Oligo targeting for profiling acoziborole resistance mutations in *Trypanosoma brucei*

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Current drug treatments for human African trypanosomiasis require hospitalisation and disease stage diagnosis by painful lumbar puncture. However, recent phase 2/3 trials for an oral dose of acoziborole showed efficacy against both early and late-stage disease. Benzoxaboroles, including acoziborole, target a subunit of the cleavage and polyadenylation specificity factor complex (CPSF3). We previously developed a simple oligo targeting method for rapid and precision editing of drug targets in otherwise wild type trypanosomatids. Here we report progress in scaling up this approach for saturation mutagenesis of CPSF3. Of >1000 introduced mutations within 5 Å of the predicted binding site of acoziborole in the catalytic pocket of CPSF3, only those encoding Asn^{232}His conferred resistance to 1 or 3 µM acoziborole. Furthermore, none of the mutations yielded resistance to higher doses of acoziborole selection (9 or 27 µM). This may reflect a restrictive mutational space in the CPSF3 catalytic pocket and demonstrates limited scope for acoziborole resistance conferring mutations within CPSF3.