MALARIA, one of the top three infectious diseases in the world, CAN ONLY BE ERADICATED WITH THE CRUCIAL CONTRIBUTION OF AN EFFECTIVE VACCINE [1]. Although some limited success has recently been achieved using sub-unit vaccines consisting of a single, or a limited number of malaria-parasite proteins/antigens expressed in heterologous systems, A FULLY EFFECTIVE ANTI-MALARIA VACCINE REMAINS A PRESSING AND, AS YET, UNMET NEED [2].

Our proposal looks to address this need by genetically modifying the malaria parasite, Plasmodium, to express antigens such that these parasites can be used to provide potent stage-transcending protection against malaria. This is in complete alignment with the recently renewed WHO STRATEGIC GOAL that calls for the DEVELOPMENT OF A MALARIA VACCINE THAT SIMULTANEOUSLY PREVENTS DISEASE, DEATH AND TRANSMISSION [3]. Such a tool requires the combination of antigens from pre-erythrocytic liver stages (PE), asexual blood stages, sexual and mosquito stages of parasite development in a single multi-component, multi-stage vaccine that IMPACTS ALL LIFE STAGES OF THE PARASITE (Fig.1).

Studies in animal models and in humans have shown that WHOLE-ORGANISM PRE-ERYTHROCYTIC (WOPE) VACCINES ARE, SO FAR, THE SOLE STRATEGY CAPABLE OF CONVEYING COMPLETE PROTECTION AGAINST MALARIA [4]. Such approaches are based on the generation of immunity by live sporozoites, the parasite form injected by mosquitoes into vertebrate hosts, rendered safe by attenuation or chemoprophylaxis [5]. Subunit vaccines have lower efficacy than their WOPE counterparts because the former generate immune responses to only a limited number of antigens, which may have many different isoforms in the population. Moreover, Plasmodium proteins are notoriously difficult to express in common protein expression systems, failing to fold or to be correctly modified post-translationally [6]. On the contrary, WOPE vaccines provide a vast array of correctly folded antigens. However current WOPE vaccine candidates target solely the PE stages of parasite development, and do not take advantage of blood-stage driven immunity to reduce parasite burden and protect against disease, nor of a transmission-blocking immunity to increase herd immunity [7].

Our HYPOTHESIS is that the parasite itself is the most appropriate vehicle to express Plasmodium antigens and that the protective immunity induced by WOPE vaccination will be enhanced if sporozoites express correctly folded and modified Plasmodium proteins that are important vaccine targets for blood and transmission vaccines (Fig.1).

We propose to use an innovative strategy for genetic modification (GM) of parasites TO SUBSTANTIALLY EXPAND THE RANGE OF SPECIFIC ANTIGENS PRESENTED BY WOPE VACCINES. By introducing in rodent or human parasites used for WOPE vaccination a broad selection of major vaccine candidate antigens from the P. falciparum (Pf) asexual, sexual and mosquito stages (PfASM), we aim to ultimately ENSURE SIMULTANEOUS BLOCKAGE OF ALL STAGES OF THE PARASITE LIFE CYCLE BY WOPE VACCINES (Fig.1).

Our project stems from A SOLID ARRAY OF PRELIMINARY RESULTS demonstrating that: (i) our GM strategy ensures robust expression of Pf antigens during sporogonic and PE development; (ii) GM parasites used in WOPE immunization are able to induce a strong immune response which recognizes and binds Pf; (iii) the immune response induced by GM parasites can actively inhibit Pf infection.

The PROJECT'S STRATEGIC AIMS are to: (i) optimize the expression of PfASM antigens in GM parasites for WOPE malaria vaccines; (ii) evaluate the immunogenic potential of newly generated GM parasites as multi-stage WOPE vaccines; (iii) assess functional blockage of Pf development and transmission upon multi-stage WOPE vaccination with GM parasites.

To achieve these aims, we will employ STATE-OF-THE-ART METHODOLOGIES available at the host and collaborating institutions. These methods will be applied to established rodent models, as well as to vitro cultures of human malaria parasites, covering all stages of the parasite's life cycle [8].

The project combines AN UNIQUE SET OF EXPERTISES, bringing together the expertise of the Prudencio Lab (IMM, Portugal) at unveiling mechanisms for the establishment of efficient immune responses against malaria [8, 9], and those of our world renowned collaborators, which include pioneers in the genetic modification of Plasmodium parasites [10] and experts in WOPE vaccines [5] (Dr. Chris Janse & Dr. Shahid Khan, LUMC, Netherlands); world experts in Plasmodium blood stage development and vaccine discovery [11] (Dr. Gavin J. Wright & Dr. Cecile Crosnier, WT Sanger Inst., UK); and the world's reference laboratory for functional assessment of malaria vaccines [12] (Dr. Carole A Long & Dr. Kazutoyo Miura, NIH/NIAID, USA).

We are certain this project will set a NEW STANDARD IN THE DEVELOPMENT OF MULTI-STAGE MALARIA VACINNES that may bring hope to millions of people to whom this disease poses an unbearable burden.