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Turning the tide: targeting PfCRT to combat drug-resistant *P. falciparum*?

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Abstract

Resistance to the current first-line antimalarials threatens the control of malaria caused by the protozoan parasite *Plasmodium falciparum* and underscores the urgent need for new drugs with novel modes of action. Here, we present the argument that the parasite's chloroquine resistance transporter (PfCRT) constitutes a promising target to combat multidrug-resistant malaria.

Plasmodium falciparum malaria continues to have an enormous global health impact, causing over 200 million cases and an estimated 405,000 deaths in 2018 ([World malaria report](#)). For decades, treatment depended on the highly efficacious, safe and affordable drug chloroquine. The emergence and global spread of *P. falciparum* resistance to chloroquine, driven by region-specific mutations in the gene encoding the *P. falciparum* chloroquine resistance transporter (PfCRT), ultimately led to the global implementation of artemisinin-based combination therapies. These therapies combine a fast-acting artemisinin derivative with a mechanistically distinct, longer-acting partner drug, primarily lumefantrine or amodiaquine in Africa or piperaquine in Southeast Asia. Artemisinin-based combination therapies have helped decrease the global malaria burden by 37% from 2000 to 2015. Unfortunately, partial resistance to artemisinin has emerged and spread throughout Southeast Asia. More recently, these strains have also acquired high-level resistance to piperaquine, leading to treatment failure rates averaging ~50% across the region and attaining up to 87% in northeastern Thailand¹. Overcoming resistance in Southeast Asia and preventing it from affecting Africa and other malaria-endemic regions remains a key priority².

PfCRT, a member of the superfamily of drug and metabolite transporters, is located on the membrane of the intra-erythrocytic digestive vacuole of the parasite. This acidic lysosome-like organelle mediates the digestion of endocytosed host haemoglobin to provide globin-derived amino acids, which are then exported into the parasite cytosol for parasite protein synthesis. This process liberates membrane-lytic haem species in the digestive vacuole, which are detoxified via their incorporation into chemically inert haemozoin crystals.

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Competing interests

The authors declare no competing interests.

Chloroquine, amodiaquine and piperazine, all 4-aminoquinolines, concentrate to low micromolar levels in the digestive vacuole and bind β -haematin dimers, thereby preventing haem detoxification. Variant isoforms of PfCRT were earlier shown to mediate chloroquine resistance by drug efflux out of the digestive vacuole, away from the drug site of action. These findings led to the proposal that overcoming chloroquine resistance might be achievable by directly inhibiting PfCRT-mediated transport of either drug or its natural substrates, postulated to include globin-derived peptides³.

Two recent findings have refocused attention on PfCRT: epidemiological, gene editing and clinical studies have revealed that novel amino acid mutations in the gene encoding this transporter are driving high-grade resistance to piperazine across Southeast Asia^{1,4}; and the structure of PfCRT was solved to a resolution of 3.2 Å, using single-particle cryo-electron microscopy applied to purified protein that was stabilized as a complex with a bound antibody fragment⁵. Molecular epidemiological data from western Cambodia, the epicentre of multidrug resistance, indicated that these novel piperazine resistance-conferring *pfcr*t mutations increased in frequency from <10% in 2011 to >90% by 2016 (REF⁴). These studies also revealed that editing individual mutant residues into a South American PfCRT isoform was sufficient to confer piperazine resistance in parasites from that region. At the structural level, PfCRT comprises ten transmembrane helices arranged as five antiparallel pairs and a large central cavity of ~3,300 Å captured in an open-to-digestive vacuole conformation. Binding of the antibody fragment was localized to this cavity, which can also accommodate chloroquine or pip-erazine. Most of the mutations that contribute to parasite resistance to these drugs line the central cavity of the transporter, where presumably they enable drug-binding events to be converted into transport across the digestive vacuole membrane. Biochemical studies with proteoliposomes containing PfCRT revealed that transport was pH gradient and membrane potential dependent⁵.

These genetic and structural data reveal an intricate molecular process that requires specific combinations of 4–9 amino acid substitutions, compared with the conserved drug-sensitive wild-type isoform, to produce chloroquine resistance via a gain of drug efflux. High-level piperazine resistance in Southeast Asia arose by the selection of specific single amino acid substitutions introduced into the regionally predominant chloroquine-resistant PfCRT isoform (that harbours eight mutations). Binding studies with purified protein provided evidence that in addition to their inhibition of haem detoxification, both drugs exert antiparasitic activity, in part, by directly inhibiting PfCRT's native function³. Importantly, most mutations that mediate piperazine resistance lead to a loss of chloroquine resistance and to an increased susceptibility to amodiaquine. In light of these findings, we propose that it is time to once again consider PfCRT as an attractive drug target.

A PfCRT-specific inhibitor that binds the central cavity of the drug transport-competent isoforms and restores chloroquine and piperazine sensitivity could enable the clinical reimplementation of one or both of these effective and inexpensive antimalarials in areas of multidrug resistance. This combination could also prevent the development of further resistance to either compound by creating opposing selective pressures, whereby a gain of resistance to one compound would collaterally sensitize parasites to the other PfCRT-interacting inhibitor. This concept is similar to a previously proposed population biology

trap for inhibitors of the *P. falciparum* dihydroorotate dehydrogenase enzyme⁶. Earlier studies identified multiple chloroquine resistance-reversal compounds, including verapamil, amantadine, imipramine and chlorpheniramine; however, their clinical use for malaria has been prevented by issues of low efficacy in vivo, poor pharmacokinetic properties, or toxicity³. More recent ‘reversed chloroquine’ molecules, which combine a chloroquine-like quinoline ring with moieties designed to inhibit efflux, constitute a promising therapeutic avenue⁷. PfCRT structural analysis and binding and transport assays^{3,5,8} could aid the design of highly-specific PfCRT inhibitors. By utilizing molecular modelling, compound libraries could also be virtually screened against drug-sensitive and drug-resistant isoforms. Another approach would be to perform a whole-cell compound library screen against drug-resistant *P. falciparum* parasites with conditionally downregulated *pfcr*t expression, which would enable the identification of compounds that display increased potency in comparison with their activity against wild-type parasites. This strategy would build on the earlier demonstration that the metabolite desethyl-chloroquine displayed increased potency against parasites whose expression of *pfcr*t was conditionally downregulated using a TetR-aptamer system⁹.

Pursuing resistance drivers as drug targets is a validated concept in antimicrobial chemotherapy. The development of β -lactamase inhibitors salvaged the class of β -lactam antibiotics, although drug-resistant β -lactamases have also emerged. Researchers in the fields of antibacterial and antimycobacterial resistance or multidrug resistance in cancer are now turning to targeting efflux pumps as innovative targets to combat resistance¹⁰. PfCRT has low homology to human proteins and is essential for parasite survival, which are favourable attributes for a target irrespective of its role in resistance. We now have the tools to design and test highly-specific PfCRT inhibitors, which may be able to overcome some of the off-target effects that are likely to contribute to the toxicity seen with earlier chloroquine resistance-reversal agents. While the antimalarial drug discovery and development pipeline is rich, *P. falciparum* resistance is a significant threat². By drawing inspiration from our colleagues in bacterial and tumour resistance who are actively pursuing efflux pump inhibitors, we propose that targeting PfCRT could be an effective way to restore existing drugs and create new combinations with higher barriers to resistance.

References

1. van der Pluijm RW et al. Determinants of dihydroartemisinin-piperaquine treatment failure in *Plasmodium falciparum* malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. *Lancet Infect. Dis* 19, 952–961 (2019). [PubMed: 31345710]
2. Phillips MA et al. Malaria. *Nat. Rev. Dis. Primers* 3, 17050 (2017). [PubMed: 28770814]
3. Summers RL, Nash MN & Martin RE Know your enemy: understanding the role of PfCRT in drug resistance could lead to new antimalarial tactics. *Cell. Mol. Life Sci* 69, 1967–1995 (2012). [PubMed: 22286067]
4. Ross LS et al. Emerging Southeast Asian PfCRT mutations confer *Plasmodium falciparum* resistance to the first-line antimalarial piperaquine. *Nat. Commun* 9, 3314 (2018). [PubMed: 30115924]
5. Kim J et al. Structure and drug resistance of the *Plasmodium falciparum* transporter PfCRT. *Nature* 576, 315–320 (2019). [PubMed: 31776516]
6. Ross LS et al. Identification of collateral sensitivity to dihydroorotate dehydrogenase Inhibitors in *Plasmodium falciparum*. *ACS Infect. Dis* 4, 508–515 (2018). [PubMed: 29336544]

7. Gunsaru B et al. Simplified reversed chloroquines to overcome malaria resistance to quinoline-based drugs. *Antimicrob. Agents Chemother* 61, e01913–01916 (2017). [PubMed: 28193646]
8. Callaghan PS, Hassett MR & Roepe PD Functional comparison of 45 naturally occurring isoforms of the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT). *Biochemistry* 54, 5083–5094 (2015). [PubMed: 26208441]
9. Goldfless SJ, Wagner JC & Niles JC Versatile control of *Plasmodium falciparum* gene expression with an inducible protein-RNA interaction. *Nat. Commun* 5, 5329 (2014). [PubMed: 25370483]
10. Lamut A, Peterlin Masic L, Kikelj D & Tomasic T Efflux pump inhibitors of clinically relevant multidrug resistant bacteria. *Med. Res. Rev* 39, 246020132504 (2019).