Resistance to antimalarials - a pharmacogenomics approach for both parasite and human host.

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Objectives: To identify new genetic causality for Plasmodium falciparum resistance against present and in the pipeline antimalarials, considering interactions with the malaria patient pharmacogenetics. Ultimately, to identify new parasite molecular markers of drug suspetibility and human pharmacogene variation that fuel resistance development.

Methodology: The PhD project will be developed in three phases, constituting in all a full range training in malaria research with a omics approach as the driving force.

1. Northwest Angola has been recently recognized as the first robust focus of clinical resistance to Artemether-lumefantrine, the global mainstay for the management of malaria. We propose the performance of clinical trial at the Centro de Investigação em Saúde e Angola at Caxito, 80 Km North of Luanda, co-managed by the Calouste Gulbenkian Foundation . This trial will be specifically designed for the detection of resistance, and isolation of key biological/clinical material. In field determination of ex vivo susceptibility will be performed in an appropriate subset of the data, as well as PCR-based correction of treatment success (pfmsp1/2, glurp, WHO guidelines).

Training output: clinical trial design, field experience, ex-vivo drug resistance testing.

2. gDNA and RNA will be isolated from patients peripheral blood, upon sampling before and after treatment. The subset of recrudescent infections will have its exome sequenced (30x) at Karolinska Institutet in direct link with the Swedish National Genomics Infrastructure (Stockholm, Sweden) . Potential resistance markers will be then analysed in the remaining infections through ultra-deep focused sequencing (300x) for omics based analysis of infection population structure.

Day 7 plasm antimalarial drug levels will be determined through LC-MS-MS methodologies at Sahlgrenska Academy, Göteborg University, Göteborg, Sweden (Prof. M. Ashton) , through ongoing collaborations with Karolinska Institutet. Such pharmacologic phenotype will be key for purposeful association analysis –with the unveiled molecular marker candidates. Further, a full set of ADME genes (phase 0-III, plus drug targets, n= ≈150) will be NG sequenced for relevant pharmacogenetics/pharmacokinetic/pharmacodynamics associations.

Training output: pharmacogenomics and transcriptomics methods and analysis, including a novel approach for the evaluation of mixed infections in malaria with applications in other diseases.

3. Relevant parasite markers of resistance will be in vitro tested through allele modification approaches with novel CRISPR based methods adapted for Plasmodium falciparum genomics (ICVS, Universidade do Minho). Resulting parasites will be tested (in vitro, high throughput cell sorter based IC50 determination) for a large range of antimalarial, including compounds presently at phase II/III clinical trial testing (e.g. KAF156, KAE609 and OZ439).

Training output: cut of the edge genetic modification technology for Plasmodium falciparum. Training on drug resistance evaluation in vitro.

Type of fellowship: Mixed (Portugal and abroad): BioISI (Lisbon, Portugal), CISA/FCG (Caxito, Angola), Karolinska Institutet (Stockholm, Sweden); Sahlgrenska Academy - Göteborg University (Göteborg, Sweden); Universidade do Minho (Braga, Portugal).