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 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.

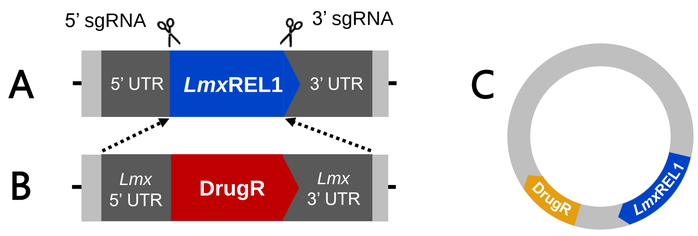

Fig 2. LeishGEdit CRISPR/Cas9 system[5]. (A) Single guide (sg) RNAs specify sites for Cas9 to introduce double-stranded breaks in the target gene. **(B)** Drug resistance cassette replaces region removed by Cas9. **(C)** If the target gene is essential, addback plasmid expresses an ectopic copy of the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

- Knockouts could only be achieved in the presence of an ectopic *Lmx*REL1 copy.
  → **Strong evidence that REL1 is essential in *L. mexicana* promastigotes[6].**

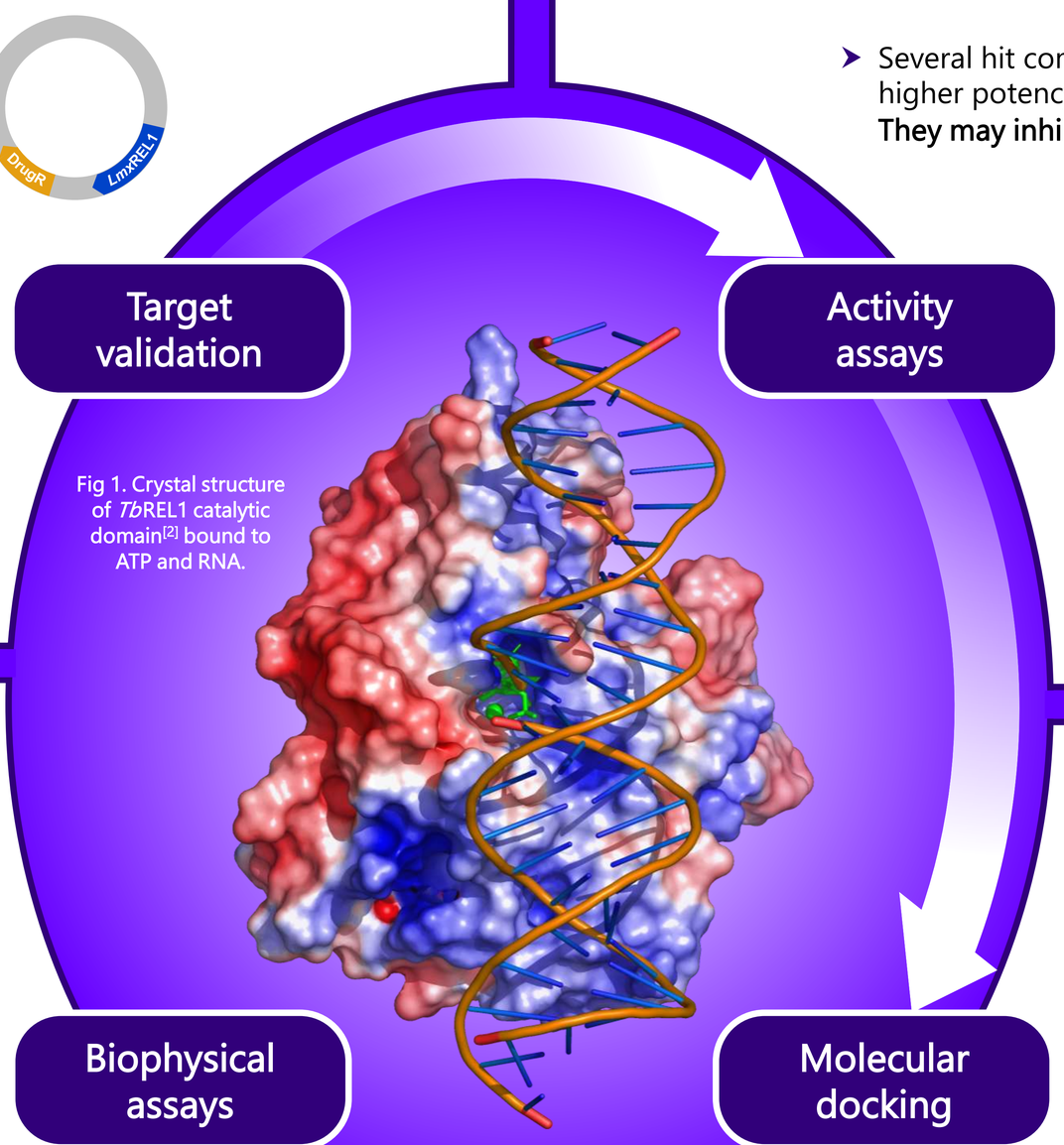

Fig 1. Crystal structure of *Tb*REL1 catalytic domain[2] bound to ATP and RNA.

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.***

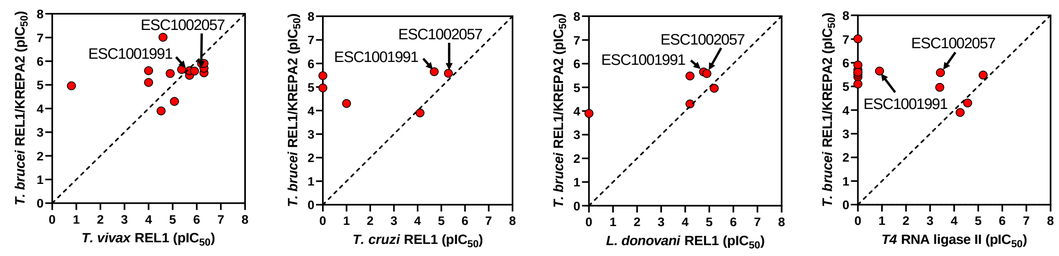

Fig 3. Comparison of pIC_{50} values of promising hit compounds against REL1 from *T. brucei, T. vivax, T. cruzi, L. donovani* and T4 RNA ligase II. Reduction in REL1 activity was measured in a FRET-based ligase activity assay[7]. T4 RNA ligase II, a close homologue of REL1, was included to test the 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*.**

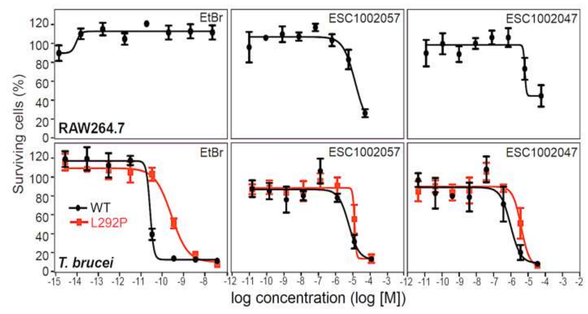

Fig 4. Cellular effect of two promising hit compounds on mammalian macrophages (RAW264.7), wild-type (WT) and mtDNA-independent (L292P) bloodstream form *T. brucei.* Ethidium bromide (EtBr) was included as a control. Viable cells were quantified in Alamar Blue assays[8] (n=3).

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

Aim 4: Develop a biophysical assay based on SPR to confirm target engagement.

Fig 6. Principle of SPR experiment[10]. 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[11].

Fig 7. 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 8. High KCl levels reduced *Ld*REL1 activity. High KCl levels increased background fluorescence of RNA substrates (n=3).

Some REL1 inhibitors are predicted to bind to the ATP binding pocket with high affinity.

Aim 3: Predict and confirm the binding mode of promising REL1 inhibitors.

- Extensive hydrophobic interactions with V88, F209, A243 & V286.
- Water displacement from pocket deep end.
- Optimisation of crystallisation conditions under way.

Fig 5. Proposed binding mode of ESC1001991 (red) and ESC1002057 (blue). ATP binding pocket surface and *Tb*REL1 residues are shown in green. Hydrogen bonds are represented by dashed lines. Calculated with AutoDock Vina[9].

What's next?

- Optimise **SPR-based assay** to investigate REL1-inhibitor interactions.
- Confirm REL1 inhibition *in vivo* by measuring **effects on uridylyl insertion/deletion mRNA editing** in *Trypanosoma brucei* cell lines.
- Generate recombinant **REL1 mutants** to corroborate binding predictions.
- Solve the **crystal structure** of REL1 orthologues with and without compounds.

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]** Jones *et al.* (2018). *ACS Infect Dis.* 4(4): 467-477. **[7]** Zimmermann *et al.* (2015). *NAR.* 44(3): e24. **[8]** Dean *et al.* (2013). *PNAS.* 110(36): 14741–46. **[9]** Trott & Olson (2010). *J Comput Chem.* 31: 455-461. **[10]** Patching (2014). *Biochim Biophys Acta*, 1838: 43-55. **[11]** Fairhead & Howarth (2015). *Method Mol Biol.* 1266: 171-84.