RNA editing ligase 1 as a drug target: On the road to lead generation

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Introduction

- Trypanosomatid parasites cause **devastating human and veterinary diseases**, for which current treatment is largely insufficient[1].
- **RNA Editing Ligase 1 (REL1)** is essential for uridylyl insertion/deletion mRNA editing, a unique and extensive form of RNA editing in the mitochondria of trypanosomatids[2].
- REL1 is an attractive **drug target**, since it is essential in *Trypanosoma brucei*[3], has no mammalian homologues[4], and can be inhibited by drug-like molecules.

Is REL1 also essential in *Leishmania* parasites?

Aim 1: Investigate the essentiality of REL1 in *L. mexicana* using CRISPR/Cas9.

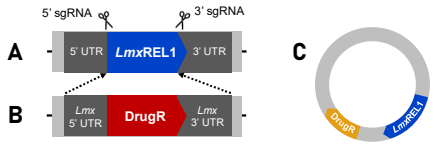

Fig 2. LeishGEdit CRISPR/Cas9 system[5]. **(A)** Single guide (sg) RNAs specify sites for Cas9 to introduce double-stranded breaks in the target gene. **(B)** Drug resistance cassette replaces region removed by Cas9. **(C)** If the target gene is essential, addback plasmid expresses an ectopic copy of the gene that allows cells to survive.

- Very few drug-resistant clones emerged after transfection as they may have retained copies of *Lmx*REL1 → If further attempts to generate null mutants fail, **this suggests REL1 is essential**.
- Growth rescue was not observed as the addback plasmid contained a truncated *Lmx*REL1 gene sequence.

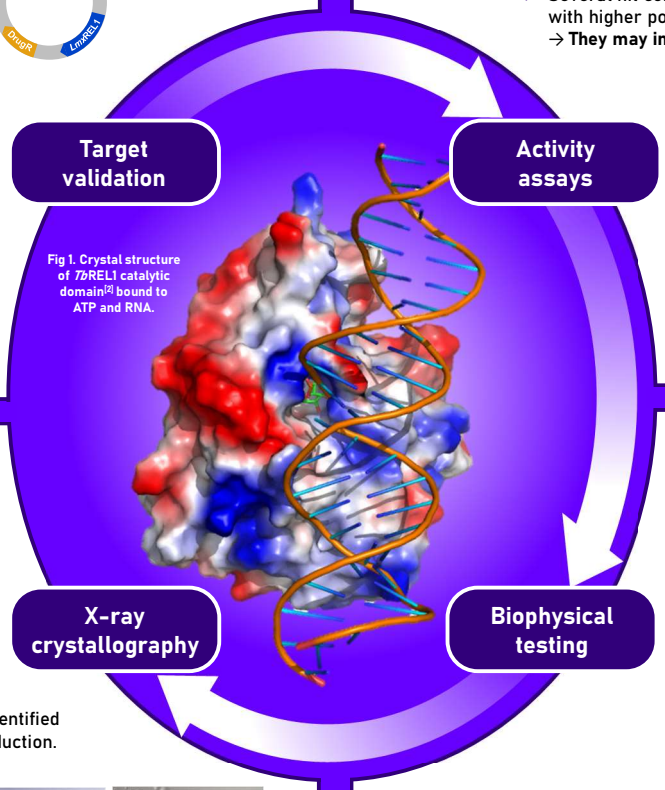

Fig 1. Crystal structure of *Tb*REL1 catalytic domain[2] bound to ATP and RNA.

Can we use X-ray crystallography to explore Structure-Activity Relationships (SARs) of REL1 inhibitors?

Aim 4: Solve the structure of protein-inhibitor complexes to study their interactions at the atomic level.

- Ongoing crystallisation efforts may have identified suitable conditions for *Ld*REL1 crystal production.

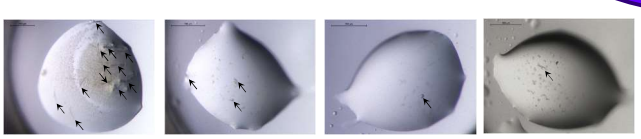

Fig 8. Sitting drops from vapour diffusion crystallisation experiments. Potential *Ld*REL1 crystals are indicated by black arrows.

What's next?

- Clone **full-length *Lmx*REL1 sequence** into CRISPR/Cas9 addback plasmid.
- Confirm REL1 inhibition *in vivo* by measuring **effects on uridylyl insertion/deletion mRNA editing** in *Trypanosoma brucei* cell lines.
- Optimise **SPR-based assay** to investigate REL1-inhibitor interactions.
- Optimise **crystallisation conditions** to solve full-length *Ld*REL1 structure.

Previous high-throughput screens identified promising REL1 inhibitors.

Aim 2: Identify compounds with high efficacy and specificity *in vitro* and *in vivo*.

- Several hit compounds inhibited **various REL1 orthologues *in vitro*.**

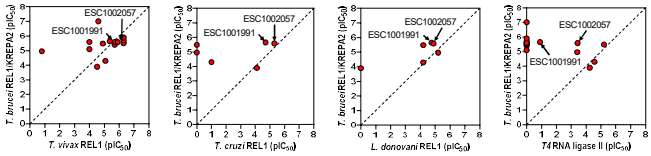

Fig 3. Comparison of pIC_{50} values of promising hit compounds against REL1 from *T. brucei*, *T. vivax*, *T. cruzi*, *L. donovani* and T4 RNA ligase II. Reduction in REL1 activity was measured in a FRET-based ligase activity assay[6]. T4 RNA ligase II, a close homologue of REL1, was included to test the degree of REL1 specificity.

- Several hit compounds inhibited the growth of *T. brucei* with higher potency for mitochondrial DNA-dependent cells → **They may inhibit *Tb*REL1 *in vivo*.**

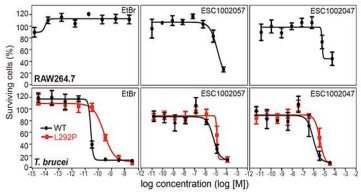

Fig 4. Cellular effect of two promising hit compounds on mammalian macrophages (RAW264.7), wild-type (WT) and mtDNA-independent (L292P) bloodstream form *T. brucei*. Ethidium bromide (EtBr) was included as a control. Viable cells were quantified in Alamar Blue assays[7] (n=3).

Can we use Surface Plasmon Resonance (SPR) to study REL1-inhibitor interactions?

Aim 3: Develop a biophysical assay based on SPR to confirm target engagement.

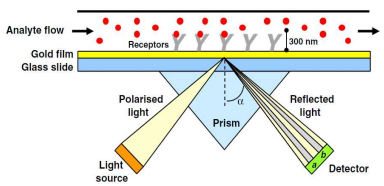

Fig 5. Principle of SPR experiment[8]. Protein of interest is immobilised on a sensor chip and potential ligands are flowed over the surface. Polarised light is directed to the chip, generating surface plasmons that absorb light at a critical angle. Refractive index varies proportionally to the amount of material bound on the surface.

- ***Ld*REL1 was immobilised** on the SPR chip through a biotin-based approach[9].

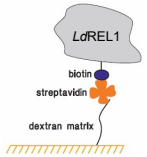

Fig 6. Biotinylated *Ld*REL1 capture on streptavidin chip.

- **There was no evidence of binding with ATP.** *Ld*REL1 was virtually inactive in SPR assay conditions (i.e. 100 mM KCl).

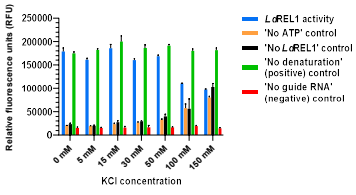

Fig 7. High KCl concentrations reduce *Ld*REL1 activity. High KCl levels increase background fluorescence of RNA substrates (n=3).

References **[1]** Altamura *et al.* (2020). *Drug Dev Res.* **[2]** Read *et al.* (2016) *WIREs RNA.* 7(1): 33–51. **[3]** Schnaufer *et al.* (2001). *Science*, 291(5511): 2159–62. **[4]** Deng *et al.* (2004). *JMB.* 343(3): 601–13. **[5]** Beneke *et al.* (2017). *R Soc Open Sci.* 4(5): 170095. **[6]** Zimmermann *et al.* (2015). *NAR.* 44(3): e24. **[7]** Dean *et al.* (2013). *PNAS.* 110(36): 14741–46. **[8]** Patching (2014). *Biochim Biophys Acta*, 1838: 43–55. **[9]** Fairhead & Howarth (2015). *Method Mol Biol.* 1266: 171–84.