Genome plasticity, an essential adaptive mechanism that drives drug resistance in *Leishmania* spp.

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Discovering how small molecules function to kill *Leishmania* parasites can result in the identification of new "chemically validated" targets. By generating promastigote stage parasites resistant to the clinical antileishmanials amphotericin B and miltefosine we have, via whole-genome sequencing coupled with other metabolomic and lipidomic approaches, identified mutations associated with drug resistance *in vivo*. Implementing this pipeline has also allowed us to interrogate other structurally distinct antileishmanial candidates whose mode of action (MoA) was previously unknown, including for validation of the IPC synthase as a target of the orphan drug clemastine-fumarate. This MoA was supported by reduced drug susceptibility and accumulation of lipid species in a sphingolipid-deficient mutant *L. major,* generated via gene knockout using homologous recombination. To begin to understand how these parasites survive in the absence of 'essential' sphinglopids we further explored the genome of this historic ΔLCB2 (loss of the catalytic subunit of serine palmitoyltransferase) knockout cell line which demonstrated a complete loss of sphingolipid biosynthesis. While this mutant remained viable and infective in mice, whole genome sequencing revealed a number of SNPs and structural changes such as CNVs and gene deletions, including of a putative ABC3A sterol transporter. Importantly, simultaneous deletion of this ABC3A gene facilitated LCB2-targeted knockout in the model *L. mexicana,* suggesting a compensatory effect. We are currently expanding the tools employed in this pipeline to characterise a series of new molecules with activity against *Leishmania* and *T. cruzi*. Whole genome sequencing supported with other analytical tools have provided essential insights into gene function and proven useful in revealing mutations and other structural changes that play a role in promoting resistance in *Leishmania* spp. Furthermore, we emphasize the necessity to re-examine the many other historical *Leishmania* spp knockout lines where genes, such as LCB2, were previously deemed non-essential.