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Spotlight

A promising pipeline of preclinical drug candidates for leishmaniasis and chronic Chagas' disease

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The drug discovery pipeline for leishmaniasis and trypanosomiasis has been filling with novel chemical entities with known mechanisms of action. González et al. and Braillard et al. report a cytochrome bc_1 complex inhibitor as another promising preclinical candidate for visceral leishmaniasis (VL) and, in combination with benznidazole, for chronic Chagas' disease (CCD).

There is a pressing need to develop new effective treatments for leishmaniasis and Chagas' disease; however, the inherent complexities of these infections further complicate this endeavor as both diseases are caused by parasites with complex life cycles that can exist in multiple forms and organs within the human body. As current therapies require extensive periods and frequently provoke severe side effects, patient adherence to treatment might often be reduced. Consequently, the desired target product profile for both diseases sets a high bar [1]. Despite these obstacles, concerted efforts are being made to overcome these complications and develop safe and effective oral antiparasitic treatments.

In the past decade, high-throughput phenotypic screenings have been the go-to method for discovering new antiparasitic agents, providing a powerful tool to identify compounds that kill parasites without prior knowledge of specific targets. The subsequent target deconvolution of a hit accelerates drug development by enabling the optimization of these compounds for efficacy and selectivity. Considering that the current care standards for VL and CCD are far from ideal, this acceleration in the discovery of alternative therapies is critically needed.

Therefore, developing new chemical entities (NCEs) with clinical potential for single or combined therapies is a significant breakthrough in treating these illnesses. Examples are studies identifying inhibitors of the proteasome [2,3], topoisomerase II [4], cyclin-dependent kinase 12 [5], or CPSF3 [6]. Now, González *et al.* [7] and Braillard *et al.* [8] have recently published their findings on an unbiased phenotypic screening of the GSK compound collection. Their work identified related drug-candidate molecules, the pyrrolopyrimidine series, that effectively inhibit *Trypanosoma cruzi* and *Leishmania donovani* growth *in vitro* by inhibiting cytochrome b (cytb) (key summary of findings are depicted in Figure 1). Mutations in the Q_i site of cytb confer resistance of the parasites to the compounds, suggesting cytb as the primary target. Multiple copies of the cytb gene in *Leishmania* and *T. cruzi* make it challenging to perform genetic manipulation of the parasites to directly confirm this; however, inhibition of complex III of the electron transport chain by the compounds in parasite lysates provides additional evidence.

The cytochrome bc1 complex plays a pivotal role in the mitochondrial respiratory chains of numerous eukaryotic microorganisms. Historically, there have been recurring challenges that have hampered bringing Q-cycle inhibitors to patients. These include a rapid propensity for mutation leading to drug resistance, drug–drug interactions, and safety concerns during clinical trials [9]. Previously, these authors have shown that a wide range of chemotypes can target the cytb Qi site of both *L. donovani* and *T. cruzi*. Hence, strategies were put in place to prevent over enrichment of compounds targeting the Qi site of cytb [10] for VL and CCD drug development programs. So, why are these inhibitors now deemed to be promising clinical candidates?

Primarily, this is because high-quality chemistry has delivered compounds with potent *in vivo* activity. One of the compounds, DNDI-6174, is effective in animal models of VL and it has the appropriate safety and PK-PD characteristics for clinical development. A closely related compound cannot completely clear chronic murine infections of *T. cruzi* as a solo agent, but when used in combination with a lowered dose of the current first-line treatment, benznidazole, significantly reduced the treatment duration with no relapse after immunosuppression. This is exciting news: shorter CCD treatment regimens for benznidazole could improve safety, lower drop-out rates, and impact public health by lessening the burden on healthcare systems.

The *in vivo* performance of the current pyrrolopyrimidine leads has the potential to challenge the prevailing notion about cytb inhibitors as antiparasitic agents. Some further work must be done, however, before putting this mechanism of action at the drug development frontline. For instance, a better understanding of the binding mode beyond *in silico* predictions might help to identify a suitable chemical optimization strategy, while preserving metabolic stability and safety. Although the inhibition of cytb shortens and reduces benznidazole treatment, the exact mechanism behind its synergistic effect is not yet understood. One possibility is that this combination causes DNA instability by accumulating free radicals and disrupting the parasite's mitochondrial

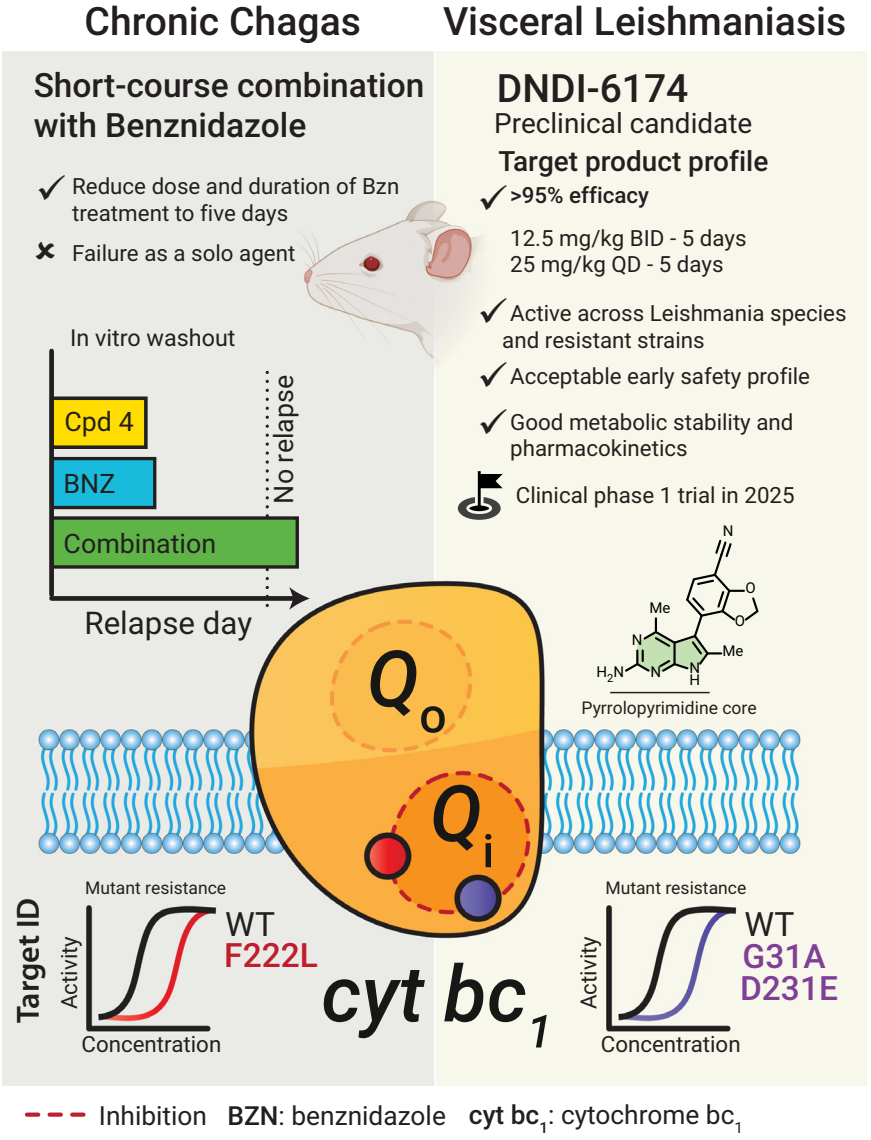

Figure 1. Pyrrolopyrimidine compounds in chronic Chagas' disease (CCD) and visceral leishmaniasis (VL). Upper left: Short course combination *in vitro* demonstrates that lead compound 4 fails to prevent relapse as a solo agent; however, when administered with a low concentration of benznidazole, it prevents relapse with only 5 days of combined treatment. This result was also translated *in vivo* using a mouse model of CCD [7]. Upper right: DNDI-6174 fulfils the criteria for progressing to preclinical development for VL [8]. Lower panel: Mutations in the Q_i site of cytochrome b confer resistance to pyrrolopyrimidine series. In *Trypanosoma cruzi*, F222L (red) mutation disrupts ligand binding, yielding high resistance. Similarly, *Leishmania donovani* bearing G31A or D231E mutations has a high resistance to DNDI-6174.

function; indeed, recent studies have suggested that rapid and irreversible DNA damage can rapidly cure trypanosome infections [4].

As the authors acknowledge, there are limitations in translating the efficacy of these compounds in animals to the clinic. The mouse is inherently resistant to VL and does not adequately reproduce human disease, whilst for CCD the murine model relies on a single strain of *T. cruzi*, CL Brener, which is just one of seven discrete typing units. In a financial environment where funds are limited, prioritizing which of these promising NCEs should enter clinical trials will be challenging. Considering the populated pipeline of NCEs in clinical development for VL, it would be worthwhile to assess whether cytb inhibitors could also be a potential treatment for other types of leishmaniasis, such as cutaneous leishmaniasis, as Braillard and colleagues present data revealing that this compound retains activity across different *Leishmania* species.

Overall, the outstanding performance of cytb inhibitors in preclinical studies, as a single agent for VL or in combination for CCD, represents an important advancement in trypanosomatid drug discovery.

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Declaration of interests

The authors declare no competing interests.

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