Deep mutational resistance profiling of an anti-trypanosomal proteasome inhibitor

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We recently reported the development of oligo targeting for profiling drug resistance mutations in the parasitic trypanosomatids. This simple, and Cas9-independent, method allows for rapid and precise editing in otherwise wild type trypanosomatids. Improving our understanding of mutations associated with drug resistance is a priority given that several new anti-trypanosomal drugs, with known targets, are currently in clinical development. Accordingly, we have scaled-up oligo-targeting for deep mutational scanning and have applied the approach to the *Trypanosoma brucei* proteasome, using a promising proteasome inhibitor (EC_{50} 4 nM). Using cryo-EM structural data, we identified 20 proteasome β5 subunit residues within 5 Å of the drug-binding pocket. A set of codon-mismatched oligonucleotides was used for site saturation mutagenesis at these sites and to generate a pooled library of 1280 *T. brucei* mutants. Amplicon sequencing was used to validate library complexity and for codon variant scoring following drug selection, which revealed >100 distinct resistance conferring base-edits. The digital data was used to derive virtual dose-response curves for >45 distinct amino acid edits. We are assembling a panel of mutants for validation and are investigating structure-activity relationships using cryo-EM structural data and computational modelling. We hope to provide unprecedented insights into proteasome-inhibitor interactions, to facilitate assessment of resistance potential, and to improve prospects for future drug design.