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Reply to "Drug Susceptibility of Genetically Engineered *Trypanosoma cruzi* Strains and Sterile Cure in Animal Models as a Criterion for Potential Clinical Efficacy of Anti-*T. cruzi* Drugs"

Amanda Fortes Francisco,[a] Michael D. Lewis,[a] Shiromani Jayawardhana,[a] Martin C. Taylor,[a] Eric Chatelain,[b] John M. Kelly[a]

Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, London, United Kingdom[a]; Drugs for Neglected Diseases Initiative (DNDi), Geneva, Switzerland[b]

We appreciate the interest of Professors Urbina and McKerrow in our recent paper (1) and welcome the opportunity to discuss the work further. In our study, we found that benznidazole consistently cured both acute and chronic *Trypanosoma cruzi* infections in mice, whereas posaconazole was much less effective, data consistent with the results of a recent clinical trial (2).

In response to specific comments by our colleagues, we point out the following. (i) Contrary to what is stated in the letter from Professors Urbina and McKerrow, our paper does not include experiments where mice in the acute stage of *T. cruzi* infection were treated with benznidazole for 5 or 10 days. (ii) Previous studies on the efficacy of 20 days of benznidazole treatment against infections with the *T. cruzi* CL strain (see references 7 to 10 in the letter from Professors Urbina and McKerrow) have produced a range of results, which may reflect the differing mouse models, timing of treatment start points, and choice of drug vehicle. Indeed, we notice in a paper by Professor Urbina that treatment of Swiss mice with 100 mg/kg orally for 20 days resulted in a 100% cure (see Table 1 in reference 8 of the letter from Professors Urbina and McKerrow). (iii) The bioluminescent CL clone used in our study was not "hypersusceptible to benznidazole." We would have been careless not to test for this. The 50% inhibitory concentration for intracellular amastigote forms was 1.2 μM (unpublished data), not significantly different from that for the wild type and well within the normal range reported elsewhere for *T. cruzi* CL and other diverse strains (3). (iv) Our treatment data are consistent with findings reported in a recent paper (4). Using PCR to assess drug efficacy, these authors find that treatment of *T. cruzi* CL-infected mice with 100 mg/kg benznidazole for 20 days results in a complete cure. Furthermore, they also find that posaconazole has a limited curative effect, even when treatment is extended to 40 days.

Investigating whether subcurative treatment with posaconazole leads to improved clinical outcomes is a complex issue and was not an objective of our study. However, it is unlikely that any new treatment for *T. cruzi* infection will be licensed unless it can achieve a sterile cure. Unfortunately, based on *in vitro* studies (3), *in vivo* assessment (1, 4), and a clinical trial (2), posaconazole does not seem to meet this benchmark.

As outlined by Professors Urbina and McKerrow, benznidazole is far from an ideal drug and the search for more effective alternatives must remain a research priority.

REFERENCES

1. **Francisco AF, Lewis MD, Jayawardhana S, Taylor MC, Chatelain E, Kelly JM.** 2015. The limited ability of posaconazole to cure both acute and chronic *Trypanosoma cruzi* infections revealed by highly sensitive *in vivo* imaging. Antimicrob Agents Chemother **59:**4653–4661. http://dx.doi.org/10.1128/AAC.00520-15.
2. **Molina I, Gómez i Prat J, Salvador F, Treviño B, Sulleiro E, Serre N, Pou D, Roure S, Cabezos J, Valerio L, Blanco-Grau A, Sánchez-Montalvá A, Vidal X, Pahissa A.** 2014. Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. N Engl J Med **370:**1899–1908. http://dx.doi.org/10.1056/NEJMoa1313122.
3. **Moraes CB, Giardini MA, Kim H, Franco CH, Araujo-Junior AM, Schenkman S, Chatelain E, Freitas-Junior LH.** 2014. Nitroheterocyclic compounds are more efficacious than CYP51 inhibitors against *Trypanosoma cruzi*: implications for Chagas disease drug discovery and development. Sci Rep **4:**4703. http://dx.doi.org/10.1038/srep04703.
4. **Khare S, Liu X, Stinson M, Rivera I, Groessl T, Tuntland T, Yeh V, Wen B, Molteni V, Glynne R, Supek F.** 2015. Antitrypanosomal treatment with benznidazole is superior to posaconazole regimens in mouse models of Chagas disease. Antimicrob Agents Chemother **59:**6385–6394. http://dx.doi.org/10.1128/AAC.00689-15.

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Address correspondence to John M. Kelly, john.kelly@lshtm.ac.uk.

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