Anti-Leishmanial Drug Discovery: a Model Based on Industrial Partnerships & OMICS Tools

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Problem: current antileishmanial treatments have many limitations: (1) chemotherapy is toxic and increasingly ineffective with rising resistance; (2) there is no vaccine. **Aim:** to identify a new therapeutic target in *Leishmania* as a primer for target-based drug discovery in cutaneous leishmaniasis. **Model approach:** cutting-edge OMICS tools and industrial partnerships to deconvolve the MoA of a novel antileishmanial compound, 'C4', identified by GSK in a phenotypic screen (Pena et al,. 2015). Our model builds on our previous work (Mina et al., 2021). **Tools & partnerships:** genomics (Mottram, York University), proteomics (Trost, Newcastle University), metabolomics and lipidomics (Barrett, Glasgow University), and CRISPR/Cas9 gene-editing. **Future work:** This work will contribute to the WHO's aim under the NTD roadmap for 2021-30: to 'develop and scale up an easy-to-administer oral or topical treatment' for cutaneous leishmaniasis.

Physiochemical characterization of compound C4

Tables. Physiochemical properties of the compound as found in the literature and predicted by the SwissADME webtool.

Formula	ChEMBL_Ref	MW (Da)	Polarity (TSPS (Å²))	Lipophilicity (Log $P_{o/w}$)	Water solubility Log S (ESOL)
$C_{21}H_{22}F_3N_5O_3$	TCMDC-143486	449.43	102.16	3.3	-4.61

Lapinski violations (druglikeness)	HIA	P-gp substrate	PAINs alerts	Brenk alerts	Leadlikeness violations
0	High	Yes	0	0	1

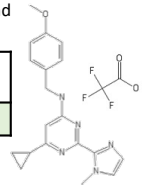


Fig 1. Chemical structure of C4.

In vitro evolution & screening

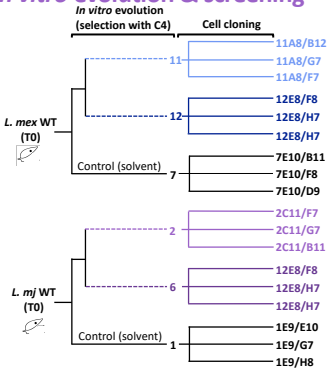


Fig 2. *In vitro* evolution of *L. major (L.mj)* and *L. mexicana* (*L.mex*) promastigotes against C4 resulted in resistant clonal lines.

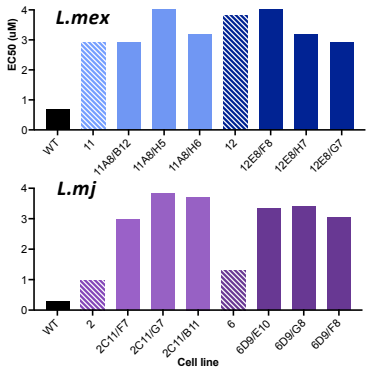
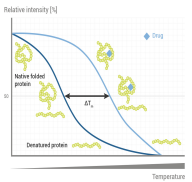


Fig 3. Bar charts showing EC50s of WT and C4-resistant cell lines against C4.

CRISPR/Cas9 (genetic target-validation)

KO of IPCS (proposed target of the recently identified antileishmanial tamoxifen (Trinconi et al., 2018) yielded increased tamoxifen sensitivity but no morphological phenotype (Zimbres, unpublished).

Thermal Proteomic profiling



Metabolomics & lipidomics

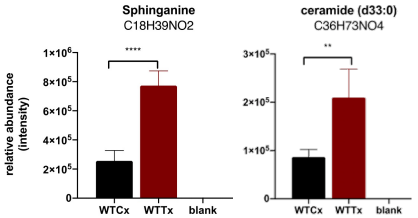


Fig 6. Relative abundance of metabolites in WT *L.mj* promastigotes treated with clemastine (Mina et al., 2021)

Genomics (NGS & mutation identification)

num.	gene ID	abbreviation	single nucleotide polymorphisms (SNPs) cl.C	cl.D	total
1	LmjF.34.3740	SPT	1	3	4
2	LmjF.35.0320	SPT-like	3		3
3	LmjF.35.0330	3-KSR		1	1
4	LmjF.31.1780	CerS			
5	LmjF.26.1670/80	CerD	1	1	2
6	Nd	CerAse			
7	LmjF.35.4990	IPCS	2		2
8	LmjF.08.0200	ISCL	2		2
9	Nd	CerK			
10	Nd	CerP			
11	LmjF.26.0710	SK	(2*)	2	4
12	LmjF.32.2290	SPPAse	2 (1*)	3	6
13	LmjF.30.2350	SPLY			
14	LmjF.18.0440	PAF	3 (1*)	2	6
		total	18	12	30

Fig 4. SNPS and CNVs observed in cloned clemastine-resistant *L.mj* promastigotes (lines cl.C and cl.D) following NGS (Mina et al., 2021).

Cross-resistance (resistant lines vs drugs)

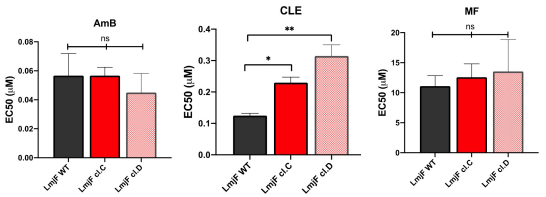


Fig 5. Cross-resistance profiles of cloned clemastine-resistant *L.mj* promastigotes (lines cl.C and cl.D) (Mina et al., 2021).

References: Mina et. al., *ACS Infect. Dis.* 7 (2021), 1, 47–63, December 8, 2020 | Peña, et. al., *Sci Rep.* 5 (2015) 8771| Trinconi et al., *IJP: Drugs and Drug Resistance* 8 (2018) 475–487