CONCLUSIONS

1. Malaria parasites can acquire resistance to BS by epigenetic changes in the expression of *clag3* genes. These results are a proof of principle that parasites can acquire resistance to toxic compounds by epigenetic changes in gene expression.

2. Expression of alternative *clag3* genes results in differences in the transport efficiency of some solutes through PSAC, altering parasite sensitivity to drugs. Resistance to high drug concentrations requires simultaneous silencing of both *clag3* genes, an unexpected expression pattern that had not been previously described.

3. Selection of parasites with *clag3* transcriptional patterns that confer a better fitness in a given situation plays a role in the adaptation of malaria parasites to the presence of toxic compounds, which provides an example of adaptation by bet-hedging strategy in malaria parasites.

4. *P. falciparum* parasites in natural infections express one of the two *clag3* paralogues, consistent with the mutually exclusive expression property previously observed in lab-adapted parasites. Additionally, parasites preferentially express the same paralogue in human infections: *clag3.2*, at least in the studied population.

5. *clag3* epigenetic memory undergoes a reset after parasites go through transmission stages; when parasites exit the human liver they express either one or the other paralogue. Natural selection favours the survival of those parasites expressing the paralogue that confers the best fitness in the blood environment: *clag3.2*. Whether there is any natural condition in which expression of *clag3.1*, both or none of the *clag3* genes is more favourable is yet to be determined.

6. We have identified paralogue-specific conserved regions at the N-terminal end of CLAG3 sequences (NtCR). These regions may contribute to the preferential expression of CLAG3.2 in human infections.

7. The presence of the same toxic compound in the media selects for parasites expressing either *clag3.1* or *clag3.2,* depending on the parasite line. This observation suggests that the different transport efficiency for specific solutes between CLAG3 proteins is determined by the genetic background of the parasite, being probably defined by the most polymorphic regions (HVR).

8. Additionally to BS, uptake of other antimalarial compounds, such as T3, requires expression of *clag3* genes; thus, it could be affected by the drug resistance mechanism described here. Other

compounds that are suspected to use PSAC can reach their target despite the absence of CLAG3 proteins, suggesting that other genes, such as other *clag* family members, are involved in the activity of the channel.