Inhibition of *Pf*CLK3 interferes with malaria parasite RNA-splicing and explains the mechanism of action of a new class of antimalarial candidates.

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Next generation antimalarials must be active across different *Plasmodium* species and life cycle stages with prophylaxis, cure and transmission blocking potential. Our lab employed chemogenetics, adaptive evolution, whole-genome sequencing, and standard membrane feeding assays to validated *Pf*CLK3 as a novel antimalarial target conserved across *Plasmodium* species. Inhibition of PfCLK3 blocks sporozoites invasion of hepatocytes, providing prophylaxis; kills all stages of asexual blood stage parasites, offering cure; and prevents gametocyte maturation and mosquito infection thereby blocking transmission.

PfCLK3 is closely related to human kinases PRPF4B and CLK2, both established as essential players for efficient RNA splicing in eukaryotes. We therefore hypothesise that the homology between PfCLK3 and these human kinases suggested that the parasiticidal activity of *Pf*CLK3 inhibitors results from disruption of RNA processing. To investigate this, we performed whole-transcriptome RNA sequencing of trophozoite-stage parasite samples (30 hours post invasion, 30 hpi) from wild type (WT) and inhibitor-resistant mutant (G449P) lines treated for 1 hour with the selective PfCLK3 inhibitor TCMDC-135051.

RNA-sequencing data revealed extensive RNA-splicing defects in WT whereas G449P mutant showed no detectable splicing deficiency. Selective *Pf*CLK3 inhibition affected 2,039 splice-junctions across 1,125 genes that were mis-spliced in treated WT parasites compared to controls. Importantly, functional analysis of the mis-spliced transcripts indicated disruption of numerous essential parasite processes spanning multiple life cycle stages, including multiple aspects of RNA metabolism (mRNA transcription and splicing), invasion, and DNA replication. This effect has been validated as being specific to CLK3 inhibition across different stages of the parasite life cycle.

In summary, we established *Pf*CLK3 as a key regulator of spliceosome activity and RNA splicing in *P. falciparum* parasites and confirmed the mechanism of action of TMCDC-135051. This system now provides a powerful platform to further investigate RNA splicing mechanism in *Apicomplexa.* Most importantly, our group is optimising selective *Pf*CLK3 inhibitors as next-generation radical cure antimalarials with prophylaxis, curative and transmission blocking potential.