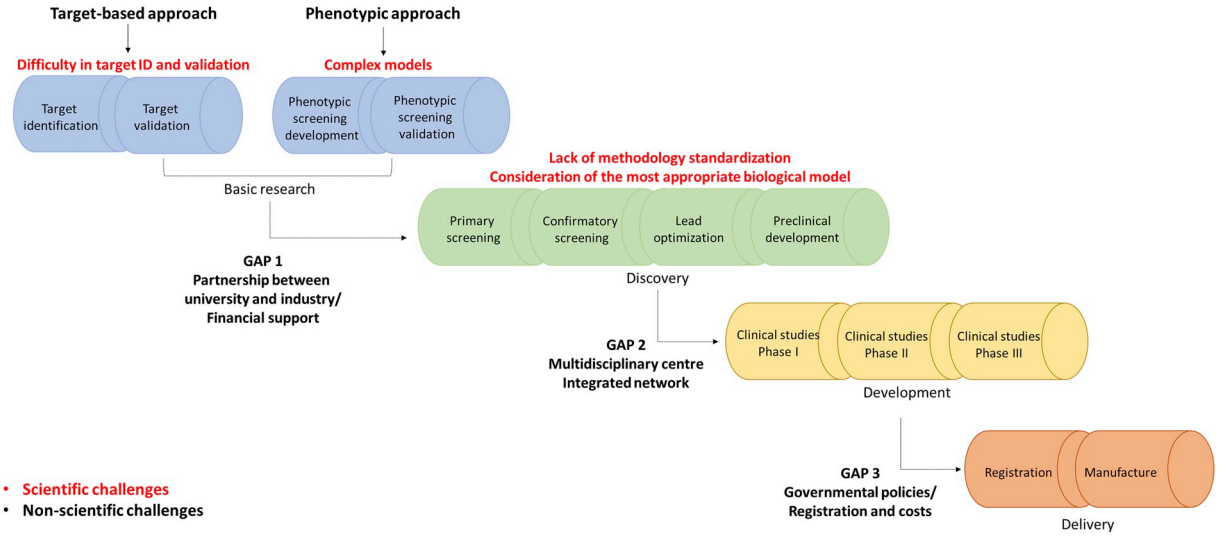

Fig. 1. Classical pipeline for drug discovery highlighting scientific and non-scientific challenges. The process often starts with basic research in order to (i) identify and validate molecular/biochemical targets (target-based assays) or (ii) develop and validate phenotypic assays (cell-based assays), in which compounds are tested against the whole parasite or a given biological system. Medicinal chemistry experts will then optimize selected compounds (*hits*). Next steps consist in testing candidates in animal models and assessing their performances by determining pharmacokinetics and pharmacodynamics properties. Finally, a compound is targeted to clinical trials in humans and, once showing a satisfactory profile, it is defined as a drug candidate. The last steps of the pipeline include registration and manufacture of the medicine.