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Progress with *Plasmodium falciparum* sporozoite (PfSPZ)-based malaria vaccines

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Abstract

Sanaria Inc. has developed methods to manufacture, purify and cryopreserve aseptic *Plasmodium falciparum* (Pf) sporozoites (SPZ), and is using this platform technology to develop an injectable PfSPZ-based vaccine that provides high-grade, durable protection against infection with Pf malaria. Several candidate vaccines are being developed and tested, including PfSPZ Vaccine, in which the PfSPZ are attenuated by irradiation, PfSPZ-CVac, in which fully infectious PfSPZ are attenuated *in vivo* by concomitant administration of an anti-malarial drug, and PfSPZ-GA1, in which the PfSPZ are attenuated by gene knockout. Forty-three research groups in 15 countries, organized as the International PfSPZ Consortium (I-PfSPZ-C), are collaborating to advance this program by providing intellectual, clinical, and financial support. Fourteen clinical trials of these products have been completed in the USA, Europe and Africa, two are underway and at least 12 more are planned for 2015–2016 in the US (four trials), Germany (2 trials), Tanzania, Kenya, Mali, Burkina Faso, Ghana and Equatorial Guinea. Sanaria anticipates application to license a first generation product as early as late 2017, initially to protect adults, and a year later to protect all persons >6 months of age for at least six months. Improved vaccine candidates will be advanced as needed until the following requirements have been met: long-term protection against natural transmission, excellent safety and tolerability, and operational feasibility for population-wide

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administration. Here we describe the three most developed whole PfSPZ vaccine candidates, associated clinical trials, initial plans for licensure and deployment, and long-term objectives for a final product suitable for mass administration to achieve regional malaria elimination and eventual global eradication.

Keywords

Malaria vaccine; *Plasmodium falciparum*; Sporozoite; PfSPZ Vaccine; PfSPZ-CVac; PfSPZ Challenge

1. Background

The *Plasmodium* sporozoite (SPZ), transmitted to the human host by female *Anopheles* mosquitoes, is an attractive vaccine candidate. If immune responses induced by such a vaccine could kill the SPZ during its journey from the mosquito proboscis to the liver or during development in the liver (pre-erythrocytic stages of the parasite life cycle), there would be no blood stage infection and no production of gametocytes. This would address an urgent public health priority, namely protecting people in endemic areas from clinical malaria, especially those susceptible to severe disease such as infants and young children. A pre-erythrocytic stage vaccine would also benefit travelers, obviating the difficulties of prophylactic drug compliance and side effects. Ultimately, a highly effective pre-erythrocytic stage vaccine, serving as a vaccine to interrupt malaria transmission (VIMT), would be the ideal tool to eliminate malaria and contain the spread of malaria parasites from defined geographic regions, leading to global eradication [1].

1.1. Targeting the sporozoite

Several approaches have been taken to induce protective immunity against SPZ. Forty years ago injection of attenuated whole SPZ was shown to protect rodents against malaria infection, followed quickly by proof-of-concept that the same approach works in humans (Box 1). However, at that time SPZ had to be administered to humans by mosquito bite, limiting feasibility as a vaccine approach. The alternative, producing large numbers of infected mosquitoes, harvesting the SPZ, and purifying them from contaminating salivary gland antigens, also appeared to be difficult if not impossible, especially as the purified sporozoites would have to be aseptic for injection. All in all, immunization with whole sporozoites was felt to be "too crude and impractical to produce a vaccine for wide application" [27].

1.2. Whole sporozoites for parenteral injection

Sanaria Inc., a biotechnology company in Rockville, Maryland, spent the last 10 years addressing the challenges of production, purification and cryopreservation, and now routinely manufactures vials of highly purified, aseptic *P. falciparum* (Pf) SPZ that are in compliance with regulatory standards for purity, potency, safety and consistency [28]. 2155 doses of PfSPZ (doses as high as 2.2 million PfSPZ) have now been administered to 824 adults by the intradermal (ID), subcutaneous (SC), intramuscular (IM), intravenous (IV) or direct venous inoculation (DVI) routes, and have shown excellent safety and tolerability. In blinded, randomized, placebo-controlled trials, the PfSPZ have not caused any detectable systemic reactogenicity (Sissoko et al., unpublished; Mordmüller et al., unpublished) [29] or allergic responses, indicating that PfSPZ may be suitably safe for mass administration campaigns for malaria elimination.

Because *Plasmodium* is a eukaryotic organism, the PfSPZ need to be cryopreserved and stored in liquid nitrogen vapor phase (LNVP) to maintain viability, like other cellular therapies or products (mammalian sperm, eggs, embryos, cellular cancer vaccines), as well as eleven veterinary vaccines including the *Theileria parva* vaccine used in Africa. Indeed, LNVP storage may prove to be advantageous, enhancing delivery to remote areas since no electricity is needed to maintain the cold chain (compared to refrigerated vaccines), and the vaccine can remain stable for weeks to months in a free-standing container. LN is widely available across Africa and other tropical areas, with infrastructure in place to support veterinary vaccine applications, artificial insemination of cattle, oil and mining applications, and the brewing industry. Distribution of the vaccine in LNVP is feasible in malaria endemic countries using a hub and spoke model, and projected costs are roughly equivalent to those required for adding a new vaccine to current distribution networks for refrigerated (2–8 °C) vaccines [30].

PfSPZ, the core Sanaria product, are manufactured in accordance with Title 21 of the Code of Federal Regulations (21 CFR) and in accordance with International Conference on Harmonization (ICH) guidelines. Manufacturing of PfSPZ products is performed following current Good Manufacturing Practice (cGMP) guidelines where processes are defined and controlled to ensure consistency and compliance with specifications. PfSPZ products have excellent stability profiles even when stored for more than 4 years. Sanaria maintains Biologics Master Files with the US FDA, and all clinical trials are conducted with US FDA oversight under investigational new drug applications (IND), and with appropriate host nation regulatory oversight.

2. Products

Sanaria's first PfSPZ products are based on the NF54 strain of Pf, isolated in 1981 from a Dutch farmer living near Amsterdam's Schiphol Airport and originating in Africa [31,32]. Pf NF54 strain is chloroquine sensitive. More recently, Sanaria has manufactured PfSPZ derived from a clone of a Brazilian isolate, 7G8 [33], as well as PfSPZ from a Cambodian clone, NF135.C10 [34].

Controlled human malaria infection (CHMI) and exposure to natural transmission have been used to test the efficacy of Sanaria's vaccine products. CHMI has been performed by mosquito bite, using mosquitoes infected with NF54, 3D7 (a clone of NF54) or 7G8, or by the direct injection of infectious PfSPZ (NF54) manufactured by Sanaria. When NF54 or 3D7 are used, the CHMI is considered homologous, since the parasite is identical or highly similar to the vaccine. When other strains are used to assess efficacy, the CHMI is considered heterologous, and a potentially better predictor of efficacy under conditions of natural transmission, where mosquitoes harbor heterogeneous populations of Pf.

2.1. PfSPZ Vaccine

The first PfSPZ product developed using Sanaria's manufacturing technology was Sanaria® PfSPZ Vaccine, which is composed of aseptic, purified, live (metabolically active), radiation-attenuated, cryopreserved PfSPZ. Several clinical trials of PfSPZ Vaccine have been completed in the USA. In the first trial, conducted at the Naval Medical Research Center (NMRC), the University of Maryland Baltimore, Center for Vaccine Development (UMB CVD), and the Walter Reed Army Institute of Research (WRAIR), the vaccine was administered ID or SC, was poorly immunogenic and protected at best only 2 of 16 volunteers at one dosage against CHMI [35]. A concurrent study in non-human primates (NHP) at the NIH Vaccine Research Center (VRC) administered the vaccine SC, as in the clinical trial, and by direct venous inoculation (DVI), as done in the original rodent experiments (Box 1). DVI induced far superior immune responses: 3.2% of CD8+ T cells in the livers of the three NHPs immunized by DVI responded with IFNγ production to PfSPZ stimulation, compared with low to undetectable frequencies in SC-immunized monkeys [35]. This finding provided the proof of concept for the second trial, conducted at the VRC, where the vaccine was given IV through an in-dwelling catheter. This trial escalated through five increasing doses of the vaccine, administering four to six injections to the volunteers over the course of 20–26 weeks. Sterile protection in 6/6 (100%) subjects receiving the highest total dose (Table 1) demonstrated that the PfSPZ were potent and provided the critical proof-of-principle that the model of protection by mosquito bite with radiation-attenuated SPZ (Box 1) could indeed be translated into a human injectable product. There was a clear dose-threshold effect, with the three lower doses showing limited, but dose-related protection, and the highest dose conferring high-grade sterile immunity (Table 1). There was a dose response for antibody and cellular immune responses, and antibody responses to PfSPZ were associated with protection. The most significant association was with antibodies to PfSPZ measured by the inhibition of sporozoite invasion into hepatocytes (ISI) assay [36].

2.2. PfSPZ Challenge

Sanaria® PfSPZ Challenge is manufactured identically to PfSPZ Vaccine except that the PfSPZ are not irradiated, and are therefore fully infectious. CHMI with injectable PfSPZ can replace traditional CHMI by the bite of mosquitoes to measure vaccine and drug efficacy, as well as to increase understanding of factors (genetic, immune) that affect Pf infectivity. The optimal dose and route for administering PfSPZ Challenge were worked out in a series of eight clinical trials conducted from 2010 to 2014, with the first five testing ID and IM routes. These were conducted at Radboud University Medical Center (RUMC), Nijmegen, the Netherlands [37], the University of Oxford, Oxford, UK [38], the Ifakara Health Institute (IHI), Bagamoyo, Tanzania [29], UMB CVD [51], and the Kenya Medical Research Institute (KEMRI), Nairobi, Kenya [39,40]. The aim of these studies was to administer a dose of PfSPZ Challenge that reproduced the infectivity and prepatent period of five PfSPZ-infected mosquitoes, namely 100% of volunteers infected and a prepatent period by thick smear microscopy of <12 days. ID administration has achieved 100% infection rates, but not the target prepatent period. IM administration of 7.5×10^4 PfSPZ achieved both attributes [41]. IV inoculation using an in-dwelling intravenous catheter was first tested at the University of Tübingen, Germany, and achieved 100% infection rate after administering only 3.2×10^3

PfSPZ [42]. The geometric mean (GM) prepatent period was 11.2 days (range 10.5–12.5 days). These results were then reproduced at the Barcelona Centre for International Health (CRESIB), Spain [41,42], establishing 3.2×10^3 PfSPZ of PfSPZ Challenge as the new gold standard for "mosquito-free" CHMI. In Barcelona the PfSPZ were administered by DVI, inserting the 25 G needle of a 1 mL syringe directly into a vein and rapidly injecting the PfSPZ in a volume of 0.5 mL (Fig. 1). This standard dose and DVI administration were used by the Lambaréné Centre for Medical Research in Gabon to study the effect of naturally acquired immunity and sickle cell trait on the growth rate of Pf *in vivo* (Lell et al., unpublished). These studies were performed using different PfSPZ Challenge lots, some of which were manufactured years apart. In summary, these studies of PfSPZ Challenge demonstrated that the purified, cryopreserved PfSPZ produced by Sanaria were potent (infectious) and stable, and that IV and DVI routes were the most efficient means of administration.

Having established a standard route and dose, injections of 3.2×10^3 PfSPZ of PfSPZ Challenge, administered by DVI, have now been used successfully in seven CHMIs in Tanzania and Germany to test the efficacy of PfSPZ Vaccine and PfSPZ-CVac respectively, infecting all control volunteers ($n = 43$) (Mordmüller et al., unpublished; Shekalaghe et al., unpublished). CHMI by PfSPZ Challenge reduces costs and streamlines the logistics compared with CHMI by mosquito bite. It also allows repeated CHMIs and adaptive clinical trial designs, since the timing of CHMI is independent of the complex process needed to produce a batch of infected mosquitoes. Since each inoculation is identical, PfSPZ Challenge also standardizes the dose of infectious PfSPZ, which cannot be done with CHMI by mosquito bite.

2.3. PfSPZ-CVac

In a seminal study of chemoprophylaxis with SPZ (CPS) conducted at RUMC in Nijmegen [43], 3 × 12–15 bites from non-irradiated *Anopheles stephensi* mosquitoes harboring PfSPZ administered to malaria-naïve adults concurrently taking chloroquine resulted in 100% sterile protection against CHMI (10/10 volunteers protected). The protection persisted for at least 28 months in the majority of volunteers undergoing a second CHMI, with 4/6 sterilely protected and 2/6 showing prolonged prepatent periods [44]. The Sanaria team and collaborators reasoned that PfSPZ Challenge should be able to substitute for the mosquito bites, and accordingly, PfSPZ Challenge was tested as the immunogen in a CPS vaccine approach called Sanaria® PfSPZ-CVac (CVac = Chemoprophylaxis Vaccine).

The first PfSPZ-CVac trial was conducted at RUMC; PfSPZ Challenge was administered ID with chloroquine as the drug partner. Disappointingly, but in hindsight not unexpectedly, three or four ID administrations of 75,000 PfSPZ induced minimal immunogenicity and little or no protection [45]. Once the superiority of the IV route was demonstrated for PfSPZ Vaccine in the VRC312 trial, however, there was justification for a second trial of PfSPZ-CVac, which was conducted at the University of Tübingen. PfSPZ were administered by DVI rather than ID, chloroquine was retained as the partner drug, and this time the outcome was dramatically reversed [Mordmüller et al., unpublished]. The transformation from low- to high-grade immunogenicity and efficacy mirrored the similar transformation for PfSPZ

Vaccine when the route of administration was changed from ID or SC to IV. The finding that IV or DVI administration was needed to reveal the potency of PfSPZ for immunizing volunteers parallels experience with PfSPZ Challenge for which the IV or DVI route was by far the most efficient for infecting volunteers. Thus for each objective – inducing protective immunity with PfSPZ Vaccine or the PfSPZ-CVac approach, or inducing infection with PfSPZ Challenge – there was a multifold difference in potency between the IV/DVI routes of administration and the more traditional ID, SC and IM routes.

2.4. Comparative potency of PfSPZ Vaccine and PfSPZ-CVac

Our cumulative experience using PfSPZ Vaccine shows that it requires several hundred thousand PfSPZ to induce high-grade protection, while the same or better can be achieved using the PfSPZ-CVac approach using a fraction of the dose. This parallels earlier experience using mosquito bite immunization: with CPS, it took exposure to ~45 PfSPZ-infected mosquitoes to achieve durable, high-level protective efficacy. In contrast, it required exposure to the bites of at least 1000 mosquitoes carrying radiation-attenuated PfSPZ to consistently achieve high-level protection (Box 1). The likely reason for this is that radiation-attenuated PfSPZ invade hepatocytes and begin the process of development, and although they express ~1000 proteins their replication arrests early in liver stage development. In contrast, the infectious PfSPZ in PfSPZ-CVac invade hepatocytes, but then replicate 10,000–40,000-fold and express ~4500 different proteins, including blood stage proteins. Thus there are dramatically more parasites and antigens presented to the immune system per PfSPZ injected with PfSPZ-CVac than with PfSPZ Vaccine.

2.5. PfSPZ-GA1

Sanaria® PfSPZ-GA1 consists of purified, aseptic, cryopreserved Pf sporozoites (NF54 strain) genetically attenuated by removal of the *b9* and *slarp* genes to halt development in the early liver stages [46,47]. The parasite line was generated by the Leiden University Medical Center (LUMC) and RUMC in collaboration with Sanaria. PfΔ*b9*Δ*slarp* parasites invade hepatocytes but are incapable of sustaining liver stage development, similar to radiation-attenuated PfSPZ. The potential advantages of PfSPZ-GA1 are that (1) the PfSPZ are homogenous and have a precisely characterized genetic basis for attenuation; (2) manufacturing PfSPZ-GA1 cannot result in accidental exposure of staff to infectious parasites, thereby simplifying the approach to safety of operators and lowering costs. PfSPZ-GA1 also has the potential to induce more efficient protection than the radiation-attenuated PfSPZ Vaccine due to a different pattern of developmental arrest and antigen expression.

2.6. Safety and tolerability

To date, 2155 doses of cryopreserved PfSPZ from Sanaria products have been administered to 824 adult volunteers in 17 clinical trials via a variety of routes (Sanaria, unpublished). Several of these trials included randomized, double-blind allocation to PfSPZ or normal saline (NS) placebo, and data are available from three such trials, involving 97 PfSPZ recipients and 68 placebo recipients in total. The two groups were compared in each trial after unblinding the adverse event data; there were no differences in adverse event profiles between volunteers receiving PfSPZ and placebo during the first seven days after injection [29] (Sissoko, Healy et al., unpublished; Mordmüller et al., unpublished). Moreover, there have been no allergic reactions to PfSPZ, nor any serious adverse events attributed to PfSPZ. Experience includes 30 IV injections of 9×10^5 PfSPZ and 36 IM injections of 2.2×10^6 PfSPZ (Lyke, Seder et al., unpublished). The absence of clinically significant side effects linked to PfSPZ administration applies also to laboratory abnormalities, including liver function tests.

DVI is rapid and efficient (Fig. 1). If the veins in the arm are suitable for obtaining blood via standard venipuncture, they are suitable for administration of PfSPZ by DVI. The procedure involves the insertion of a 25 G needle into the vein, a slight withdrawal on the plunger to demonstrate blood flashback, loosening of the restricting tourniquet and immediate injection of 0.5 mL of diluted PfSPZ (or placebo), typically taking 10–15 s. More than half (in some trials >90%) of subjects have rated the injections as painless on a four-point scale (painless, mild pain, moderate pain, severe pain). DVI may cause slight bruising at the injection site if there is extravasation of blood from the vein, but there are no persistent local signs or symptoms such as tenderness, erythema or induration, since the vaccine is dispersed on injection. ID and SC injections are also well tolerated, indicating that if any inoculum is deposited into the surrounding tissues during DVI, it does not affect tolerability. The vaccine contains no adjuvant or pro-inflammatory material.

The demonstrated safety of PfSPZ at doses up to 9×10^5 PfSPZ by DVI has enabled plans for further dose escalation, in order to maximize the degree and duration of sterile immunity, and this will be done for both PfSPZ Vaccine and PfSPZ-CVac. An interesting aspect of the latter approach when using chloroquine as the antimalarial is that 5.5–7 days after injection merozoites are released into the blood and are detectable by qPCR, providing a transient low-grade parasitemia that is rapidly cleared by chloroquine. The kinetics of transient parasitemia allow an estimate of the number of infected hepatocytes, which can be used to correlate immunogen dose (number of sporozoites infecting hepatocytes) with protective efficacy.

3. Clinical development plan

3.1. PfSPZ Vaccine

Springboarding off the two published trials of PfSPZ Vaccine [35,36], particularly following the high-grade protection in VRC 312 and ongoing studies, there are five new trials completed or underway of this product that constitute “Stage 1” of the PfSPZ Vaccine clinical development plan (CDP) (Fig. 2). Each of these trials was initiated by the primary performing institutions (Table 2), which also provided funding and developed protocols in close partnership with Sanaria. These studies have reproduced the high-grade efficacy seen in VRC 312, demonstrated that PfSPZ Vaccine induces heterologous and durable (12 month) protection against CHMI and against naturally-transmitted malaria, and that a three-dose regimen can be highly protective. We have also learned that malaria-naïve individuals in the U.S. respond better to the vaccine than malaria-exposed individuals in Africa after receiving an identical dose and regimen, exhibiting multifold higher titers of antibodies to PfCSP by ELISA. This indicates that increased doses of PfSPZ, and potentially interval changes between doses, will be required to achieve high-level immunogenicity and sterile protection in malaria-exposed individuals. This difference in responsiveness may result from the immune modulation caused by repeated malaria infections. The specific results from these trials will be published by the investigators.

Stage 2 of the clinical development plan, launched in late-2015, will address the following objectives:

1. Demonstrate high-grade sterile protection in malaria-naïve adults, including durable (≥6 month) protection against heterologous CHMI following a simplified (e.g. three dose) regimen. These studies aim to finalize the regimen for licensure to protect travelers, including military personnel, during stays in malarious areas.
2. Demonstrate high-grade sterile protection in malaria-exposed African adults, including durable (≥6 month) protection against naturally-transmitted malaria following a similarly simplified regimen. These studies aim to define a regimen that can be used in malaria elimination campaigns to halt infection and transmission.
3. Evaluate the tolerability, immunogenicity and efficacy of truncated regimens: 0, 1, and 4 weeks; 0 and 1 week; 0, 2, 4 and 6 days or even a single immunization. These studies aim to improve the operational feasibility of using the vaccine for all indications.
4. Demonstrate safety, immunogenicity and protection in African infants 5 months or older and in young children. These studies aim to protect the most vulnerable age groups from malaria and will optimize dose of vaccine with respect to age and body weight. We hypothesize that because African infants have had limited exposure to malaria, vaccinations will result in better protective responses than for African adults thereby providing an effective vaccine for the most vulnerable populations.
5. Demonstrate safety in the elderly and in HIV-infected individuals. These studies aim to show that screening for diminished health or immunodeficiency will not be required when conducting mass administration campaigns. Since radiation-attenuated parasites cannot replicate, they should prove safe in all individuals, including the immunocompromised.
6. Evaluate efficacy against *P. vivax* by CHMI and natural transmission. Pv and Pf share tens of thousands of minimal T cell epitopes [48,49], and PfSPZ may induce cross-protective cell mediated immunity.
7. Establish immunological correlates of infection; all immune responses measured in these trials will be assessed as potential correlates.
8. Continue with operational research in preparation for phase 3 clinical trials and elimination campaigns with the licensed vaccine.

There are seven funded trials that will address these objectives in the USA, Germany, Tanzania, Mali, Burkina Faso, Kenya and Equatorial Guinea (Table 2), plus one additional trial of a new strain of PfSPZ Challenge. A program for studying PfSPZ Vaccine in pregnancy is in the planning stages.

Stage 3 of the CDP will include expanded safety testing and large scale CHMI trials in malaria-naïve adults using one or more heterologous parasites for CHMI, and will also include large-scale field efficacy trials in malaria endemic areas including all age groups older than six months. These studies are planned for 2016–18. The studies in malaria-naïve adults will support the targeted submission of a biologics license application (BLA) in 2017–18 for a traveler's vaccine, and the studies in endemic areas will support an additional indication for use in endemic areas subsequently. Operationally we intend to initially target infants, age 6–12 months, to reduce morbidity and mortality, and mass administration projects intended to achieve halting of transmission and elimination of malaria will follow.

3.2. PfSPZ-CVac

The CDP for PfSPZ-CVac parallels that of PfSPZ Vaccine with the added necessity of optimizing the administration, dose and regimen of the partner drug. Development is being prioritized and accelerated because of PfSPZ-CVac's increased potency compared to PfSPZ Vaccine. One clinical trial is ongoing (TÜCHMI-002 trial at the University of Tübingen), one clinical trial has just started at the U.S. National Institutes of Health (NIH) Clinical Center, and three additional trials are planned for 2015–2016 (Table 3). These include trials of condensed regimens (as few as 3 doses in 10 days) and alternative drug partners (atovaquone/proguanil, azithromycin, pyrimethamine). Close attention is being paid to safety considerations, since the PfSPZ-CVac approach includes injecting healthy individuals with a human pathogen, notwithstanding the fact that PfSPZ Challenge (NF54) is highly sensitive to chloroquine and other antimalarials. Any regimen will require ingestion and retention of a protective drug before the PfSPZ are administered. This will be done under direct observation. The challenge during development is to demonstrate that vaccination with PfSPZ-CVac is as safe as any other approach to vaccination. PfSPZ-CVac may be most appropriate for use in elimination campaigns in endemic areas, where the population is already exposed to natural malaria transmission.

3.3. PfSPZ-GA1

Clinical lots of PfSPZ-GA1 have been manufactured. A proposal for the first trial has been submitted for funding. The first step will be to establish adequate attenuation and to compare protective efficacy with PfSPZ Vaccine. A vaccine based on genetically attenuated Pf designed to arrest development late in the liver stage or immediately after release of parasites into the blood is also being actively pursued, as such parasites will mimic PfSPZ-CVac without the need for administering anti-malarial drugs.

3.4. International SPZ Consortium

A key aspect of the CDPs for PfSPZ Vaccine, PfSPZ-CVac and PfSPZ-GA1 is their reliance on an informed and proactive consortium of research and funding institutions that together constitute the International PfSPZ Consortium (I-PfSPZ-C) (Table 4). Members meet periodically to share and critique data, and to discuss plans. The most recent meetings were held 11–12 March 2015, 9 September 2015 and 29–30 October 2015 in Tübingen, Basel and

Philadelphia, respectively (Box 2). The enthusiasm and aggressive research strategies of the I-PfSPZ-C have propelled innovative approaches and greatly accelerated the development and testing of PfSPZ-based products. Sanaria acts as sponsor for nearly all the trials, which are conducted under US FDA oversight, as well as the oversight of regulatory authorities in the countries outside the US where the trials are conducted. Sanaria also serves as lead organizer for the I-PfSPZ-C and as a repository for information. Of particular importance is the dissemination of clinical data among sites, to provide alerts in case of adverse events, and to inform all partners regarding outcomes which may impact the design and management of other trials. In the case of the meeting in Tübingen, results of five clinical trials were reported to members long before publication in a scientific journal.

3.5. Malaria elimination campaigns

A remarkable aspect of the PfSPZ development story has been the financial support by African partner countries, first Tanzania, then Equatorial Guinea and, most recently, Ghana; this is an important milestone in malaria vaccine development. A striking example is occurring in Equatorial Guinea, which will support four trials of PfSPZ Vaccine and associated operational research in Equatorial Guinea and Tanzania to optimize vaccine administration for later use in malaria elimination campaigns. The Equatorial Guinea Government, Marathon Oil Corporation, Noble Energy, AMPCO, Medical Care Development International (MCDI) and Sanaria have partnered to conduct the four trials and are aiming to conduct a malaria elimination campaign after a PfSPZ-based vaccine is licensed for this indication. The collaboration in Equatorial Guinea involves not only Equatorial Guinea Ministry of Health and Social Welfare and Sanaria investigators, but also a team from the Ifakara Health Institute in Bagamoyo, Tanzania, and collaborators from the Swiss Tropical and Public Health Institute, MCDI, and La Paz Medical Center. The first phase of the demonstration will target elimination of malaria from Bioko Island. The population of >250,000 endure ongoing malaria transmission and significant malaria-related morbidity and mortality despite a well-funded and executed malaria control program [50]. Plans for this campaign will be developed during the coming years, anticipating launch of the campaign immediately after licensure.

4. Conclusions

With the development of PfSPZ-based products for parenteral injection, the field of malaria vaccines is returning to principles of highly protective immunization first established in birds in the early 1900s, in mice in the 1960s and in humans in the 1970s (Box 1). The focus has been to reproduce the same durable protective immunity using an injectable product that is safe for human use. This approach, unencumbered by *a priori* restrictions on vaccine design, has led to rapid progress, and should translate into a more thorough understanding of the immunological mechanisms underlying protection. Moreover, the whole organism approach mirrors that of many other live, attenuated vaccine products, nearly all of which are highly protective.

The fact remains, however, that there are no vaccines licensed to protect humans against parasites, which are far more complex than viral or bacterial pathogens. It is therefore to be expected that numerous innovations have been required, and these have included novel manufacturing process steps for the production of highly PfSPZ-infected, aseptic mosquitoes and purifying and cryopreserving the PfSPZ. On the clinical side, it has been necessary to develop new immunization regimens, and to develop DVI as a method for efficient PfSPZ administration. Further process refinements are anticipated in the coming years after licensure of the first generation PfSPZ vaccine(s), and these include *in vitro* development of PfSPZ from sexual stage parasites. Although the worldwide need for a malaria vaccine can be met using current methods for manufacture, such innovations will simplify scale-up and reduce the cost of goods.

The rapid progress achieved by the I-PfSPZ-C would not have been possible without the open-minded and creative approaches adopted by Sanaria and its collaborators. Members of the I-PfSPZ-C have provided leadership in key developments including DVI administration, condensed immunization regimens, and novel vaccine concepts such as PfSPZ-CVac. Remarkably, our clinical experience has demonstrated excellent safety and tolerability, regardless of route of administration. This allows for the testing of higher doses, which appear needed to achieve our objectives in those with prior malaria exposure. As new technologies for manufacturing, formulation, cryopreservation and administration are developed, and as indicated by the results of ongoing clinical testing, optimized vaccine candidates and immunization regimens will be advanced under appropriate regulatory guidance. The long-term goal is durable, cross-strain, sterile immunity in >90% of vaccine recipients with the lowest numbers of PfSPZ in the least numbers of doses in the shortest period of time. The target product must also demonstrate operational, safety and tolerability characteristics suitable for use in mass administration campaigns. The I-PfSPZ-C is working toward these long-term objectives, aiming for a PfSPZ vaccine to be the cornerstone for malaria elimination and eradication.

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Box 1

Targeting the SPZ

Whole SPZ were one of the first malaria immunogens tested as a malaria vaccine, described by Sergent and Sergent in a report published in 1910 by the Academy of Sciences, Paris, on the avian parasite *Plasmodium relictum* [2]. In the early 1940s, Mulligan, Russell and Mohan at the Pasteur Institute in Coonoor, India partially protected fowl against another bird parasite, *P. gallinaceum*, by administering five intravenous injections of a crude preparation of dried, ground thoraces from infected mosquitoes (220 thoraces per fowl) [3,4]. Vaccinated animals and controls were challenged by the bites of two infected *Aëdes albopictus* mosquitoes, and while all immunized fowl developed asexual blood stage infections (thus protection against infection was nil), prior SPZ vaccination halved the parasite density in the blood, and reduced mortality from 55% to 30% or less. Similar results were obtained with SPZ dissected from salivary glands and inactivated by a 30-min exposure to ultraviolet light. The partial protection by killed SPZ in the avian model was confirmed in subsequent studies [5]. It was shown that protected fowl were still susceptible to blood stage challenge, indicating that the partially effective immune response induced by whole killed SPZ vaccination targeted the pre-erythrocytic stages.

A significant improvement in protection was reported by Nussenzweig and colleagues in 1967 by using attenuated, rather than killed SPZ, this time in a rodent model. X-irradiation was used to damage *P. berghei* SPZ dissected from infected *Anopheles stephensi* mosquitoes [6]. SPZ receiving this sublethal irradiation remained metabolically active and motile, could invade hepatocytes and round up (a morphological change signifying the earliest stage of development in the hepatocyte), but could not develop into hepatic trophozoites or schizonts. When mice immunized by IV administration of 75,000 SPZ irradiated with 80–100 gray (Gy) were challenged by IV injection of non-irradiated SPZ two weeks after immunization, infection rates were 37% compared to 90% in controls, indicating that more than half had sterile immunity against infection. Those animals becoming infected despite vaccination showed delays in the prepatent period. Eleven protected mice were re-challenged at 36 days, and most were again protected, indicating a degree of immune durability and also ruling out non-specific protection mediated by the innate immune system. The murine model thus established that attenuated, metabolically active parasites could induce sterile immunity to malaria.

The radiation-attenuated SPZ approach was rapidly translated to humans in a series of studies in the early 1970s carried out by Clyde and colleagues [7–11], and Rieckmann and colleagues [12–14] and to non-human primates [15]. A review of these studies and subsequent work by the US military [16,17] and the University of Maryland [18,19] by Hoffman et al. [16] concluded that administration of more than 1000 bites from irradiated, infected mosquitoes consistently protected >90% (13/14) of recipients undergoing CHMI by mosquito bite within 10 weeks of immunization, and 5/6 volunteers were still protected on repeat CHMI up to 42 weeks later. Furthermore, 4 subjects underwent 7 CHMIs with heterologous strains of Pf, and there was 100% protection. 150

Gy was established as a sufficiently attenuating radiation dose to prevent blood stage infection without killing the parasites.

This work provided proof-of-concept for high-grade protection against parasitemia in humans by targeting the SPZ (and/or the early liver stage parasites they become), and inspired efforts to develop pre-erythrocytic stage malaria vaccines. It was reasoned that if the antigenic components of whole SPZ could be identified and suitably formulated as vaccines, it should be possible for subunits to reproduce the high-grade protection induced by whole SPZ. The report in 1984 of the identification and cloning of the major surface protein of the *P. falciparum* (Pf) SPZ, the circumsporozoite protein (CSP) [20], galvanized this effort, and led to the clinical testing of a series of recombinant and synthetic constructs [22,23], the most successful being RTS,S, a recombinant protein containing a portion of PfCSP fused to hepatitis B surface antigen, produced in yeast, assembled into virus-like particles, and formulated in adjuvant. RTS,S has been extensively tested in Africa, and the results of a large Phase 3 trial recently reported [24]. In addition to RTS,S, several other subunit approaches using pre-erythrocytic stage antigens are being pursued, including gene-based approaches; some, like RTS,S, have sterilely protected volunteers against CHMI [25,26]. While these subunit approaches are promising, they present the immune system with only a small number of pre-erythrocytic immunogens, and have provided only modest protection. With the development by Sanaria of technologies for the manufacture and administration of whole SPZ, many in the malaria vaccine development field have begun to direct their efforts back toward this more empirical whole organism approach (see Box 2).

Box 2

The International PfSPZ Consortium

The International PfSPZ Consortium (I-PfSPZ-C) has evolved to integrate the planning and governance of development among the organizations collaborating to take PfSPZ Vaccine and PfSPZ-CVac through to licensure. The I-PfSPZ-C has an inclusive structure in which all individuals, groups, institutions and funding organizations involved in studies with Sanaria's PfSPZ-based products participate in the planning of trials, and reviewing data on safety, immunogenicity and protective efficacy of PfSPZ vaccines. They also contribute to the efforts to raise funds to support the program. The I-PfSPZ-C has met two to three times per year since November 2011, including sessions organized in association with the annual meeting of the American Society of Tropical Medicine and Hygiene (ASTMH). The first meeting of 2015 was held in March at the University of Tübingen, Germany, and was attended by 81 individuals from 35 different institutions based in 14 different countries in North America, Europe and Africa. More recently, a meeting was held in October in association with the annual meeting of the ASTMH in Philadelphia, and was attended by 106 individuals from 43 different institutions based in 16 different countries. In addition to clinical investigators, participants included representatives from African governments, policy agencies, regulatory authorities and funding agencies.

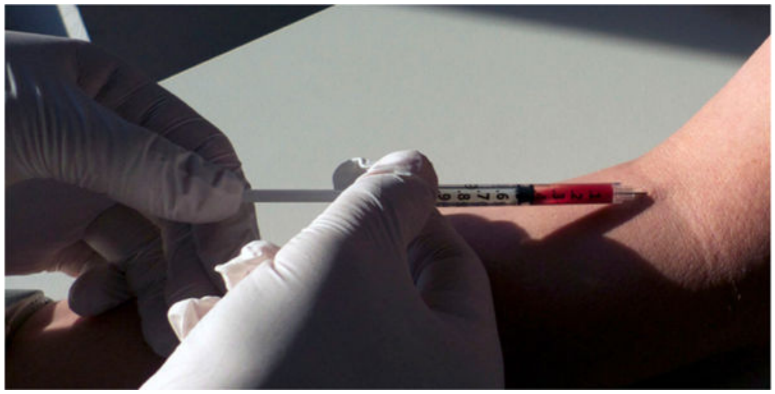

Fig. 1.

Direct venous inoculation (DVI) of PfSPZ [42]. Once the injection site is prepped with an antiseptic such as 70% isopropyl alcohol, needle insertion, loosening the tourniquet and vaccine injection take only about 10–15 s. Note the small size of the needle (25 gauge) and syringe (1 mL), and blood flashback confirming successful puncture of, in this case, the median cubital or cephalic vein. Veins in the back of the hand or side of the wrist may also be used. Photograph by B. Mordmüller.

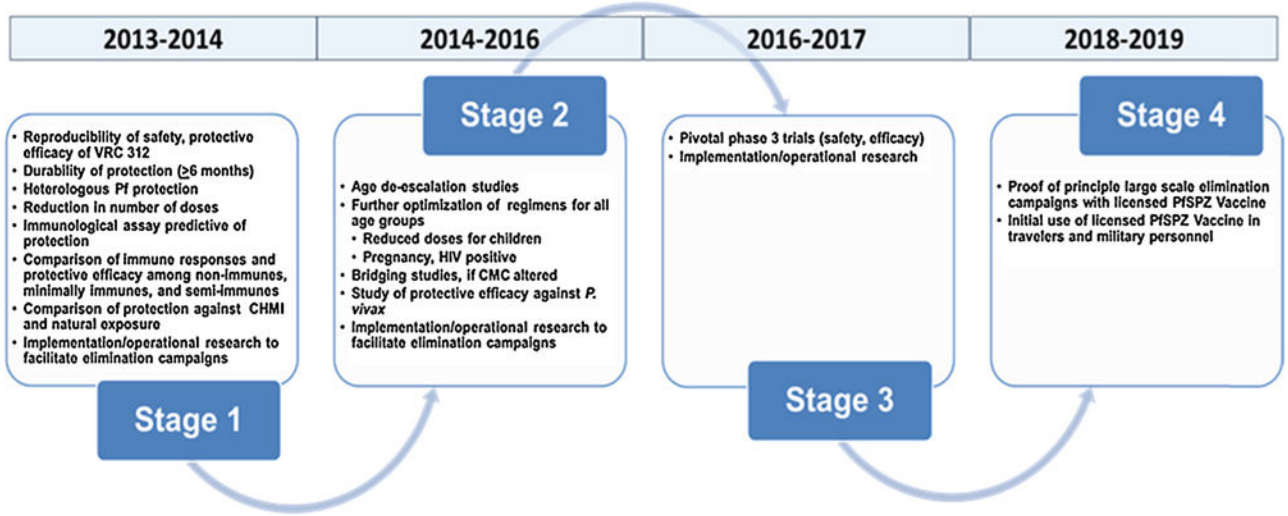

Fig. 2.

Clinical development plan for PfSPZ Vaccine. Current activities fall into Stage 2.

Table 1

Summary of protective efficacy and antibody responses in volunteers immunized in the VRC 312 clinical trial with PfSPZ Vaccine [36].

Dosage regimen			Anti-PfCSP Antibodies (OD 1.0)[a]	Number of volunteers		Protective efficacy
Dose (PfSPZ × 10^5)	Number of doses	Maximum total dose (PfSPZ × 10^5)		Challenged	Protected	
0.075	4 or 6	0.45	13	6	0	0%
0.3	4 or 6	1.8	324	11	1	9%
1.35	4	5.4	3454	9	6	60%[b]
1.35	5	6.75	6716	6	6	100%

[a] Anti-PfCSP antibody level 2 weeks after the last dose and before CHMI by ELISA (geometric mean of the inverse of the serum dilution at which the optical density (OD) was 1.0).

[b] 5/6 controls developed parasitemia.

Table 2

Summary of Stage 1 and Stage 2 PfSPZ Vaccine clinical trials. The performing institutions are core members of the expanding International PfSPZ Consortium (see Table 4).

Stage 1			
Country	**Protocol name**	**Primary performing institutions**	**ClinTrials.gov Identifier**
USA	VRC 314	NIAID Vaccine Research Center (VRC), University of Maryland Baltimore, Center for Vaccine Development (UMB CVD)	NCT02015091
Mali	14-I-N010	Malaria Research & Training Center (MRTC), University of Bamako NIAID Laboratory for Malaria Immunology and Vaccinology (LMIV)	NCT01988636
Tanzania	BSPZV1	Ifakara Health Institute (IHI) Swiss Tropical and Public Health Institute (Swiss TPH)	NCT02132299
USA	WRAIR 2080	Naval Medical Research Center (NMRC) Walter Reed Army Institute of Research (WRAIR)	NCT02215707
Equatorial Guinea	EGSPZV1	Equatorial Guinea Ministry of Health and Social Welfare (MOHSW) IHI Swiss TPH Medical Care Development International (MCDI) La Paz Medical Center, Malabo, EG	NCT02418962
Stage 2			
Country	Primary performing institutions	Objectives	Efficacy assessment
Germany	University of Tübingen	Condensed regimens in adults	CHMI – PfSPZ Challenge
Tanzania	IHI Swiss TPH	Age de-escalation to infants and efficacy in adults	CHMI – PfSPZ Challenge
Mali	MRTC LMIV	Dose escalation and efficacy in adults	CHMI – PfSPZ Challenge + Natural exposure
Burkina Faso	Centre National de Recherche et de Formation sur le Paludisme (CNRFP) UMB CVD	Dose escalation and efficacy in adults	Natural exposure
USA	NMRC UMB CVD WRAIR	Finalized regimen for adult travelers	CHMI – Mosquito bite
Equatorial Guinea	Equatorial Guinea MOHSW	Efficacy in adults Comparison with PfSPZ-CVac Age escalation to 65 Age de-escalation to infants Safety in HIV positive subjects	CHMI – PfSPZ Challenge
Kenya	Kenya Medical Research Institute (KEMRI)	Age de-escalation from 10 year olds to infants	
	US Centers for Disease Control (CDC) NIAID VRC	Safety and efficacy in infants	Natural exposure

Table 3

Summary of current and planned PfSPZ-CVac clinical trials.

Country	Primary performing institutions	Objectives	Efficacy assessment
Germany	University of Tübingen	Condensed regimens	CHMI – PfSPZ Challenge
(current)	(ClinicalTrials.gov NCT02115516)	Alternative drug partner – azithromycin	
USA	NIAID Laboratory of Malaria Immunology and Vaccinology (LMIV)	Alternative drug partner – pyrimethamine	CHMI – PfSPZ Challenge
(current)	(ClinicalTrials.gov NCT02511054)		
USA	NIAID Division of Microbiology and Infectious Diseases (DMID) Group Health Research Institute Seattle BioMed	Dose escalation, condensed regimen	CHMI – PfSPZ Challenge
Ghana	Kintampo Health Research Centre US Navy	Dose escalation	CHMI – PfSPZ Challenge
Germany	University of Tübingen	Alternative drug partners – atovaquone/proguanil	CHMI – PfSPZ Challenge

Table 4

Members of the International PfSPZ Consortium. Partners and funding organizations are listed by country and do not necessarily match left to right.

Location	Collaborative and funding partners	Funding organizations
USA	Naval Medical Research Center (NMRC), Department of Defense (DoD) Walter Reed Army Institute of Research (WRAIR), DoD NIAID Vaccine Research Center (VRC)[a] NIAID Laboratory of Malaria Immunology and Vaccinology (LMIV)[a] University of Maryland Baltimore, Center for Vaccine Development (UMB CVD) Centers for Disease Control and Prevention (CDC)[a] Medical Care Development International (MCDI)	Military Infectious Disease Research Program (MIDRP) US Navy Advanced Medical Development Program US Army Medical Materiel Development Activity (USAMMDA) NIAID Division of Microbiology and Infectious Diseases (DMID) PATH Malaria Vaccine Initiative (MVI) (funded by Bill & Melinda Gates Foundation) (BMGF) Marathon Oil Corporation Noble Energy Atlantic Methanol Production Company (AMPCO) Institute for OneWorld Health (funded by BMGF)
Europe		European Vaccine Initiative
Switzerland	Swiss Tropical and Public Health Institute (Swiss TPH)[a]	Swiss State Secretariat for Education, Research and Innovation
Germany	University of Tübingen[a]	German Centre for Infection Research
The Netherlands	Radboud University Medical Center (RUMC), Leiden University Medical Center	Top Institute Pharma
Spain	ISGlobal, Barcelona Centre for International Health Research (CRESIB)[a]	CRESIB, Spanish Government
UK	Jenner Institute, Oxford University[a,b]	The Wellcome Trust
Africa		
Tanzania	Ifakara Health Institute (IHI)	Tanzania Commission on Science and Technology (COSTECH)
Equatorial Guinea	Ministry of Health and Social Welfare	Government of Equatorial Guinea Equatorial Guinea LNG
Kenya	Kenya Medical Research Institute (KEMRI) Wellcome Trust Laboratories Centre for Research in Therapeutic Sciences (CREATES)	
Gabon	Centre de Recherches Médicales de Lambaréné, Albert Schweitzer Hospital	
Mozambique	Manhiça Health Research Center (CISM)	
Ghana	Kintampo Health Research Center	Ghana Ministry of Health
Mali	Malaria Research and Training Center, University of Bamako (MRTC)	
Burkina Faso	Centre National de Recherche et de Formation sur le Paludisme (CNRFP)	
Asia		
Indonesia	Eijkman-Oxford Clinical Research Unit (EOCRU), Jakarta	

[a]Invested institutional funds in clinical trials of PfSPZ-based products.

[b]Only involved with PfSPZ Challenge.