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Transmission blocking malaria vaccines: Assays and candidates in clinical development☆

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ABSTRACT

Stimulated by recent advances in malaria control and increased funding, the elimination of malaria is now considered to be an attainable goal for an increasing number of malaria-endemic regions. This has boosted the interest in transmission-reducing interventions including vaccines that target sexual, sporogenic, and/or mosquito-stage antigens to interrupt malaria transmission (SSM-VIMT). SSM-VIMT aim to prevent human malaria infection in vaccinated communities by inhibiting parasite development within the mosquito after a blood meal taken from a gametocyte carrier. Only a handful of target antigens are in clinical development and progress has been slow over the years. Major stumbling blocks include (i) the expression of appropriately folded target proteins and their downstream purification, (ii) insufficient induction of sustained functional blocking antibody titers by candidate vaccines in humans, and (iii) validation of a number of (bio)-assays as correlate for blocking activity in the field. Here we discuss clinical manufacturing and testing of current SSM-VIMT candidates and the latest bio-assay development for clinical evaluation. New testing strategies are discussed that may accelerate the evaluation and application of SSM-VIMT.

1. Introduction

The World Malaria Report 2014 documented major progress with a considerable reduction of the malaria burden in several countries. In sub-Saharan Africa, average infection prevalence in children aged 2–10 years showed a decline of 48% since the year 2000 with similar decreases in malaria-attributed mortality [1]. In addition to the prevention and treatment of clinical cases, reduction of malaria transmission forms a fundamental basis of malaria control and elimination. As new tools are developed, their impact on malaria parasite transmission can be quantified and malaria elimination may become a realistic endeavor for an increasing number of settings. Transmission reduction may thereby become a key metric in measuring the impact of new tools and combinations of intervention methods. Vaccines interrupting malaria transmission (VIMT), introduced in the malaria vaccine roadmap (reviewed in [2]), that effectively interrupt transmission from humans to mosquitoes have the potential to fill a critical gap in the malaria vaccine portfolio. Such vaccines aim to reduce the spread of the parasites among humans by preventing infection of *Anopheles* vectors and represent highly relevant tools for the accomplishment of malaria elimination and eradication. Traditionally only sexual- and sporogonic antigens have been considered transmission blocking vaccine targets but more recently pre-erythrocytic vaccines have been included because these – if highly effective – prevent parasitaemia and thus generation of gametocytes and onward transmission [2,3]. Pre-erythrocytic vaccines have the advantage that they confer direct personal protection while only delayed personal protection can be expected from vaccines directed exclusively against sexual-sporogonic-mosquito stages (SSM-VIMT).

Already in the 1950s, it was shown that immunization of chickens with a mix of asexual-and sexual stages of *Plasmodium gallinaceum* blocked parasite infectivity [4]. Twenty years later, it appeared that antibodies against target antigens on sexual stages were responsible for the observed transmission blocking effects in this model and that these antibodies acted after ingestion by mosquitoes [5,6]. Antibodies can destroy gametes and zygotes up to several hours after a mosquito blood meal and can completely prevent infectivity to mosquitoes. The first description of mosquito feeding assays, that form a cornerstone of assessments of transmission blocking immune responses, dates from the 1950s [7]. In the decades that followed, experimental mosquito membrane feeding systems were optimized and monoclonal antibody technology became available. This resulted in the development

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of the standard membrane feeding assay (SMFA) and a series of sexual stage proteins were defined as targets for transmission blocking antibodies ([8–10], reviewed in [11]). The genes encoding Pfs48/45, Pfs230 and Pfs25 in *Plasmodium falciparum* as well as its ortholog Pvs25 in *Plasmodium vivax*, were isolated and these target proteins remained the major candidates for clinical development of transmission blocking vaccines for already for the past three decades [12–15]. During this period, several workshops have been organized by, amongst others, the World Health Organization, the United States National Institutes of Health (NIH), Malaria Vaccine Initiative and the Bill & Melinda Gates Foundation to discuss clinical trial design and endpoints, efficacy evaluation assays, regulatory aspects and application strategies [2,3,16–18]. Major challenges in this endeavor have been (i) incomplete/inadequate conformation of vaccine proteins and the production of clinical grade material; (ii) absence of validated standards and assays for clinical efficacy; (iii) absence of a rapid pipeline of proof-of-principle human trials for testing lead candidates. Here, we will discuss the latest progress in clinical development of SSM-VIMTs and evaluation of biological endpoint measures with suggestions for future steps.

2. SSM-VIMT candidates in clinical development

Transmission blocking immunity relies on functional antibodies against surface membrane proteins on sexual/sporogonic stages or mosquito midgut antigens. Pre-fertilization antigens are expressed in gametocytes in the human host but only become accessible for functional antibody binding once gametocytes emerge from the red blood cells to form gametes in the mosquito midgut. Naturally acquired- or experimentally-induced antibodies can bind to gametes, thereby preventing fertilization and zygote formation. Pre-fertilization antigens expressed in humans can induce antibodies after natural infection thereby creating the possibility to boost and/or enhance vaccine induced antibody titers and longevity [19–21]. Post-fertilization transmission blocking target antigen expression occurs in the mosquito midgut in zygotes and ookinetes [10,22]. There is currently no evidence that these proteins are expressed in gametocytes in the human circulation and therefore the human immune system is not exposed to these proteins and naturally acquired antibodies are not detectable [23,24]. Although a substantial number of targets have been identified and tested in preclinical studies over the past decades, the lead vaccine candidates have not changed and include pre-fertilization proteins Pfs48/45 [8,10] and Pfs230 [25] and post-fertilization antigen Pfs25 [10]. Recombinant P25 proteins have been successfully generated for both *P. falciparum* and *P. vivax* [26,27]. In a very different approach, mosquito derived targets have been identified that are involved in egress of the ookinete stages from the midgut represented by AnAPN1 [28], also forming a starting point for SSM-VIMT development.

2.1. Pfs48/45

*Pfs*48/45 is expressed in gametocytes once the parasite undergoes sexual differentiation in the human host and plays a critical role in male gamete fertility [29]. *Pfs*48/45 is a member of a protein family defined by a disulfide bonding pattern of six conserved cysteine residues and glycosylphosphatidylinositol (GPI) anchored in the membrane [30]. The transmission blocking B-cell epitopes fully depend on tertiary structures [31] with an N-terminal domain with epitope V, a central domain that comprises epitopes II and III, and a C-terminal domain containing the most potent transmission blocking epitope I [32]. The most effective target for transmission blocking antibodies is epitope I, while antibodies against epitopes II and III are less potent but show complementary functionality in suppression of infectivity to mosquitoes [33,34]. Although a well-established SSM-VIMT since the gene was cloned in 1993, *Pfs*48/45 has proven to be a difficult target for production of appropriate conformers in multiple heterologous expression systems [13,35–38]. Significant progress was made with production of the 10-C fragment, containing 10 cysteines (residue 159–428) in fusion with Maltose Binding Protein in *Escherichia coli* [35]. While generating appropriate conformers and inducing transmission blocking antibodies in mice, the yield remained low after purification [35]. These challenges were partly overcome by a correctly-folded functional fragment of *Pfs*48/45 (10C) as a chimeric antigen fused in frame with a section of GLURP (GLURP.RO) in *Lactococcus lactis*. Purified R0.10C induced functional antibodies in rats showing strong transmission blocking activity [37]. A fully current good manufacturing process (cGMP) compatible production and downstream purification process of R0-10C has been established at industrial scale in collaboration with Gennova Biopharmaceuticals (India) (Sauerwein, unpublished). Formulation conditions are currently optimized in preparation for clinical testing.

2.2. Pfs230

Production of Pfs230 starts in immature gametocyte stages as a 363-kD precursor protein of 70 cysteine residues, which is subsequently processed into 300- and 307-kDa fragments [14,39]. In the apparent absence of a GPI anchor, these fragments are expressed in stable complex with Pfs48/45 on the parasitophorous membrane of gametocytes [30,39–41]. Once these parasite forms are activated and transformed into gametes, Pfs230 is readily accessible on the membrane for binding to specific antibodies. Functionality of anti-Pfs230 Mabs is dependent on complement fixing isotypes [25,42,43]. A study by Read et al. demonstrated that Mabs of a complement-fixing isotype prevented infectivity of *P. falciparum* to mosquitoes in the presence of complement whilst none of the tested Mabs of non-complement fixing isotypes had transmission reducing effects [44]. Due to its complexity, recombinant expression of full-length Pfs230 has never been achieved. Pfs230 fragment C (residues 443–1132) contains epitopes for at least partial transmission reduction and subsequently became the focus for clinical vaccine development [45,46]. A purified portion of Pfs230 domain C, 230CMB, (residue 444 to 730) expressed in a plant-based expression system induced fully blocking activity in the presence of complement in rabbits qualifying as potential SSM-VIMT vaccine candidate for clinical development [47].

2.3. Pfs25

Pfs25 has been in the lead position since 1983 when specific and potent mAbs were shown to block transmission [10]. Pfs25 is a 25-kDa GPI-anchored surface protein containing four epidermal growth factor (EGF)-like domains with 22 cysteines and 11 disulfide bonds [12]. The protein is shed from the membrane and likely involved in ookinete adhesion and subsequent penetration of the midgut [10,12]. The potent transmission blocking activity of a panel of Mabs recognizing (linear and conformational) epitopes has greatly boosted subunit vaccine development [26,48]. Recombinant Pfs25 as well as *P. vivax* ortholog Pvs25 proteins expressed in yeast, have been the only SSM-VIMTs tested in clinical trials, first as TBV25H that later became Pfs25H [49]. The first human phase 1 trial occurred in 1994 with *P. falciparum* TBV25H absorbed to Alum where 1/8 volunteers experienced a hypersensitivity reaction. Antibody titers were low with consequently unsatisfactory results in the SMFA (Kaslow, unpublished) [49]. In a second trial, TBV25H/alum was used to boost a prime induced by the virally vectored multi-antigen NYVAC-Pf7 that included Pfs25, resulting in at least 75% oocysts reductions in the SMFA in 3/9

volunteers [49]. Many modifications were employed to overcome problems related to homogeneity and conformational integrity of Pfs25H [50]. To increase potency a different adjuvant platform was explored resulting in a clinical trial using Montanide ISA 51 rather than Alum [51]. However, this trial had to be terminated due to occurring erythema nodosum reactions likely related to this specific antigen/adjuvant combination and possibly also the case for the observed transient leukemoid reactions. Sera from volunteers who completed two scheduled doses of Pfs25/ISA51 showed functional activity in mosquito feeding assays. The first clinical *P. vivax* transmission blocking vaccine trial using recombinant Pvs25 formulated with Alhydrogel also induced too low levels of functional blocking antibodies and oocyst reductions by >75% was not obtained [52]. The major challenge for P25-based SSM-VIMT seems the conditional induction of sustained high antibody titers. The exact concentrations of anti Pfs25 abs in human serum required for >75% oocyst reduction are not known but likely far more than 100 μg/ml [10]; in contrast 15–50 μg/ml of Pfs25 Mab 32F81 is sufficient for near complete blockade in laboratory and field-based mosquito feeding assays [53]. More recently some of these limitations may have been successfully addressed. Potent transmission blocking activity was obtained in pre-clinical immunization studies using purified codon-harmonized Pfs25 expressed in *E. coli* [54], with Pfs25 produced in chloroplasts of algae [55], with Pfs25 in Chad63/modified vaccine virus [Kapulu et al. *submitted*, 2], with Pfs25-IMX313 nanoparticles in *Pichia pastoris* [56] or with Pfs25 fused to a modified lichenase (LicKM) carrier in *Nicotiana benthamiana* plants [57]. Another promising approach for enhancing Pfs25 immunogenicity is conjugation to carrier proteins with known high immunogenicity e.g. the outer membrane protein complex (OMPC) of *Pseudomonas aeruginosa* [58]. The group at NIAID that pioneered this approach with Merck scientists has subsequently moved a Pfs25 protein conjugate vaccine into the clinic. Phase 1 human trials of Pfs25 that is chemically conjugated to *E. coli*-expressed ExoProtein A (EPA), the latter entity being a detoxified form of ExoToxin A of *P. aeruginosa* is advancing to a Phase 1 trial by the NIAID group in collaboration with the University of Bamako in Mali.

2.4. Mosquito antigen AnAPN1

Antivector SSM-VIMT can inhibit parasite development by eliciting antibodies that prevent ookinete interaction with mosquito midgut ligands. The Anopheline Alanyl aminopeptidase N (AnAPN1) is originally isolated from the apical brush border microvilli fraction of *Anopheles gambiae* midguts [28,59]. Next to presence of a transmission-blocking epitope, the C-terminal AnAPN1 fragment [60] also contains an immune-dominant decoy epitope inducing non-blocking antibodies. This has delayed the clinical development of AnAPN1. Based on the crystal structure of the near-full length APN1[unpublished], a structure-guided construct has been expressed in *E. coli* and is currently under investigation in transmission-blocking studies (Dinglasan R, *personal communication*).

3. Biological endpoints of SSM-VIMT

Functional transmission blocking activity relies on antibodies targeting surface membrane antigens on sexual/sporogonic stages or mosquito midgut targets. Next to specificity, other aspects have not been fully determined such as antibody concentration and quality e.g. the capacity to fix complement or mediate opsonization [43,61]. There is an obvious need for an immune correlate that will mimic field conditions and reliably predict functionality of induced antibodies against genetically diverse *Plasmodium* and *Anopheles* variants.

3.1. The standard membrane feeding assay

The initial validation and prioritization of SSM-VIMT candidates is guided by antibody tests and functional assays that prevent infection of mosquitoes. The latter is envisaged to be part of the critical pathway of testing efficacy in SSM-VIMT vaccinated communities. This is a major advantage of SSM-VIMT vaccines over other malaria life-stages vaccines for which there is no evident biological endpoint. The standard membrane feeding assay (SMFA) is the most widely used assay to determine the functionality of transmission-blocking antibodies [62,63]; in vitro cultured gametocytes are mixed with serum or purified antibodies and fed through membrane feeders to laboratory-reared, uninfected *Anopheles* mosquitoes. Two outcome measures of the SMFA are the proportion of infected mosquitoes (oocyst prevalence) and the infection burden (oocyst density) in mosquitoes. These outcomes are commonly determined by microscopical examination of mosquito midgut but can also be determined by the detection of parasite DNA by molecular methods [64,65] or parasite protein by immuno-assays [65,66]. Recently, fluorescence and luminescence-based SMFA approaches have been proposed that use *P. falciparum* strains expressing green fluorescent protein [67] and firefly luciferase protein [68]. Luciferase activity in the SMFA closely relates to oocyst prevalence and density [68]. The SMFA has traditionally been evaluated in terms of the reduction in oocyst density compared to controls [69] which gives reproducible results for potent antibody concentrations [70,71]. However, high densities of gametocytes as used in the SMFA and related oocyst densities are not representative for natural infections [70–75]. It is, therefore, difficult to directly translate SMFA outcomes on a density scale to a predicted impact on malaria transmission in the community [74,76]. In order to reduce malaria transmission in a vaccinated human population an SSM-VIMT will have to reduce oocyst prevalence – and not just mean oocyst densities – in mosquitoes feeding on the vaccinated population. This is because even mosquitoes with only one oocyst will produce salivary gland sporozoites [65] and may be (equally) infectious, therefore, as mosquitoes with many oocysts. Therefore, reducing oocyst densities may have little effect on overall malaria transmission unless it also results in significant reduction in oocyst prevalence in the mosquitoes Although oocyst prevalence and density are positively associated in experiments, substantial reductions in density are needed to obtain lower prevalence rates [70,71,77]. As a result, a relatively large number of mosquitoes is needed for precise estimates of transmission reducing activity at the scale of mosquito infection prevalence [77]. The SMFA can be adapted to make these estimates on the prevalence scale more attainable. This can be achieved by diluting gametocyte concentrations in feeders so that <50% of control mosquitoes is infected [77] and by using scalable assessments of mosquito infection status that obviate the need for mosquito dissection and microscopy, such as the above described luciferase-based SMFA [68].

An SMFA that is designed to obtain precise estimates of the reduction in oocyst density appears fully justified, provided outcome measures are presented on both the density and prevalence scale [77,78]. For selected SSM-VIMT candidates, it is of great value to test their efficacy against a range of different oocyst densities in control mosquitoes for a predicted biological efficacy that is most closely related to its public health endpoint i.e. reducing the probability that transmission from a human subject to a mosquito.

3.2. Immunological assays

Antibody concentrations against recombinant vaccine candidates, as measured by conventional enzyme-linked immunosorbent assays (ELISA) have been shown to associate with SMFA

outcome for anti-Pfs25 [24,79,80], Pvs25 [80], Pfs230 [24], Pfs48/45 antibodies [37]. For pre-fertilization antigens, the ability of sera to recognize the native form of proteins can be further tested by quantitative ELISA where gametocyte extract is used for coating [37] or by semi-quantitative immunofluorescence assays that use fixed extracellular gametes (IFA) or live intact gametes in suspension (SIFA) [35–37]. The quantification of antibody recognition in these assays has recently been improved by using luminescence rather than fluorescence as read-out (Roeffen, unpublished observations).

4. Public health impact of SSM-VIMT

Evidence from the field and transmission models indicate that the public health impact of SSM-VIMTs can be expected over all levels of transmission intensity [75,81]. This will lead to a reduced incidence of infections in all endemic areas and can accelerate the path to elimination in low endemic settings (e.g. EIR <8 infectious bites/year) [16] or an incidence of infection below 0.2/person/year [82]). The minimum efficacy of a SSM-VIMT to achieve these reductions in transmission intensity, and even the exact definition of efficacy, has recently been debated. Original workshops agreed that high efficacy in biological endpoints, ≥80% reduction in oocyst intensity in the mosquito midgut [3,75], is needed to take candidates forward to clinical development and that higher efficacy will likely be only achieved by a multi-antigen vaccine. Recently these assumptions were challenged by a population transmission-model using rodent *Plasmodium berghei* and *Anopheles stephensi* mosquitoes to determine the impact of transmission blocking interventions over multiple transmission cycles in different populations with parasite prevalences that reflect different levels of transmission intensity [76]. Using a transmission blocking drug, this model has convincingly shows that the current go/no-go efficacy thresholds for an effective SSM-VIMT of ≥80% reduction in oocyst density [3,75] may be too stringent. Vaccine candidates may be discarded that induce lasting antibody levels and thereby contribute to malaria elimination over several infection cycles [76]. This model further shows that the efficacy of SSM-VIMT strongly depends on the local transmission intensity [75,76,81,82]. Important questions for future SSM-VIMT application are the minimum vaccination coverage and the duration of vaccine efficacy. Unlike vaccines that aim to protect high risk groups from (severe) disease, SSM-VIMT aim for high coverage in the entirety of the human population that contributes to malaria transmission. This reservoir comprises all age groups [63,83]. SSM-VIMT application will in a way resemble mass drug administration campaigns for which high coverage over repeated rounds has been shown to be a considerable challenge [84,85]. The required high and long-lasting antibody titers will stress the need for optimized delivery platforms and adjuvants.

5. Biological endpoints of clinical trials in non-endemic settings

5.1. Immunological and mosquito feeding assays in phase I trials

Sera from vaccinated individuals can be tested for immunogenicity but also for efficacy in the SMFA and the direct membrane feeding assays (DMFA). In the DMFA procedure, venous blood from naturally infected gametocyte carriers is used as source of gametocytes and offered to locally reared mosquitoes in a membrane feeder system similar to that used for the SMFA [86]. The DMFA can support Phase I trials in non-endemic settings by allowing assessment of functional activity of sera against gametocyte infections from endemic settings [73]. This approach was elegantly illustrated in a Phase 1 clinical trial with Pfs25 that was conducted in the US and where transmission blocking activity was induced in a minority of vaccinees, as detected by SMFA [51]. One serum sample was further tested in the DMFA using gametocyte isolates and mosquito colonies from Burkina Faso and Thailand [87]. These DMFA experiments confirmed the transmission blocking potential of vaccination against a range of genetically complex, naturally circulating parasite strains and illustrated the richness of information obtained with these biological assays early in clinical testing.

6. Biological and public health endpoints of trials in endemic settings

6.1. Immunological and mosquito feeding assays

The broader application of immune- and bioassays in Phase 1 and 2 studies in malaria endemic settings can directly quantify the transmission potential of vaccinated gametocytaemic individuals. In addition to SMFA and DMFA, Direct Feedings Assay (DFA) are possible. In DFA laboratory-reared non-infected mosquitoes are allowed to feed directly on the skin of gametocyte carriers; the assay is more sensitive than DMFA, represents a more natural method but is subject to ethical considerations that limit the number of mosquito observations, repeated assessments of infectivity and the use in very young participants [86]. The most obvious design of phase 2 studies in endemic settings would be an individual randomized trial where vaccinated and non-vaccinated individuals are compared for safety, immunogenicity and biological efficacy of vaccination. An alternative or complementary design could involve a comparison of endpoints in trial participants before and after vaccination. This would be particularly valuable if individuals can be identified who are chronic carriers of infectious gametocytes and whose infectiousness can be determined before and after vaccination. The biological endpoints of Phase 2 clinical trials in endemic settings will allow determination of: (i) antibody concentration and dynamics; (ii) dynamics of functional transmission blocking activity in the SMFA with cultured gametocytes; (iii) dynamics of functional transmission blocking activity in the DMFA with naturally infected gametocyte donors; (iv) in a subset of vaccinees who become gametocytaemic, functional transmission blocking activity can be assessed by DMFA and DFA with naturally acquired gametocytes from the vaccinee. For a fully efficacious vaccine, evidence of (near) complete prevention of transmission and long-lived antibody responses make a strong case for accelerated approval where the vaccine can be registered based on surrogate (biological) endpoints from mosquito feeding assays and where evidence for an impact on public health endpoints is collected shortly after vaccine implementation.

6.2. Cluster-randomized trials for public health endpoints

The ultimate objective of SSM-VIMT is to reduce the number of incident infections in a population. There is currently no agreement on whether surrogate endpoints of transmission measures would be satisfactory for accelerated approval, especially if vaccine efficacy is sub-optimal [82]. Two relevant shortcomings of mosquito infection outcomes are that they fail to quantify the effect of the SSM-VIMT over repeated generations, thereby plausibly underestimating efficacy in reducing incident infections [76] and the poorly parametrized association between reductions in the likelihood that a mosquito becomes infected and the level of malaria exposure experienced by a community. The conventional approach would include Phase 1 and Phase 2 trials followed by a large scale population based Phase 3 trial. This Phase 3 trial will involve a cluster-randomized design and outcome measures based on PCR-detected infection incidence with clinical endpoints and safety evaluations as secondary objectives. The assumptions,

outcome and design of a cluster randomized trial for SSM-VIMT were recently reported as outcome of a series of expert meetings [83]. The power and require sample size strongly depend on the chosen settings and their characteristics in terms of transmission intensity (ideally intermediate intensity transmission; ~0.6 incident infections/person/year), stability of transmission, other malaria interventions, homogeneity of transmission between clusters and migration of unvaccinated individuals into intervention areas.

7. Conclusions and perspectives

Stimulated by recent advances in malaria control and increased funding, the elimination of malaria is now considered to be an attainable goal for an increasing number of malaria endemic regions [88]. There is currently an unsurpassed momentum for the development of SSM-VIMT to support elimination initiatives. Despite very slow progress in the last decades, the first clinical trial with Pfs25-EPA is currently being undertaken in endemic settings and, regardless of the efficacy outcome of this trial, will lead to considerable progress in our knowledge on how to conduct and evaluate SSM-VIMT clinical trials. An alternative that has never been fully explored is passive immunization with transmission blocking monoclonal antibodies for preventing malaria transmission. The most potent monoclonal antibodies show full blocking activity at micrograms per milliliter and achieving blocking concentrations in humans may be feasible. Such an approach could serve as a general proof of principle for SSM-TBV in endemic settings and may also be of value as an intervention in specific circumstances such as malaria epidemics where SSM-TBV may be of particular value [23].

The assays to evaluate the biological efficacy of SSM-VIMT have been under scrutiny in recent years, leading to a better parametrization of different feeding assays, higher throughput systems, a range of qualitative and quantitative tools for infection detection and more robust analytical approaches [77]. Importantly, there has been a timely investment in discussing the pathway of SSM-VIMT development from pre-clinical to population studies. In addition to assays with cultured gametocytes (SMFA) or naturally acquired gametocytes (DMFA, DFA), it is conceivable to induce gametocytaemia in the Controlled Human Malaria Infection (CHMI) model [89]. This model has been shown to be a powerful tool for evaluation of antimalarial drugs [90], blood stage and pre-erythrocytic vaccine candidates [91,92]. Early evidence for the induction of gametocytes after CHMI was provided when mRNA of Pfs16, which is one of the earliest expressed gametocyte-specific protein, was detected 48 h after the first detection in the blood circulation of the 18s rRNA of asexual parasites [93]. Blood-stage challenges in volunteers more recently provided evidence that mature gametocytes, detected by microscopy and Pfs25 mRNA, can be induced following drug treatment (McCarthy, 64th Annual Meeting of the American Society of Tropical Medicine & Hygiene, New Orleans 2014). If these gametocytes are found in sufficiently high concentrations to infect mosquitoes, this will pave the way to use CHMI-transmission studies as part of the developmental pathway for SSM-VIMT development. Cluster-randomized trials for SSM-VIMT are considered as an ambitious but achievable approach. Because of the nature of activity of SSM-VIMT and the changing malaria epidemiology in many African and non-African settings [94], cluster-randomized trials for SSM-VIMT will be amongst the most challenging intervention trials in terms of implementation and monitoring. A careful consideration of the optimum design of preceding clinical trials in relation to a case for accelerated approval remains of great importance.

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