Two Decades of Commitment to Malaria Vaccine Development: GlaxoSmithKline Biologicals

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Abstract. GlaxoSmithKline Biologicals (GSK) is committed to the development of a safe and effective malaria vaccine. Its research program in this field was initiated in 1984 and has been continuously active to this day, making it unparalleled within the vaccine industry. Although it works in partnerships with several leading organizations from the public sector, this effort has required GSK to invest major financial and human resource commitments, and its partners rely heavily on the company's global infrastructure and expertise in research, advanced clinical development, regulatory, large-scale manufacturing, and commercialization. Through GSK's pioneering business model and working in partnership with global vaccine funding agencies, the company is committed to seeing that, once approved, a safe and effective malaria vaccine will be available to everyone that needs it.

INTRODUCTION

GlaxoSmithKline Biologicals (GSK), one of the world's leading vaccine manufacturers, is devoted to discovering new vaccines that are cost-effective, convenient, and designed to prevent infections that cause serious medical problems in both the developing and developed world. In 2006, GSK distributed 1.1 billion doses of vaccines, 75% of which went to the developing world. Through pioneering approaches such as tiered-pricing and public-private partnerships, it insures that the vaccines it produces reach the people that need them the most. It should not be surprising that GSK is the only global manufacturer developing vaccines for the world's "big three" infectious diseases: malaria, HIV, and tuberculosis. This review focuses on the company's > 20-year quest to develop a safe and effective malaria vaccine and shows how innovative technology, committed scientists, and dogged persistence have come together in pursuit of this important goal. It is a story that is not yet over.

EARLY RESEARCH AND DEVELOPMENT

As with many of its vaccines, GSK's strategy to develop a malaria vaccine was based on extensive research conducted in the academic sector beginning in the 1960s, which revealed that hyperimmunization with radiation-attenuated sporozoites could protect rodents, primates, and ultimately human volunteers from malaria infection.1–3 The circumsporozoite protein (CSP), a sporozoite surface antigen, was identified as the likely target of the protective immune responses, and the gene encoding the CSP of Plasmodium falciparum was cloned and sequenced by scientists at the US National Institutes for Health and the Walter Reed Army Institute of Research (WRAIR), thereby ushering in the era of subunit vaccine development for malaria.4–6

In early 1984, WRAIR entered into an important collaboration with GSK to produce a malaria vaccine using GSK's recombinant Escherichia coli expression systems. Although efforts to produce a full-length CSP were unsuccessful, a series of alternative constructs were produced.7–12 Studies using synthetic peptides had mapped the epitope of protective monoclonal antibodies to the central repeat region of the P. falciparum CSP,13 and four constructs based on these sequences were expressed and purified to preclinical grade: R16tet32, R32tet32, R48tet32, and R32LR.7 All four were tested for immunogenicity in animals, and R32tet32 was selected for clinical development as the world's first malaria vaccine candidate. A Phase I/IIa challenge trial of R32tet32 formulated with alum (FSV-1) evaluated a dose range of 10–800 µg CSP. The vaccine was shown to be safe, but it was poorly immunogenic. Nevertheless, the team decided to carry out an experimental sporozoite challenge of a subset of these volunteers.14 Although the overall efficacy was disappointing, one volunteer was completely protected, marking the first time a human was protected from malaria using a subunit vaccine. Believing that it would ultimately be necessary to test this vaccine under field conditions, WRAIR developed its first malaria vaccine testing site in Saradidi, a small village in western Kenya, where FSV-1 was tested for safety and immunogenicity in malaria experienced adult volunteers. Significant anti-CS responses were observed in a majority of subjects,15 but more importantly, this marked the beginning of the Walter Reed Project laboratories that continue to be active in malaria research to this day.

Over the next several years, a series of P. falciparum, P. vivax, and P. falciparum/P. vivax combination constructs were produced by GSK that incorporated the NS1<sub>1341</sub> protein from influenza to provide T-cell help: R32NS<sub>1341</sub>, R32V20NS<sub>1341</sub>, V20R32NS<sub>1341</sub>, and NS<sub>1341</sub>V20. All four antigens were tested in preclinical models for immunogenicity at WRAIR, R32NS<sub>1341</sub> and NS<sub>1341</sub>V20 were selected for further clinical development, and a Phase I trial for NS<sub>1341</sub>V20 was conducted.16 However, immunogenicity was low, and no further clinical development was done. The NS<sub>1341</sub>V20 antigen was used for primate studies and seroepidemiology studies of human P. vivax infection.17–19

In parallel, WRAIR did a Phase I/IIa challenge trial of R32NS<sub>1341</sub> on alum (FSV-2). FSV-2 was more immunogenic than FSV-1, but it failed to protect any of the volunteers.20 Believing that stronger adjuvantation would make a difference, a Phase I trial of R32NS<sub>1341</sub> in liposomes plus 3-deacylated monophosphoryl lipid A (MPL) and a Phase I/IIa challenge trial of FSV-2 formulated with the Ribi Detox adjuvant (a lyophilized oil drop emulsion consisting of the cell wall and cytoskeleton of Mycobacterium phlei and MPL) were conducted, but again the results were disappointing.20,21

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Preclinical studies suggested that CS epitopes lying outside the central repeat region might also be involved in protection mediated by cellular immunity. Therefore, a “repeletless” version of CS NS1agRLF was produced and formulated with liposomes plus MPL, but the vaccine showed no protection in a challenge trial.

During this same period, GSK, WRAIR, and the Swiss Serum Institute collaborated to determine whether better immunogenicity could be achieved with a conjugate vaccine. Thus, recombinant R32LR was conjugated to cholera toxin (CT) or ToxA from *Pseudomonas aeruginosa*. Preclinical results favored the R32ToxA construct on alum, and a Phase I/IIa trial of R32ToxA was done. To rule out the possibility that the experimental challenge might be underestimating efficacy in the field, Phase Ib field trials of R32ToxA were conducted in Thailand and Kenya, but neither study showed vaccine efficacy, and the candidate was abandoned.

Meanwhile, GSK and WRAIR continued to work on the basic research front to pursue alternative strategies. Several constructs were produced for preclinical studies using *P. berghei* and *P. yoelli* CS and the asexual stage antigens GP195 and GPB. DGIgM, a human monoclonal derived from the first protected volunteer immunized with FSV-1, was also produced for preclinical studies. Efforts to convert DGIgM into an IgG for prophylaxis were unsuccessful. GSK also collaborated to develop an oral malaria vaccine based on recombinant *Salmonella typhimurium* expressing the CSP of *P. berghei*. Although protection was seen in mice, a stable *P. falciparum* *Salmonella* candidate could not be produced.

In 1987, the GSK malaria vaccine program was transferred from its laboratories in Philadelphia, PA, to its vaccine division in Belgium to benefit from the research and development expertise in the field of vaccinology that existed in the Belgian division.

**MORE RECENT RESEARCH AND DEVELOPMENT**

GSK Biologicals scientists pioneered the use of the hepatitis B surface antigen (HBsAg) as a carrier matrix for the central repeat region of the *P. falciparum* CSP. An initial construct, R16-HBsAg, was abandoned after Phase I testing, and a more promising CSP-HBsAg fusion protein was developed that also incorporated the CSP C-terminal region containing B- and T-cell epitopes into a chimeric gene expressed in *Saccharomyces cerevisiae*. This construct was named “RTS,S,” indicating the presence of the CSP repeat region (R) and T-cell epitopes (T) fused to the hepatitis B surface antigen (S), co-expressed and self-assembled with unfused S antigen.

Although the particulate nature of RTS,S was an important advance, it was the development of GSK’s innovative adjuvant systems that was critical to the success of this vaccine. The first clinical trial of RTS,S evaluated two formulations, RTS,S with alum and RTS,S with alum plus MPL adjuvant system (AS04), for safety and efficacy in malaria-naïve adults at WRAIR. Both vaccines proved to be safe and well tolerated, but RTS,S/AS04 was more immunogenic. After sporozoite challenge, zero of six RTS,S/alum vaccines and two of eight RTS,S/AS04 vaccines were protected. GSK and WRAIR then initiated a comparative preclinical safety and immunogenicity evaluation of six GSK proprietary adjuvant systems with RTS,S in rhesus monkeys to improve both humoral and cell-mediated immunogenicity. An adjuvant system formulation made up of an oil-in-water emulsion plus MPL and QS21 (AS02A) showed the best antibody and CMI (delayed-type hypersensitivity) responses. In 1996, RTS,S formulated with AS04, AS03 (oil-in-water emulsion alone) or AS02A were tested in a human challenge trial. The two emulsion-based formulations were equally immunogenic in terms of humoral response, but the AS02A formulation was the most effective against sporozoite challenge, protecting 67 volunteers. A rechallenge 6 months later found one of five previously protected RTS,S/AS02A-immunized volunteers were still protected. Dose optimization studies at WRAIR confirmed that two or three doses of RTS,S/AS02A consistently confer complete (sterile) or partial (significant delays in the prepatent period) immunity in most vaccinees who undergo experimental challenge. Immunologic analyses of protected and non-protected subjects were consistent with the hypothesis that both functional antibody responses and CD4+ T cells expressing interferon (IFN)-γ play an important role in protection.

Stability testing revealed a limited shelf life of liquid RTS,S in the AS02A adjuvant, so RTS,S was reformulated as a lyophilized antigen for reconstitution with AS02A. A comparative trial showed equivalent safety and immunogenicity of the lyophilized and lyophilized formulations, and the lyophilized formulation provided equivalent protection against experimental malaria challenge. Subsequent trials of RTS,S have used the lyophilized version.

During initial trials, RTS,S/AS02A was administered on a 0-, 1-, 6- to 9-month schedule. Later, a Phase 2a trial at WRAIR compared the safety, immunogenicity, and efficacy of lyophilized RTS,S formulated with AS02A on two different accelerated schedules. Forty volunteers received a full dose of RTS,S/AS02A on a 0-, 1-, 3-month schedule or on a 0-, 7-, 28-day schedule. After sporozoite challenge, 45% and 38%, respectively, of vaccinees were protected against malaria, and there was no increase in reactogenicity versus historical controls (KE Kester, unpublished results).

A Phase I trial of RTS,S/AS02A on a 0-, 1-, 3-month schedule in The Gambia in healthy adult males showed that the vaccine was safe and immunogenic. Afterward, a Phase Ib efficacy trial showed a 34% reduction in the incidence of first parasitemia (95% CI, 8–53%; P = 0.014) over a 16-week follow-up period. Subsequently, in 2001, GSK and the Malaria Vaccine Initiative at PATH (MVI) entered into a partnership for the pediatric clinical development of RTS,S/AS02A. Initial studies by the Medical Research Council in The Gambia and Centro de Investigação em Saúde da Manhiça (CISM) in Mozambique showed the vaccine to be safe and immunogenic in children 1–11 years of age. A Phase 2b efficacy study conducted by CISM and the University of Barcelona in Mozambique, showed that, over a 6-month period in 1- to 4-year-old children, RTS,S/AS02A conferred 30% (95% CI, 12–45%; P = 0.004) efficacy against first clinical episodes and 58% (95% CI, 16–81%; P = 0.019) efficacy against severe malaria caused by *P. falciparum*. Efficacy was maintained up to 18 months after Dose 3. These results were a major milestone because they were the first conclusive demonstration of sustained protection against malaria combined with substantial prevention of severe malaria by a malaria
vaccine. These children will be followed for safety, immunogenicity, and efficacy until at least 45 months after Dose 1.

More recent studies have compared safety and reactogenicity of RTS,S/AS02A with new adjuvant systems using animal models. In studies in rhesus monkeys, one alternative adjuvant system, AS01B (liposomes, MPL, QS21), induced a sustained and improved RTS,S-specific IFN-γ ELISPOT response and an equivalent antibody response to that induced by RTS,S/AS02A. Additionally, a Phase 2a trial in 104 (3D7 and FVO) and in 291 subjects after primary challenge (KE Kester, personal communication). 3D7 MSP-142 was subsequently studied in a Phase I/IIa challenge trial including a co-administration group that also received RTS,S/AS01B than recipients of RTS,S/AS02A and in subjects who were protected compared with non-protected subjects after primary challenge (KE Kester, personal communication).

Evaluation in adults continued with a Phase 2b trial of RTS,S/AS01B and RTS,S/AS02A (versus control) in 255 adults living in a malaria-endemic area of Kenya. Again, RTS,S/AS01B was significantly more immunogenic than RTS,S/AS02A for anti-CS antibodies while retaining a good safety profile. Both vaccines were equally efficacious against malaria (ME Polhemus, personal communication). After these encouraging results in adults, RTS,S/AS01E, a version of the vaccine specifically reformulated for administration to children as part of the Expanded Program on Immunization of the WHO, is undergoing a series of Phase II clinical trials with partner institutions in Africa (Figure 1) in preparation for a major Phase III pediatric program to begin in 2008, with the aim of regulatory filing by 2011.

**ALTERNATIVE ANTIGENS FOR COMBINATION AND PRIME-BOOST STRATEGIES**

In an attempt to improve on RTS,S, combination vaccines incorporating RTS,S with one or more additional pre-erythrocytic or blood stage antigens also have been under examination.

Thrombospondin related anonymous protein (TRAP), a sporozoite and liver stage antigen with promising preclinical data in rodent models, was expressed at GSK using a baculovirus system. A manufacturing process was developed leading to the production of good manufacturing practice lots of the TRAP antigen. Preclinical studies focused on coformulation with RTS,S/AS02A, and safety and immunogenicity studies were performed in primates. Parallel to a Phase I safety and immunogenicity trial conducted in Belgium, a Phase I/IIa challenge trial comparing TRAP alone and TRAP coformulated with RTS,S was done at WRAIR. No protection was seen among volunteers receiving TRAP alone, and there was a significant loss of protection in the RTS,S coformulation group (KE Kester, personal communication).

Pfs16, a pan-blood stage antigen that seems necessary for successful gametocytenogenesis, was used to create particulate structures using the same HBsAg expression system as for RTS,S and was found to be immunogenic in preclinical studies. However, there was no transmission blocking activity, and research and development work halted. Recent findings on the biology of Pfs16 suggest that this antigen may deserve additional study.

Additionally, GSK, MVI, and WRAIR have developed a pre-erythrocytic recombinant vaccine based on a liver stage antigen (LSA-1). Preclinical toxicology studies with both AS01B and AS02A were performed, and a Phase I/IIa challenge trial has recently been conducted, in which both formulations were tested. However, neither vaccine showed efficacy (J Cummings, personal communication).

GSK assisted WRAIR in the development of E. coliexpressed blood stage antigens that might be combined with RTS,S. To date, two allelic forms of MSP-1e2 (3D7 and FVO) and two allelic forms of AMA-1 (3D7 and FVO) have been produced by WRAIR and formulated with GSK adjuvants. The 3D7 MSP-1e2 vaccine was studied preclinically and in a Phase I clinical trial. In a subsequent Phase I/IIa challenge trial including a co-administration group that also received RTS,S/AS02A, no interference with RTS,S was observed, but 3D7 MSP-142 did not protect against infection in malari naive subjects (J Cummings and C Ockenhouse, personal communication). 3D7 MSP-142 was subsequently studied in a...
Phase Ib trial in adults [ref below: Withers] and a Phase IIb proof-of-concept trial supported by MVI and USAID in Kenya.

Phase I trials of AMA-1/AS02A have been completed at WRAIR and in Mali, and a Phase Ib trial is underway in Mali, partially supported by the NIH. A Phase IIa challenge of AMA-1–immunized subjects is underway, partially supported by MVI. If protection (sterile or delay) is observed, co-formation studies would be the next step.

GSK has also studied “prime-boost” regimens involving CS delivered by different platforms, including DNA vaccination (in collaboration with the US Navy Medical Research Center) or recombinant MVA (modified vaccinia virus, Ankara; with Oxford University). In both cases, heterologous priming did not result in significant improvements in immune responses, and no evidence of enhanced efficacy was observed in a challenge trial involving recombinant MVA and RTS,S/AS02A.

More recently, a collaboration between Crucell, GSK, and WRAIR tested an adenovirus 35 CSP vaccine directed at P. falciparum and a combination of Ad 35 CS and RTS,S in a prime/boost regimen. Preclinical evaluations of Ad 35 CS alone and in combination with RTS,S in the rhesus monkey safety and immunogenicity model at WRAIR revealed impressive priming by Ad 35 CS for CD4+ IFN-γ T-cell responses that were boosted by RTS,S/AS01B.

**TOWARD THE FUTURE**

Despite the enormous progress achieved with RTS,S, GSK and its partners continue to work to evaluate potential new antigens for use as improved formulations with the RTS,S antigen or as vaccine candidate antigens in their own right. Equally important, working in partnership with MVI and others, GSK is strengthening the clinical trials capacity at a number of potential study sites in sub-Saharan Africa. In the process, many bright young African scientists are gaining valuable experience working on malaria vaccine trials, and a new generation of African researchers, fully capable of leading their own research programs, is emerging. Certainly this must be one of the most rewarding outcomes of all.

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Disclosure: W. Ripley Ballou and Conor Cahill are employees of GlaxoSmithKline Biologicals. W. Ripley Ballou holds shares in GlaxoSmithKline. Conor Cahill is a professional medical writer at GSK Biologicals. W. Ripley Ballou is listed as an “inventor” of patented malaria vaccines; however, he does not hold a patent for a malaria vaccine.

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