Status, challenges and regulatory strategies to develop a malaria vaccine

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List of Abbreviations

ACT	Artemisinin-combination therapies
ADCI	Antibody-dependent cellular inhibition assay
AEFI	Adverse event(s) following immunization
AIDS	Acquired Immune Deficiency Syndrome,
AMA	Apical membrane antigen
AVAREF	African Vaccine Regulatory Forum
BLA	Biological license application
CBER	Center for Biologics Evaluation and Research
CHMI	Controlled human malaria infection
CHMP	Committee for Medicinal Products for Human Use
CIOMS	Council for International Organizations of Medical Sciences
CMI	Cell-mediated immunity
CSP	Circumsporozoite protein
DALY	Disability-adjusted life years
DB-RCT	Double-blinded, randomized controlled trial
DCVRN	Developing Countries Vaccines Regulatory Network
DNDi	Drugs for Neglected Diseases initiative
DNA	Deoxyribonucleic acid
DTP3	Diphtheria-tetanus-pertussis vaccines
EC	European Community
EEA	European Economic Area
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EOI	Expression of interest
EPI	Expanded Program on Immunization
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendment Act
GCP	Good clinical practice
GDP	Good distribution practice
GIA	Growth inhibition assay
GLP	Good laboratory practice
Glurp	Glutamate-rich protein
GMP	Good manufacturing practice
Hib	Haemophilus influenzae type b
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonisation
IFN	Interferon
IgG	Immunglobulin G
IM	Intramuscularly
IMPD	Investigational medicinal product dossier
IND	Investigational new drug
ITN	Insecticide treated net
IPT	Intermittent preventive treatment of malaria
IRS	Indoor residual spraying
i.v.	intravenous
IVB	Immunizations, Vaccines and Biologics
IVR	Initiative for vaccine research
LLIN	Long lasting insecticide treated bed nets
MAA	Marketing authorisation application
MALVAC	Malaria Vaccine Advisory Committee
MCTA	Malaria Clinical Trials Alliance
ME-TRAP	Multi-Epitope Thrombospondin related adhesive protein

MSP	Merozoite surface protein
MVA	Modified vaccinia virus Ankara
NCA	National competent authorities
NRA	National regulatory authority
ODA	Orphan Drug Act
P.	*Plasmodium*
PfSPZ	*Plasmodium falciparum* sporozoite
PfGAP	*Plasmodium falciparum* Genetically Attenuated Parasite
PFS	Product Summary File
PPC	Preferred Product Characteristics
PQP	Prequalification of Medicines Programme
PSF	Product Summary File
PSPQ	Programmatic suitability of vaccines for prequalification
PSUR	Periodic safety update report
RA	Regulatory authority
RCT	Randomized controlled trial
RDT	Rapid diagnostic test
RTS,S	Not an acronym. Scientific name of a malaria vaccine candidate
SAGE	Strategic Advisory Group of Experts
SMFA	Standard membrane feeding assay
TBV	Transmission blocking vaccine
TRAP	Thrombospondin related adhesive protein
s.c.	subcutaneous
SAE	Serious adverse event
SPC	Summary of product characteristics
UK	United Kingdom
UN	United Nations
UNICEF	United Nations Children's Fund
UniTAID	Not an acronym. Organization cooperating with WHO and others on the WHO millennium goals
USA	United States of America
VIMT	Vaccines that interrupt malaria transmission
VVN	vaccine vial monitors
VWP	Vaccine Working Party
WHO	World health organization

1. Introduction

Malaria is a severe and potential fatal disease which poses a major health threat to humans since the Neolithic revolution around 10.000 years ago till to date [1]. It was long thought to be caused by "bad air" (in medieval Latin *"mala aria"*) arising from the marshes[2]. As early as 1677 the extracts of the cinchona tree, containing quinine, were documented as a successful treatment in the London Pharmacopoeia[3]. In 1882 it could be confirmed that malaria is caused by a parasite that later was described as *Plasmodium*[4], a protozoan[5]. Despite many efforts malaria continued to be of huge impact for human health and still is a risk for around 40 % of the world population today[6]. Main risk is that malaria can worsen to a life threatening status causing malaria based death that globally accounted for estimated 600.000 - 1 million deaths in 2010. Mortality is especially high in people not protected sufficiently by an acquired immunity, such as young children, pregnant women and migrants or travellers originating from a region malaria is not found. Although malaria is not listed as a neglected disease by the WHO[7], drug development has been adopted strongly by governmental, private and public funding as well as global health plans, like the WHO and the Bill & Melinda Gates Foundation[8] reflecting a strong political and social willingness to strengthen the development of anti-malaria measures, including a malaria vaccine.

Vaccines have been developed successfully for many diseases and have supported to eradicate smallpox[9] and to reduce the burden of diseases like measles, polio and tetanus[10]. For parasites being protozoan and metazoan organisms, however, only few vaccines have been successfully developed so far. These include vaccines against protozoa, helminths and ticks that were developed for veterinary use[11]. The key challenge for vaccines against parasites generally is seen in the complexity of the organisms. *Plasmodium* species consist of around 5300 genes with many of those involved in immune evasion and host-parasite interactions[12]. This includes shading against the host by resting inside of red blood cells or a high rate of gene polymorphism. A complex pattern between different sexual and asexual forms of *Plasmodium* additionally complicates the immune reaction against the parasite. The parasite´s mechanisms are counteracted by an acquired immunity evolving upon continuous malaria exposure. This form of immunity has lead to the understanding that also a vaccine should be able to trigger an immune answer. In the last decades, the malaria vaccine development and research has been encouraged by several results showing that it is feasible to induce an immune response against *Plasmodium*, moreover that high levels of protection can be achieved by injecting irradiated forms of the parasite[13]. Many of the candidates, however, failed in preclinical and clinical development or had to be redesigned requiring further evaluation. A first candidate has reached a phase III trial and first efficacy data have been released. Depending on the final results and pending evaluations as required, a first malaria vaccine may be available in the near future[14].

2. Aim of the master thesis

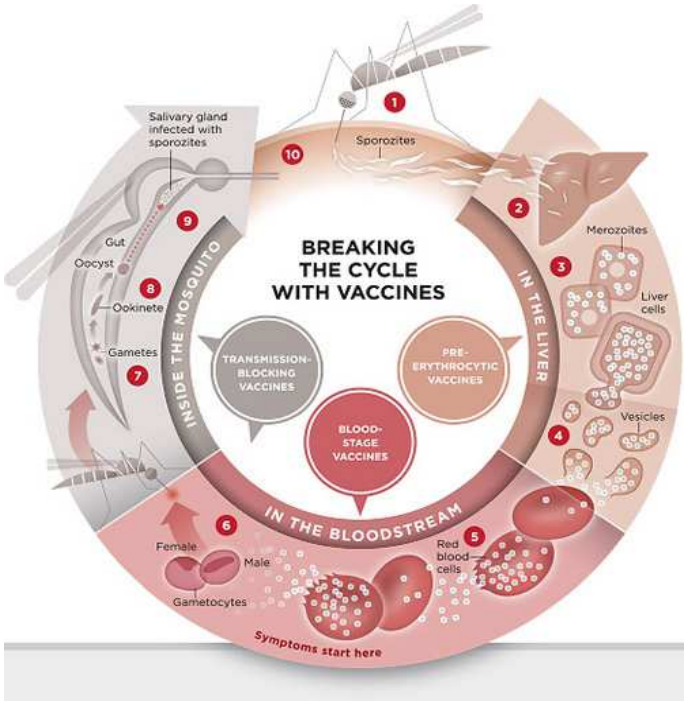
The aim of this master thesis is to evaluate the scientific ground and the regulatory environment that are the basis for developing a vaccine against malaria in humans. It shall assess the need for a malaria vaccine beyond the epidemiological background and current prevention and treatment options. Presenting the current vaccine candidates in development, the applicable regulatory bases and settings for market implementation are discussed. Related to the vaccines under development the regulatory grounds related to quality, safety and efficacy assessments of preclinical and clinical development are evaluated. The main characteristics of malaria vaccine development summarizing key aspects will be discussed.

3. Malaria disease, epidemiology and therapy / prophylaxis

3.1. The *Plasmodium* life cycle

Malaria is caused by monocellular organisms belonging to the genus *Plasmodium*, with its species *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovalae*, in seldom cases in Asia also *P. knowlesi*. They are transmitted via the mosquito *Anopheles* represented by about 20 different species[15]. Characteristic for *Plasmodium* is a life cycle involving two different hosts and several subsequent stages the parasite is represented by unique characteristics. This is reflected by the nomenclature for each *Plasmodium* stage such as sporozoites, merozoites or trophozoites. The *Plasmodium* life cycle is shown in Figure 1.

Figure 1: The *Plasmodium* life cycle[16]



With a bite by an infected mosquito, sporozoites are injected into the human blood (1). The sporozoites are transported via the circulation to the liver and invade hepatocysts (2). Here they undergo numerous divisions, thus, multiply in numbers, and pass through development stages of schizonts into merozoites (3). Through rupture of the hepatocytes, merozoites are first released into vesicles that circulate in the blood stream. In the lung they finally disintegrate and release the merozoites in the blood stream (4). The merozoites invade red blood cells (5), perform multiple divisions passing stages of trophozoites and schizonts and are released into the blood by rupturing of the red blood cells (6). This blood cell cycle repeats and is the basis for regular fever symptoms. Few merozoites eventually develop into gametocytes (7) that get ingested by a

mosquito (8). In the mosquito gut, *Plasmodium* gametocytes, as ingested with the human blood, develop into gametes (7), which fuse to form a zygote that gets motile (ookinete, 8) and invades into the stomach lining. Here, oocysts develop into sporozoites that evade the gut and enter the mosquito's salivary glands (10). Related to the life cycle, vaccines under development are classified according to their mode of action, either to target the pre-erythrocytic stages represented by sporozoites and liver stages (brown colour), blood stage vaccines represented by the merozoite invading red blood cells (red colour), and by transmission blocking vaccines represented by the sexual blood forms in the human host and in the mosquito (grey colour).

3.2. Epidemiology of malaria

Malaria is observed mainly in tropical and subtropical regions, particularly in Africa south of the Sahara, South-East Asia, and the forest fringe zones in South America[17]. As reported by the WHO, it is estimated that about 219 million cases of clinical malaria and an estimated 660.000 malaria caused deaths occurred globally in 2010[18]. Integrating further data about the clinical history, the cases of death have been calculated to be around 1.2 million in 2010[19]. Sub-Saharan Africa accounts for the main burden of overall malaria incidences (80 % of cases), overall malaria deaths (91 % of cases) and deaths in the age group 0-5 years (86 % of cases), that are mostly caused by *P. falciparum* (98 % of all cases)[20]. *P. vivax* caused malaria is found in Asia, Latin America, and in some parts of Africa, and is known to be rarely fatal. It can form dormant liver stages ("hypnozoites") that can get active even months or years after the infecting mosquito bite[21,22]. In Africa, malaria incidence and death cases are focussed in 15 and 13 countries, respectively, and have been correlated to poverty and people living in rural areas[23]. Based on an acquired immune answer against malaria developing within years under malaria exposure, older children and adults remain with a low risk against malaria while complete protection is not observed[24]. However this immunity declines and migrants returning to malaria-endemic regions again have a high risk to develop clinical malaria. Beside children, pregnant women belong to the groups under risk, as an acquired immunity is not functioning in pregnant women, especially in the developing placenta, during first pregnancies. This results into increased morbidity and mortality of the mother and child through occurring miscarriages, premature delivery, up to 25 % of maternal death, a low-birth-weight of neonates as well as neonatal death[25]. Also, an impaired immune system as in HIV patients, or a malaria naïve immune system as in travellers, accounts for an increased incidence of malaria cases and severity in these groups[26,27].

In former times, and partially till the 20th century, malaria was also frequent in Europe with the northernmost country affected being Norway[28]. Interventions on land structure disabling breeding of the mosquitos are thought to be the main cause of malaria eradication in most parts of Europe. It needs to be noted that *Anopheles* is still endemic in Europe, as for example in Italy[29]. In 2011, in Germany overall 562 cases were reported originating mostly from a stay in African regions. The same year, 40 indigenous *P. vivax* infections have been reported from Greece[30].

Globally the land area that enables malaria has been reduced by half in the 20th century. However, population at risk has increased in relative as well as in absolute terms since 1994 due to population growth in malaria-endemic regions[31]. Climate change and global warming have frequently been discussed as contributors to a further malaria distribution and incidence. Conversely, when projections were performed to analyse the situation in Africa, it was rather seen, that some areas will show an increase whereas other regions will show a decrease of malaria[32]. Moreover, global warming is not judged to be the dominant parameter and geographical extension of malaria is thought to be stronger impacted by ecological, social, political and economical aspects than by climate changes[33]. To monitor potential changes seeing that reoccurrence of malaria in Europe cannot be excluded, the effects of global warming on the transmission of infectious diseases are subject to increased attention by the European Commission[34].

3.3. Clinical signs and symptoms of malaria

The dominant clinical sign of uncomplicated malaria is fever that can occur intermittently and has given malaria the name “remittent fever”. The species *P. malariae and P. ovale* as well as *P. vivax* cause fever that repeats every 72 hours (Malaria quartana) and 48 hours (Malaria tertiana), respectively. Fever caused by *P. falciparum* instead occurs irregularly and does not follow the remittent fever cycles. Further symptoms of malaria are headache, chills and vomiting, as well as speech difficulties, deafness or blindness. Non-severe malaria is well curable based on a correct diagnosis and with appropriate therapy as recommended[35].

In contrast to the milder forms *P. falciparum* infections may develop, if not treated within 24 hours after occurrence, to severe malaria[36]. Severe malaria is defined as “Acute falciparum malaria with signs of severity and/or evidence of vital organ dysfunction“[37] and is the main cause of the observed mortality in the high risk groups. These risk groups include young children, pregnant women, immuno-compromised patients (like HIV patients), international travellers from non-endemic areas and immigrants from endemic areas returning to endemic areas[38]. Occurrence of severe malaria is an emergency situation considered to requiring the highest clinical care also in an emergency unit and applying a parenteral therapy[39]. With first occurrence of symptoms and even if treatment is initiated immediately, mortality rates are already as high as approx. 10-20 %. Any delay or non-treatment leads to a drastic increase of mortality[40]. The symptoms of severe malaria include severe anaemia, respiratory distress in relation to metabolic acidosis, cerebral malaria resulting in coma or multi organ failure, kidney failure, seizures, pulmonary oedema and bleeding due to blood clotting. Cerebral malaria and coma have been attributed to long term neurological sequelae in children as well as in travellers[41].

3.4. Natural and vaccine induced immunity against malaria

The human immune system with its cellular and humoral defence mechanisms is the basis to generate an immune answer against pathogens. As a response to continuous malaria exposure, people develop a so called acquired immunity[42]. With a first infection, non-

immune individuals develop clinical malaria based on a very low parasitemia that is at risk to progress to severe malaria. With further infections an anti-disease immunity is emerging showing a reduced risk of severe malaria while the parasite burden is still high. If infections continue, an immunity protecting also against mild forms of malaria is achieved. The individuals are healthy and asymptomatic and have only low numbers of parasites in the blood. This stage is thought to be a steady state having a clinical immunity of the individual on one side, however the benefit of the parasite that is able to continue transmission on the other side. Sterilizing immunity of the human host preventing transmission seems not to be achieved in natural immunity[43]. It is unclear if immunity is consisting of solely anti-parasitic responses or also of mechanisms to reduce inflammation that reduce clinical symptoms. The acquired immunity is normally developing in children in the ages between 6 months and 5 years. Infants up to approximately 6 months are immune through antibodies and growth-inhibitory factors provided in the milk of the mother. Immature immunity is causally responsible for the high incidence of severe disease and mortality of children up to an age of 5 years in sub-Saharan Africa. Acquired immunity needs regular re-challenge and is getting lost if people move to non-malaria regions for more than approximately 6-12 months. The immunity is stronger in high transmission areas compared to low transmission areas and is also strain and species specific[44].

It is thought that all stages of *Plasmodium* cause an immune answer with the asexual blood stages of merozoites being the main immune stimulators. When antisera of immune competent individuals containing antibodies were given to immune incompetent individuals a marked reduction in parasitemia was observed showing that the so called humoral immune response with B cells and antibodies is contributing, however alone not sufficient for protection[45]. They also showed that a passive immunization through transfer of serum of an immune competent individual to another is not likely to be useful when developing a malaria vaccine. Rather, malaria vaccination needs to target an active immunization that requires an induction of a functional immune response of the vaccinated individual. The second important branch to establish an immune response, the so called cell mediated immune response through cells like T cells or phagocytes, was shown to strongly contribute to protective immunity in mouse models and in human natural immunity[46]. The key components have been specified in more detail. However, the kind of immunity differs strongly between individuals and common mechanisms for all immune individuals have not been found. Accordingly a surrogate endpoint for efficacy in clinical trials based on an immune correlate is not yet available[47]. Current malaria vaccine research has started to build on a broad cell mediated as well as a humoral immune response. Unravelling the immune mechanisms of innate as well as vaccine inducted immunity is seen to be essential to be able to develop a vaccine assuring a high and long lasting efficacy[48].

To note is that as result of a strong natural selection provoked by malaria, human genetic mutations have evolved that mean a benefit for the carrier against clinical malaria giving raise to innate immunity[49]. Well known is the haemoglobin beta gene mutation, causing sickle cell trait in the homozygous situation. Heterozygously, the mutation is beneficial for the carrier, who have a 90 % protection against severe and, thus, life threatening malaria[50]. A further gene is the duffy protein encoding gene, that, when mutated, prevents

P. vivax invasion of red blood cells. Here, the carrier of the mutation, known to be frequent in West and Central Africa[51], are protected from all forms of clinical malaria[52]. Accordingly, the assessment of the genetic background of study participants might be to be considered when testing vaccine efficacy in clinical trials.

3.5. Unmet needs beyond current malaria prevention and treatment practice

Today a range of options exist to prevent mosquito bites, to implement a medical prophylaxis and to apply diverse treatment options in case a malaria infection occurred. An easily manageable disease, with prevention measures functioning on a high quality and long term level, would likely not result into efforts to develop a vaccine for global implementation. To assess the unmet need in malaria prevention that is not covered by the existing measures, however could be fulfilled potentially by a vaccine, current malaria prevention and treatment practice is presented here.

Ways to mechanically prevent mosquito bites and thus a malaria infection are to use insecticide treated bed nets (ITNs), especially long lasting insecticide treated bed nets (LLIN), indoor insecticide spraying (also called “indoor residual spraying”, IRS) or mosquito control in the natural habitat. In sub-Saharan Africa around 35 % of the population are sleeping under an ITN, a percentage that is judged to be far from universal coverage targets[53]. The bed nets can be used effectively over three years before they need to be exchanged. However, the supply and resupply is not thought to be assured, mainly due to funding issues[54]. Indoor residual spraying has recently reached 11 % of the population at risk in Africa. It is assumed that high costs limit any further increase of spraying measures[55]. Moreover, several studies indicate emergent resistances against the insecticides, as in Mozambique, Ghana and Uganda[56,57,58]. Similarly to emerging insecticide resistances, resistances against larvicides used in land control programmes has been observed. This indicates that existing measures have a high risk to be ineffective in the near future and new compounds are required[59].

Medical prophylaxis is based on medicinal products used in monotherapy or in a combination therapy. The products chosen depend on the targeted geographical region and local resistances. Short term travellers are thought to have a broad prophylaxis regimen that is judged to be effective and impacted only by mild side effects and lacking compliance in the intake. People living in endemic areas or long term travellers, who in total represent the majority of people under risk, have a limited possibility to perform medical prophylaxis. For endemic regions WHO recommends a preventive treatment for pregnant women and children in high transmission areas and for children of less than 5 years of age in areas of seasonal malaria[60].

Main therapies of clinical malaria include monotherapy with quinines and artemisinin-combination therapies (ACT) as recommended by the WHO[61]. Today, based on the current therapies, the WHO considers malaria to be a preventable, curable disease[62]. This however requires quality drugs are available globally. Instead, ACT availability is impacted by supply constraints, quality issues and counterfeits. In South-East Asia, for example, it was estimated that around 30-35 % of ACTs are counterfeits[63]. A WHO enabled task force provides support to minimize ACT shortcomings[64]. As reported in 2012, it is estimated that

in the African and South-East Asian region only 55 % and 73 % of malaria patients coming to public facilities could be treated as needed[65]. Moreover, artemisinin resistance has been observed in 4 Asian countries and is monitored very carefully by the WHO as a new threat to current malaria treatment standards[66]. Current practice is also to advice ACT treatment when fever occurs, however malaria has not been firmly diagnosed[67]. Overall it is judged that ACTs is not available as needed and is not used as indicated.

The global decrease in incidence and mortality from 2001 to 2010 is seen to reflect that 274 million cases of clinical malaria and 1.1 million of malaria caused death could be prevented by ACT treatment, usage of IRS and bed nets. Ten countries with the highest burden of malaria have contributed more than half to this number[68]. In field studies in diverse countries the impact of measures was analysed more specifically. In Zanzibar for example, implemented ACT treatment was correlated with a 71 % reduction of malaria incidence[69]. Introduction of ACT treatment plus indoor residual spraying resulted into a 99 % decrease of malaria incidence in a defined area within South-Africa[70]. The analyses show that measures of insecticides treated nets, vector control, indoor residual spraying and preventive treatment lead to promising results locally. Conversely to the promising results it was also seen that a shift in the peak of clinical malaria to older children occurred, implying the need to carefully monitor any interference with the natural course of malaria[71]. The under-coverage of measures globally and the need to replace currently used drugs and chemicals due to emerging resistances are understood as a gap in malaria control globally necessitating better solutions.

Preventive vaccines are known to have a good safety profile that is better than therapeutic treatments and they are judged to be the most efficient tools for promoting individual and public health[72]. Main characteristics are that preventive vaccines can provide a durable prevention against a disease and accordingly prevent burden that would occur through a clinical manifestation and the recommended treatment with its side effects, required treatment schedules and costs. Although smallpox is so far the only disease that has been eradicated through the implementation of vaccines, local elimination of other diseases like measles or mumps is reported, overall resulting in estimations that almost 6 millions deaths per year can be prevented by vaccines[73]. Vaccination in general terms is described to make good economic sense, however especially to meet the need to care for the weakest members of societies. One aspect is the reduction of child mortality through vaccines as such being a moral obligation for the international community[74]. Beyond this background it is understood that a preventive malaria vaccine could be a potent, effective and affordable tool to reduce malaria incidence and mortality[75] and to lead to huge savings of costs currently correlated to malaria[76]. Scientifically it was seen that immune mechanisms are part of the natural defence strategy and moreover that they can be raised artificially in animal modes and in human beings, making It reasonable and feasible to develop a protective vaccine against malaria[77]. Newly established malaria vaccines with a changed mode of action and targeting transmission of the parasite even add a new dimension of effectiveness when targeting malaria eradication[78].

4. Expectation on a malaria vaccine

WHO, with its Malaria Vaccine Advisory Committee and coordinated by the WHO initiative for vaccine research (IVR), established a malaria road map to strengthen the development of a malaria vaccine by defining goals related to timelines, safety and efficacy of a quality malaria vaccine according to international standards. The efforts are supported widely by foundations, governmental organizations as well as by vaccines and clinical trial initiatives. The phrased roadmap defines the following goal and landmarks related to efficacy of a future malaria vaccine[79]:

"Strategic goal: By 2025, develop and license a malaria vaccine that has a protective efficacy of more than 80 % against clinical disease and lasts longer than four years
Landmark: By 2015, develop and license a first-generation malaria vaccine that has a protective efficacy of more than 50 % against severe disease and death and lasts longer than one year."

Adapted to changing epidemiology and evolving knowledge, WHO and funding agencies discussed an update of the vision in March 2013 to include *P. vivax* vaccines. Moreover, the vision is broadened to also target *Plasmodium* transmission to finally enable malaria eradication[80]. Accordingly the following strategic goals have been added (draft status, pending final publication)[81]:

"Strategic goal: By 2030, license Vaccines targeting P. falciparum and P. vivax and encompassing the following two goals, for use by the international public health community:
– Malaria vaccines with a protective efficacy of at least 70-80 % against clinical malaria, suitable for administration to appropriate at-risk groups in malaria-endemic areas.
– Malaria vaccines that reduce transmission of the parasite and thereby substantially reduce the incidence of human malaria infection. This will enable elimination in multiple settings."

Requirements on safety of a future malaria vaccine need to take into account, that preventive vaccines are targeting normally healthy individuals, among those also mostly children. This is limiting the accepted risks almost too non-serious, short term side effects[82]. For a malaria vaccine the desired safety profile is described to be non-inferior to that of currently licensed paediatric vaccines, and ideally superior to that of current vaccines[83].
In order to provide further guidance to vaccine developers on the desired characteristics, the WHO, with input from the Strategic Advisory Group of Experts (SAGE), will develop two sets of "Preferred Product Characteristics (PPC)" in the coming years 2013-2014. These will be aligned continuously to scientific and regulatory progress and thus do not represent static documents. Most guidance for vaccine developers is thought to be needed in defining the target population against clinical disease and transmission, respectively, and also how to determine efficacy by appropriately designed clinical trials.

5. Malaria vaccines in clinical development

Malaria vaccines under development belong to the group of vaccines causing an active immunization through induction of an immune response by the vaccinated individual. Based on the stage of *Plasmodium´s lifecycle,* the genes or cells used to establish a vaccine are active, the vaccines can be subdivided into pre-erythrocytic stage vaccines, blood stage vaccines and transmission blocking vaccines (see also section 3.1). The different subgroups are thought to establish a specific kind of protection predetermined through their mode of action related to the life cycle.
The subunit vaccines in development are either the products of antigens expressed in bacteria or yeast or are vectors containing the subunit coding DNA and allowing antigen expression in the human host. Whole cell vaccines comprise the entire *Plasmodium* cell and are administered either as intact, inactivated or attenuated forms that are expected not to cause a breakthrough infection.
Key vaccines of the different subgroups are described below with key features of preclinical and clinical testing and observed efficacy, concluded with an overview on trends in the vaccine development generally.

5.1. Pre-erythrocytic vaccines

Vaccines composed of specific antigens of sporozoite or merozoite stages or of whole cell preparations of the sporozoite represent pre-erythrocytic vaccines. By establishing immunity against these targets, pre-erythrocytic stage vaccines are expected to prevent migration of the sporozoite from the injection site through the circulation to the liver, the invasion of the liver cells and the parasite development within the liver cells. This mode of action is expected to prevent clinical disease and finally transmission[84]. As these vaccines prevent occurrence of blood stages of the parasite and thus may interfere with the development of naturally acquired immunity, the efficacy should be long lasting and achievable with a short term immunization scheme. The vaccines are supposed to elicit antibodies that can trap the migrating parasite and to generate cellular immune responses that interfere with parasite development in the liver cells. The number of parasites after a first infection is still low and thus might represent a good target for a trained immune system, conversely the time to elicit an immune response is limited and thus the vaccine induced immunity needs to be effective not needing any further potentiating processes.

5.1.1. Subunit antigens

One key candidate antigen is the circumsporozoite protein (CSP) that is ubiquitously and strongly expressed by the sporozoite on its cell surface during the time the sporozoite migrates to the liver[85]. The protein is involved in the sporozoite adhesion and invasion of the hepatocyte. In the 1980´s it was shown that antibodies against CSP inhibit malaria infection and *Plasmodium* invasion of cultured cells[86]. Moreover CSP was determined to be a key component to transfer sterile immunity upon infection of genetic or radiated attenuated sporozoites, confirming its key role in developing a vaccine[87]. However, first

preclinical and clinical attempts with CSP did not show the targeted efficacy. In further attempts, a recombinant protein consisting of a CSP domain causing antibody responses was fused to a hepatitis B virus surface protein. This protein enables the formation of virus like particles as a carrier. The CSP-hepatitis construct was added with other CSP domains found to elicit T cell responses. The resulting vaccine RTS,S/AS01 formulated with the adjuvant AS01 represents the most advanced vaccine in development. The proteins are expressed in yeast where they co assemble to the virus like particles that represent the final vaccine. The CSP gene has been used to develop other vaccines, that are currently in phase I and II clinical trials. Here, CSP is combined with other subunit antigens, like AMA1 and Multi-epitope-Thrombospondin related adhesive protein (ME-TRAP), and additionally different delivery systems and carriers are tested[88]. Also a new combination of CSP with a viral vector has started in phase I preparing for potential future use to improve a CSP based vaccine with another vector system[89].

Another pre-erythrocytic antigen is the protein TRAP. It becomes exposed at the surface of the sporozoite when the parasite comes in contact with host cell and is required for sporozoite motility and host cell invasion[90,91]. The presence of IgG-antibodies against TRAP correlated with a decreased risk for infection similar to a presence of anti-CSP-antibodies[92]. TRAP is also targeted by cell mediated immunity being involved in inhibition of infection[93]. The antigen TRAP has been combined with multiple epitopes ("ME") that are known to elicit a stronger antibody and T cell response, giving rise to the construct ME-TRAP. This construct has been tested in combination with several different vectors, that are not only carrying the antigen DNA, but are known to contribute to the immunogenicity of the resulting vaccine. Vectors tested for delivery and expression of ME-TRAP included a DNA vector, a fowl pox vector, the modified vaccinia virus Ankara (MVA) and chimpanzee adenovirus vector 63 (ChAd63). It was seen that in malaria-naïve individuals, a partial protection could be observed for some of the tested constructs. However, the results did not translate into efficacy in individuals in malaria-endemic regions with the conclusion drawn that the T cell response was not sufficient. Further improvement of the ME-TRAP antigen immunization was assessed by a heterologous prime-boost immunization scheme involving the vectors MVA and ChAd63[94]. It involves priming immunization with ChAd63-ME-TRAP followed by boosting with MVA-ME-TRAP. To be able to compare malaria naïve versus malaria-exposed individuals the trials were conducted in similar settings in the UK and Gambia and Kenya. The data show that the vaccines are safe and well tolerated. A strongly increased answer of IFN-γ secreting T cells over a time period up to 9 months was observed in this vaccination settings showing that the targeted increase of T cell mediated immunity was achieved. A phase I/IIb trial to assess efficacy of the heterologous prime-boost strategy is currently recruiting children in Burkina Faso[95]. The efficacy is currently further tested in adults in Kenya and in Senegal in ongoing phase IIb studies with the aim to also define correlates of efficacy and immunity[96, 97]. Moreover MVA-ME-TRAP and ChAd63 ME-TRAP have been combined with other pre-erythrocytic antigens, like CSP or AMA-1, to further increase immunogenicity. Also, ME-TRAP is tested for increased immunogenicity when combined with an adjuvant[98].To note is, that many viral vector vaccines and prime-boost immunization regimes are, beside malaria, still in development for HIV, cancer or influenza[99]. Few viruses as vaccine vectors

have been licensed for human use so far, reflecting that malaria vaccine development is applying state of the art research that will need to be based on state of the art regulatory processes as well.

5.1.2. Sporozoite based whole cell vaccines

Whole cell vaccine approaches have been tried since early in the 1970s using pre-erythrocytic sporozoites with promising signs of efficacy in animal as well as in human healthy volunteer studies. A major breakthrough was achieved in a study performed in mice showing that irradiated sporozoites, transferred through mosquito bites, conferred a partial protection against malaria[100]. Later on, this was confirmed also in primate models and in human volunteers showing that even sterile protection can be achieved with irradiated sporozoites[101]. A sterilizing protection against infectious mosquito bites was also achieved when the volunteers received whole cell immunization under prophylactic chloroquine treatment[102]. However, sporozoite production and delivery to the host as a vaccine could not be achieved adequately to support a clinical development of these whole cell vaccines[103]. The experiments, however, were continued as they also gave important insights into natural immunity. Moreover in form of the controlled human malaria infection (CHMI) they served as a test model for other malaria drugs in development[104]. It could be shown, that genetically attenuated sporozoites arresting in late liver stages provide a better protection than genetically or radiation attenuated sporozoites that arrest in early liver stages[105].

Attenuation is an essential aspect during development of attenuated vaccines needing to show the resulting vaccine is a homogenous product, is safe and not causing break through infection of the vaccinated individual instead of their prevention or a reversion to virulence. Additional concern are a reversion to an infective organism or a possible exchange of genetic information with wild type or other micro organisms[106]. In the experiments with *Plasmodium*, attenuation of the sporozoites has been achieved through radiation, chemical manipulation and genetic means. Essential for the attenuation, that on the one hand sporozoites should retain sufficient viability up to a liver stage state, as non-viable forms do not confer protection. On the other hand attenuation should prevent any break through to a clinical manifestation of malaria caused by the vaccine. Radiation or chemical treatment results in random DNA breaks that can interfere with development of the sporozoites. In contrast to this, genetic ablation targets specific genes that are essential for the sporozoite and that can be stably kept in form of a *Plasmodium* strain. Genetically attenuated *Plasmodium* strains therefore represent a homogenous genetic population. Deleting a gene involved in fatty acid synthesis or in liver stage specific expression, respectively, break through infections were observed, showing that the attenuation by deleting one gene was not sufficient. The double attenuated strain containing both mutations instead of only one showed a complete attenuation[107].

Mosquito bites to achieve sporozoite infection are not the appropriate method in order to achieve standardized, sterile infection on a large scale basis. Moreover, an appropriate dosing route needs to be developed for an approvable vaccine based on whole *Plasmodium* cells. Recently, a sporozoite production method has been developed that

allows producing infectious aseptic, purified, vialed and cryopreserved sporozoites. These sporozoites can be injected with syringe and needle and were shown to be viable based on emerging parasitemia in healthy individuals, thus omitting any mosquito bites[108]. Using this technique, it was shown that intradermal and subcutaneous applications of sporozoites were safe, but resulted in only weak immune responses in the tested animals and human trial participants, respectively. In mouse experiments, for example, it was shown that an intradermal injection resulted into a 7-13 % protection against malaria, whereas a 90-100% protection was achieved through an intravenous injection of the sporozoites[109]. As a next step, the intravenous dosing of the sporozoites as described had been tested for safety, tolerability and efficacy in a phase I trial with malaria naïve adults[110]. The data show that the vaccine was safe, well tolerated and efficacious in a dose dependent manner with a higher efficacy if more sporozoites per dose and more injections were given. In the highest dose group a completed protection against infection was seen in all six volunteers. Moreover also the elicited cellular and humoral immune response was found to be dose-dependent. Future studies to assess duration of protection, degree of protection against other *Plasmodium* strains and the underlying immune response are planned.

5.2. Blood stage vaccines

Blood stage vaccines targeting the asexual stages are aimed to prevent multiplication of merozoites in the blood cycles and thus should reduce overall parasite burden resulting into less severe and less frequent clinical disease targeting ideally complete prevention of clinical disease. Blood stages of *Plasmodium* are characterised by a high proportion of gene polymorphisms. This is thought to prevent complete efficacy of blood stage vaccines and may conversely promote further escape mechanisms by the parasite resulting potentially into *Plasmodium* forms that are more pathogenic than the existing forms[111]. Presented examples of asexual blood stage antigens in development are the Apical membrane antigen (AMA-1) and the merozoite surface antigens MSP-1 and MSP-3.
The AMA-1 protein is involved in the hepatocyte invasion by the sporozoite[112], but mainly known to inhibit merozoite invasion of the red blood cell[113], and is therefore classified as a blood stage vaccine. AMA-1 based subunit vaccines have achieved protection against malaria challenge in animal models and anti-AMA-1 antibodies have been showed to inhibit *P. falciparum* growth *in vitro*[114]. Anti-AMA-1 antibodies have been further detected in malaria immune individuals, and it was found that the antibody titer correlated with malaria protection[115]. AMA-1 antigen as a vaccine target turned out to be implicated by the high polymorphism of this protein and strain specific solutions or restriction to a conserved domain of the AMA-1 protein have been analysed. Similarly to TRAP, AMA-1 has been tested related to different vector constructs and combination with other antigens, like CSP and ME-TRAP are ongoing. Recently, in a phase I study with a CHMI read out to assess for protection, 27 % of sterile immunity was observed in malaria naïve adults after a heterologous prime-boost vaccination containing the antigens CSP and AMA-1 placed to a DNA- and a adenovirus vector, respectively[116].

The merozoite surface protein 1 (MSP-1) is involved in the merozoite invasion of the erythrocyte and intracellular merozoite development[117]. First attempts with a MSP-1 containing subunit vaccine were poorly immunogenic, caused hypersensitivity reactions or anti-MSP-1 antibodies did not show efficacy[118,119]. However antibodies against one domain of MSP-1 were correlated with a lower malaria incidence[120,121]. A phase I study testing a strain specific MSP-1 allele showed no clear efficacy as tested in the GIA as a surrogate for efficacy[122]. A cross reactivity against heterologous alleles might argue in favour of further trials involving the MSP-1, also overall development of this antigen seems to be seen controverse[123].
Merozoite surface protein 3 (MSP-3) is involved in the invasion of hepatocytes as shown by *in vitro* assays. Moreover immunization of *Saimiri sciureus* monkey controlled parasitemia to various degrees[124]. A phase I study (not designed to assess any efficacy), revealed also hints of efficacy of a MSP-3 vaccine[125] and antibodies against the C-terminus of MSP-3 were correlated with a lower malaria incidence[126]. Currently a vaccine consisting of MSP-3 is assessed in combination with the protein Glutamate-rich protein (GLURP)[127] assessing if a synergistic effect can be verified in a clinical trial[128]. A proof-of-concept trial is ongoing in parallel[129] assuming that a new vaccine candidate may progress in development if the results are positive.

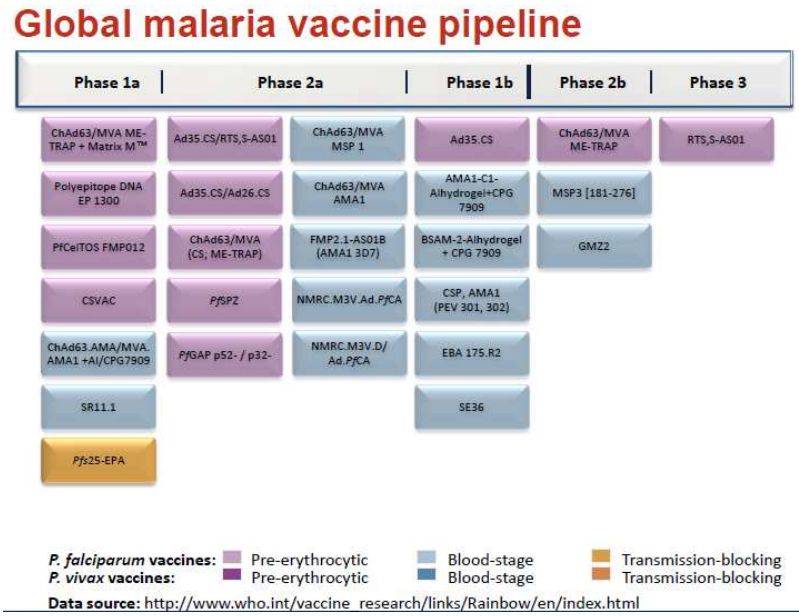
5.3. Transmission blocking vaccines

Sexual blood and mosquito stages are expected to prevent transmission of *Plasmodium* from the human to a mosquito reducing, in a first step, parasite prevalence in the mosquito and, in a second step, reducing transmission intensity. This kind of vaccine is also named transmission blocking vaccine (TBV). By preventing transmission, this type of vaccine is meant to reduce overall burden of malaria in a specific geographical region and on a population level, but not for the individual who is vaccinated. This is a significant characteristic having impact on the regulatory strategy of clinical development and licensing.
So far several proteins have been identified that are candidates for transmission blocking antigens. As immune pressure by the host did not select for polymorphism, these antigens are not or only little polymorphic[130]. Mechanism of killing the parasite is mainly antibody driven and allowed the development of an assay assessing antibody responses elicited by vaccines, the Standard membrane feeding assay (SMFA)[131] (see also section 9.2). Using this assay in a phase I clinical trial, it could be shown that a vaccine carrying the gene coding for the ookinete specific surface protein, named Pfs25, elicits a transmission blocking activity[132]. Currently a phase I trial assessing Pfs25 together with detoxified form of *Pseudomonas aeruginosa* exoprotein A to increase the immune responses, is ongoing[133,134]. Combining the antigen with the pre-erythrocytic antigen CSP long lasting immune responses in mice could be elicited, showing that combination vaccines may be feasible to target different stages of the *Plasmodium* cycle[135].

5.4. Conclusions on malaria vaccines in development

Malaria vaccines in development belong to diverse subunit antigens related to all stages of the *Plasmodium* life cycle and use either single genes or the whole cell assessing diverse vectors and adjuvants, with a focus on *P. falciparum* pre-erythrocytic and blood stage vaccines. An overview on current vaccines in development is published by the WHO in form of the WHO rainbow table integrating regular updates. This table lists key preclinical vaccine projects as well as clinical projects related to the type of subunit vaccines with a graphic overview on clinical projects shown in Figure 2.

Figure 2: Global malaria vaccine pipeline[136]



Legend/Reference to key vaccines described in the text:
CSP derived vaccines: *Ad35CS, RTS,S-AS01, CSVAC*
TRAP derived vaccines: *ME-TRAP*
AMA-1 derived vaccines: *AMA1-C1, AMA1 3D7, AMA1, ChAd63- AMA*
MSP-1 derived vaccines: *BSAM-2*
MSP-3 derived vaccines: *GMZ2*
Whole cell vaccines: *PfGAP p52-/p32 (genetic attenuation), PfSPZ (attenuation by radiation)*

Interestingly the rainbow table also keeps record of projects that have failed either during preclinical or during clinical development reflecting the aim to present malaria vaccine research in a transparent manner. To note is that currently a single project assessing a TBV is in clinical development. Conversely, *P. vivax* vaccines and a vaccine targeting malaria in pregnancy are still in preclinical development[137,138]. Taking into account that a high proportion of vaccine candidates will fail during development, main targets of the malaria roadmap covering special populations like pregnant women, or targeting eradication applying TBVs, likely will not be achieved in the near future.

6. Regulatory basis for the development of a malaria vaccine

With around 40 % of the world population at risk, a malaria vaccine is a matter of global interest. Accordingly the World Health Organization (WHO), defined to be “the directing and coordinating authority for health within the United Nations system“, is actively „shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries and monitoring and assessing health trends“[139]. A malaria vaccine with a medical demand on a global level is to be seen is this regulatory and quality frame set by the WHO. In its function, the WHO is providing the path for developing countries to finally perform local licensing. Leading regulatory authorities like the FDA and the EMA are engaged additionally to strengthen efforts to improve global health status. To be able to do so, specific legal bases have been phrased to take regulatory and licensing conditions of the malaria-endemic countries into account. The regulatory bases of WHO, FDA and EMA, that are essential for the development of a malaria vaccine in its quality, safety and efficacy requirements to target licensing in malaria-endemic, however also in malaria non-endemic countries, are presented in the following. The involvement of NCA (national competent authorities) for licensing of a malaria vaccine in the malaria-endemic regions is discussed in the global framework. Their input into clinical trial authorisation is shown in section 6.4.2.

6.1. World Health Organization (WHO)

The WHO is the agency of the United Nations (UN) that is concerned with international public health within the WHO. The department IVB (Immunizations, Vaccines and Biologics) is the main organ taking care of the broad range of vaccine aspects, including quality, research and development, vaccine supply and immunization financing and system strengthening and optimal use[140]. Reference material, guidelines and recommendations developed by the WHO, with its associated groups, serve as a basis to achieve global quality, safety and efficacy of biological medicines and diagnostic tests and are meant to provide guidance to national regulatory authorities and to vaccine manufacturers. Any guideline may be adopted as such as a national regulatory document and in case it is adapted locally, it should not deviate from the established standards to assure finally a safe and efficacious product. Beside setting standards, the WHO defines its own role in global health, to provide leadership, to shape the research agenda, to articulate ethical and evidence-based policy options, to provide technical support and to monitor the global health situation and trends[141].

In 2001, the WHO started to offer the assessment of quality, safety and efficacy, including adherence to GMP and GCP, of medicinal products, that are purchased by global agencies like UNICEF, the Global Fund and UNITAID meant to be for distribution in resource-limited countries[142]. The aim is to give an independent advice on the assessed products and to assure they are safe, effective and suitable for the target populations at the recommended immunization schedules and with appropriate concomitant product[143]. The programme is called WHO Prequalification of Medicines Programme (PQP) that is a

UN programme managed by the WHO. With adequate data on quality, safety and efficacy WHO policy recommendation and the WHO process of prequalification will finally trigger the global implementation of vaccines. Policy recommendation and prequalification are described in detail in sections 6.1.1 and 6.1.2.

The following main WHO recommendations and guidelines apply to preclinical and clinical aspects as well as to prequalification of a malaria vaccine:

Table 1: Key relevant WHO guidance documents

WHO documents	Document reference
Quality	
WHO good manufacturing practices: main principles for pharmaceutical products. In: Who Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fifth report. World Health Organization, 2011, Annex 3.	WHO Technical Report Series, No. 961, 2011
Good manufacturing practices for pharmaceutical products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-second report. Geneva, World Health Organization, 1992, Annex 1.	WHO Technical Report Series, No. 823, 1992
Guidelines on stability evaluation of vaccines. Geneva, World Health Organization, 2006	Document WHO/BS/06.2049
WHO good distribution practices for pharmaceutical products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fourth report. Geneva, World Health Organization, 2010, Annex 5.	WHO Technical Report Series, No. 957, 2010
Guidelines on the international packaging and shipping of vaccines	WHO/IVB/05.23, December 2005
Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities. Points to consider for manufacturers of human vaccines.	WHO, November 2012
Guidelines for Independent Lot Release of Vaccines by Regulatory Authorities.	WHO, 2012, Technical Report Series (in press) (ECBS 2010)
Non-clinical development	
WHO guidelines on nonclinical evaluation of vaccines.	WHO Technical Report Series, No. 927, 2005
Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines.	Proposed guideline, WHO/BS/2013.2214
Clinical development	
Clinical considerations for evaluation of vaccines for prequalification. Points to consider for manufacturers of human vaccines.	WHO, October 2010
Guidelines for good clinical practices (GCP) for trials on pharmaceutical products. In: WHO Expert Committee on the Use of Essential Drugs, Sixth report. Geneva, World Health Organization, 1995, Annex 3.	WHO Technical Report Series, No. 850, 1995
Guidelines on clinical evaluation of vaccines. Regulatory expectations. Annex 1 in: WHO Expert Committee on Biological Standardization. Fifty second report. Geneva, World Health Organization, 2004.	WHO Technical Report Series, No. 924, 2004
Global vaccine safety blueprint.	WHO/IVB/12.07 WHO February 2012
Definition and Application of Terms for Vaccine Pharmacovigilance. Report of CIOMS/WHO Working Group on Vaccine Pharmacovigilance.	Council for International Organizations of Medical Sciences (CIOMS) 2012
Guidelines for the evaluation of *Plasmodium falciparum* vaccines in populations exposed to natural infection.	TDR/MAL/VAC/97 WHO 1997
Guidelines on the quality, safety and efficacy of recombinant malaria vaccines targeting the pre-erythrocytic and blood stages of *Plasmodium falciparum.*	New guideline, WHO October 2012
Guidance on the evaluation of *Plasmodium vivax* vaccines in populations exposed to natural infection. Mueller I, Moorthy VS, Brown GV, Smith PG, Alonso P, Genton B; WHO Malaria Vaccine Advisory Committee (MALVAC).	Vaccine. 2009 Sep 18;27(41):5633-43. doi: 10.1016/j.vaccine.2009. 07.018.
Prequalification	
Procedure for assessing the acceptability, in principle, of vaccines for purchase by United Nations Agencies.	WHO/BS/10.2155. WHO 2010

Draft guidance on reporting variations to a prequalified vaccine V1. February 2013.	In Draft, open form comments by 29 March 2013
Assessing the Programmatic Suitability of Vaccine Candidates for WHO Prequalification.	WHO/VB/12.10. WHO October 2012
Processes for local regulatory authorities	
Vaccine Introduction Guidelines. Adding a vaccine to a national immunization programme: decision and implementation.	WHO/IVB/05.18 WHO November 2005
Procedure for expedited review of imported prequalified vaccines for use in national immunization programmes.	WHO/IVB/07.08. WHO September 2007
Annex 2: Guidelines for national authorities on quality assurance for biological products.	WHO Technical Report Series, No. 822, 1992
Regulation and licensing of biological products in countries with newly developing regulatory authorities. In: WHO Expert Committee on Biological Standardization. Forty-fifth report. Geneva, World Health Organization, 1995, Annex 1.	WHO Technical Report Series, No. 858, 1995

6.1.1. WHO Policy recommendation

In the form of a WHO policy recommendation, the WHO gives a statement that a vaccine, beyond the given background of epidemiology, economical, legal, ethical and social aspects involving also supply questions, should be targeted for further implementation. The policy recommendations are the basis for the WHO to prioritize vaccines in the WHO prequalification programme[144].

The need for a WHO policy recommendation can be expressed by members of the WHO, however also by interested parties submitting requests, including researchers and product developers, who should submit evidence to support a policy recommendation. If the evidence is accepted by the WHO, working groups are established, that are collating background information on the vaccine and the disease, summaries of evidence and a proposed recommendation. The presented material is used by the WHO´s Strategic Advisory Group of Experts on Immunization (SAGE) to discuss risks and benefits of the vaccine. The SAGE will come up with a final recommendation that will be published in form of a position paper. This position paper is written involving review of the relevant stakeholders, like SAGE, WHO regional offices, external experts and others. A position paper is published in the Weekly Epidemiological Record. The coordinated steps to develop a WHO policy recommendation starting from the defined need till its publication last approx. 2 years[145.] The minimum every 2 years the position paper undergoes review for an update.

The process to develop a policy recommendation can be started early during vaccine development. It will be continued when the vaccine is close to be submitted for a marketing authorisation and a final meeting to discuss the position paper is held when the information for submission has been completed[146]. After a position paper has been set up, additional data may be requested by the SAGE or any advisory group on any aspect like quality, safety, efficacy or cost-effectiveness. Depending on the kind of request, a policy recommendation can be set up, while some data are still pending. A policy recommendation may be only issued, once requested data or settings have been improved as requested. For a pneumococcal vaccine for example, funding and supply issues had to be solved before a global policy recommendation was granted[147.]

A list of malaria vaccine key considerations on safety and efficacy of the vaccine, but additionally also on costs and other public health interventions and local implementation questions have been drafted, that are expected to be discussed related to a policy recommendation (attached to Appendix)[148]. Vaccine developers thus should be prepared to present data allowing broad discussions about pharmaceutical, however also logistical and monetary aspects. With expected release of critical data of the pivotal RTS,S/AS01 phase III study end of 2014, the WHO has forecasted a plan to present a policy recommendation for 2015[149].

6.1.2. WHO Prequalification

To assess quality, safety and efficacy of medicinal products, that are purchased by global agencies like UNICEF or the Global Fund the WHO conducts the WHO Prequalification of Medicines Programme (PQP). After medicinal products are prequalified, they need to undergo a local licensing in the country the product.

One requirement of the programme is that the national regulatory authority of the manufacturer's country is functional as defined and is collaborating with the WHO in the process. Also, the requirements of WHO guidelines and recommendations should be met to be able to receive the status "Prequalified". The aim is also to meet the requirements of packaging and presentation specifications of the relevant UN agency. In 1987 the procedures for evaluating vaccines by the WHO has been established and has since undergone updates and refinements. Today many countries are using the list of prequalified medicines as a basis to buy bulk ware of medicinal products [150]. Out of the prequalified vaccines, it was found that they were used to immunize 53 % of the global birth cohort against 19 infectious diseases [151] reflecting the central role of prequalification for global health. Out of the prequalified medicinal products, so far 29 quinine or artimisinin based products against malaria have been prequalified. Manufacturers are companies based mostly in China, India and Morocco, but also in Korea, Germany, Uganda and USA[152].

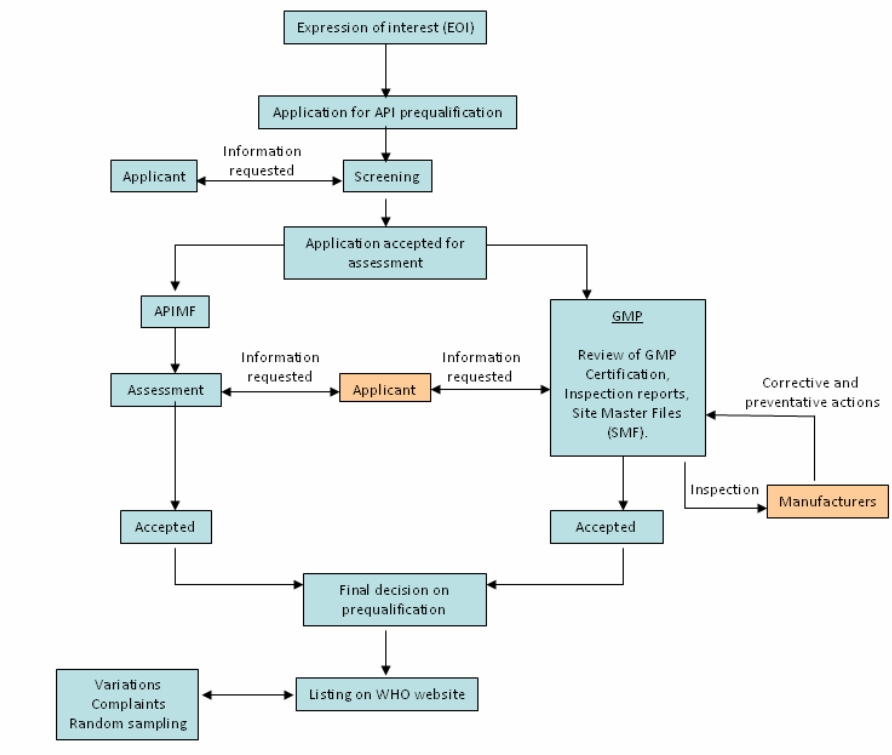
A vaccine can be accepted for prequalification, if it is listed on the current priority list of UN, the NRA has been considered to be "functional", and a marketing authorisation has been granted by the relevant NRA or a positive scientific opinion has been given by the EMA[153].

For a prequalification, data on manufacturing, quality, safety and efficacy will be assessed by appointed WHO experts including experts from developing countries. Manufacturing and clinical trial sites may be inspected and samples of the pharmaceutical product may be tested. The WHO is verifying that the rules of GMP, GLP and GCP, and relevant guidelines and standards are adhered to. The WHO may collaborate with the national medicines regulatory authorities in the country of manufacture that should be informed by the applicant about the planned prequalification process.

A manufacturer will be invited by the PQP, an UN agency or UNITAID to send an expression of interest (EOI) to the WHO to apply to the programme. When the application is accepted, the Product Summary File (PSF) will be sent to the WHO as well. In a first step, the submitted information is screened by the PQP secretariat to see if the product is

suitable for the prequalification program as defined. Any critical characteristic found during this step needs to be assessed by the Programmatic Suitability for Prequalification (PSPQ) that can recommend, if needed after consultation of the manufacturer, to either accept the submission for further evaluation or to reject it. If all criteria are met as required, the evaluation of the PSF is initiated. Audits of the manufacturing site, a consultation of the national regulatory authority of the manufacturer and tests on samples of the vaccines are performed. If concerns are raised during the review, this may be discussed in an *ad hoc* committee meeting that may result in additional action by the manufacturer. The process will be terminated when the requirements cannot be fulfilled. However, when the review is satisfactorily passed, the manufacturer is informed, a letter to UN Agencies is set up and the vaccine is listed on the publicly available product list of prequalified medicinal products. In the years 2006-2009, the timelines for prequalification improved from approx. max. 4 years for some compounds to around one year or less in year 2009 being below the target of one year for WHO internal assessment time[154]. Based on defined prioritization rules the WHO seeks to shorten review timelines for high priority medicinal products[155]. An overview of the process is shown in Figure 3.

Figure 3: WHO Prequalification process [156]



It is thought that WHO prequalification has strongly accelerated patient access to medicinal drugs have an urgent need in developing countries, such as HIV and also malaria. Moreover it supports to build local regulatory capacities, through involvement of local regulators in the prequalification process and fellowship programmes[157]. The time period to achieve a prequalification, however, being on average 2 years, and 31 months for vaccines, has been judged to be rather long[158]. Also, costs for prequalification of vaccines have to be considered. One sensible point is also, from which countries and how many reviewers are engaged. Whereas an engagement of many reviewers may delay the process, the engagement of just a few of these may rather restrict local capacity building of non-involved countries[159].

6.2. European Medicines Agency (EMA)

The EMA is the European Regulatory Authority of the European Union, responsible for the scientific evaluation of medicines developed by pharmaceutical companies for use in the European Union. They are responsible for the evaluation of marketing authorisations for human and veterinary medicines in the centralised procedure laid down in Regulation (EC) No. 726/2004 and for coordinating the European pharmacovigilance system. Additionally they provide scientific advice to developers of medicinal products. The agency is the centre of the European medicines network comprising the national regulatory authorities, the European Commission, the European parliament and other decentralised EU agencies [160]. To note is that the authorisation of clinical trials is performed by the NCA where the clinical trial is planned to be conducted. Only in aspects of scientific advice as well as protocol assistance related to phase III trials aiming at marketing authorisation of an orphan drug the EMA will get involved in single clinical trials.

The EMA, with its Vaccine Working Party (VWP), is involved in matters related to vaccines and has established numerous specific guidance documents supporting preclinical and clinical development as well as marketing authorisation of preventive vaccines in the community. To be able to also evaluate medicinal products, that are intended exclusively for marketing outside of the community, EMA, with article 58, Regulation (EC) No. 726/2004, has set the legal frame to perform an evaluation resulting in a scientific opinion, however not in a marketing authorisation that would also require to place the approved medicinal product on the market within 3 years as per Sunset Clause. Thus, the process allows the evaluation of the medicinal product by the EMA while marketing authorisation in the community is not targeted by the vaccine developer. As described by the EMA, "*Medicines eligible for this procedure are used to prevent or treat diseases of major public health interest. This includes vaccines used in the WHO Expanded Programme on Immunization or for protection against a public health priority disease, as well as medicines for WHO target diseases such as HIV/AIDS, malaria, or tuberculosis*"[161]. By providing a scientific evaluation of the medicinal product as it is done for any product in the centralised procedure, EMA is providing support to developing countries and vaccine developers. The aim of EMA to "*Stimulate medicines development in areas of unmet medical needs, neglected diseases ….."* to address public health needs and to increase the number of article 58 processes performed, has just recently been expressed*[162].*

As described rare malaria cases in Europe occur due to travelling or *P. vivax* endemic infections. Any medical prevention or treatment within Europe requires the appropriately tested and approved drugs. The processes to obtain a marketing authorisation for a malaria vaccine within the EU are described in section 6.2.2.

Prior to an article 58- or marketing authorisation application the EMA and national regulatory authorities in the European countries might be involved at several aspects during the development of the vaccine. Clinical trials of a malaria vaccine involve assessments in human volunteers that are frequently performed in Europe or in the US and thus undergo the required clinical trial authorisation processes (not subject of this thesis). Briefly, through the clinical trial authorisation processes the NCA as well as the ethic committees get into contact with documents presenting the current knowledge about

quality, safety and efficacy of the product, such as Investigational medicinal product dossier (IMPD), Investigator´s Brochure or Clinical Study Protocol undergoing submission as required. For the development and as for any other medicinal product it is advisable to seek scientific advice from the regulatory authority during the development. This may include protocol assistance in case of an orphan medicinal drug. Through clinical trials and scientific advice the vaccine developer present and have the option to discuss malaria vaccine development with the authorities either as a stand alone option or considering any future article 58 procedure.

The following main EMA legal basis, guidelines and guidance documents apply to preclinical and clinical aspects of safety, quality and efficacy of a vaccine as well as to marketing authorisation of a malaria vaccine development:

Table 2: Key relevant EMA legal bases and guidance documents

Legal basis	Document Reference
Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency.	NA
Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 December 1999 on orphan medicinal products	NA
Preclinical development	
Note for Guidance on preclinical pharmacological and toxicological testing of vaccines.	CPMP/SWP/465/95
Clinical development	
Note for Guidance on the clinical evaluation of vaccines.	CHMP/VWP/164653/2005
VWP Conclusions from the Workshop on Co-administration of Vaccines held on 31 Jan-1 Feb 2006	EMEA/CHMP/VWP/14684/2007
Concept paper in the development of a committee for human medicinal products (CHMP) revised guideline on clinical evaluation of new vaccines	CHMP/VEG/1820/04
Further guidances	
Guideline on adjuvants in vaccines for human use	EMEA/CHMP/VEG/134716/2004
Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines	EMA/CHMP/VWP/141697/2009
Environmental risk assessments for medicinal products containing, or consisting of, genetically modified organisms (GMOs) (Module 1.6.2)	EMEA/CHMP/BWP/135148/2004
Article 58 process	
Guideline on procedural aspects regarding a CHMP scientific opinion in the context of cooperation with the World Health Organization (WHO) for the evaluation of medicinal products intended exclusively for markets outside the community,	EMEA/CHMP/5579/04, Rev.1
Related Q&A document: European Medicines Agency procedural advice on medicinal products intended exclusively for markets outside the Community under Article 58 of Regulation (EC) No 726/2004 in the context of cooperation with the World Health Organization.	EMEA/534107/2008

6.2.1. EMA Article 58 procedure

The article 58 procedure, laid down in Regulation (EC) No. 726/2004 basically follows a marketing authorisation application of a centralised procedure, through reference to article 6-9 within the Regulation, till an adoption of an opinion by the Committee for Medicinal

Products for Human Use (CHMP), called scientific opinion in the article 58 procedure. The evaluations include, beside assessment of quality, safety and efficacy, also the risk benefit ration and the appropriateness related to the conditions in the developing world.
For the article 58 procedure an EMA product team leader and rapporteur/co-.rapporteur, as well the experts as proposed by the rapporteur/co-rapporteur, will be appointed. Within a 210 days procedure, including a clock stop at day 120 if questions to the applicant arise, the CHMP adopts a scientific opinion and the CHMP assessment report. As for the centralised procedure or for any other marketing authorisation application (MAA) procedure, GMP- or GCP-inspections may be indicated. Under the article 58 procedure, full complete, full/mixed, well established use, new fixed combinations, informed consent, generic, hybrid and similar biological applications can be done with dossiers accordingly built. Also, the applicant or their contact point must be established in the European Economic Area (EEA). The applicant may also use scientific advice to consult the authority in the development phase of the product, prior to application to the CHMP or after the scientific opinion has been adopted. After adopting of the opinion, follow up requirements that can be fulfilment of conditions posed by the CHMP, update of data resulting in a variation of the opinion, pharmacovigilance requirements incl. reporting of serious adverse event (SAEs) and periodic safety update reports (PSURs), should be fulfilled.
Essential and unique to the article 58 procedure is, that WHO and EMA form a partnership. To fulfil this partnership, the WHO can provide experts or can appoint representatives from national regulatory authorities (NRA) who may follow the EMA plenary sessions and who may participate in inspections of the manufacturing facilities. This collaboration shall also facilitate listing of products, after a positive scientific opinion by EMA has been given, to the list of prequalified products of the WHO[163]. Beyond the specific assessments in the article 58 procedure a main interest is to promote regulatory capacity building by involving local regulatory authorities additionally to WHO.
Some steps of the article 58 process differ from a centralised procedure. Firstly, the applicant should pose a request for classification as an article 58 medicinal product at least six months prior the planned application or, if a scientific advice meeting is considered to be prior the submission, the request should be in line with the scientific advice meeting. At that time point the applicant may also request an acceleration of the process based on a given justification. The assessment of classification is done individually by WHO and EMA, respectively, and the EMA and WHO opinions are returned individually and separately from each other to the applicant. Also the applicant should inform the EMA about the intention to make a submission according to article 58 process at least six months before the actual submission and including basic information like draft summary of product characteristics (SPC), intention to submit a vaccine antigen master file or manufacturing sites[164]. For a submission according to article 58, paediatric requirements according to Regulation (EC) No. 1901/2006 as amended are waived and the sponsor does not need to have a paediatric investigation plan in place at time of marketing authorisation submission. However candidate products are likely to be targeted for use in children, and applicants are encouraged to discuss paediatric questions as part of a scientific advice.

As marketing is planned completely outside the community, no invented name is needed. Also the product information needs to be submitted in English only and the user testing is optional as well. Finally, after the scientific opinion has been adopted, there is no decision making process on a marketing authorisation, as the product is meant to be exclusively marketed outside the EEA. If requested by the opinion holder, a Certificate of a Medicinal Product confirming, the medicinal product has been evaluated for quality, safety and efficacy by the EMA, can be issued. If a marketing is later on planned in the community, a complete submission may be performed[165].

The article 58 procedure, based on Regulation (EC) No. 276/2004, has been established in 2004. So far five medicinal products have been fully evaluated as part of the article 58 procedure[166] . Beside three medicinal products indicated for the treatment of HIV and one hexavalent paediatric combination vaccine (Hexaxim®)[167], also Pyramax®, an artesiminin combination to treat malaria, has received a positive scientific opinion. The vaccine candidate RTS,S/AS01 in development is planned to be submitted via an article 58 procedure as well[168]. To allow a smooth process, WHO and EMA are seeking alignment to keep the time minimal from issuing a positive scientific opinion by the EMA till a WHO prequalification can be started[169]. For this, a simplified procedure has been established, that enables the WHO to obtain the documentation of the scientific opinion with its Annexes, the certificate of analyses of the consistency lots and the reports from the relevant inspections. Based on this information, the WHO will only perform only those additional reviews that fall into prequalification specific further questions, like review of stability data according to the needs of the immunization programmes[170].

Different to the prequalification process, the article 58 process has not been chosen often by the developing companies developing medicinal products for developing countries. This was mainly addressed to the lack of incentives, offering neither market exclusivity nor orphan drug approval. To improve the process it was suggested to allow European market access of an article 58 processed medicinal product by a single bridging study to apply to European population. Another option discussed involve merging an article 58 process to an orphan drug approval thereby opening the process to market exclusivity and incentives while receiving also the status “Prequalified” by the WHO.

6.2.2. EMA marketing authorisation

In the EU incl. Norway, Iceland and Liechtenstein, medicinal products can be authorised by the centralised, the mutual recognition, the decentralised procedure or locally in one country via a national procedure. The marketing authorisation procedure for a malaria vaccine is predetermined by the targeted disease as well by the characteristics of the vaccine.

Malaria as a severe and potential life threatening disease shows a very low incidence in Europe, which meets the limit of < 5/10.000 affected persons in the community set by the orphan drug Regulation (EC) No. 141/2000. Also, it is understood that return of investment in the community will not balance the investment needed for development and marketing. These characteristics both qualify a future malaria vaccine as an orphan drug that, as per the mandatory scope of Annex 1 of Regulation (EC) No. 726/2004, has to be authorised

under the centralised procedure. To receive the designation of an orphan drug, the applicant should send an application for designation of the orphan drug status prior to the MAA[171] and once the designation is given, can benefit from incentives, like reduced charges for protocol assistance[172] or longer market exclusivity rights compared to a non-orphan medicinal product as per Regulation (EC) No. 141/2000.
Subunit vaccines are produced biotechnically by recombinant means combining the targeted gene sequence with genes of other antigens (example RTS,S/AS01), added to a viral or bacterial vector. With these characteristics they are to be authorised by the centralised procedure based on these product intrinsic characteristics. To be consistent with the classification, it should be mentioned that as per optional scope any medicinal product containing a new active substance not marketed yet, as well as any new scientific, therapeutic or technical innovation or being in the interest of patients any malaria vaccine qualifies for the centralised procedure. A new malaria vaccine, either as subunit or as attenuated whole parasite vaccine, would be able to be described as such as well.

6.3. Food and Drug Administration (FDA)

In the US, the FDA is an agency within the U.S. Department of Health and Human Services. Within FDA, the Center for Biologics Evaluation and Research (CBER) is responsible for the regulatory oversight of quality, safety and efficacy of vaccines. The FDA is the central agency to perform evaluations of MAAs in the USA. Similarly as for other products, in the US the developers need to submit an Investigational New Drug (IND)-Application before start of a first clinical trial or before the new drug shall be transported in the US. During this process the clinical trials submitted as covered in the IND will undergo FDA review and thus is a chance to discuss the specific trial design with the regulatory authority. During pre-clinical and clinical development of a medicinal product the agency is providing scientific advice to developers at specific timepoints, like as a Pre-IND meeting or an End-of-Phase I meeting to well prepare for example a biological license application (BLA) of a vaccine. The settings allow discussing malaria vaccine development with the authority in an continuous manner.
Per Food and Drug Administration Amendment Act (FDAAA), the ground is laid to approve vaccines to be used against diseases that are a threat for developing nations. Complementing applicable Acts and Guidances for vaccine development in preclinical, clinical and post-approval processes (summarized in[173]), the guidance document “General principles for the Development of Vaccines to Protect Against Global Infectious Diseases” applies to a malaria vaccine[174]. It is stated that a vaccine, meant to prevent a disease that is non-endemic in the US, can be licensed in the US applying the same regulatory processes as those vaccines that are targeting US endemic diseases. For licensure, data of clinical trials performed entirely outside of the US are accepted. Based on the FDAAA, the approval of a medicinal product used to treat a neglected tropical disease, including malaria, is connected with the incentive of a priority voucher given to the developer. With this voucher any other future medicinal product will be reviewed within a 6 months priority timeframe compared to the standard 10 months review time. This voucher may be also transferred or sold. In the US malaria drugs like ACT or quinine have frequently received

an orphan drug status and have been licensed as such[175]. As per Orphan Drug Act (Orphan Drug Act (ODA), Public Law 97-414) the Orphan drug status is designated, if a vaccine will be administered to fewer than 200.000 persons or, if given to more than 200.000 persons, it is expected that return of investment will not be able to achieved once the product marketed. Similar to the European situation, incentives, such as waiving the BLA submission fees or benefits related to market exclusivity are granted[176]. To note is that efforts to develop a malaria vaccine originate also from the need of globally acting US military personnel and thus target FDA approval accordingly[177].

Actual FDA BLA experiences with a malaria vaccine are pending and actual similarities or differences in the implementation for US endemic versus non-endemic vaccines remains to be seen. For example, traditionally US vaccines needed to show 80 % efficacy for licensure, whereas a malaria vaccine as expected from preliminary phase III data will be clearly below this level[178]. Also the risk benefit balance of a vaccine described to support a marketing authorisation relies on the population looked at. When the risk benefit balance of a vaccine is judged to be acceptable in malaria-endemic countries with a high burden, it may not be acceptable in a country where the disease is rather rare. The risk is seen that the FDA will look differently to products for developing countries versus for developed countries. Furthermore, the FDA does not explicitly describe that representatives from local countries will be involved in the evaluation of a BLA[179].

For a malaria vaccine, it is expected that a significant progress in the treatment of the disease as well as the priority review of the FDA can speed up the FDA review time down to around 6 months compared to 13-15 months of a standard review[180]. To note is for the general categorization that prophylactic vaccines currently are not recognized as specified biotechnology products in Title 21 *Code of Federal Regulations* §601.2[181] .

The FDA with its experts is actively contributing to WHO initiatives and processes, such as the Global Advisory Committee on Vaccine Safety, the SAGE, African Vaccine Regulatory Forum (AVAREF) and Developing Countries Vaccines Regulatory Network (DCVRN)[182]. Also, medicinal products having received a full or tentative approval by the US FDA are automatically placed on the prequalified product list of the WHO[183].

Table 3: Key relevant FDA guidance documents

	Date / Reference
Legal basis	
Food and Drug Administration Amendment Act (FDAAA)	Public Law 110-85
Orphan Drug Act (ODA)	Public Law 97-414
Guidance documents	
Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications	February 2010
Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications	November 2007
Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications	February 2006
Guidance for Industry: General principles for the Development of Vaccines to Protect Against Global Infectious Diseases	December 2011
Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials	September 2007
Guidance for Industry for the Evaluation of Combination Vaccines for Preventable Diseases: Production, Testing and Clinical Studies	October 1997

6.4. Regulatory authorities in malaria-endemic countries

6.4.1. Marketing authorisation in malaria-endemic countries

Vaccines released for a local market and public distribution need to be evaluated properly against WHO standards of quality and safety by a national regulatory agency[184]. As per the WHO guideline, minimum requirement for a NRA is to be able to perform licensing and post marketing surveillance in the situation the vaccine is obtained from a UN agency. As the vaccine has been prequalified by the WHO, the packaging and shipping conditions under a cold chain has been assured starting from supply by the manufacturer till delivery in the country. In this situation, the obligation of the NRA is to verify the shipping conditions and the batch release certificate issued by the NRA of the manufacturer. To support NRAs, the WHO has set up a guidance document providing the ground for an expedited NRA review and approval of imported, prequalified vaccines[185].
If the vaccine is not supplied through an UN agency, but procured directly by the country, also lot release should be performed by the national NRA. If applicable, laboratory testing should be available as well. Also, related documentation should be reviewed to be able to testate vaccine quality, incl. product file with safety and efficacy data, packaging and shipping documents. When a vaccine is manufactured in a specific country, the respective NRA is also required to perform GMP inspections and an evaluation of the clinical performance. The functions, a regulatory authority should be able to fulfil depending on the way of sourcing of a vaccine, is shown in Table 4.

Table 4: Relation of critical functions of a national regulatory authority implementing a vaccine to a national immunization programme[186]

Vaccine source	Licensing	Surveillance	Lot release	Laboratory access	GMP inspections	Clinical evaluation
UN agency	x	x				
Procured	x	x	x	x		
Produced	x	x	x	x	x	x

Further to local licensing the decision to include a new vaccine into a national immunization programme depends on a multi-factorial decision supposedly involving NRA, but also national policy makers etc. The decision to include a new vaccine is based on data on disease burden, public health priority, characteristics of quality, safety, efficacy as well as of its formulation and presentation of the new vaccine, considerations of the overall intervention program, the supply chain and lastly economical and financial aspects.
In developing countries the status and organization of regulatory authorities varies greatly and has been found to be insufficient in many countries. Assessing local authorities in sub-Sahara in the years 2002-2009 clear gaps in a variety of drug related areas, like production, import or surveillance were observed[187]. Related to new innovative products, a category that malaria vaccines would logically fall into, it was stated that "*The capacity to assess applications for new innovator products was almost non-existent in most countries*"[188]. To strengthen the capacity of NRA, WHO facilitated networks have been set

up, like the DCVRN[189] as well as regional initiatives like the African Vaccine Regulatory Forum (AVAREF)[190]. And also, as described before, WHO prequalification and EMA article 58 procedure are aimed to support and strengthen local regulatory capacities. To be able to assess quality, safety and efficacy of drugs according to international standards, the need to establish own function regulatory process is seen to be a must[191].

6.4.2. Clinical trial authorisation in developing countries

Laid down in WHO guidelines "Guideline on clinical evaluation of vaccines: regulatory expectations"[192] and in guideline on the quality, safety and efficacy of recombinant malaria vaccines targeting the pre-erythrocytic and blood stages of *Plasmodium falciparum*"[193] , with reference to WHO guidelines on GMP[194,195] and GCP[196], clinical trials should be approved by the relevant national regulatory authority and should undergo review by an ethic committee, as laid down in the Declaration of Helsinki[197].

In African countries this involves the submission of the clinical trial to regulatory authorities and ethic committees in each of the targeted countries as per national requirements. A WHO assessment for 26 countries in the sub-Sahara region showed that most countries do control clinical trials, but with a focus on ethical considerations (18 out of 26 countries)[198]. With involvement of an ethic committee, the integration of the regulatory authority however seems to be strongly reduced. Moreover, adherence to GLP and GCP is not a requirement in 22 out of the 26 countries and also GCP guidelines were only available in two countries. Aligned to the development of the RTS,S and other malaria vaccine and supported by diverse initiatives to stimulate clinical research in less developed countries progress has been achieved in setting up quality trial sites and staff[199]. Improved processes are strongly supported by the WHO, who is providing templates for guidance documents for clinical trial submission[200] and review process[201] as well as import of investigational medicinal products[202].

Facilitated by the WHO as well, the African Vaccine Regulatory Forum (AVAREF) has been founded to build capacities for the evaluation of clinical trials by national regulatory authorities and ethic committees. To align the processes in different African countries, a 2 step process for clinical trial authorisation consisting of submission and approval, with integration of inspections, was established. The clinical trial is submitted to the NRAs using a defined template. This is followed by a joint meeting involving the WHO, the NRAs and the vaccine developer and trial sponsor. After the evaluation the NRAs send their observations and questions to the trial sponsor. The trial sponsor sends the response to the NRAs and each NRA is performing an own review and approval of the clinical trial. During conduct of the clinical trial, members from the involved NRAs and EC as well as participants from the WHO, perform joint inspections of clinical trial sites. The AVAREF owned process has up to date resulted in joined reviews of three clinical trials, including the clinical RTS,S/AS01 phase III trial, and joined clinical site inspections[203]. The first experiences also raised the need to further improve the processes, for example based on further specified information in the submission documents, and timelines for submission and approval will be also one aspect. The review and evaluation of clinical trials in multiple countries poses the issue that different and divergent responses are given, resulting into

the risk that one common trial protocol might not be feasible in the multinational setting. The AVAREF based joint review of clinical trials thus represents an important answer on how to best perform multinational clinical trials. One similar process of harmonisation, also part of an overall different regulatory environment, can be seen in the Voluntary Harmonisation Procedure established in the EU, allowing a joint review of a clinical trial by the regulatory authorities[204].

6.5. Global strategies to implement a malaria vaccine

Bringing a malaria vaccine to all people who can benefit from it, marketing authorisations are needed in each country in which the vaccine should be used. Diverse strategies are possible to achieve this goal. The EPI programme using WHO prequalified vaccines has most successfully brought vaccines to many people globally. As per guidance "Assessing the Programmatic Suitability of Vaccine Candidates for WHO Prequalification" prequalification can be started after a positive article 58 scientific opinion has been granted or a national MAA has been granted in the country of vaccine manufacturing. If the manufacturing company is a US company, a FDA orphan drug marketing authorisation can be applied first and the authorised vaccine is then automatically listed as a prequalified vaccine. In case the manufacturer is in the EU, the prequalification follows a positive scientific opinion after an article 58 process.

Table 5: Strategies to globally implement a malaria vaccine

Vaccine Manufacturer	**Global Process Plan Outline**
EU	Article 58 > Prequalification → National processes Orphan Drug * → Prequalification → National processes Orphan Drug * → Prequalification >* National processes
US	FDA ODA * = Prequalification → National processes
Country other than US/EU	Manufacturer country approval → Prequalification → National processes
Potential parallel processes: Orphan Drug Approval US-EMA ± Local RA, EMA Article 58 - FDA ODA, Prequalification – Local RA	

-→ Next step follows when approval given > Accelerated prequalification after article 58 positive scientific opinion
= Automatic prequalified status if FDA approved * as simultaneous process FDA/ EMA possible
>* Expedited review and approval after prequalification [205]

In the context of introducing medicinal products against neglected diseases, other regulatory strategies have been considered that may be applicable also to a future malaria vaccine. This includes parallel approvals by a stringent, "western" RA and a national RA, although in practice local RA often seem to wait till an approval by a stringent RA has been given[206]. Also a twinned review by a stringent RA with a local RA might be an option. This offers the advantage that there is consultation between the RA and expertise on vaccine development on the one hand and expertise on local needs and requirements on

the other hand can be beneficial. The latter has been tested by a WHO organized joint review of an artesunate-amodiaquine product with a joint review by EMA and the DNDi[207]. Furthermore a malaria medicinal product can first be authorised by the EMA or the FDA, followed by local RA submission being in parallel to the start of the prequalification process. An example is Eurartesim® (Dihydroartemisinin-Piperaquine), a chemical artesiminin product that has received an EU marketing authorisation as an orphan medicinal product in 2011[208]. While the WHO prequalification process is still ongoing[209], the product has recently been registered in Ghana by the local RA, and further African countries will follow[210]. The benefit of this strategy can be to minimize the time until people gain access to a medicinal product in selected countries.

7. A brief overview to cost-effectiveness of a malaria vaccine

One answer to the question, why to develop a malaria vaccine, is often that a vaccine will be a cost effective measure taking the planned global need and the costs of other and existing anti-malaria measures into account. Therefore an overview shall be briefly outlined in the context of this thesis taking into account that pricing is an essential aspect of market implementation and for overall development of medicinal products.
Numerous cost-effectiveness calculations have been done to project malaria prevention and treatment costs[211] and shall be only briefly outlined in the context of this thesis. The cost calculations have been also considered as a basis for national policy committees to alleviate the implementation of the vaccine in a country, knowing that budgetary conditions are a main factor for implementing immunization strategies[212]. A common unit to present cost-effectiveness is the unit of disability-adjusted life years (DALY). For cost projections the avoidance of DALYs through an intervention has been used as a unit. The costs for malaria prevention has been forecasted to be on average US $ 3.6 billion annually for indoor residual spraying, long lasting insecticide bed nets and intermittent preventive treatment. Among the costs, indoor residual spraying represents the most costly prevention method contributing to 55 % of the annual budget needed[213]. Cost projections for a vaccine providing a partial effectivity have named around annually US $ 533 million and accordingly approx. less than 20 % of the overall preventive measures. Presenting the costs as DALY, all malaria prevention measures were found to cost between 2 and 24 $ per DALY averted, being the second most cost effective measure following overall child immunization measures overall (1-5 $ per DALY averted). To note is that the RTS,S/AS01 vaccine is expected to cover the costs including a return of investment of 5 percent[214].

8. Quality requirements for a malaria vaccine

The quality of a vaccine is paramount to assure safety and efficacy to the vaccinated individuals. The WHO New guideline on recombinant malaria vaccines clarifies aspects on the quality of subunit malaria vaccines, including aspects of production, quality control, characterisation and stability. Reference is given to applicable general guidelines and standards of medicinal products in common and to biological products as well. As noted, the WHO guidances are meant to complement and not to invalidate applicable guidelines and standards, which, like the International Conference on Harmonisation (ICH)-Guidelines on Quality, are the current standard for any new medicinal product and remain fully valid. A summary outline shall be given on specific quality aspects to be considered for malaria vaccines based on WHO, EMA and FDA requirements.
As per WHO guideline aspects of the control of source materials, fermentation, single harvest, control of purified antigen bulk, final bulk, filling and containers, control test on final lots, records, retained samples, labelling, distribution and transport as well as stability testing, storage and expiry date are given[215]. To fulfil the WHO prequalification requirements, several aspects like for example approval of the cell bank, data on stability of the expressions system, fermentation media or intermediate hold times of intermediates are to be approved by the national regulatory authority (NRA) of the vaccine manufacturer´s country. With no authorised malaria vaccine yet, the guideline provides also details regarding production and testing of RTS,S/AS01 meant to be a guidance for the licensing NRA on the required quality review of the dossier.
Main requirements set by the WHO prequalification program being compulsory ("mandatory" or "critical" (subject to PSPQ standing committee recommendation)) to an EPI programme vaccine, reflect, as one aspect of quality, the stability requirements of vaccines in developing countries[216]:

- Anti-microbial preservatives should be used for ready to use presentations of vaccines and multi-dose containers of more than two doses per vial.
- Temperature: No storage below +2 °C > 6 months, no required storage < - 20°C.
- Antigenic stability: Should show antigenic stability also in multi-dose presentations for 28 days after reconstitution.

To prevent microbial growth inactive agents or preservatives are used as additives. They might be added during the production process or to the final drug product, respectively. One widely used compound is thiomersal, a product containing mercury that has been brought into connection with autism and brain development disorders in the general public. This correlation could not be verified by many authorities and organizations including the WHO[217], however has decreased the acceptance for thiomersal-containing vaccines. For any new malaria vaccine like any other vaccine, the question how to prevent microbial growth in the targeted formulation and dosing plans should be one important consideration from early preclinical development onwards.

A storage in temperature ranges of +2 to +8°C or < 0 °C pose specific needs on the shipment and storage conditions of those vaccines, that are targeted for subtropical and

tropical areas in developing countries. Falling into the requirements of a “Good distribution practice (GDP)” it requires a cold chain maintaining defined temperature conditions without any interruption during all steps in the supply chain from the manufacturing site till the point of usage. Since 1997, the vaccine vial monitors (VVM) with thermocrome labels identifying non-usable vaccines, have been widely introduced[218]. The temperature requirement of a vaccine should match to ranges of the available VVM profiles, as defined in form of a critical parameter during prequalification[219] assuring the verification of a correct storage. Furthermore refrigerators and freezers are subject to prequalification by the WHO and a set of guidance documents has been developed to also specify the equipment subject to diverse power supply conditions[220]. Additional vaccine quality is a focus of WHO offered training material[221].

Quality aspects of viral vector based vaccines are subject of Guidelines and Recommendations by the WHO[222], by EMA[223] and by the FDA, with the general guidance to use applicable information on individual vaccines, including Ph.Eur information[224].

A draft set of requirements for a specific type of malaria vaccines, the transmission blocking vaccines, has been proposed[225]. This involves the presentation of the vaccine in multi-dose vials, a preferably liquid formulation and a shelf life of at least 2 years. Storage should be preferably at ambient temperatures or minimally at 2-8°C with a packaging requiring minimal storage.

Compared to subunit vaccines, whole sporozoite vaccines need to be based on newly developed standards as for sterility, dosage or storage as well as sufficient attenuation. These vaccines have reached clinical trials and recently safety and immunogenicity could be shown in a phase I clinical trial applying attenuated, aseptic, purified, cryopreserved sporozoites[226]. To keep viability of the sporozoites, the vaccines are stored in liquid nitrogen vapour phase at temperatures below -140 °C and need a distribution through a special cold chain. Examples of the coast fever vaccine distribution in East Africa[227] or use of veterinary vaccines show that this is logistically feasible[228], and also the financial feasibility has been shown[229]. It remains to be seen if the vaccine will show the expected efficacy. If it does, this may need to trigger strategies to implement liquid nitrogen capabilities in malaria-endemic countries, distribution outside of the EPI programme[230] and, moreover, update of WHO prequalification requirements that currently obligatorily involve storage not below - 20°C.

One important quality step is the lot release of each individual vaccine lot by the regulatory authority. The WHO is supporting lot release testing with a guideline and providing standards and training[231]. Minimally the assessment is based on the data obtained from the production protocol of the manufacturer. Further documents undergoing review may be the release certificate from the responsible NRA and national control laboratory, and may be further supplemented by an independent lot testing performed additionally to the control testing of the manufacturer. If a vaccine is procured or produced by a country, the regulatory authority should be capable of performing a lot release. To note is, that if the vaccine is purchased via the UN, a lot release is not a requirement for the NRA (see also section 6.4.1).

9. Non-clinical development

Preclinical testing is a prerequisite to clinical testing that finally should show safety and efficacy of the vaccine in the human population. Although many details about acquired immunity and vaccine induced immune reactions in the human body are known, a clearly defined immune correlate that is applicable more generally has not yet been identified. As described by the preclinical evaluation group in 2004, development of a vaccine remains to some extent empiric despite all *in vitro* and *in vivo* preclinical assessments, and also needs constant back validation of surrogate parameters in ongoing clinical trials[232]. Whole cell vaccines or viral vector vaccines are associated especially with risks unique of life vaccines. It needs to be verified that the attenuated parasites will not be able to revert to an infective agent or a virus cannot recombine resulting into a new virulent genotype[233]. Accordingly the assay systems and animal models need to be chosen to properly test the safety of these life vaccines. Assessing attenuated and viral vector based vaccines, it is essential to verify that no recombination with field viruses occur resulting in a preversal to an infective virus or to an infective protozoan can occur. Being part of early clinical trials in addition to preclinical assessments, *in vitro* assays should be able to predict potential efficacy of a vaccine for later confirmatory clinical trials. With insufficient knowledge about the human immune correlate, preclinical and any later *in vitro* assessments are even more difficult due to the high polymorphism of the parasite per se and further variability arising from the characteristics of the different *Plasmodium* species. Emphasis was therefore put on the approach to use “best science available” for each step along the vaccine development pathway.

9.1. Toxicology

Toxicology tests should be done to identify potential toxic effects of the vaccine prior starting clinical trials and according to WHO guidelines on nonclinical evaluation of vaccines[234] . Further guidance from WHO, FDA or EMA as applicable for vaccines or specific subtypes of vaccines apply, as for example ICH guideline S6 (R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals relevant for recombinant DNA protein vaccines[235]. Beyond general non-clinical safety assessments, the toxicity studies should be able to identify toxic effects like precipitation of immune complexes or humoral or cell-mediated immune response against the host[236]. The studies include systemic as well local tolerance studies. Developmental and pharmacokinetic studies are normally not needed, however may be indicated if pregnant women are supposed to receive the vaccine, or if novel routes for administration are planned. Also genotoxicity or carcinogenicity studies may be only needed if adjuvants or additives may require the assessments.

9.2. *In vitro* assays

Whereas general assessments of the immune system can benefit from existing and standardized assays used, like for example enzyme-linked immunosorbent assay (ELISA)

assays, the tests that are meaningful to *Plasmodium* need to be developed specifically. Progress for malaria specific tests was made when *in vitro* culture of *Plasmodium* species was achieved[237]. *In vitro* assays presented here are used to complement animal studies as well as clinical trials analyses. The following i*n vitro* assays are fundamental for malaria research, although they are not judged to represent a surrogate of protection[238]:

Growth inhibition assay (GIA):

In this assay *P. falciparum* invasion of red blood cells or their growth within the red blood cells after infection is measured in the presence of test sera from animals or human individuals who have been exposed to the test vaccine. Inhibition of invasion or parasite growth gives a measure that vaccine-elicited antibodies are capable of interfering with *Plasmodium* development. This test has been widely used and some data on correlation between *in vitro* and *in vivo* assays exist. It is discussed that a clear decision can be only drawn from any proven lack of inhibition, whereas any inhibition shown in the test may not translate into the clinic setting[239] due to insufficient sensitivity and consistency as well as lack of reliable correlation to *in vivo* protection[240].

Antibody-dependent cellular inhibition assay (ADCI):

P. falciparum parasites are cultivated *in vitro* under supplement of monocytes. Growth inhibition by added test sera gives a measure of effectivity of the antibodies elicited by a vaccine. Positive controls used are sera of immune competent individuals living in malaria-endemic regions. In addition to the *in vitro* test, as an *in vivo* test, test sera and their impact on parasitemia levels can be assessed similarly in immunocompetent mice[241].

ELISA specific for anti-CSP[242]

Anti-CSP antibodies represent a measure of humoral immune response elicited by a vaccine having circumsporozoite as the antigen. The assay was validated according to ICH Guidelines[243] and will allow comparing immunogenicity profiles of diverse anti-CSP vaccines.

In Vitro Assay in *P. vivax*[244]

Recently an *in vitro* assay has been established allowing to test the effects of drugs on a *P. vivax–hepatocyte system*. The cultivation of sporozoites as well as the reproducible infection of hepatocytes was achieved. Infection of late liver stages could be also reduced by prymiquine that is specifically killing the hypnozoites of *P. vivax*. This test may be a “high through put assay to screen for drug inhibiting liver stages” and further basis to assess *P. vivax* specific vaccines[245].

Standard membrane feeding assay (SMFA)

A mixture of cultured *P. falciparum* gametocytes, human red blood cells and test antisera is fed to mosquitos through a membrane feeding apparatus. One week later the mosquitos are dissected and the number of oocysts in the midguts within the different test groups is. The result is presented as a transmission-reducing activity of test sera against the control.

This test is widely used in preclinical and clinical settings and is key to assess the effectiveness of TBVs. It allows determining the effect of antibody responses elicited by a vaccine, either applied to the animal or to humans, and that are still active against *Plasmodium* in the mosquito. In the test it was seen that reproducibility of the results may be impacted by the assay settings and thus reducing the value of the test[246]. The recently

published qualification of the SMFA according to ICH guideline Q2(R1)[247] is a step to improvement of the assay.

9.3. *In vivo* assays – animal models

Beside basic research about disease pathology and immunity, the main aim of animal studies is to pre-assess the vaccine as best as possible prior to human exposure. Thus, animal studies represent a major tool for Go / No Go decision in vaccine identification and development. Although it is well accepted that animal studies cannot replace the human situation, an animal model should as close as possible resemble the human situation studied.

In malaria research several animal models have been used for toxicity studies as well as for studies on natural *Plasmodium* biology in the context of a host and on the effects of vaccination. Requirement is that prior start of clinical studies extensive product characterisation, immunogenicity, safety testing and proof-of-concept studies has been done in animals[248]. In the context of malaria, rodent and non-human primate animal models have commonly been used. Rodents are used to assess the immunogenicity or the efficacy of the vaccine albeit a strong limitation of relevance is seen in the different signs of disease or immunological responses. Non-human primates are judged to be more similar to humans based on a general susceptibility to human malaria and are used to study immunogenicity, efficacy of vaccines or specific aspects of disease features. The advice is not to give non-human primates studies priority to other animal studies. Instead specific non-human primate species are known to be commonly used study defined processes of malaria, like blood stage infection studies or *P. vivax* infections.

To use the animal models for studies of toxicity, pathophysiology of the disease or test of novel vaccines upon artificial *Plasmodium* infection, infection techniques have been developed to either inject sporozoites or parasitized red blood cells, as obtained from standardized *Plasmodium* laboratory cultures, thus omitting the mosquito infection. Depending on the model, the animal can be infected with the naturally matching *Plasmodium* species, with a *Plasmodium* species adapted to laboratory use or also the species causing human malaria. The mouse model commonly used and connected with the development of the RTS,S/AS01 vaccine is infected with *P. berghei,* which naturally does not infect mice, but rats. The mouse strain CBA/Ca infected with the ANKA strain of *P. berghei* is used as a model for human severe malaria developing cerebral symptoms, although this model is seen controversial in the research community[249]. So far there is no small animal model to study *P.vivax* malaria, instead there are attempts to develop better *in vitro* assays[250] additionally to the studies in *Saimiri* monkeys[251].

The *Aotus* and *Saimiri* monkeys can be infected with *P. falciparum* or *P. vivax*, but the resulting malaria shows some features that differ from the human variety. Assessments of safety and immunogenicity have been performed preferably in the rhesus monkey, thought to be more closely related to humans than the mouse model[252].

To better assess the human situation immunodeficient mice have been transfected with human red blood cells and hepatocytes together with *P. falciparum* to create humanized mouse models. However, these models have long been questioned for their relevance to

humans, but were judged to be the best available models[253]. Other objections against the model were a limited reproducibility of parasitemia and a high variability between the animals. Recently a new immunodeficient mouse model was introduced that showed improved characteristics[254]. Other mouse models are tested by injecting human bone marrow or liver cells. It is also tried to establish completely new models starting from wild thick rats[255] following the suggested priority to re-invest newly into non-human primate models[256].

An overview of the commonly used species with their characteristics is shown in Table 6.

Table 6: <u>Overview on animal models in malaria vaccine development</u>[263]

Animal	Plasmodium	Utilisation related to malaria specific assessments	Known limitations
House mouse *Mus musculus* Strains: BALB/c mice Strain: ANKA	*P. yoelii* *P. berghei* *P. chabaudi* *P. berghei*	Antigen immunogenicity (cellular and humoral response) w/o P. infection Heterologous immunization challenge model Protection against *Plasmodium* challenge, incl. heterologues *P. falciparum* challenge Screening of delivery systems ANKA Model for cerebral malaria	Relevance to humans is low
Wistar rats *Rattus norvegicus*	*P. berghei*	Parasitemia and up to 100 % mortality occurs upon infection and can be reduced by chloroquine treatment. The model is thought to be used to test malaria drugs. [257]	
New World Monkey *Aotus* Species: *- A.l.lemurinus,* *- A. nancymai* *- A. vociferans* *- A.l.grisemembra*	*P. falciparum* *P. vivax* *P. malariae* *P. falciparum* *P. vivax*	Safety signal detection, Immunogenicity, options to develop this model to verify *in vitro* assays Sporozoite infection (incl. heterologous challenge with diverse P. strains) Efficacy related to antigen form, expression system, formulation (decision point prior clinical production) Mosquito transmission and susceptibility Liver stages studies	Limitation to relevance: - Fast acquisition of effective immunity - Life threatening anaemia - need of splenectomy High standards in animal care make these models costly Restriction to few *P. falci-parum* strains
New World Monkey *Saimiri* Species: *- Saimiri b.bolivensis* *- S.sciureus*	*P. falciparum* *P. vivax* *P. malariae* *P. simium*	Screening of delivery systems Blood stage infection and related antigens Pre-erythrocytic vaccines (best combination*: Aotus l. grisemembra – P. falciparum and Saimiri b. boliviensis – P. P. simium*)	
Rhesus monkey *Macaca mulatta*	*P. knowlesi*	More similar to human than *Aotus* and *Saimiri.* Animal models used since the 1970s for immunization and vaccine studies. Efficacy of vaccines after parasite challenge with trial end point death. [258], Develop also semi-immunity, chronic infections and relapses. Test model for immunogenicity, formulation selection, safety of vaccines Model for human South-East Asian *P. knowlesi* malaria [259]	No infection with *P. falci-parum* possible
Baboon *Papio anubis* [260]	*P. knowlesi*	Largest non-humane primate model fully susceptible to P. knowlesi infection and developing mild and severe malaria, immunological characterisation is ongoing [261]	
Rhesus monkey *Macaca mulatta*	*P. cynomolgi*	Mimics biology and pathogenesis of *P. vivax*, allows to study the dormant hypnozoite forms[262]	

10. Clinical development

Clinical testing is a long, expensive process to test a drug candidate for safety and efficacy with the final goal to have a product, whose benefit-risk profile is positive and sufficiently established to allow licensing. Most malaria vaccines are still in early development with only few candidates in phase II b and one, the RTS,S vaccine, in phase III.

All trials should adhere to the WHO guidelines on clinical evaluation of vaccines: regulatory expectations[264], the WHO guidelines for good clinical practice[265], Good Clinical Practice (ICH-GCP)[266], Declaration of Helsinki[267], local requirements and applicable guidelines on overall clinical development as well as vaccine requirements for quality, safety and efficacy as applicable to fulfil the accepted standards. The vaccine manufacturer and developer should consider that, due to the novelty of the vaccines against malaria, open points remain. Thus, state of the art, ongoing consultation with regulatory authorities and integration of actual scientific basis is key for the pharmaceutical development.

Schematic clinical development pathways have been depicted for malaria and are shown schematically in Table 7. It is thought that specific development plans need to be defined for the different target populations of children living in stable transmissions areas, pregnant women as well as non-immune people exposed to malaria in a short or long term manner[268].

10.1. Safety and immunogenicity studies

A vaccine comprising a new antigen, a new formulation or a new whole organism component will be started in clinical development with early clinical trials, mainly in phase I, assessing safety and immunogenicity. The aim for these early studies is to verify the vaccine is safe and immunogenic. In this context the optimal formulation, the optimal dose and the primary immunization schedule related to the target population as a basis for later confirmatory studies are explored. In these early studies the formulation is tested and if needed improved, to verify the medicinal product is stable and safe also in the clinical setting. During phase I and II studies and prior start of phase III the optimal formulation should have been found, the vaccine should be fully characterised and the final manufacturing process, specifications and batch release testing procedures should have been established[269]. For example any new adjuvant or a considerable change in the adjuvant needs to be confirmed to be better than the formerly used vaccine formulation (“superiority studies”). Phase I and also phase II clinical trials are performed with low subjects numbers, and thus data are preliminary and also limited, needing data basis on a higher subject number and confirmation of the data in later trials. As part of the early clinical trials the foreseen target population should be tested. If needed, the trials should start in a population showing less risk before high risk groups are tested, for example related to age. Here adults should be tested first, and age de-escalation steps should be introduced to finally reach the youngest population targeted for the vaccine[270].

After safety assessments of the single product have been performed in the targeted population of children, the combination with other vaccines is important to test if the integration into the expanded programme on immunizations (EPI) is targeted. If integrated, vaccination against malaria can benefit from a good global immunization coverage of EPI[271].

It needs to be shown that the established single or multiple vaccines in use will be as safe, immunogenic and effective with the malaria vaccine as they would be without it ("non-inferiority studies"). The EPI today covers vaccination against tuberculosis, diphtheria-tetanus-pertussis (DTP3), polio, measles, yellow fever, hepatitis B and Haemophilus influenzae type b (Hib). The scheduled vaccination should be performed at birth and ages of 6, 10 and 14 weeks as well as at age of 9 months[272]. The most advanced malaria vaccines like RTS,S/AS01, have been tested in combination with EPI. A malaria vaccine however might also to be explored beyond the EPI schedule, for example for mass campaigns, annual boosting or infants older than 9 months, if needed to achieve the targeted efficacy[273].

10.1.1. Safety assessments

The first clinical trial, the "First-in-Man Study", is a key critical step as the plethora of *in vitro*, *in vivo* and animal test cannot completely rule out risk for human volunteers. For these trials the guideline "Guideline on strategies to identify and mitigate risk for First-In-Man human clinical trials with investigational medicinal products"[274] should be considered in addition to general and vaccine specific guidelines. The precaution measures will increase with decreasing similarity of the new entity (e.g. antigen, new vector, new adjuvant or any other compound in the formulation) to any previously tested vaccine. Also a new route of dosing to be tested like the intravenous dosing for a whole cell vaccine of P. *falciparum* will need high attention to risk minimization. Identified safety and immunization schedules will be needed to be verified in later confirmatory studies, which may possibly also assess protective efficacy.
Preventive vaccines are targeting normally healthy individuals, among those also mostly children, limiting the accepted risks almost to non-serious, short term side effects[275]. Main minimum safety target of a malaria vaccine has been drafted along with the updated malaria roadmap[276]. The target is that the safety profile is non-inferior to that of currently licensed paediatric vaccines, and ideally superior to that of current vaccines.
In vaccine clinical trials safety is assessed in terms of so called adverse events following immunization (AEFI). They are defined as "*Any untoward medical occurrence which follows immunization and which does not necessarily have a causal relationship with the usage of the vaccine. The adverse event may be any unfavourable or unintended sign, abnormal laboratory finding, symptom or disease*"[277]. The definition is further specified, depending if it is caused by product characteristics, by quality issues, by inappropriate handling, prescription or administration, through anxiety of the vaccinated individual or by a coincidental event. Considering the target population of children and infants, frequent other childhood associated diseases may occur. This mandates well powered studies to clearly determine the causality to the vaccine under evaluation[278].
First and early clinical trials will enrol a low number of adult study participants, in a range of ten participants per group. Related to the low number of participants there is a high chance to detect the most common AEFIs, but a lower chance to identify rare AEFIs. This relation is especially strong for vaccine development that starts with 10-50 participants in clinical trials, and may end up with a huge portion of the world population being finally vaccinated with the marketed product. Rare AEFIs occurring in less than one out of 100.000 vaccines will therefore mostly depend on data obtained after licensing[279]. According to the goals at the

different time points of development, safety and tolerability will be the primary endpoint in early studies. The studies should be designed in a way to allow the detection of severe local and systemic adverse reactions[280]. In malaria clinical trials, once the safety in adults is judged to be adequate, an age de-escalation of study participants is to be performed in phase Ib trials to finally be able to test the vaccine's safety in the target population of infants[281]. In later studies assessing efficacy as a primary endpoint, safety will be kept as an essential data basis as secondary endpoints. The vaccine safety data from the clinical trials need to be adequate to allow WHO prequalification or any marketing authorisation. In case the data are not judged to be adequate, the WHO or any regulatory authority will request phase IV studies to assess the safety questions. Furthermore, a pharmacovigilance plan is an essential part of the PSF and updates on safety data are required in form of a PSUR in defined frequencies.

With the name of a "Global Vaccine Safety Blueprint", in 2011 an initiative was started to support RA of low and middle income countries to set up vaccine pharmacovigilance activities[282]. Especially related to newly developed vaccines the capacities for vaccine safety shall be supported. This is supposed to include national data bases and reporting forms for AEFIs as well as strengthening safety monitoring and evaluation of safety signals. It can be expected, that a future malaria vaccine will benefit from this initiative on top of phase IV and regular pharmacovigilance activities. Notably, a data base kept by the Uppsala Monitoring Center serves as a global AEFI repository as part of the WHO Programme for International Drug Monitoring.

As per WHO GCP and ICH-GCP requirements, safety data in vaccine clinical trials need to adhere to documentation and reporting requirements. The standardization of AEFIs that will allow comparability of vaccines is crucial for vaccine safety. This includes definitions for clinical assessments, for example the body site used for body temperature measurements to assess fever or the time after immunization for which AEFI assessment should be performed. A commonly used classification system is the "Brighton Collaboration case definitions"[283]. Recently, the data basis and reporting of randomized, controlled vaccine clinical trials, including malaria vaccine trials, conducted in developing countries has been analysed. Albeit case definitions for specific AEFIs exist, only few trials had implemented these standards, and the AEFI term "Fever" has shown the highest variability between the trials. As a conclusion the authors propose to more stringently implement the standards for reporting of safety data, case definitions and to also harmonise publishing of the data[284].

Typical vaccine related adverse events include local reactions at the injection site, generally symptoms in the first few hours after immunization like swelling, headache or fever. Serious induced AEFIs known from vaccines include seizures, allergic reactions or anaphylaxis[285]. Local side reactions at the injection site and fever have also been reported for the vaccine RTS,S. It was found that site reactions occurred less frequently after RTS,S vaccine immunization compared to immunization with the EPI programme vaccine DTP-HepB/Hib vaccine[286]. Beside the requirement that any malaria vaccine should be at least as safe as other paediatric vaccines, other specific safety assessments have been drafted in 2010, based on an overall acceptable safety profile and a positive evaluation by WHO. These include that malaria incidence is not enhanced after vaccination through a "Rebound

Table 7: Schematic view of clinical aspects of a malaria vaccine clinical development pathway [289, 290, 291]

	Phase Ia	Phase Ib	Phase II	- Phase II a	- Phase IIb	Phase III	Phase IV
Main Study population	Healthy, malaria naïve adults to avoid interference of natural infection	▪ Healthy malaria non-naïve adults ▪ Age de-escalation adults to infants	▪ Malaria naïve adults ▪ Healthy malaria non-naïve target population	Human experimental challenge studies in Malaria naïve adults	▪ Natural exposure, malaria-exposed population ▪ Population as for phase III/ licensing	Target population of licensing • Different malaria transmission patterns • Influence of parasite / human genetic factors	Subgroups not included in label
Primary endpoint	Safety Immunogenicity	Safety Immunogenicity	Safety, immuno-genicity or efficacy Optimal dosage and schedule	Efficacy: Prevention of infection Optimal dosage and schedule	Efficacy Optimal dosage and schedule	Efficacy	Safety
Main secondary endpoints	Further immunogenicity aspects not covered by primary endpoint	Correlates of efficacy	Efficacy: revention of infection Safety/ immuno-genicity; optimal dosage / schedule	Safety, immunogenicity	Safety, immunogenicity	Safety, immunogenicity	Monitoring of efficacy / effectiveness Duration of protection
No. of participants	Small (Tens up to less than hundred)	Small (Less than hundred)	Medium (Several hundreds up to few thousands)	Medium (Several hundreds up to few thousands)	Medium (Several hundreds up to few thousands)	Medium (500-4000 per group up to 30.000 per group)	Large (N= x 10.000 – x 100.000)
Trial duration	Up to one year	Up to one year	Two or more years	Two or more years	Two or more years	Three to five years	Up to four to six years
Trial design	Open label trial, uncontrolled trials DB-RCT	Open label trial, uncontrolled trials DB-RCT	DB-RCT	DB-RCT	DB-RCT: 1 or 2 studies Definition of Go/No Go for phase III before trial start	DB- RCT Licensed vaccine as comparator accepted to avoid a placebo	RCT Observational studies
Specificities of VIMT [287]	Safety and imunogencity, identification of doses and schedules across a wide range of ages. Robust assays of a surrogate efficacy endpoint on individual level may allow identifying populated based efficacy correlate. If possible Phase IIb and Phase III trial may follow as depicted above, if not possible may follow as depicted specifically for VIMT.				Identification of surrogate efficacy endpoint on individual level	Validated surrogate efficacy endpoint agreed with RA basis for conditional approval	Endpoint: Efficay Community scale trials with endpoints on population level
Comments	Use of validated IgG ELISA. Further assays undergoing standardization [288]	Approaches to combine phase Ia/Ib studies prior phase II are ongoing to accelerate development	Phase II or Phase II a trial in malaria naïve people may be done prior phase Ib studies or as com-bined phase I/II study	Essential trial for pre-erythrocytic vaccines Negative results of blood stage vaccines are not a decision not to proceed	Smaller trials with less population variance Interaction study to test other vaccine used in schedule	Primary analysis: Earliest 12 months after last subject / last dose OR event driven (Number of malaria incidence in defined timeframe)	Trials for Long term Follow up beyond phase III data
	Establishment of formulation (preservatives, excipients, adjuvants), vaccination regimen (antigen doses, schedule), incorporation to vaccination programs (EPI)			**Proof-of-concept trials**		**Pivotal efficacy trial**	**Post licensure safety / effectiveness trials**

* DB-RCT: Double-blinded, randomized controlled trial.

effect", other prevention and treatment strategies are not impacted and that safety in immunologically compromised groups is acceptable[292].

10.1.2. Immunogenicity assessments

A malaria vaccine is supposed to raise immune responses that should be characterised for amount, kinetic, specificity and quality of antibodies. They should be tested for functionality to interfere against the target and for forming cross-reactive antibodies. Additionally it should be tested if the humoral immune answer is influenced by immunological status of the vaccinated individual like for example maternal antibodies present in young infants till an age of around 6 months. Cell-mediated immunity (CMI) is an essential part of an immune reaction as well and, moreover, thought to be essential for malaria natural immunity. Thus, it needs to be assessed and the way this is done needs to be justified in the application dossier. Tools are for example the quantity and quality of T cell responses. When there is no established correlate for efficacy, as it is the case for a malaria vaccine, every effort should be made to establish such a correlation. For malaria an essential test currently is the CHMI for vaccines interfering with the human *Plasmodium* infection and the SMFA assessing TBVs. After establishing basic immunological profiles, the immune responses are further defined in different subpopulation like specified age or ethnic groups, in different formulations and compositions with adjuvants and additives or with other vaccines.
Any natural exposure to the parasite occurring additionally to initial safety and immunogenicity assessments is thought to make the analyses too complex[293]. For this reason, early trials termed phase Ia studies are performed in malaria-naïve population in sites located in malaria-free regions. The observed vaccine effects then undergo a confirmation as part of phase Ib studies that are conducted in malaria-exposed individuals.

10.2. Efficacy and effectiveness studies

Clinical disease preventing and transmission blocking vaccines are targeted to achieve efficacy and effectiveness, respectively, according to the following definitions[294]:

- Vaccine (protective) efficacy
  The reduction in the chance or odds of developing clinical disease after vaccination relative to the chance or odds when unvaccinated
- Vaccine effectiveness
  The protection rate achieved through vaccination in a specified population. Vaccine effectiveness measures both direct and indirect protection (i.e. protection of non-vaccinated persons by the vaccinated population).

Thus, whole cell and pre-erythrocytic as well as asexual blood stage subunit vaccines will target primarily efficacy, whereas sexual blood stage vaccines and TBVs target effectiveness.
After safety and immunogenicity has been shown in phase Ib studies, proof-of-principle studies will be first conducted in malaria naïve populations as phase IIa studies. They are followed by essentially similar studies in malaria-exposed population studies as phase IIb studies. Along the path, the data may show that the vaccine should be improved to achieve

a better formulation, schedule, safety or efficacy. This may trigger the need to repeat certain phase I and II studies in the changed settings.

At a predetermined decision point the data basis should be robust to start phase III trials. This point is a critical check point in the development path, as phase III studies are lengthy in time, need the enrolment of a high number of study participants and accordingly are very expensive. This time point is also essential to involve WHO and regulatory authorities in scientific advice meetings and End-of-Phase II-meetings, respectively. As defined by the WHO, one or more controlled studies are needed to provide definitive evidence of protection. This shall include at least one pivotal phase III study of efficacy[295].

To be able to obtain phase II data, that have a high change to translate into positive safety and efficacy phase III data, well defined Go/No Go criteria are needed out of phase II studies. In the absence of a clear immunological correlate of either natural or vaccine based immunity, the human experimental challenge infection as read out of phase IIa studies has been key to define such Go/No Go decision point[296]. Efficacy endpoints commonly used are the occurrence of infection, level of parasitemia, occurrence of clinical disease, severe disease and death. The endpoints are tested in randomized controlled clinical trials mostly with a placebo control group[297]. The endpoints of clinical studies testing *P. vivax* vaccines need to reflect that the specific hypnozoites may rest in the liver in dormant stages for long times up to years. Accordingly trial design with *P. vivax* will need to consider late follow up measures[298].

The efficacy time period can be assessed by asking for the time to first and only clinical disease, as it has been implemented in the RTS,S/AS01 phase III as a result of a WHO consultation. With availability of statistical methodology to assess the endpoint “total number of episodes over time period x” this is expected to be implemented in future clinical trials better reflecting the public health needs[299]. The assessment over a defined time period will be essential to determine, when vaccine efficacy might wane. To analyse the effect of a booster dose, a study may be designed to foresee this additional treatment for the participants showing a defined decrease of efficacy.

The characteristics of transmission blocking vaccines have implications for pharmaceutical development strategies. Most importantly, the success of the vaccines will become evident on population level rather than in the individual in the decrease of overall *Plasmodium* burden and infectivity. Consequently, the vaccinated individual will not directly profit from the vaccination and this requires special considerations related to study effectiveness endpoints of clinical trials, beside ethical questions to enrol subjects not expected to benefit. Beyond the expected and substantial public health interest, a sole population benefit is accepted by regulatory authorities and has been implemented several times, for example concerning traveller vaccination to prevent a disease that is non-endemic in a certain area[300]. However the impact for development of TBVs is that regulatory strategies are expected to be more complex than for vaccines targeting clinical disease[301] (see also Table 7). Beneficial for development seems to be that transmission interfering immunity is mediated entirely by the humoral immune response and that the efficacy as part of animal test as well as of human clinical trials can be sufficiently performed with a laboratory assay, the SMFA; (see section 9.2)[302].

Endpoints in trials assessing TBVs may require differentiating between surrogate endpoints that are validated to reflect long term effectiveness or those surrogate endpoints that need a true validation on a population level after safety and immunogenicity has been shown. Phase III efficacy data based on a surrogate endpoint could be the basis for a conditional marketing authorization, requiring the proof-of-clinical efficacy in phase IV post-licensure trials[303]. The endpoints might be the reduction of parasitemia in the mosquito assessed in the SMFA as a surrogate endpoint of phase III and the reduction in effective parasite reduction in the population like in defined geographical areas. The identification of an immunological correlate of efficacy that is essential for pre-erythrocytic and blood stage vaccine is therefore also essential to alleviate clinical development of TBVs.

Recently the concept of TBVs has been broadened to also include vaccines against the mosquito vector or high effective pre-erythrocytic and blood stage vaccines. These are named “vaccines that interrupt malaria transmission” (VIMT) to differentiate them from the TBV targeting sexual or mosquito *Plasmodium* stages. This concept keeps into account that a less effective vaccine against a pre-erythrocytic target may not necessarily reduce transmission as a high effective vaccine that will more likely also reduce the sexual stages enabling transmission[304]. Thus, future vaccines interfering with transmission may evolve having different targets in the *Plasmodium* lifecycle than the established concept of TBVs.

10.2.1. Controlled human malaria infection (CHMI)

A controlled infection of malaria-naïve volunteers can be performed to test efficacy of pre-erythrocytic vaccines and of blood stage vaccines with the aim to demonstrate proof-of-concept before going to larger clinical trials[305,306,307]. Parasitemia will only occur, if pre-erythrocytic, hepatic and erythrocytic stages have developed. If after vaccination parasitemia is not observed, the grade of vaccine efficacy can be described related to number and time point of occurring parasitemia. A differentiation between liver stage and blood stage parasite development may also be done using genetic tests of *Plasmodium* DNA, allowing specifically assessing the pre-erythrocytic vaccine efficacy. However these trials represent unique situations, with the given advice to discuss clinical development plans involving such a trial with the local regulatory authority[308].

In the experiment malaria naïve volunteers are exposed to parasitemic mosquitos in a standardized way. Mosquitos are fed in the laboratory with defined *Plasmodium* strains and after a defined time period the salivary glands of the animals are explored for existing parasites. The volunteers are exposed for 5 or 10 minutes to bites from five mosquitos located in a small chamber. After the challenge the volunteers are densely controlled for developing parasitemia, occurring within 7-20 days after infection. With the first detection of infection, effective malaria treatment is initiated. The challenge model using *P. falciparum* is used since 1986 and has been shown to be safe in about 1300 participants. Side effects are judged to be mild and tolerable and emerging malaria symptoms can be treated in all cases. So far results of one positive efficacy and two failed efficacy outcomes could be confirmed in later efficacy studies involving malaria-exposed individuals thus confirming, also on still few data, the value of this test[309].

The mosquito borne human challenge is thought to have some difficulties related to standardization and quality. The number of infected sporozoites is variable, also their viability can be only determined retrospectively when dissecting the mosquitos after the challenge. Compared to a natural infection, a laboratory *Plasmodium* strain is used, so that any efficacy seen might not transfer into efficacy in field conditions. The complicated handling of *Plasmodium* and mosquitos in the laboratory restrict these experiment to few, specialised sites.

To avoid mosquito borne malaria challenge experimental infection using infected red blood cells has been evaluated. Using a master cell bank of infected red blood cells efficacy of pre-erythrocytic and blood stage vaccines can be tested. It is disadvantageous that the liver stage is circumvented, an immune response might be boosted by the longer time the parasites are allowed to develop and parasite burden of the used red blood cells are variable. As this test allows analysing vaccine efficacy over several cycles of red blood cell multiplication this is judged to be a useful tool to assess asexual blood cell efficacy in malaria naïve volunteers[310].

Recently aseptic sporozoites, that are genetically diverse, were produced for intravenous injection to be used for development of whole cell vaccines, but to also improve the human experimental challenge[311]. Currently this is tested in several clinical trials to standardize this technique aimed to reach consistent and reliable infection and to overcome the disadvantages of mosquito borne or red cell based challenge studies[312].

In a modified way the sporozoite challenge test is used to test for vaccines against *P. vivax* infections, although experiences are much more limited. Here *P.vivax* is taken from blood from infected patients. To prevent the hypnozoite stages, primaquine is added to the treatment scheme of the volunteers[313].

10.3. Specific aspects of clinical development of a malaria vaccine

10.3.1. Considerations for trial sites in malaria-endemic regions

Trial site location is a critical factor for trial design and conduct. This relates for example to malaria burden caused by the different *Plasmodium* species, mainly *P. vivax* versus *P. falciparum*, and the genetic predisposition of the trial participants or the transmission intensity.

The level of transmission or disease burden is a parameter to be assessed when study sites are chosen. In areas of high transmission, natural immunity is expected to develop faster than in low transmission areas. Accordingly the study sites may be restricted to a certain area to enrol either participants from low or high transmission area. Conversely, in areas of low transmission trial duration and number of participants should be planned as needed to assure a statistically sound proportion of participants who will have undergone mosquito bites. In addition to clinical symptoms of malaria triggering a malaria specific test, it might be to considered to regularly test all participants for infection to assure a proper analysis of vaccine efficacy. During the course of the trial it might be useful to check for multiplicity of infections. The considerations likely mean that in areas of low or seasonal transmission more volunteers will be needed to be recruited or recruitment times should be restricted to

seasonal times of transmissions. It may also be considered, if clearing of parasites through medical treatment should be implemented before trial treatment of a volunteer starts.

The parameters assessed to determine the endpoints need to be well defined in time and kind. For example the assessment of the parasite count in malaria-endemic regions may require clearing the blood of the study participants from existing parasites prior to vaccination[314]. However pre-treatment may also have an impact of the vaccination. Accordingly pharmacological characteristics and the half-life of the used medicinal product for pre-treatment should be taken into account. During trial treatment effective anti-malaria medication should be available for the trial participants and all treatments and malaria measures like usage of bed nets should be well documented. As defined in the WHO guideline on pre-erythrocytic vaccines, clear case definitions on clinical diagnosis and case ascertainment exist. One aspect is to clearly define measurable limits to discriminate between non-severe and severe malaria. The diagnosis may need to be verified by trained study staff, however may also involve home visits to achieve an acceptable compliance to study assessments. Depending on the local context, it is to be considered to also have assessments performed by health care providers, who are not necessarily study staff, but other medical staff being trained adequately.

To define the study population appropriately, the frequency of mutations conferring a natural resistance should be considered. This may mean to perform genotyping of volunteers DNA to check for mutations and, thus, should be planned in the study design and participant information.

Changes of disease burden over time may convert high transmission sites into low transmission sites over time. Changing epidemiology may require that the target group of the vaccinated individuals for example related to age has to be adapted. Additionally changing *Anopheles* biting behaviours as a response to efficient treatment options can impact the effectiveness of other malaria measures, like usage of bed nets or indoor residual spraying. To allow appropriate analyses the usage of the additional anti-malaria measures are supposed to be documented sufficiently.

Aligned to the recent progress of the RTS,S/AS01 vaccine and other subunit vaccines clinical trial sites in endemic regions in Africa have been set up supported by a Malaria Clinical Trials Alliance (MCTA) assuring the required quality requirements on site. Most of these sites also participated in the RTS,S/AS01 phase III trial and adherence to international requirements of GCP or the Declaration of Helsinki has been taken seriously. Essential for clinical trials is the informed consent process is performed according to the standards. This includes considerations on illiterate participants, involvement of witnesses in the informed consent process and in the required signatures of the patient information and informed consent forms[315]. To keep and further increase the achievements as main basis to perform clinical trials in malaria-endemic regions, funding issues have been identified as a major issue[316]. Also more specialised sites for example for phase I will be needed as the pipeline of malaria vaccines is supposed to be increasing in the next years.

10.3.2. Overview of RTS,S clinical development

The research on the RTS,S vaccine has started in 1984 resulting in the most advanced malaria vaccine to date. While CSP was chosen due to its abundance of expression, multiple preclinical and clinical attempts were needed to develop the current RTS,S-construct. It consists of different *P. falciparum* CSP domains that are fused to a domain of the hepatitis B virus [317]. In the experiments it was found to be essential that B- as well as T-cell immune answers were elicited giving rise finally to the CSP-Hepatits B construct. Due to the composition the RTS,S vaccine is a bimodal vaccine against the malaria and the hepatitis antigen and can be submitted as such based on adequate hepatitis data gained [318]. The vaccine is combined with the AS01 adjuvant system that was proven safe and most immunogenic compared to other adjuvants[319].

Diverse phase II studies have been performed to assess safety, immunogenicity and efficacy in terms of clinical malaria. Important milestones were a proof-of-concept of efficacy study in Mozambiquanian infants and children at age of 1-4 years. Here, RTS,S with the adjuvant AS02A showed a 35 % efficacy against clinical malaria and 49% efficacy against severe malaria over 18 months[320,321]. In a further RTS,S/AS01E proof of efficacy study enrolling infants at age of 5-17 months, a efficacy of 53 % against clinical malaria was observed over 8 months[322,]. In a longer follow up of this study, a decline of efficacy was seen within 4 years after vaccination[323]. Further phase II studies conducted in infants in Sub-Saharan African confirmed the vaccine is safe and an immune response is seen[324,325,326,327].

Prior to phase III, safety data, pooled from diverse phase II studies, gave a further proof the vaccine is safe in use. Furthermore, compatibility with EPI regimen was shown in several trials. In these trials, efficacy was detected to be in ranges of 50-60 % over 3 to 12 months. Additionally, a study comparing RTS,S/AS01 and RTS,S/AS02 showed, that the addition of AS01 to RTS,S elicited a better immune response and accordingly was chosen for the following phase III study.

The pivotal phase III trial is a 3-arm, randomized, controlled, multicentre, participant- and observer blind study. This study has been developed integrating support and discussions by WHO expert groups like the WHO MALVAC[328] and a WHO assigned Study Group on Measures of Malaria Vaccine Efficacy[329], as well as consultation of EMA, FDA and national African regulatory authorities[330].

Infants of ages 6-12 weeks and 5-17 months were enrolled and outcomes are presented separately for each group. In- and exclusion criteria were defined to have a study population closest possible to the natural population targeted by the malaria vaccine. However, few groups have been excluded mainly for safety reasons, like infants with advanced HIV stage of disease. For different medical and operational reasons different vaccines have been integrated as controls for the younger and older age groups, respectively. For the analyses, parameters like bed net usage or administered doses of an intermittent preventive treatment as well as distance to an inpatient health facility will be integrated to draw conclusions of factors contributing to malaria incidence and data surveillance as well. If the rate of mosquito attacks turned out to be less than expected the enrolment could be prolonged to reach the required sample size related to the transmission quote. For stringent safety documentation field workers conducted monthly visits at the infants' homes to assure complete SAE

documentation. Regular blood samples were taken to assess safety and immunogenicity of the vaccine. Importantly, the trial is conducted as required according to ICH-GCP, Declaration of Helsinki and local requirements[331].

The co-primary outcomes are efficacy against clinical malaria over 1 year each in the groups of infants aged 6-12 weeks and aged 5-17 months after first vaccination, respectively. Multiple data points were collected to be able to analyse detailed measures of efficacy, immunogenicity and safety. This includes data related to disease characteristics like parasite burden, effects in subgroups like HIV infection children and efficacy of the vaccine over time after vaccination. Results related to the period of 14 months after first vaccination have been published for the two age groups[332,333]. For both age groups it was shown that the vaccine is safe. Anti-circumsporzoite antibodies titers were lower in the younger age group than in the older age group. Efficacy in terms of first/only malaria incidence in the per-protocol population was 55.8 % in the older age group and 31.3 % in the lower age group. Similarly the efficacy against severe malaria was higher in the older age group (47.3%) versus the younger age group (36.6%). Final analyses based on 32 months after first vaccination are expected to be available in 2014.

Based on phase II data the lower efficacy of the young age group compared to the older group was not expected. However, the vaccine is still thought to confer modest efficacy. After final data will be released, RTS,S/AS01 vaccine is planned to undergo an article 58 process, followed by a WHO policy recommendation and WHO prequalification[334].

Further phase III trials are foreseen, e.g. in order to test the malaria vaccine with the new emerging EPI vaccines Rotavirus and *Streptococcus pneumoniae*. Furthermore, lot to lot consistency tests are planned as part of a phase III trial[335] and pharmacovigilance and vaccine effectiveness analyses are planned as part of a post-approval program[336].

11. Discussion

Malaria vaccine research up to now has resulted in a variety of candidates that are at different early and late clinical development stages, with the RTS,S/AS01 vaccine about to conclude phase III. The subunit vaccines in development show the focus of research on pre-erythrocytic and blood stage vaccines targeting *P. falciparum* caused malaria. The vaccine candidates in development might be changing based on the recently updated strategic goals to include transmission blocking and *P.vivax* specific vaccines. With positive results seen for the RTS,S vaccine and other subunit antigen based vaccine, it can be expected that a second generation vaccine may follow by combining subunit antigens targeting different stages of the *Plasmodium* lifecycle. One example might be to combine pre-erythrocytic and blood stage subunit antigens in a single combinatorial vaccine preventing clinical disease while prohibiting break through infections as well[337]. Additionally, through improvement of whole cell vaccines, the antigen delivery systems and the used adjuvants, further progress is likely. The RTS,S vaccine, having progressed towards licensing, shows a modest efficacy, however seems to be crucial in reducing severe morbidity of young children. Seeing that many parameters in vaccine formulation individually are undergoing preclinical and clinical tests, a high complexity is eminent. In case malaria vaccine development will progress only stepwise to more efficacious vaccines, vaccine development may not hold the promises for disease reduction and eradication in the coming years. Remarkable in the field is the chance and the willingness to explore unbeaten path, as seen, when a new animal model is looked for based on a species from the field or the intravenous route of application of whole cell vaccines is assessed. Also, viral vector vaccines and prime-boost immunization regimes are, beside malaria research, still in development, as in HIV, cancer or influenza clinical development[338]. Few viruses as vaccine vectors have been licensed for human use so far, reflecting that malaria vaccine development is applying state of the art research that will need to be based on state of the art regulatory processes as well.

Developing a vaccine to a complex organism like *Plasmodium* faces scientific questions. So far the basis of naturally acquired immunity has not been dissected. This is a scientific challenge impacting clinical research, as no correlate of immunity is known and proof-of-efficacy has to be based on a long term clinical follow up. Based on increasing knowledge gained through ongoing, however also failed vaccine projects, it is promising to see that several vaccines have shown some grade of efficacy. A high proportion of medicinal products fail between phase I and III and beyond, showing that assumptions of early development do not necessarily correlate to the conditions in the real clinical situation. This is aggravated in malaria research, where the assumptions of *in vitro* and *in vivo* tests have just started to be reassessed and validated in the clinical situation. Also, first signs became apparent that the parasite seeks new ways out of increased selection pressure. This was evident in mosquitos that bite at different times of the day or an increased malaria incidence in older age groups of infants. It is also possible, that the parasite, adapted to be a versatile organism during its co-evolution with human beings, will become more pathogenic as a result of the new selection pressure caused by a vaccine[339]. In addition to pharmacovigilance plans to record side effects, that have not turned up in the clinical trial population, post marketing as well as risk management plans will need to prepare for any

change in the parasite's biology. This will logically also include foreseeing scenarios how malaria may return to areas that are currently considered to be non endemic for malaria.
Supported by a malaria road map the malaria vaccine development is guided by long term strategic goals and landmarks. The recently updated goals show that different types of vaccines targeting reduction of clinical disease and transmission are foreseen. Moreover, related to the new goal, different age groups and global disease settings are considered. In drug development, this requires different clinical development plans related to the type of vaccine as well as the target population. Each clinical development plan will be based on unique clinical trial strategies consisting of specific characteristics, like target population, endpoints, efficacy measurements or trial durations. Furthermore, once approved, a specific vaccine may not be used globally, but adapted to areas with a low or high disease transmission, local medicinal care conditions or the political situation. It could require that in some regions a vaccine targeting clinical disease is chosen first for implementation. Only as soon as the transmission intensity has been lowered to a defined threshold, the transmission preventing vaccine may be indicated. Thus, the clinical development of malaria vaccines today show multiple facets that each will require separate considerations and accordingly, guidance and interaction of vaccine developers with the regulatory authorities.
Thus, in aspects of parasite biology, pharmaceutical development of a vaccine and clinical development strategies, malaria vaccine development is challenging. Accordingly new aspects, questions and discussions are raised related to preclinical, clinical and licensing requirements and guidance by regulatory authority processes. So far only few regulatory documents exist that specifically address malaria vaccines. The recent WHO guideline on a malaria vaccine is addressed to subunit vaccines, but is advised to be used where applicable for other topics of malaria vaccine development, like the controlled human malaria infection studies. The intended PPC will be one important tool to provide guidance to vaccine developers as well as to single regulatory authorities. Posed by the global need of the malaria vaccine with a challenging road map the involvement of all stakeholders and regulatory authorities worldwide pose a challenge. Interactions between the regulatory authorities aligned to the global development plans of a malaria vaccine are an asset to promote the development in this field. Current practice of WHO and EMA to interact, for example in the article 58 procedure or the interaction of EMA and FDA with the WHO are one basis. It needs to be noted that local regulatory authorities provide essential input related to the local conditions and requirements such as the target population of the vaccine or local supply and storage conditions. Ignored local conditions may lead to a longer and more cost, time and resource needing drug development, however may also lead to a failure. An overarching collaboration of WHO, FDA and EMA with local RAs is supposed to strengthen the process even more. Furthermore, other agencies for this global topic like Japan, Asian countries, Australia and Latin America so far are only marginal player, if at all. Thus, it is in the best interest for malaria vaccine development to have a tight interaction between vaccine developers, the industry manufacturing the vaccine and the regulatory authorities to be able to commonly progress in developing high quality, safe and efficacious malaria vaccines.

12. Summary

Today, malaria is judged to be a preventable and curable disease, although yearly around 1 million deaths and 219 million clinical malaria cases occur. To cover unmet needs, malaria vaccines are in development since decades, but the most advanced compound is just about to conclude in a phase III trial. In this thesis the status, the scientific grounds and the regulatory environment of developing a malaria vaccine meeting quality, safety and efficacy standards as well as strategies of a market implementation are evaluated.

Plasmodium, the parasite causing malaria, passes a complex life cycle involving mechanisms to evade a natural occurring immunity. Recently global strategies target malaria eradication additionally to reducing mortality and morbidity, resulting in diversifying vaccine types rather than having a single one. The current data show that subunit vaccines composed of single or multiple *Plasmodium* antigens are mostly safe, and several candidates warrant further development due to proven immunogenicity or signs of efficacy. In early development different vector targets and adjuvants are evaluated to improve the vaccine prior late stage clinical trials. Especially viral based vectors show that most recent technology is applied. Whole cell vaccines are long known to elicit a high rate of efficacy. An Evolved manufacturing technology allowed testing of attenuated *Plasmodium* in a phase I clinical trial showing it is safe, immunogenic and able to elicit a complete protection against malaria. It implies new paths of development to be explored, like an intravenous vaccine dosing or storage requirements at -70 °C.

By targeting mainly developing countries, malaria vaccines are subject for a WHO vaccine prequalification followed by a marketing authorisation in the respective local countries. Laid down in Regulation (EC) No. 726/2004, article 58, EMA is facilitating a scientific advice for such a vaccine that is not targeting a EU marketing authorization, furthermore the FDA is promoting development of medicines for developed countries. Currently the national competent authorities show a diverse pattern of marketing authorisation capabilities ranging from being fully functional up to relying strong on marketing authorisations granted in other countries. It can be seen that malaria vaccine development efforts resulted in streamlined local regulatory processes for clinical trials. In a similar manner the input of local regulatory authorities in future marketing authorisation processes is seen to be essential to better reflect the local requirements. The integration of regulatory expertise on a global level involving regulatory authorities of developed as well as less developed countries is thought to further increase support malaria vaccine development. However, as a clinical correlate of natural immunity has not yet been identified, also surrogate parameter and clinical endpoints for clinical trials are not firmly established yet and still require validation in the target population. Key biological assays are currently exploratory only or have just undergone validation to meet regulatory standards. Clinical development plans, clinical trial characteristics and target product profiles are discussed in the scientific community. Preferred product characteristics meant to be a guidance to vaccine developers are planned to be established. Regulatory support has already been strengthened with a new malaria vaccine specific WHO guideline that integrated recent expertise of the most advanced products. Thus, malaria vaccine development relies strongly on the need to integrate most recent scientific results and a strong and early interaction with regulatory authorities.

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14. Appendix

Draft list of key data considerations information malaria vaccine decision making based on documentation and WHO interviews

Source: *Malaria Journal* 2010, **9**:182 doi:10.1186/1475-2875-9-182

Safety and efficacy in relevant populations	Safety An acceptable safety profile Freedom from "rebound" effect, that is, enhancing disease incidence in target groups following use: needs follow-up population monitoring Positive evaluation from WHO GACVS No significant adverse impact on other malaria prevention and treatment strategies (i.e. increasing adverse events from another product) or on response to concomitantly administered vaccines Safety evaluated in immunologically compromised groups, e.g. HIV-infected
	Efficacy Acceptable level of reduction of disease-related morbidity and/or mortality in target populations Efficacy demonstrated in different malaria endemicity settings Delivery schedules, dosing and administration route feasible and consistent with burden of disease in target countries
Implications for costs and population health	Supply, financing, and cost-effectiveness issues Availability of product under the regulatory oversight of a fully functional regulatory authority and/or prequalification Available supply related to anticipated demand Affordability Means of monitoring impact to feed into cost-effectiveness assessment Prospects for competitive vaccine market
	Impact on other public health interventions Vaccine delivery strategies to reach desired target groups (e.g., catch-up immunization where relevant) Impact of vaccine use on compliance with other interventions, e.g. ITN Community perception of given malaria vaccine products given their likely characteristics Impact of the vaccine demonstrated in the context of other malaria control strategies
Localization of data	Local applications of the intervention Evidence sufficient for local decision making available to the appropriate in-country groups (such as Immunization Advisory Committee, Interagency Coordinating Committee, etc), including, as relevant, national stakeholders and decision makers and key partners Ability to deliver vaccine through local cold chains Specific evidence for unique epidemiological situations available, if applicable Information from demonstration projects available particularly where new target groups or specific product acceptance issues are involved

Hiermit erkläre ich an Eides statt, die Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

Frankfurt am Main, im September 2013