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Development of the RTS,S/AS malaria candidate vaccine

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ABSTRACT

A vaccine against malaria which complements existing control tools is an urgent medical need. RTS,S/AS, a pre-erythrocytic candidate vaccine, which targets the circumsporozoite protein, is the most advanced in clinical development. The safety, immunogenicity and efficacy of this candidate vaccine have been investigated in a series of trials in children and infants in endemic African countries. The vaccine shows promise for providing important public health benefits and a multicenter Phase III trial has started in Africa, aiming to further characterize the efficacy of the candidate vaccine and generate the regulatory data required for the licensing approval of the vaccine.

1. Malaria vaccine: an unmet medical need

The availability of a malaria vaccine would contribute greatly to the efforts aimed at controlling this major threat to global health. It is estimated that in 2007, more than 2.37 billion people were living in areas where *Plasmodium falciparum* malaria transmission occurs [1]. Sub-Saharan Africa carries the highest burden of the disease, with an estimated 250 million cases and nearly 1 million deaths each year [2,3]. Malaria represents a huge burden on health care systems and economical losses [4]. Over 80% of fatalities occur in African children under the age of 5 [4] justifying the development of a vaccine targeting primarily this vulnerable population.

Recently, hopes for the possibility of long term control, elimination and even eradication of the disease have re-emerged, encouraged by important reductions in malaria burden in countries, such as Rwanda, Zambia, Madagascar, Zanzibar and The Gambia where large scale malaria control programs have been implemented [3]. These recent successes are great news, but no reason for complacency. Currently implemented malaria control strategies include interventions such as insecticide-treated net, insecticide residual spraying, intermittent preventive treatment to pregnant women, and Artemisin based combination treatment. Unfortunately, both the malaria parasite and the transmitting mosquitoes have shown a very high capacity to develop pharmaceutical escape mechanisms and drug resistance, as demonstrated by the previously failed World Health Organisation (WHO) malaria eradication program, also based on malaria drugs and mosquito control. The addition of a vaccine to the anti-malaria arsenal is an urgent medical need, and may possibly contribute to reduce the risk of emergence of parasite drug resistance, thereby increasing the sustainability of existing malaria control strategies.

2. The RTS,S/AS malaria candidate vaccine construct

The most advanced malaria vaccine candidate is RTS,S formulated with GSK proprietary Adjuvants Systems (AS) AS01 or AS02. This candidate vaccine has been in development for over 20 years, and since 2001 under the leadership of a public-private product development partnership between GSK Biologicals and the PATH Malaria Vaccine Initiative (MVI), with support from the Bill and Melinda Gates Foundation. The overall objective of the RTS,S/AS program is to reduce the burden of disease related to *P. falciparum* malaria in infants and children residing in sub-Saharan African malaria-endemic countries. The vaccine would ideally be implemented through the existing infant vaccine delivery program, the Expanded Program on Immunization, in conjunction with other malaria control interventions.

The vaccine candidate targets the pre-erythrocytic phase of the parasite life cycle, comprising the sporozoite and liver stages. Sporozoites, which are injected in the circulation following a bite from an infected mosquito, rapidly target the liver, and invade hepatocytes. The parasite then develops into a schizont containing 10,000–30,000 merozoites, which are later released from the liver into the blood, whereby the disease-associated erythrocytic phase of the infection is initiated.

The target antigen is the circumsporozoite protein (CS), a 412 amino acids protein abundantly associated with the sporozoite surface, also expressed by liver forms and exported in the cytoplasm of hepatocytes. It has a characteristic central conserved NANP repeat region and non-repeat flanking regions with both conserved and

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variable segments. This protein is thought to play a role in the entry of the parasite in the liver through an interaction with liver sinusoidal heparan sulphate proteoglycans, and interference with the hepatocyte cellular processes, down regulating NF-KB mediated inflammation, thereby creating a favourable niche for the parasite and allowing the development of the liver stage [5].

The feasibility of developing a pre-erythrocytic vaccine was demonstrated in the late 1960s, when it was shown first in rodents then in humans that immunization with irradiated sporozoites can induce complete protection against experimental sporozoite challenge [6,7]. In this immunization model, the CS has been shown to be a major target of protective immunological effectors [8]. Both CS-specific antibodies and T cells have been shown to be implicated in protection [9]. Anti-CS antibodies can block sporozoite liver cell invasion in vitro [10] and prevent experimental infection in animals [11]. Passive transfer of both CS-specific CD4 and CD8 T cells can protect animals from experimental infection [11,12]. In humans the activation of CD4+ T cells and cytotoxic CD8 T cells has been reported [13–15].

Initial candidate vaccine constructs targeting only the central repeat region of the CS protein failed to provide substantial protection [16,17]. The recruitment and activation of T cells through the development of innovative technologies became a key objective of the team working collaboratively at the Walter Reed Army Institute of Research and GSK Biologicals in the 1980s. A new vaccine construct was generated [18] based on a large segment of CS (Amino Acids 207–395 of the CS from the NF54 *P. falciparum* strain) that included 19 NANP conserved repeats and the C-terminal part of the non-repeat region (excluding the hydrophobic membrane anchor) known to contain T cell epitopes, and covalently bounded to the HBs antigen (adw serotype), the hepatitis B vaccine antigen. When recombinantly expressed in *Saccharomyces cerevisiae* the fusion protein (RTS), together with a free transcript of the hepatitis B antigen (S), spontaneously assemble to form virus-like particles, known to favour antigen presentation to the T cell compartment [19,20].

The vaccine construct was formulated with two Adjuvant Systems, AS01 and AS02, shown to induce both strong humoral and cellular immune responses. Both include the immunostimulants MPL and QS21. The monophosphoryl lipid A molecule MPL consists of a chemically detoxified form of the parent lipopolysaccharide (LPS) from the Gram negative bacterium *Salmonella minnesota*. QS21 is a natural saponin molecule purified from the bark of the South American tree, *Quillaja saponaria*. AS02 contains in addition an oil in water emulsion while AS01 contains a liposomal suspension [21].

3. Early RTS,S/AS02 evaluation in adults

Early clinical development of the RTS,S malaria candidate vaccine was initiated in studies in malaria-naïve adults in collaboration with the Walter Reed Army Institute of Research (WRAIR). The first challenge study demonstrated the importance of the adjuvant in the generation of protective immunity. Volunteers vaccinated with three different adjuvant formulations of RTS,S and controls were subjected to experimental sporozoite challenge. Out of seven volunteers vaccinated with the RTS,S/AS02 formulation, six were protected against challenge, while the two other formulations protected one of eight and two of seven participants. Immunological analysis showed that as compared to the other formulations the RTS,S/AS02 induced a high level of anti-CS and anti-HBs antibodies as well as IFN-γ producing T cells. When re-challenged 6 months later, out of seven protected volunteers in the initial challenge experiment (across the three vaccine formulations tested) who accepted to participate to a second challenge, two were protected again [22–24]. Across several challenge studies conducted over the years, it was consistently shown that RTS,S/AS02A vaccination provides protection against sporozoite challenge of a magnitude of 40%. It was observed that in addition to providing full protection (sterile protection, characterised by the absence of parasitemia after challenge) in some of the vaccinated individuals, the development of parasitemia in the breakthrough cases was delayed for 48 h or longer compared to non-vaccinated controls. It is hypothesized that this delay reflects a diminished merozoite release from the liver. Such a decrease in the parasite load initiating the blood stage may allow the immune system to better cope with the growing parasitemia, with a possible impact on the development of associated symptoms, and decrease the risk of progression towards the more severe forms of the disease [25].

The results of the challenge studies led to evaluation of RTS,S/AS02 in malaria-endemic regions.

Study designs in malaria efficacy trials, in conditions of natural malaria exposure

Several study designs can be used to assess efficacy of a malaria candidate vaccine in conditions of natural malaria exposure. Evaluation of efficacy against rare endpoints such as severe malaria is relevant to public health but requires large sample size and is reserved for late vaccine evaluation. Early in vaccine development programs, studies focus on more frequent events like occurrence of *P. falciparum* infection, defined as the presence of *P. falciparum* parasites in the blood, or uncomplicated malaria disease, defined as *P. falciparum* parasitemia above a specific threshold in a participant that is unwell with fever. This inclusion of a parasitemia threshold increases the specificity of the case definition [26]. In the following sections, Active Detection of Infection (ADI) will be referred to when investigators screen study participants on a regular basis, looking for parasitemia on a blood slide. Passive Case Detection (PCD) is referred to when cases are captured when a child who is unwell is brought for treatment in a health facility. Active Case Detection (ACD) is when investigators visit study participants on a regular basis, measure temperature and do a blood slide if fever is present.

The first efficacy study under conditions of natural exposure started in 1998 in The Gambia, a region of seasonal malaria transmission. The vaccine prevented 34% (95% CI: 8–53; $p = 0.014$) of infections over a surveillance period of 15 weeks during the malaria season [27]. Protection was shown not to be strain-specific [28]. When a booster dose of the vaccine was given before the subsequent malaria transmission season a year later, there was a 47% protection against infection over 9 weeks [27]. The follow-up safety evaluation, over a 5-year period, was favourable [29].

4. RTS,S/AS02 evaluation in children and infants, in malaria-endemic countries

The evaluation of RTS,S/AS02 progressed to the paediatric population initially with age de-escalation and dose optimization studies [30,31]. A large trial in Mozambican children aged 1–4 years was then started, enrolling a total of 2022 subjects. The study included two cohorts. In the first, largest cohort, efficacy against clinical malaria was assessed by PCD. Over the first 18 months of follow up the efficacy against clinical malaria was 35.3% (95% CI: 21,6, 46.6; $p < 0.0001$). The study also showed a 48.6% (95% CI: 12.3, 71.0; $p = 0.02$) reduction in the number of RTS,S/AS02 vaccinated participants having developed severe malaria, as compared to controls [32]. The follow up of study participants continued, and showed over 43 months an efficacy of 25.6% (95% CI: 11.9–37.1; $p < 0.001$) in prevention of clinical malaria episodes, and the absence of rebound in malaria or severe malaria occurrence in the vaccinated group. At the end of the 43 months post vaccination follow up, the prevalence