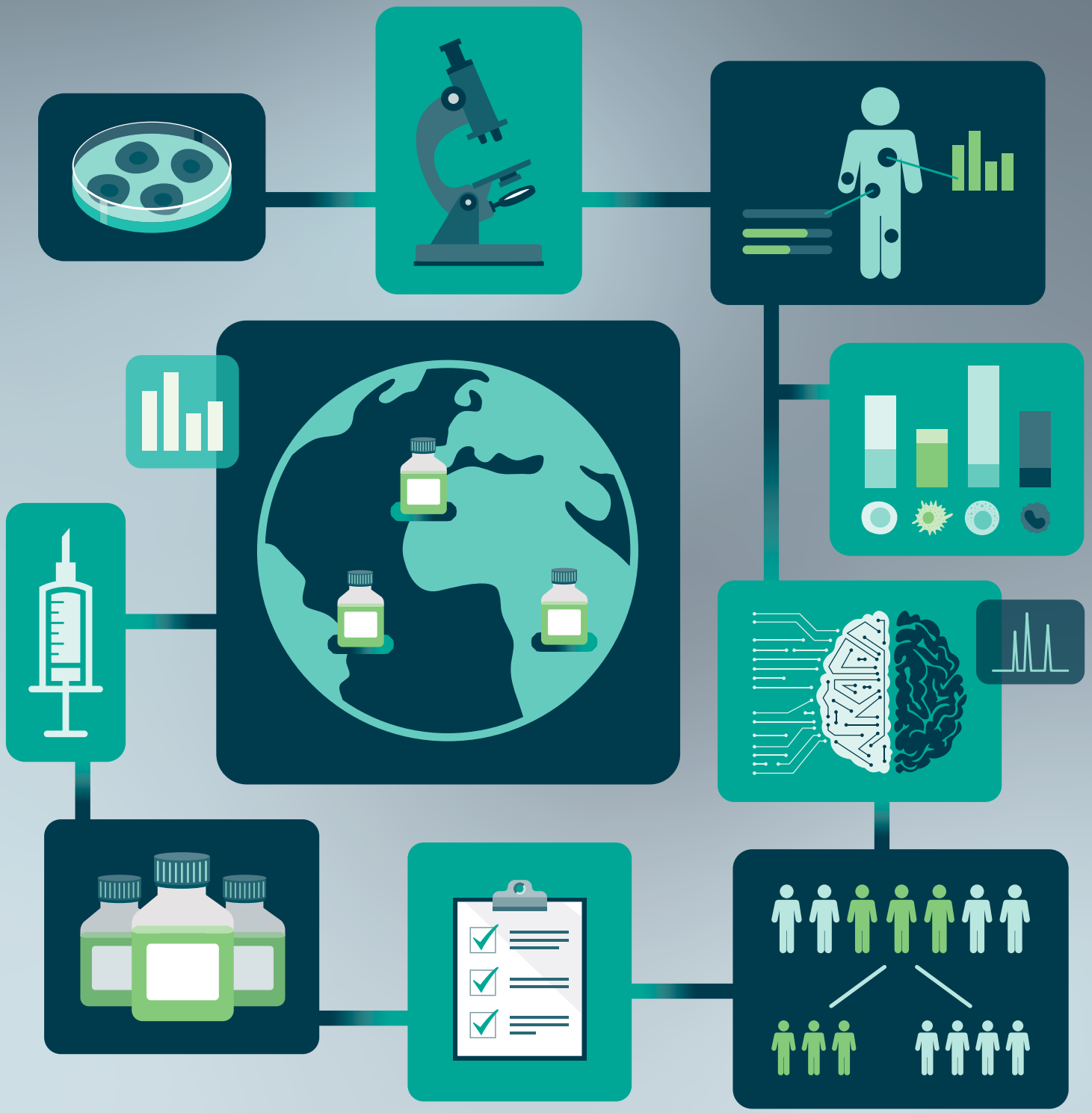
SEPTEMBER 2024 Volume 3, Issue 5

VACCINE INSIGHTS

SPOTLIGHT ON
Preclinical and clinical research



Preclinical and clinical research

VACCINE INSIGHTS

PRECLINICAL AND CLINICAL RESEARCH

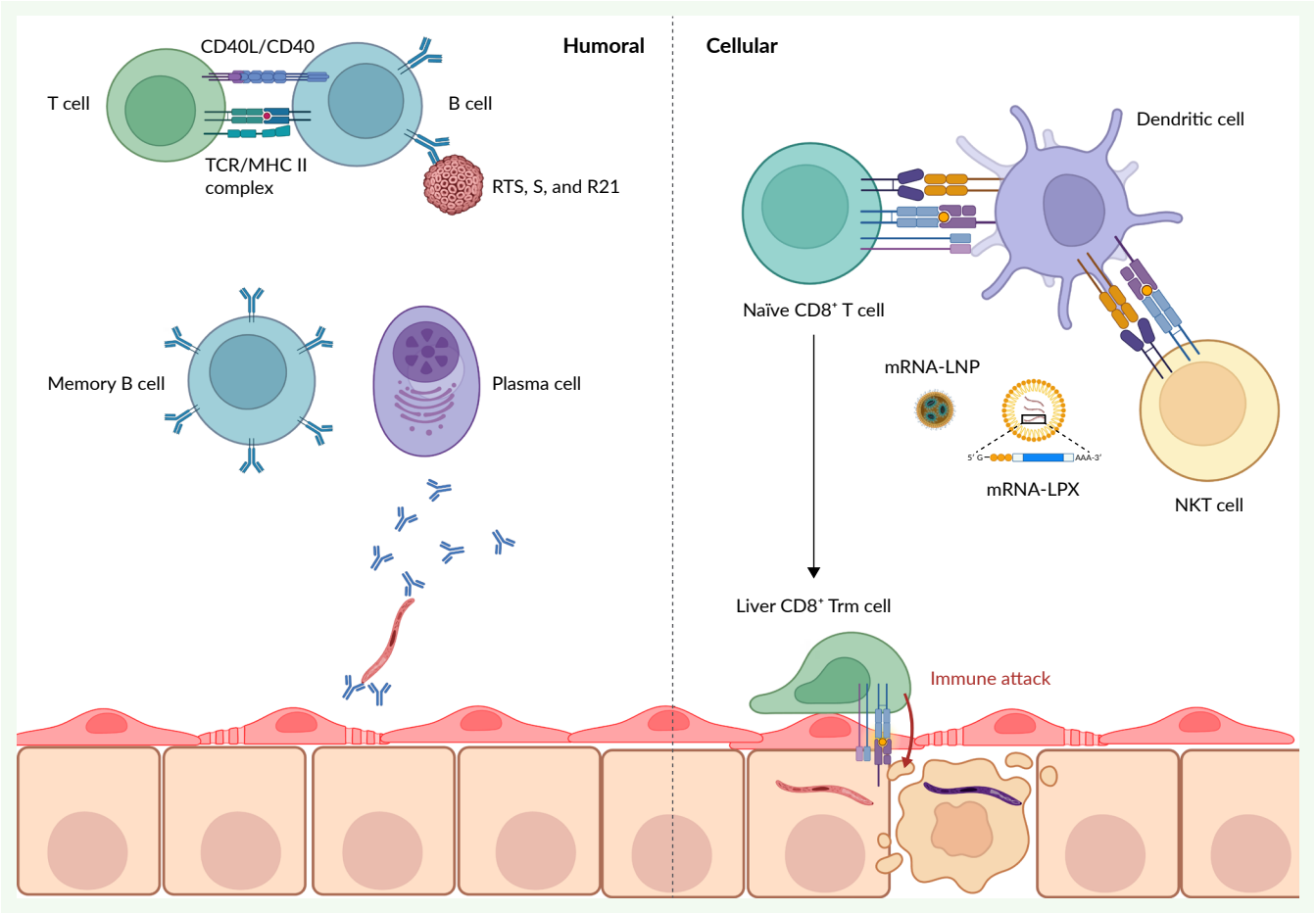
SPOTLIGHT

EXPERT INSIGHT

Immunity and vaccine development against liver-stage malaria

Mitch Ganley, William Heath, and Lynette Beattie

Malaria is still a major cause of death, with over half a million people dying every year and over 200 million infections. As of 2024, two protein subunit vaccines have gained WHO approval, the RTS,S/AS01 and R21/Matrix-M, with 30% and 75% vaccine efficacy, respectively. Despite these significant milestones, readiness for global implementation, and the potential to drastically reduce the global disease burden from malaria, there are limitations in the current approaches. This is in part due to logistical and scalability issues of the complicated subunit vaccine but also the low efficacy and requirement for 3–4 doses and a booster dose prior to the malaria season every year to maintain efficacy. Furthermore, the WHO has set the target of 90% vaccine efficacy over 12 months for future malaria vaccine development. This review will highlight the limitations to the current approaches of generating antibodies against the NANP domain of circumsporozoite protein, and the need for new approaches and technologies. Specifically, we will discuss vaccines generating the newly identified liver tissue-resident memory CD8 T cell that can provide sterile protection against liver-stage infection. Live-attenuated, protein subunit, viral vector and mRNA vaccines are considered in this review. Since the COVID-19 pandemic, mRNA vaccines have demonstrated rapid and wide-spread deployment that may overcome logistical hurdles faced by other vaccines. mRNA vaccines could be harnessed for improved generation of liver tissue-resident memory T cells and improved, long-lasting malaria immunity.



Created with Biorender®.

Vaccine Insights 2024; 3(5), 143–162

DOI: 10.18609/vac.2024.026

PLASMODIUM PARASITE AND PATHOLOGY

Malaria is a deadly and debilitating disease, caused by a single-cell eukaryote parasite of the *Plasmodium* genus. The *Plasmodium* genus is part of the Apicomplexa phylum that, along with *Toxoplasma* and *Cryptosporidium*, makes up a large group of protozoan parasites, many of which are infectious to humans. Their characteristic feature is the apical complex, essential for invasion through the host cell membrane and for performing a mode of motility, called gliding [1]. There are a number of *Plasmodium* species that afflict humans, but the majority of malaria cases are caused by five species: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* [2]. The parasites are carried by mosquitos of the *Anopheles* genus, including 40 species [3], which primarily inhabit tropical and subtropical regions, hence why the majority of malaria cases are confined to these areas. This includes 43 countries over central and south America, sub-Saharan Africa, eastern Europe, central (e.g., India) and southeast Asia; however, the majority (~88%) of cases occur in Africa, followed by southeast Asia. In 2022, approximately 608,000 deaths were caused by malaria out of 249 million infections worldwide [4]. The vast majority (76%) of fatal cases are children under the age of 5.

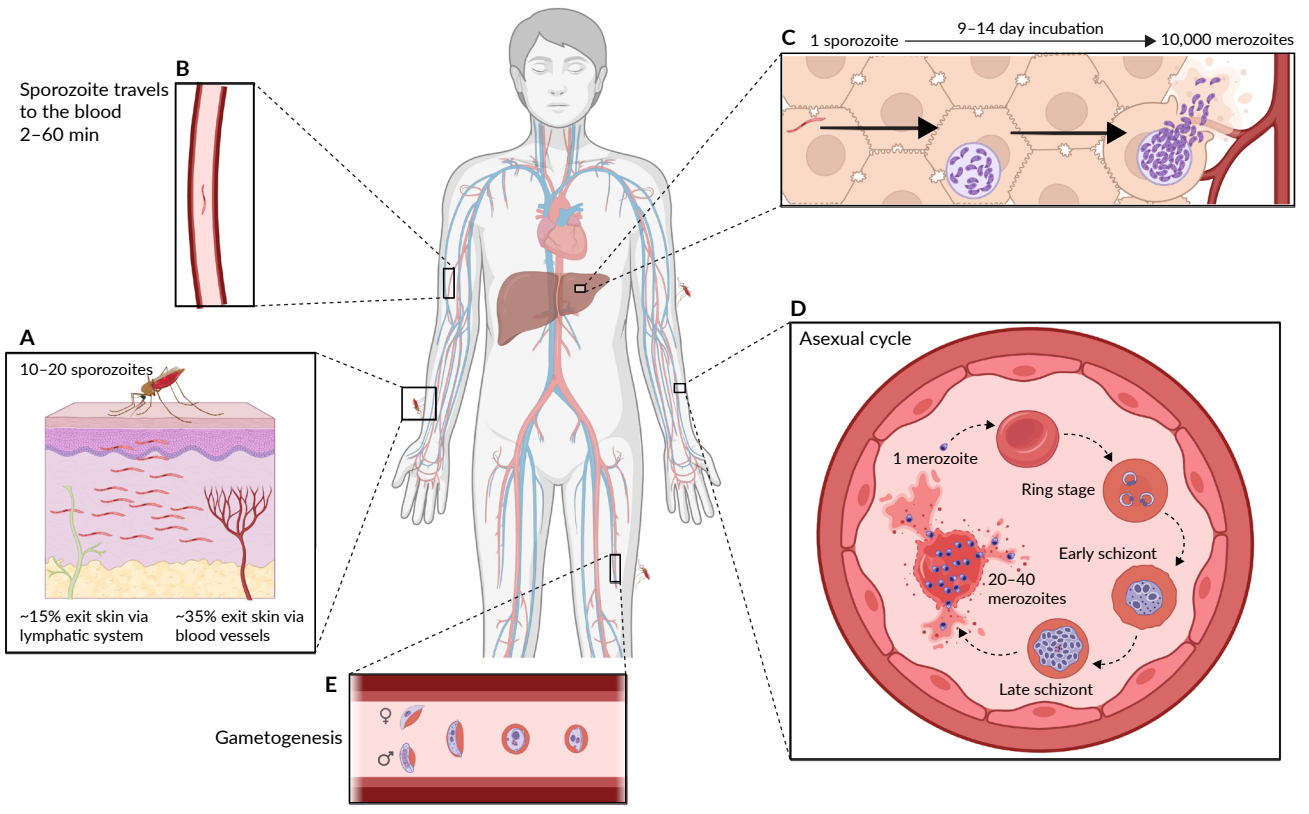
Maternal IgG antibodies (passed from mother to neonate) specific to Plasmodium antigens can protect newborns from malaria [5,6]. However, these quickly wane after 6 months, making children highly susceptible, and most children are infected by the age of 2 years in areas with high malaria prevalence [7,8].

As eukaryotes, *Plasmodium* species have a complex life cycle, with asexual and sexual stages. The sporozoite (haploid) stage of the life cycle begins in the salivary glands of infected mosquitos, which results in the depositing of sporozoites in the skin of mammals following a blood meal by female mosquitos (Figure 1A). The sporozoites travel to the liver where they infect hepatocytes and eventually develop into the merozoite stage (Figure 1B). Replication in the parasitophorous vacuole within the cytosol of infected hepatocytes takes days to weeks depending on the parasite and host species (Figure 1C). Once the merozoites break out of the liver they continuously infect red blood cells, reproduce asexually (Figure 1D), or develop into gametocytes (Figure 1E), starting the sexual reproduction cycle. During this phase, gametocytes can be taken up by a mosquito during a blood meal and fuse into a zygote (diploid) in the mosquito gut [9]. The zygote develops into an ookinete that travels through the midgut epithelial wall and undergoes meiotic recombination [10]. Following this process, an oocyst forms and then releases dozens of haploid sporozoites into the salivary gland of the mosquito, completing the cycle. The process of sexual reproduction in the mosquito takes approximately 1 week [11].

When a female mosquito has a blood meal, approximately 15–20 sporozoites are transmitted into the skin of the host [12,13], while multiple mosquito bites can increase the total number of sporozoites that enter the body. Approximately 50% of sporozoites leave the site of infection in the skin and of those, 30% enter lymphatic vessels and travel to lymph nodes while 70% travel through the blood into the liver, where they infect hepatocytes [13]. The period in which sporozoites leave the skin and travel to the liver is very short. Following an infected-mosquito blood meal, 50% of sporozoites have already left the skin after 1 hour [13]. Furthermore, sporozoites can be found in the liver just 2 min following direct intravenous injection of mice with *Plasmodium* sporozoites [14]. In humans, the development into merozoites in the liver takes longer than 1 week, depending on the *Plasmodium* species (2–3 days in mice). This lengthy incubation in the liver compared to the short period it takes sporozoites to travel to the liver from the skin has important implications when designing vaccines against *Plasmodium spp*. Furthermore, *Plasmodium ovale* and *vivax* can also enter a dormant stage in the liver, called hypnozoites, which can remain in the liver for months before causing infection in the blood [15]. When the infection progresses with normal kinetics, infection of a hepatocyte by one sporozoite results in the development of thousands of merozoites, which enter the bloodstream after exiting the hepatocyte, infecting red blood cells [16]. The merozoites can then reproduce asexually, producing dozens of merozoites from one infected red blood cell (iRBC). This is the disease-causing stage, as red blood cells are lysed during the rupture and release of more merozoites [17]. The parasite digests hemoglobin in the red blood cell, leading to the release of heme, which is toxic to the parasite [18]. This is overcome through polymerization of heme into hemozoin, a blue pigment characteristic of iRBCs. Following infection and lysis of red blood cells by *Plasmodium* merozoites, release of parasite DNA and hemozoin leads to sharp peaks in TNF-α production. This causes paroxysms, which are periods of high fever and chills, diarrhea, vomiting and headaches [19]. Hemolysis also contributes to anemia, a symptom of malaria that can develop into severe anemia, followed by organ damage and failure [20]. Apoptosis of non-parasitized RBCs and dyserythropoiesis have also been reported to contribute to anemia [21–23]. Furthermore, the lack of oxygen to various tissues, in combination with other factors, can cause metabolic

FIGURE 1

***Plasmodium falciparum* life cycle in mammals.**



(A) Female *Anopheles* mosquito blood meal leads to deposit of sporozoites. **(B)** Sporozoite travels via the blood to the liver. **(C)** Sporozoite infects one hepatocyte in a parasitophorous vacuole develops and replicates reaching up to 10,000 merozoites before rupturing into the blood. **(D)** Continuous infection and asexual reproduction of red blood cells. **(E)** Merozoite undergoing gametogenesis to form male and female gametocytes before being taken up by a mosquito for continuation of the sexual reproduction cycle. Created with Biorender®.

(lactic) acidosis [24]. *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) proteins are expressed on the surface of iRBCs, which bind to CD36, intracellular adhesion molecule-1 (ICAM-1) and endothelial protein C receptor (EPCR), all expressed by endothelial cells, mediating binding of iRBCs to endothelial cells [25,26]. This adherence causes blockage of blood vessels, leading to reduced blood flow and hypoxia [27]. This pathology leads to a range of severe outcomes including seizures, neurological damage, comas and death [28,29].

PLASMODIUM HOST INTERACTIONS AND IMMUNITY

During liver-stage infection, multiple immune cell types recognize and respond to infection, including resident lymphocytes and macrophages. For example, induction of type I interferon (IFN) in response to liver-stage parasite RNA was dependent on RNA sensing receptor melanoma-differentiation associated gene 5 (MDA5) and its downstream adaptor protein mitochondrial-antiviral signaling (MAVS) [30]. There is evidence that innate-like T cells become activated during the liver-stage infection and play a role in host defense. Natural killer T (NKT) cells can be activated by infected hepatocytes *in vitro* and directly kill the infected cells [31]. NKT cells also play a role in suppressing liver-stage infection through IFNγ production in response to type I IFN [32]. γδ T cells also play an important role in innate and adaptive defense against malaria. Mice that are deficient in γδ T cells do not develop

immunity following γ-radiation attenuated sporozoites (RAS) vaccination, due to lower T cell responses, but the mechanism was not investigated [33]. γδ T cell clones have been identified that respond to and control liver-stage infection [34,35]. There is also clear evidence of γδ T cell activation during blood-stage infection and iRBC killing via granulysin secretion and/or phagocytosis [36]. Macrophages play an important role in the innate immune response to malaria sporozoites, as they may be a gateway for sporozoites to pass the sinusoidal barrier in order to infect hepatocytes. Specialized tissue-resident hepatic macrophages called Kupffer cells line the sinusoidal endothelial wall and interact with sporozoites either through endocytosis or via direct cell invasion by the sporozoite (Figure 2). Sporozoites actively invade Kupffer cells via CD68 (Figure 2), which allows them to pass through the sinusoidal wall and infect hepatocytes [37]. Sporozoite infection studies *in vitro* demonstrate that Kupffer cells produce a myriad of cytokines, including Th1 (IFN-γ, IL-2, and IL-12), Th2 (IL-4 and IL-5), and the Th17 cytokine IL-17A [38].

Plasmodium spp. are highly adept at avoiding the immune system at every stage of the parasite life cycle. Sporozoites can drive an anti-inflammatory program in macrophages causing an increase in IL-10 expression and dampened responses to external stimuli, reducing IL-12, TNF-α, IL-6 and MCP-1 expression, and downregulating MHC I presentation [39–41]. Moreover, infection of human skin antigen-presenting cells leads to the induction of a regulatory phenotype resulting in the priming of CD8 T cells with poor function [42]. These studies highlight the ability of *Plasmodium* sporozoites to evade and manipulate the immune system to prevent liver-stage immune responses. Once merozoites break out of the liver and start infecting the blood they become virtually invisible to CD8 T cells, as erythrocytes do not express MHC I.

The development of humoral immunity against the highly defined circumsporozoite protein (CSP) in malaria-endemic areas is very inefficient following infection [43,44]. Furthermore, humoral immunity that reduces symptomatic malaria during blood-stage infection requires years of exposure. Sterile immunity, which is needed to prevent liver-stage infection prior to initiation or before progression to blood stage infection, almost never occurs. Additionally, blood-stage infection inhibits humoral immunity to the liver-stage infection, via mechanisms that have been characterized *in vivo* [45]. Blood-stage infection generates an inflammatory response that leads to IFN-γ production and release of CXCL9/CXCL10 chemokines. These chemokines disrupt germinal center formation, a transient process that is essential for humoral immunity. Blood stage infection therefore broadly inhibits the generation of effective humoral immunity. In contrast, if the development of blood-stage malaria is blocked, via use of the genetically attenuated sporozoite (*Fabb/f*$^{-}$) that arrests at the liver stage, increased CXCL13 and CCL21 production, greater germinal center formation, and enhanced memory B cell and antibody responses are observed. These data indicate that effective humoral immunity to liver-stage expressed antigens would be generated if the parasites are prevented from progressing to blood stage infection. Prevention of blood-stage infection using anti-microbials such as atovaquone could also prevent impaired B cell responses.

Controlled human malaria infection (CHMI) studies, in which volunteers are exposed to malaria (via infected mosquitos bite or via injection of sporozoites of iRBCs) followed by treatment with antimalarial drugs, have been essential in studying vaccine efficacy and the interactions of *Plasmodium* with the human immune system. Antibody subclasses IgG1 and IgG3 (high affinity with FcγRI or CD64 expressed by macrophages and dendritic cells) against CSP, have the highest association with protection from liver-stage infection [44,46,47]. In addition, Pf-specific-IgG, FcγR expression

and monocyte activation also correlate with immunity and low parasite burden in the blood [48,49].

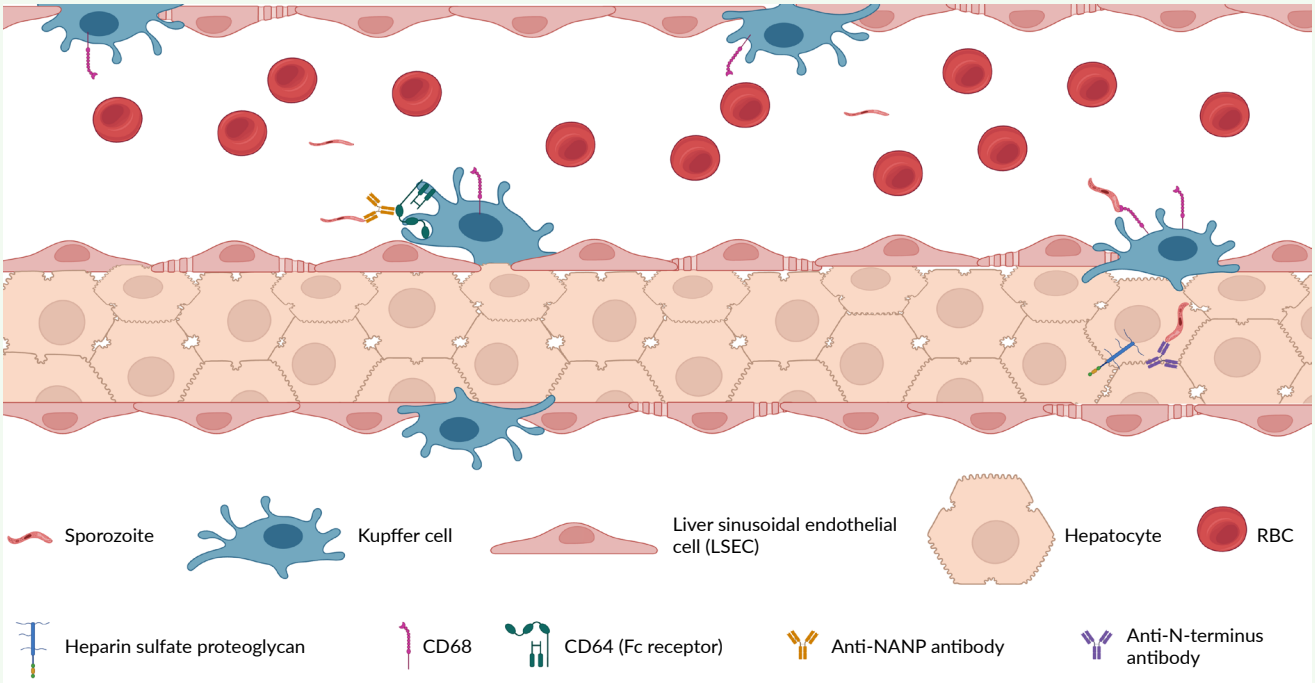
CSP and thrombospondin-related anonymous protein (TRAP) are expressed on the cell surface of sporozoites and are essential for sporozoite trafficking and infection of hepatocytes [50]. CSP contains three major domains: the N-terminal domain, central repeat domain and C-terminal domain. Within the N-terminal domain is a heparin sulfate proteoglycan (HSPG)-binding domain, which facilitates hepatocyte infection (Figure 2). Upon binding to HSPGs on hepatocytes, the sporozoite receives signals to halt trafficking [51]. The N-terminal domain of CSP undergoes proteolytic cleavage in the site just downstream of the HSPG-binding domain. In malaria-endemic areas, acquisition of anti-CSP IgG antibodies, in particular those targeting the central repeat (NANP) domain are associated with natural immunity and can mediate complement activation and sporozoite killing *in vitro* [44]. Various studies have investigated the role of antibodies to the N-terminal domain by generating and testing monoclonal antibodies (mAb), which has garnered mixed results. The 5D5 mAb is specific to the HSPG-binding/cleavage sequence region, inhibits proteolytic cleavage of the *P. falciparum* CSP and infection of mice by recombinantly expressing *Pf*CSP *P. berghei* sporozoites (Figure 2) [52]. Thai and colleagues found that the binding efficiency of 5D5 was much lower than that of the 1210 mAb (central repeat domain specific), which may be due lower relative epitope frequency [53]. It is worth noting that the combination of both 5D5 and 1210 mAbs had additive inhibitory effects [52,54]. Conversely, the C-terminal domain-specific mAb 1710 has failed to show any inhibitory efficacy [53,54]. Further studies are required to confirm the contribution of N-terminal domain-specific antibodies to protection and compare with that of the central repeat domain-specific antibodies.

PROTEIN SUBUNIT VACCINES

Since CSP was validated as a vaccine target for generating humoral immunity to prevent liver-stage infection [55], many vaccine strategies have been pursued using this antigen. The recently approved protein subunit vaccine RTS,S/AS01 (Mosquirix™) contains a CSP central **r**epeat and **T** cell domains fused to the HB**s**Ag protein in a virus-like particle (VLP) delivery vehicle, with the AS01 adjuvant system, a liposomal formulation of QS-21 and monophosphoryl lipid A (MPLA). The RTS antigen contains 19 out of the 43 repeating NANP motifs within the central repeat domain, the T cell epitope containing C-terminal domain and no N-terminal domain. This fusion protein is produced via recombinant expression in yeast, alongside unmodified HBsAg protein, and formed as a mixture of both proteins to generate VLPs. Four doses of the vaccine have moderate efficacy in preventing malaria infection, with 30% vaccine efficacy after 1 year and 5–10% after 4 years [56–59]. Although the efficacy is low and the immunological memory is short-lived, this was a huge milestone in lowering deaths and disease burden caused by malaria. CSP-specific IgG, which prevents sporozoite invasion of hepatocytes, is the strongest predictor of protection associated with RTS,S/AS01 vaccination. $CD4^+$ T cell responses to the conserved epitopes with the C-terminal T cell domain is the second strongest predictor of protection [60]. IFN-γ^+ CSP-specific-$CD4^+$ T cells were also highly correlative of protection from the RTS,S/AS02 (MPLA and QS-21 in oil-in-water emulsion) vaccine [61]. Furthermore, in the context of CHMI studies, a population of IFN-γ-producing $CD161^+$ $CD4^+$ T cells was associated with protection from malaria [49]. Further developments to this vaccine strategy include removal of the native HBsAg protein from the VLP, leaving only the RTS fusion protein to increase the antigen display on the VLP, and a new adjuvant

FIGURE 2

Liver sinusoid and sporozoite interaction with Kupffer cell and hepatocytes.



Sporozoites reach the liver and enter sinusoidal vessels. Interactions with Kupffer cells can occur through the Fc receptor (CD64) and anti-CSP (e.g., anti-NANP) antibodies, resulting in phagocytosis. Kupffer cells also express CD68, which is reported to be exploited by the sporozoite for cell invasion. These sporozoites can pass the sinusoidal endothelial barrier and infect hepatocytes. Hepatocytes express the heparin sulfate proteoglycan receptor, sporozoites interact with hepatocyte membrane via CSP and invade the cell. CSP-specific antibodies (e.g., anti-NANP or anti-N-terminus) block this interaction and prevent infection of hepatocytes. Created with Biorender®.

formulation containing QS-21, cholesterol, and phospholipid nanoparticles, now called R21/Matrix-M. In both the Phase 2 and 3 clinical trials the R21/Matrix-M vaccine reached a superior vaccine efficacy of 75% in children aged 5–36 months [62,63]. However, the younger age group (5–17 months) had a higher anti-NANP IgG titer and vaccine efficacy than the older group (18–36 months), which may be due to higher blood-stage exposure of the older age group. Furthermore, long-term memory has yet to be determined from the phase III cohort.

ATTENUATED WHOLE-PARASITE VACCINES

The first vaccine against malaria was developed in the mid-1900s using RAS, also known as the ***P****lasmodium* ***f****alciparum* **spo**roz**o**ite (PfSPZ) vaccine. The process of gamma-radiation renders the sporozoites unable to develop past the liver-stage of infection, inducing a liver-specific immune response without causing blood-stage infection. Following development of scalable methods for aseptic purification and cryopreservation of the RAS vaccine by Stephen Hoffman and colleagues at Sanaria Inc, the vaccine was tested for safety and efficacy in Phase 1/2a trials. Intravenous administration of four doses of 10^5 γ-irradiated sporozoites was well tolerated and showed no signs of breakthrough infections [64]. Vaccination-induced anti-sporozoite antibodies, $CD4^+$ and $CD8^+$ T cells, which were greater in number in protected individuals. Intravenous administration of four doses of the RAS vaccine generated Pf-specific $CD8^+$ T cells that were associated with protection (55% following CHMI after 3 weeks), and provided protection for five individuals rechallenged 59 weeks after

immunization [65]. A field trial with adult volunteers in Mali, demonstrated safety and modest efficacy (30%) at 20 weeks after vaccination [66]. A Phase 1/2a clinical trial with infants (6–12 months and 1–5 years), children (6–17 years), and adults (18–45 years) in Tanzania showed anti-PfCSP antibody responses were lower in older cohorts and lower in individuals in Tanzania compared to a parallel trial of adults in the USA [67]. Comparison of the anti-PfCSP antibody responses from trials in the USA, Mali, Tanzania and Equatorial Guinea further demonstrated lower antibody responses in the malaria endemic areas in Africa compared to unexposed individuals in the USA [68]. This may be due to previous malaria exposure and red blood cell infection in adults in malaria-endemic areas [45,69]. In the Tanzania trial, all groups except infants (6 months–5 years), had increases in Pf-specific $CD8^+$ T cells after one dose. However, $CD8^+$ T cell responses increased in 1–5-year-olds only after three doses. Although $CD8^+$ T cell responses did not increase in adults further than the levels reached after one dose. The lower response in infants has been attributed to lower numbers of γδ T cells in infants [70]. The RAS vaccine in a Phase 2 clinical trial with infants (5–12 months) in western Kenya showed no vaccine efficacy after the 6-month trial endpoint despite high anti-PfCSP antibody responses. This is probably due to the absence of T cell responses, which was attributed to a low frequency of $V\gamma9V\delta2^+$ T cells in infants [71]. Another confounding factor of this study is that the vaccine immunizations continued into the malaria season and many infants in the trial had documented parasitemia. These results are suggestive of an importance for γδ T cells in the efficacy of RAS vaccination. In another clinical trial, RAS vaccination caused γδ T cell activation and expansion that was correlated with protection [64]. Increases in NKT and γδ T cells were also observed in malaria-exposed individuals from endemic areas of Africa [49]. Furthermore, γδ T cells have been associated with IFN-γ production and malaria protection in individuals in Papua New Guinea [72]. However, in mouse models γδ T cells are essential only in CD8 T cell induction and protection but not anti-CSP IgG [73]. Activation of γδ T cells led to $CD8\alpha^+$ DC accumulation in the liver and $CD8^+$ T cell expansion, further highlighting the importance of γδ T cells and $CD8^+$ T cells in the efficacy of RAS vaccination [74].

Despite these setbacks, whole-sporozoite vaccines have the potential to elicit broad immune responses with cellular and humoral immunity against multiple antigens. In particular, $CD8^+$ T cells induced by the RAS vaccine can target antigens conserved across strains, in contrast to antibodies towards polymorphic antigens, such as CSP [75,76]. However, there are discrepancies in the heterologous protection conferred by RAS between studies, as one study demonstrated a low efficacy of 10% at a 24-week time point compared to 70% in a homologous challenge setting [77]. Furthermore, Sanaria Inc. have tested multi-dose priming with the aim of enhancing the $CD8^+$ T cell response, administering four low doses (4.5×10^5) within 1 week to prolong the time that CD8 T cells get primed and boosting 16 weeks later. This regimen generated 40% efficacy against heterologous challenge compared to the standard 8-week, 3-dose regimen of 9×10^5 or 1.8×10^6 achieving 20% and 23%, respectively [78]. However, in a clinical trial in the malaria-endemic Equatorial Guinea, the multi-dose priming regimen did not reach significant vaccine efficacy [79]. Only the regimen with vaccine administration on days 1, 9 and 29 reached a vaccine efficacy of 40%. The focus towards vaccination regimens that enhance CD8 T cell responses may have improved efficacy compared to previous larger field trials, but the efficacy is much lower than the desired efficacy of 90%. Furthermore, previous blood-stage infection and low γδ T cell frequencies in infants are likely to impact the efficacy of this vaccine platform and represent significant issues that need to be overcome to

improve the translatability of whole-sporozoite vaccines.

Genetically attenuated parasite (GAP) vaccines utilize sporozoites that have been engineered to infect the liver but not replicate further into merozoites, therefore arresting the infection in the liver. The advantage of this approach is that all the relevant antigens are present, eliciting a broad immune response (humoral and cellular) to many antigens. This contrasts RAS vaccination, where only early liver-stage antigens are expressed, as parasites do not progress fully through this stage. Genetic attenuation can be achieved by knocking out one or more essential genes in the progression of the next life cycle stage, and when compared to radiation attenuation results in more viable sporozoites, infecting more hepatocytes and increasing the antigen load. The differences between these vaccines were exemplified when comparing deletion of genes that arrest sporozoite replication in the early or late stages of hepatocyte infection to RAS vaccination. Deletion of sporozoite asparagine-rich protein 1 (SAP1), which arrests the sporozoites before substantial replication was less effective than deletion of the *FabB/F* gene that plays an essential role in type II fatty acid biosynthesis in the apicoplast of the parasite during the late (schizont) stage. Both GAP vaccines were more effective than RAS vaccination after a single dose but the *fabb/f*$^{-}$ GAP was more effective than the *sap1*$^{-}$ GAP or RAS vaccination in a homologous boosting regime designed to provide CD8^{+} T cell-mediated immunity [80]. A triple knockout (Pf *p52*$^{-}$*/p36*$^{-}$*/sap1*$^{-}$) GAP vaccine could provide sterile protection after two doses in mice and was well tolerated in a Phase 1 clinical trial [81]. Another double knockout GAP vaccine has been through a Phase 1/2a clinical trial with positive safety and modest efficacy [82]. An analogous strategy is using rodent malaria (*P. berghei*), which is naturally attenuated, and genetic engineering to introduce the *PfCSP* gene [83]. These GAP vaccine trials all utilized intravenous administration of sporozoites and have demonstrated promising results in small clinical trials with malaria-naïve individuals, but the true test will be the Phase 2/2b trials in malaria-endemic areas. Overall, GAP vaccines are promising whole sporozoite vaccine strategies that have demonstrated modest efficacy at lower doses than the RAS vaccine. This is in part due to the greater parasite viability of sporozoites attenuated genetically compared to non-replicating radiation attenuated sporozoites. While there has been concerns of potential breakthrough infections from GAP vaccination, attenuation by knocking out multiple genes will likely reduce this risk and increase safety. Furthermore, development of GAP vaccines that arrest in the late phase of liver-stage infection, such as the *fabb/f*$^{-}$ GAP vaccine may generate CD8 T cells responses to early and late-stage antigens.

Finally, chemically attenuated sporozoites involve co-administration of an anti-malarial (such as chloroquine), which prevents blood-stage infection, but allows for liver-stage growth therefore eliciting a liver stage-specific immune response, including anti-CSP antibodies [84]. Chemoprophylactic sporozoite (CPS) immunizations, also called PfSPZ-CVac, have been tested using either chloroquine or pyrimethamine in a Phase 1/2a trial with malaria-naïve individuals, and were both found to provide sterile protection from CHMI three months after immunization. Chloroquine prophylaxis was more effective than pyrimethamine, as chloroquine kills the parasites at the blood-stage infection while the latter affects the liver-stage infection. The difference in the responses is likely due to longer viability and replication in the liver for chloroquine treatment than that observed with pyrimethamine treatment. PfSPZ-CVac immunization also expanded Vγ9Vδ2^{+} T cells and anti-PfCSP antibodies were higher in protected individuals. Although chloroquine was more effective at providing sterile immunity in malaria naïve individuals than pyrimethamine prophylaxis, it has a lower safety profile as blood-stage infection still occurs. However, why immunity does not arise in

individuals who have blood-stage infection and treatment with anti-malarials is still an open question. It is possible that the observed sterile protection from CPS immunization in CHMI trials with malaria-naïve individuals, may not translate to malaria-experienced individuals. In malaria-naïve individuals, PfSPZ-CVac (chloroquine) induced pro-inflammatory CD40L$^+$ CD4$^+$ T cells against iRBCs that may contribute to immunity [85]. In this study, CSP-specific antibodies peaked after two doses, with no increase after the third dose. Another trial demonstrated how blood-stage infection can reduce efficacy of PfSPZ-CVac (chloroquine) immunization [86]. Seven-day intervals between intravenous PfSPZ administration caused a blood-stage infection period while the subsequent dose was administered, which completely abrogated the efficacy. Shortening the period between doses to 5 days solved this issue, resulting in ~75% vaccine efficacy from CHMI challenge ten weeks after final immunization. The dosage of sporozoites is lower again in the chemically attenuated sporozoite vaccine compared to RAS and GAP vaccines. However, the risk of breakthrough infections is also increased, as demonstrated in these clinical trials.

One of the limitations of the attenuated sporozoite vaccines is that, to have efficacy, they need intravenous administration [64,65]. Another drawback of RAS vaccination, as well as other attenuated sporozoite-based vaccines, is their complex manufacturing requirements, particularly at scale. This is due to the need to extract sporozoites from the salivary glands of mosquitos and the attenuation process, which lowers the virulence of the parasite, and in turn necessitates many high doses of sporozoites (10^5,10^6 for intravenous or 10^6 for intramuscular administration), increasing the scalability difficulties [64,65,67,82]. Furthermore, storage is another logistical issue as maintenance of sporozoite viability requires cryopreservation in liquid nitrogen (–150°C to –196°C), which creates limitations for vaccine deployment. While previous malaria infection is believed to be the main reason for the decreased efficacy of the RAS vaccine in malaria-endemic areas, whether this effect will apply to GAP and CPS vaccines is not clear.

AN ARGUMENT FOR AIMING FOR CD8$^+$ T CELL IMMUNITY

While antibody-based vaccines against the CSP antigen have proven effective and the modest protection provided by the RTS vaccine has reached the level required for approval and initiation of mass vaccination programs, there are limits to the antibody-based vaccine approach. Firstly, as previously discussed, the blood-stage infection will affect most people by the time they reach 2 years of age in malaria-endemic areas, and impairs the CSP-specific B cell responses to vaccination in mice, a factor that may also be important in humans [45]. There may also be inherent limitations to targeting antibody-based antigens (i.e., CSP) due to the low number of B cell epitopes in the central repeat domain. One study identified a possible mechanism for poor recall responses and boosting of CSP-specific B cells that led to the plateau of antibody levels against the immunodominant central repeat domain B cell epitopes after two doses of the RAS vaccine [87]. In this study, the researchers found that the antibodies blocked memory B cell binding and activation towards these epitopes, and the level at which this blocking occurred was below the levels required for protection. This may explain the poor memory and waning anti-CSP antibodies observed in the field, requiring boosters every year. Lastly, the time at which antibodies can act against sporozoites before they reach the liver is within minutes to hours from exposure. However, CD8$^+$ T cells can target infected hepatocytes, providing a window of days to weeks for efficacy. Growing interest in vaccines utilizing T cell immunity, particularly CD8$^+$ T cell responses, has led to potential new vaccination strategies [88].

The liver-stage of infection is a major bottleneck of the parasite's lifecycle (low parasite burden and long incubation time, i.e., 7 days in humans, 2–3 days in mice) and with the ability of infected hepatocytes to present pathogen-derived antigens on MHC I, $CD8^+$ T cells have the potential to clear malaria infection before it reaches the blood. Memory $CD8^+$ T cells generated against the CSP_{252} epitope by epitope-loaded dendritic cells (DCs) or CSP_{252}-expressing *L. monocytogenes,* provided protection against *P. berghei* in mice [89]. However, complete sterile protection was only achieved by inducing large numbers of memory $CD8^+$ T cells (i.e., $>10^6$/spleen) using heterologous boosting with both vaccine strategies. Following evidence that RAS vaccination can produce memory $CD8^+$ T cells that protect mice from malaria [90–93], various research groups have been exploring vaccination strategies that can provide $CD8^+$ T cell-mediated protection from malaria. Viral vector vaccination strategies have been employed, encoding antigens (i.e., TRAP) as a transgene, to generate antibody and $CD8^+$ T cell responses. Because of the effect that anti-vector antibodies have on the efficacy of viral vectored vaccines, heterologous boosting with **ch**impanzee **ad**enovirus (ChAd63) and **m**odified **v**accinia **A**nkara (MVA) vectors were tested. However, in Phase 1/2a clinical trials, intramuscular vaccination provided sterile protection in only 21% of malaria-naïve individuals after three doses and a 2–3-week period before challenge [94]. A Phase 2 field trial with healthy adults (aged 18–50 years) in Kenya followed vaccinated patients for 8 weeks after vaccination and antimicrobe treatment, and saw 67% vaccine efficacy [95]. However, the CD8 T cell responses to TRAP measured by intracellular staining for cytokines showed almost no difference to the control group. A parallel study in Senegal demonstrated low vaccine efficacy and taken together with the Kenya study the vaccine efficacy was 50% [96]. The results were affected by an unusually short malaria season, which resulted in the incidence of malaria being higher before the trial started, hampering assessment of vaccine efficacy. Another drawback is that antibodies generated against the MVA vector have detrimental effects on any additional boosts with the MVA vaccine. The virus needs to infect cells to express the transgene and elicit an immune response; however, neutralizing antibodies will inhibit this process.

A subset of $CD8^+$ liver-**r**esident **m**emory **T** (liver Trm) cells, are very adept at killing sporozoite-infected cells and preventing blood-stage infection [97]. As such, these T cells have become a focus in development of malaria vaccines. Trm cells are a specialized subset of memory T cells that reside permanently in peripheral tissues (i.e., liver, lung) and patrol these tissues for pathogens. Their location and behavior contrast with other memory T cell subsets, such as effector memory T cells or central memory T cells, which remain in the circulation of the lymphatic and vascular systems. The efficacy of the RAS vaccine is dependent on $CD8^+$ liver Trm cells, making them ideal targets for vaccination strategies that aim to provide protection against liver stage parasites [97,98]. However, the generation of liver $CD8^+$ Trm cells requires complicated vaccination strategies, which both promote inflammation and target antigen expression to the liver [99]. This includes the 'prime and trap' vaccination strategy, which primes CD8 T cell with a CD8 epitope fused to an anti-Clec9A antibody to target DCs. This construct is injected with CpG (TLR9 agonist) as an adjuvant to activate DCs and generate inflammation. This process is followed by the 'trap', which is a liver-trophic recombinant adeno-associated virus that drives expression of the same CD8 epitope used in the Clec9A-targetted priming strategy, in hepatocytes [97,100]. This complex vaccination system provided the proof-of-principle requirements for effective liver Trm cell generation, being effective priming in the spleen, and then inflammation and antigen in the liver. Subsequent work from our team and collaborators lead to

the development of glycolipid-peptide conjugate vaccines, which include a glycolipid NKT cell agonist and a $CD8^+$ T cell peptide epitope covalently attached via an immolative linker [101]. Testing of these vaccines revealed that the three requirements for liver Trm cell formation discussed above could be delivered in a single injection by utilizing the natural adjuvant properties of NKT cells to generate liver Trm cells that were capable and sufficient for providing sterile protection in mice [101]. NKT cells feature an invariant T cell receptor and make up a large proportion of T cells when compared to conventional $CD4^+$ or $CD8^+$ αβ T cells, particularly in the liver. When activated, they produce cytokines that promote liver inflammation, making their adjuvant properties ideal for liver Trm cell-based vaccines. Our studies showed that in addition to generating large numbers of liver Trm cells, glycolipid-peptide vaccines generated liver Trm cells with a half-life of approximately 14 months, compared to a half-life of 1 month for Trm cells generated via RAS vaccination [101]. One drawback for this peptide-based approach is that they are not directly translatable to humans due to HLA heterogeneity, which drives a need for multiple peptide epitopes to be included in order to target a single protein antigen.

As a further development, we recently showed that mRNA vaccines complexed with a cationic liposome delivery system, termed lipoplexes, with a glycolipid NKT cell adjuvant, also promoted liver Trm generation [102]. The major advantage of using this mRNA approach is the ability to encode the entire protein antigen rather than minimal peptide epitopes. Furthermore, antigens that are difficult to express and purify for recombinant protein subunit vaccines, can easily be encoded in mRNA, and administered for the immune system to translate and process for CD8 T cell activation. This unlocks new possibilities in the range of antigens that can be targeted from the *Plasmodium* liver-stage infection to fully harness CD8 liver Trm cells. Additionally, we showed that inclusion of glycolipids with 6''-modifications were superior to αGalCer as liver Trm-generating adjuvants, and these vaccine formulations provided 80% protection from a single vaccine dose. An additional, potentially significant advantage of this mRNA platform was that the liver Trm cell response was unaffected by previous blood-stage infection, when compared to the response observed in previously blood-stage exposed and then RAS vaccinated mice. Nakamae and colleagues also reported an mRNA-LNP against the CSP antigen that generated CSP-specific liver Trm cells and provided sterile protection in 50% of mice following two doses [103]. Despite the removal of the NANP repeat domain of CSP, the activity of anti-CSP IgG cannot be ruled out, as the protection only had partial CD8 T cell dependency. Overall, mRNA vaccines are a promising new strategy for vaccines against malaria in their ability to generate humoral and cellular immunity.

These studies focusing on liver Trm cell generating vaccines have utilized model CD8 T cell antigens such as ovalbumin, CSP and the recently described liver-stage antigen, ribosomal protein L6 (RPL6) [104]. This antigen is highly conserved among different *P. falciparum* strains, in comparison to TRAP, which is highly polymorphic. RPL6 and other epitopes have been identified via epitope-first approaches, which utilized TCR sequencing of CD8 T cells that respond to whole sporozoite vaccination or through mass spec of peptides eluted from MHC I and the use of bioinformatic tools to find predicted epitopes within *Plasmodium* genes expressed during liver-stage infection [105,106]. An antigen-first approach focused on conserved proteins that are expressed and exported during liver stage infection [107]. This includes the sporozoite, liver stage tryptophan-rich protein (SLTRiP) antigen, a highly conserved and immunogenic antigen that can provide protection from sporozoite challenge following peptide vaccination of defined murine epitopes [108].

Now with two clinically approved vaccines for malaria in the last 5 years, there is room

for improvement in efficacy and longevity of the immunity provided by these vaccines. Liver Trm cells provide a promising new approach for the development of vaccines for malaria, which may solve many of the issues with existing vaccines. Furthermore, the longer incubation period of liver-stage infection in human livers (≥7 days) compared to mice (2–3 days) may mean that current studies have underrepresented the potential of liver Trm cells, as longer incubation periods will allow more time for liver Trm cells to kill infected hepatocytes in humans and/or recruit other CD8 T cell subsets (i.e., effector memory T cells) to aid in clearing infection [109]. However, to realize the potential of liver Trm cells in malaria vaccine development, there is a need for the identification of new highly conserved liver-stage antigens that can be used in the next generation of malaria vaccines [104]. With the rapid advancement of new vaccine technologies, the next clinically approved vaccine for malaria may include one of these new vaccine strategies, such as genetic or chemical attenuated sporozoites, viral vector, or mRNA vaccines.

REFERENCES

1. Kappe SHI, Buscaglia CA, Bergman LW, Coppens I, Nussenzweig V. Apicomplexan gliding motility and host cell invasion: overhauling the motor model. *Trends Parasitol.* 2004; 20, 13–16.

2. Singh B, Sung LK, Matusop A, *et al.* A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet* 2004; 363, 1017–1024.

3. Bhatt S, Weiss DJ, Cameron E, *et al.* The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 2015; 526, 207–211.

4. WHO. *World Malaria Report 2023*. 2023; World Health Organisation.

5. Charnaud SC, McGready R, Herten-Crabb A, *et al.* Maternal-foetal transfer of *Plasmodium falciparum* and Plasmodium vivax antibodies in a low transmission setting. *Sci. Rep.* 2016; 6, 20859.

6. Fried M, Nosten F, Brockman A, Brabin BT, Duffy PE. Maternal antibodies block malaria. *Nature* 1998; 395, 851–852.

7. Reynaldi A, Dent AE, Schlub TE, *et al.* Interaction between maternally derived antibodies and heterogeneity in exposure combined to determine time-to-first *Plasmodium falciparum* infection in Kenyan infants. *Malar. J.* 2019; 18(1), 19.

8. Dobbs KR, Dent AE. *Plasmodium malaria* and antimalarial antibodies in the first year of life. *Parasitology* 2016; 143, 129–138.

9. Josling GA, Llinas M. Sexual development in *Plasmodium* parasites: knowing when it's time to commit. *Nat. Rev. Microbiol.* 2015; 13, 573–587.

10. Sinden RE. The cell biology of sexual development in *Plasmodium*. *Parasitology* 1983; 86, 7–28.

11. Sinden RE. Plasmodium differentiation in the mosquito. *Parassitologia 1999*; 41, 139–148.

12. Rosenberg R, Wirtz RA, Schneider I, Burge R. An estimation of the number of malaria sporozoites ejected by a feeding mosquito. *Trans. R. Soc. Trop. Med. Hyg.* 1990; 84, 209–212.

13. Amino R, Thiberge S, Martin B, *et al.* Quantitative imaging of *Plasmodium* transmission from mosquito to mammal. *Nat. Med.* 2006; 12, 220–224.

14. Shin SCJ, Vanderberg JP, Terzakis JA. Direct infection of hepatocytes by sporozoites of *Plasmodium berghei*. *J. Protozool.* 1982; 29, 448–454.

15. White NJ. Determinants of relapse periodicity in *Plasmodium vivax* malaria. *Malar. J.* 2011; 10, 297.

16. Prudencio M, Rodriguez A, Mota MM. The silent path to thousands of merozoites: the *Plasmodium* liver stage. *Nat. Rev. Microbiol.* 2006; 4, 849–856.

17. Paul AS, Egan ES, Duraisingh MT. Host-parasite interactions that guide red blood cell invasion by malaria parasites. *Curr. Opin. Hematol.* 2015; 22, 220–226.

18. Wunderlich J, Rohrbach P, Dalton JP. The malaria digestive vacuole. *Front. Biosci.* 2012; 4, 1424–1448.

19. Karunaweera ND, Grau GE, Gamage P, Carter R, Mendis KN. Dynamics of fever and serum levels of tumor necrosis factor are closely associated during clinical paroxysms in *Plasmodium vivax* malaria. *Proc. Natl Acad. Sci. USA* 1992; 89, 3200–3203.

20. Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. *Nature* 2002; 415, 673–679.

21. Totino PRR, Magalhaes AD, Silva LA, *et al.* Apoptosis of non-parasitized red blood cells in malaria: a putative mechanism involved in the pathogenesis of anaemia. *Malar. J.* 2010; 9, 350.

22. Vedovato M, De Paoli Vitali E, Dapporto M, Salvatorelli G. Defective erythropoietin production in the anaemia of malaria. *Nephrol. Dial. Transplant.* 1999; 14, 1043–1044.

23. White NJ. Anaemia and malaria. *Malar. J.* 2018; 17, 371.

24. Taylor TE, Borgstein A, Molyneux ME. Acid-base status in pediatric *Plasmodium-falciparum* malaria. *Q. J. Med.* 1993; 86, 99–109.

25. Smith JD. The role of PfEMP1 adhesion domain classification in Plasmodium falciparum pathogenesis research. *Mol. Biochem. Parasitol.* 2014; 195, 82–8.

26. Smith JD. The role of PfEMP1 adhesion domain classification in Plasmodium falciparum pathogenesis research. *Mol. Biochem. Parasitol.* 2014; 195, 82–87.

27. MacPherson GG, Warrell MJ, White NJ, Looareesuwan S, Warrell DA. Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. *Am. J. Pathol.* 1985; 119, 385–401.

28. Idro R, Marsh K, John CC, Newton CRJ. Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome. *Pediatr. Res.* 2010; 68, 267–274.

29. Ponsford MJ, Medana IM, Prapanslip P, *et al.* Sequestration and microvascular congestion are associated with coma in human cerebral malaria. *J. Infect. Dis.* 2012; 205, 663–671.

30. Liehl P, Zuzarte-Luis V, Chan J, *et al.* Host-cell sensors for Plasmodium activate innate immunity against liver-stage infection. *Nat. Med.* 2014; 20, 47–53.

31. Pied S, Roland J, Louise A, *et al.* Liver CD4(-) CD8(-) NK1.1(+) TCR alpha beta intermediate cells increase during experimental malaria infection and are able to exhibit inhibitory activity against the parasite liver stage *in vitro*. *J. Immunol.* 2000; 164, 1463–1469.

32. Miller JL, Sack BK, Baldwin M, Vaughan AM, Kappe SHI. Interferon-mediated innate immune responses against malaria parasite liver stages. *Cell Rep.* 2014; 7, 436–447.

33. McKenna KC, Tsuji M, Sarzotti M, *et al.* Gamma delta T cells are a component of early immunity against preerythrocytic malaria parasites. *Infect. Immun.* 2000; 68, 2224–2230.

34. Tsuji M, Mombaerts P, Lefrancois L, *et al.* Gamma delta T cells contribute to immunity against the liver stages of malaria in alpha beta T-cell-deficient mice. *Proc. Natl Acad. Sci. USA* 1994; 91, 345–349.

35. Tsuji M, Eyster CL, O'Brien RL, *et al.* Phenotypic and functional properties of murine gamma delta T cell clones derived from malaria immunized, alpha beta T cell-deficient mice. *Int. Immunol.* 1996; 8, 359–366.

36. Junqueira C, Polidoro RB, Castro G, *et al.* gamma delta T cells suppress *Plasmodium falciparum* blood-stage infection by direct killing and phagocytosis. *Nat. Immunol.* 2021; 22, 347–357.

37. Cha SJ, Park K, Srinivasan P, *et al.* CD68 acts as a major gateway for malaria sporozoite liver infection. *J. Exp. Med.* 2015; 212, 1391–1403.

38. Tweedell RE, Qi L, Sun Z, Dinglasan RR. Kupffer cells survive *Plasmodium berghei* sporozoite exposure and respond with a rapid cytokine release. *Pathogens* 2018; 7, 91.

39. Klotz, C, Frevert, U. *Plasmodium yoelii* sporozoites modulate cytokine profile and induce apoptosis in murine Kupffer cells. *Int. J. Parasitol.* 2008; 38, 1639–1650.

40. Steers N, Schwenk R, Bacon DJ, *et al.* The immune status of Kupffer cells profoundly influences their responses to infectious *Plasmodium berghei* sporozoites. *Eur. J. Immunol.* 2005; 35, 2335–2346.

41. Zheng H, Tan ZP, Xu WY. Immune evasion strategies of pre-erythrocytic malaria parasites. *Mediators Inflamm.* 2014; 2014, 362605.

42. Winkel BMF, Pelgrom LR, van Schuijlenburg R, *et al. Plasmodium sporozoites* induce regulatory macrophages. *PLoS Pathog.* 2020; 16, e1008799.

43. Wipasa, J, Suphavilai C, Okell LC, *et al.* Long-lived antibody and B cell memory responses to the human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*. *PLoS Pathog.* 2010; 6, e1000770.

44. Kurtovic L, Behet MC, Feng G, *et al.* Human antibodies activate complement against Plasmodium falciparum sporozoites, and are associated with protection against malaria in children. *BMC Medicine* 2018; 16, 61.

45. Keitany GJ, Kim KS, Krishnamurty AT, *et al.* Blood stage malaria disrupts humoral immunity to the pre-erythrocytic stage circumsporozoite protein. *Cell Rep.* 2016; 17, 3193–3205.

46. Seaton KE, Spreng RL, Abraha M, *et al.* Subclass and avidity of circumsporozoite protein specific antibodies associate with protection status against malaria infection. *NPJ Vaccines* 2021; 6(1), 110.

47. Moncunill G, *et al.* Antigen-stimulated PBMC transcriptional protective signatures for malaria immunization. *Sci. Transl. Med.* 2020; 12(543), eaay8924.

48. Tran TM, Guha R, Portugal S, *et al.* A molecular signature in blood reveals a role for p53 in regulating malaria-induced inflammation. *Immunity* 2019; 51, 750–765.

49. de Jong SE, van Unen V, Manurung MD, *et al.* Systems analysis and controlled malaria infection in Europeans and Africans elucidate naturally acquired immunity. *Nat. Immunol.* 2021; 22, 654–665.

50. Menard R. The journey of the malaria sporozoite through its hosts: two parasite proteins lead the way. *Microbes Infect.* 2000; 2, 633–642.

51. Coppi A, Tewari R, Bishop JR, *et al.* Heparan sulfate proteoglycans provide a signal to *Plasmodium sporozoites* to stop migrating and productively invade host cells. *Cell Host Microbe* 2007; 2, 316–327.

52. Espinosa DA, Gutierrez GM, *et al.* Proteolytic cleavage of the *Plasmodium falciparum* circumsporozoite protein is a target of protective antibodies. *J. Infect. Dis.* 2015; 212, 1111–1119.

53. Thai E, Costa G, Weyrich A, *et al.* A high-affinity antibody against the CSP N-terminal domain lacks *Plasmodium falciparum* inhibitory activity. *J. Exp. Med.* 2020; 217, e20200061.

54. Julien JP, Wardemann H. Antibodies against *Plasmodium falciparum* malaria at the molecular level. *Nat. Rev. Immunol.* 2019; 19, 761–775.

55. Nahrendorf W, Scholzen A, Bojker EM, *et al.* Memory B-cell and antibody responses induced by *Plasmodium falciparum* sporozoite immunization. *J. Infect. Dis.* 2014; 210, 1981–1990.

56. Agnandji ST, Lell B, Soulanoudijingar SS, *et al.* First results of Phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N. Eng. J. Med.* 2011; 365, 1863–1875.

57. Asante KP, Abdulla S, Agnandji S, *et al.* Safety and efficacy of the RTS,S/AS01(E) candidate malaria vaccine given with expanded-programme-on-immunisation vaccines: 19 month follow-up of a randomised, open-label, Phase 2 trial. *Lancet Infect. Dis.* 2011; 11, 741–749.

58. Mian-McCarthy S, Vansadia P, Silman M, *et al.* A Phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N. Eng. J. Med.* 2012; 367, 2284–2295.

59. Tinto H. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a Phase 3, individually randomised, controlled trial. *Lancet* 2015; 386, 31–45.

60. Moris P, Jongert E, van der Most RG. Characterization of T-cell immune responses in clinical trials of the candidate RTS,S malaria vaccine. *Hum. Vaccin. Immunother.* 2018; 14, 17–27.

61. Reece WHH, Pinder M, Gothard PK, *et al.* A CD4(+) T-cell immune response to a conserved epitope in the circumsporozoite protein correlates with protection from natural *Plasmodium falciparum* infection and disease. *Nat. Med.* 2004; 10, 406–410.

62. Datoo MS, Natama MH, Some A, *et al.* Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso: a randomised controlled trial. *Lancet* 2021; 397, 1809–1818.

63. Datoo MS, Dicko A, Tinto H, *et al.* Safety and efficacy of malaria vaccine candidate R21/Matrix-M in African children: a multicentre, double-blind, randomised, Phase 3 trial. *Lancet* 2024; 403, 533–544.

64. Seder RA, Chang LJ, Enama ME, *et al.* Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* 2013; 341, 1359–1365.

65. Ishizuka AS, Lyke KE, DeZure A, *et al.* Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. *Nat. Med.* 2016; 22, 692–692.

66. Sissoko MS, Healy SA, Katile A, *et al.* Safety and efficacy of PfSPZ vaccine against *Plasmodium falciparum* via direct venous inoculation in healthy malaria-exposed adults in Mali: a randomised, double-blind phase 1 trial. *Lancet Infect. Dis.* 2017; 17, 498–509.

67. Jongo SA, Preston Church LW, Mtoro AT, *et al.* Safety and differential antibody and T-cell responses to the *Plasmodium falciparum* sporozoite malaria vaccine, PfSPZ vaccine, by age in Tanzanian adults, adolescents, children,

and infants. *Am. J. Trop. Med. Hyg.* 2019; 100, 1433–1444.

68. Olotu A, Urbano V, Hamad A, *et al.* Advancing global health through development and clinical trials partnerships: a randomized, placebo-controlled, double-blind assessment of safety, tolerability, and immunogenicity of PfSPZ vaccine for malaria in healthy Equatoguinean men. *Am. J. Trop. Med. Hyg.* 2018; 98, 308–318.

69. Ocaña-Morgner C, Mota MM, Rodriguez A. Malaria blood stage suppression of liver stage immunity by dendritic cells. *J. Exp. Med.* 2003; 197, 143–151.

70. Zaidi I, Duffy PE. PfSPZ vaccine learns a lesson. *Med.* 2021; 2, 1289–1291.

71. Oneko M, Steinhardt LC, Yego R, *et al.* Safety, immunogenicity and efficacy of PfSPZ vaccine against malaria in infants in western Kenya: a double-blind, randomized, placebo-controlled Phase 2 trial. *Nat. Med.* 2021; 27, 1636–1645.

72. D'Ombrain MC, Robinson LJ, Stanisic DI, *et al.* Association of early interferon-gamma production with immunity to clinical malaria: a longitudinal study among Papua New Guinean children. *Clin. Infect. Dis.* 2008; 47, 1380–1387.

73. Zaidi I, Diallo H, Conteh S, *et al.* Gamma delta T cells are required for the induction of sterile immunity during irradiated sporozoite vaccinations. *J. Immunol.* 2017; 199, 3781–3788.

74. Montagna GN, Biswas A, Hildner K, Matuschewski K, Dunay IR. Batf3 deficiency proves the pivotal role of CD8α+ dendritic cells in protection induced by vaccination with attenuated *Plasmodium* sporozoites. *Parasite Immunol.* 2015; 37, 533–543.

75. Lyke KE, Ishizuka AS, Berry AA, *et al.* Attenuated PfSPZ vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. *Proc. Natl Acad. Sci. USA* 2017; 114, 2711–2716.

76. Mordmüller B, Sulyok Z, Sulyok M, *et al.* A PfSPZ vaccine immunization regimen equally protective against homologous and heterologous controlled human malaria infection. *NPJ Vaccines* 2022; 7(1). 100.

77. Epstein JE, Paolino KM, Richie TL, *et al.* Protection against *Plasmodium falciparum* malaria by PfSPZ vaccine. *JCI Insight* 2017; 2(1), e89154.

78. Lyke KE, Singer A, Berry AA, *et al.* Multidose priming and delayed boosting improve plasmodium falciparum sporozoite vaccine efficacy against heterologous *P. falciparum* controlled human malaria infection. *Clin. Infect. Dis.* 2020; 73, e2424–e2435.

79. Jongo SA, Preston Church LW, Nchama VUNN, *et al.* Multi-dose priming regimens of PfSPZ vaccine: safety and efficacy against controlled human malaria infection in Equatoguinean adults. *Am. J. Trop. Med. Hyg.* 2022; 106, 1215–1226.

80. ButlerNS, Schmidt NW, Vaughan AM, *et al.* Superior antimalarial immunity after vaccination with late liver stage-arresting genetically attenuated parasites. *Cell Host Microbe* 2011; 9, 451–462.

81. Kublin JG, Mikolajczak SA, Sack BK, *et al.* Complete attenuation of genetically engineered *Plasmodium falciparum* sporozoites in human subjects. *Sci. Transl. Med.* 2017; 9(371), eaad9099.

82. Roestenberg M, Walk J, van der Boor SC, *et al.* A double-blind, placebo-controlled Phase 1/2a trial of the genetically attenuated malaria vaccine PfSPZ-GA1. *Sci. Transl. Med.* 2020; 12, eaaz5629.

83. Reuling, IJ, Mendes AM, de Jong GM, *et al.* An open-label Phase 1/2a trial of a genetically modified rodent malaria parasite for

immunization against *Plasmodium falciparum* malaria. *Sci. Transl. Med*. 2020; 12, eaay2578.

84. Behet MC, Foquet L, van Gemert GJ, *et al.* Sporozoite immunization of human volunteers under chemoprophylaxis induces functional antibodies against pre-erythrocytic stages of *Plasmodium falciparum*. *Malar. J.* 2014; 13, 136.

85. Ibanez J, Fendel R, Lorenz FR, *et al.* Efficacy, T cell activation and antibody responses in accelerated *Plasmodium falciparum* sporozoite chemoprophylaxis vaccine regimens. *NPJ Vaccines* 2022; 7(1), 59.

86. Murphy SC, Deye GA, Sim BKL, *et al.* PfSPZ-CVac efficacy against malaria increases from 0% to 75% when administered in the absence of erythrocyte stage parasitemia: A randomized, placebo-controlled trial with controlled human malaria infection. *Plos Pathogens* 2021; 17, e1009594.

87. McNamara HA, Idris AH, Sutton HJ, *et al.* Antibody feedback limits the expansion of B cell responses to malaria vaccination but drives diversification of the humoral response. *Cell Host Microbe* 2020; 28, 572–585.

88. Kurup SP, Butler NS, Harty JT. T cell-mediated immunity to malaria. *Nat. Rev. Immunol.* 2019; 19, 457–471.

89. Schmidt NW, Podyminogin RL, Butler NS, *et al.* Memory CD8 T cell responses exceeding a large but definable threshold provide long-term immunity to malaria. *Proc. Natl Acad. Sci. USA* 2008; 105, 14017–14022.

90. Van Braeckel-Budimir N, Harty JT. CD8 T-cell-mediated protection against liver-stage malaria: lessons from a mouse model. *Front. Microbiol.* 2014; 5, 272.

91. Epstein JE, Tewari K, Lyke KE, *et al.* Live attenuated malaria vaccine designed to protect through hepatic CD8(+) T cell immunity. *Science* 2011; 334, 475–480.

92. Schofield L, Villaquiran J, Ferreira A, *et al.* γ interferon, CD8+ T cells and antibodies required for immunity to malaria sporozoites. *Nature* 1987; 330, 664–666.

93. Weiss WR, Sedegah M, Beaudoin RL, Miller LH, Good MF. CD8+ T cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. *Proc. Natl Acad. Sci. USA* 1988; 85, 573–576.

94. Ewer KJ, O'Hara GA, Duncan CJA, *et al.* Protective CD8(+) T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. *Nat. Commun.* 2013; 4, 2836.

95. Ogwang C. Prime-boost vaccination with chimpanzee adenovirus and modified vaccinia Ankara encoding TRAP provides partial protection against *Plasmodium falciparum* infection in Kenyan adults. *Sci. Transl. Med.* 2015; 7, 286re5.

96. Mensah VA, Gueye A, Ndiaye M, *et al.* Safety, Immunogenicity and efficacy of prime-boost vaccination with ChAd63 and MVA encoding ME-TRAP against *Plasmodium falciparum* infection in adults in Senegal. *PLoS One* 2016; 11, e0167951.

97. Fernandez-Ruiz D, Ng WY, Holz LE, *et al.* Liver-resident memory CD8(+) T cells form a front-line defense against malaria liver-stage infection. *Immunity* 2016; 45, 889–902.

98. Fernandez-Ruiz D, de Menezes MN, Holz LE, *et al.* Harnessing liver-resident memory T cells for protection against malaria. *Expert Rev. Vaccines* 2021; 20, 127–141.

99. Holz LE, Prier JE, Freestone D, *et al.* CD8(+) T cell activation leads to constitutive formation of liver tissue-resident memory T cells that seed a large and flexible niche in the liver. *Cell Rep.* 2018; 25, 68–79.

100. Fernandez-Ruiz D, *et al.* Liver-resident memory T cells can be harnessed for unprecedented

protection against malaria. *Am. J. Trop. Med. Hyg.* 2017; 97, 328–328.

101. Holz LE, Chua YC, de Menezes MN, *et al.* Glycolipid-peptide vaccination induces liver-resident memory CD8(+) T cells that protect against rodent malaria. *Sci. Immunol.* 2020; 5, eaaz8035.

102. Ganley M, Holz LE, Minnell JJ, *et al.* mRNA vaccine against malaria tailored for liver-resident memory T cells. *Nat. Immunol.* 2023; 24, 1487–1498.

103. Nakamae S, Miyagawa S, Ogawa K, *et al.* Induction of liver-resident memory T cells and protection at liver-stage malaria by mRNA-containing lipid nanoparticles. *Front. Immunol.* 2023; 14, 1116299.

104. Valencia-Hernandez AM, Ng WY, Ghazanfari N, *et al.* A natural peptide antigen within the plasmodium ribosomal protein RPL6 confers liver T-RM cell-mediated immunity against malaria in mice. *Cell Host Microbe* 2020; 27, 950–962.

105. Hafalla JC, Bauza K, Friesen J, *et al.* Identification of targets of CD8 T cell responses to malaria liver stages by genome-wide epitope profiling. *PLoS Pathog.* 2013; 9, e1003303.

106. Speake C, Pichugin A, Sahu T, *et al.* Identification of novel pre-erythrocytic malaria antigen candidates for combination vaccines with circumsporozoite protein. *PLoS One* 2016; 11, e0159449.

107. Jaijyan DK, Singh H, Singh AP. A sporozoite- and liver stage-expressed tryptophan-rich protein plays an auxiliary role in plasmodium liver stage development and is a potential vaccine candidate. *J. Biol. Chem.* 2015; 290, 19496–19511.

108. Quadiri, A, Kalia I, Kashif M, Singh AP. Identification and characterization of protective CD8(+) T-epitopes in a malaria vaccine candidate SLTRiP. *Immun. Inflamm. Dis.* 2020; 8, 50–61.

109. Heath WR, Holz LE, Fernandez-Ruiz D. The Batman and Robin of liver-stage immunity to malaria. *Trends Parasitol.* 2022; 38, 7–8.

AFFILIATIONS

Mitch Ganley
Department of Microbiology and Immunology,
The Doherty Institute for Infection and Immunity,
University of Melbourne,
Melbourne, Victoria, Australia

William Heath
Department of Microbiology and Immunology,
The Doherty Institute for Infection and Immunity,
University of Melbourne,
Melbourne, Victoria, Australia

Lynette Beattie
Department of Microbiology and Immunology,
The Doherty Institute for Infection and Immunity,
University of Melbourne,
Melbourne, Victoria, Australia

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Mitch Ganley and William Heath hold a patent: WO2023121483A1—Immunostimulatory Compounds.

Funding declaration: Mitch Ganley has received funding from the NZ Ministry of Business, Innovation and Employment (MBIE).

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; externally peer reviewed.

Submitted for peer review: May 23, 2024; **Revised manuscript received:** Jul 23, 2024;
Publication date: Aug 27, 2024.

VACCINE INSIGHTS

PRECLINICAL AND CLINICAL RESEARCH

SPOTLIGHT

INTERVIEW

Enhancing vaccine trials across Europe and beyond

Charlotte Barker, Commissioning Editor, *Vaccine Insights*, speaks with **Pierre Van Damme**, Professor of Vaccinology and Infectious Diseases, University of Antwerp, about challenges and strategies for streamlining vaccine trials, the role of controlled human infection models, and the importance of medical education in combatting vaccine hesitancy.

Vaccine Insights 2024; 3(5), 171–179

DOI: 10.18609/vac.2024.029

Q How did you first get involved in the vaccine field and how have your interests evolved?

PVD: My interest in preventing infectious diseases started when, as a young general practitioner in Antwerp, I began to encounter patients with HIV/AIDS. At that time, medical and therapeutic options were very limited and unfortunately I witnessed the deaths of many young homosexual men. I became interested in population health and embarked on a PhD in the prevention and transmission of hepatitis B within residents in mental institutions.

At that time, GSK had developed the first hepatitis B recombinant DNA vaccine and approached me to set up a study involving the institutionalized population. Around the same

time, GSK also approached my colleague Peter Piot to conduct a similar study with a cohort of men who have sex with men in Antwerp. These two pivotal studies demonstrated that the first recombinant DNA hepatitis B vaccine was safe, immunogenic, and protective. This experience further deepened my interest in vaccines, infectious disease prevention, and public health.

Following my PhD studies, I established the Center for Evaluation of Vaccination at the University of Antwerp. Together with a team of nurses, doctors, project managers, and coordinators, we began assessing a range of vaccines, including those targeting hepatitis A, HPV, and polio. I later became a Professor of Epidemiology of Infectious Diseases and Vaccinology, teaching the current and future generations of healthcare providers.

Q How much are healthcare providers typically taught about vaccines?

PVD: Unfortunately, vaccines and infectious disease prevention are treated as secondary in the academic curriculum at many European universities' medical schools. This approach mirrors spending on infectious disease prevention in national healthcare budgets, often receiving less than 3% of the total budget. The more funding is invested in disease prevention, the higher the return on investment will be in 10–20 years, but our politicians, hospital doctors, and deans of faculties need to pay more attention to infectious disease prevention and vaccinology in medical education, and to prevention in general. For example, we recently published the results of a survey we conducted across several countries involving various professional and student groups, termed the Vaccine Training Barometer [1]. We found that pharmacists, general practitioners, other healthcare providers, and students reported receiving little to no training in vaccinology. As a result, many healthcare providers feel unprepared to answer questions from patients or their caregivers, which can only exacerbate vaccine hesitancy in our society. This problem needs to be addressed because better education could make a huge difference: this should be reflected not only in medical educational programs but also in pharmacy, nursing, and midwifery curricula.

Q What projects are you excited about right now?

PVD: Continuing the vaccine education theme, we have been working on a new initiative with the WHO's European Office and colleagues at University of Antwerp to design a game called 'Immune Patrol'. This digital game for children aged 10–12 years includes components for both teachers and pupils. Modules cover topics in infectious diseases, such as how they spread, population or herd immunity, what vaccines are and how they work, and how to validate information. The game is now being used in several countries and has been translated into multiple languages. Currently, we are piloting 'Immune Patrol' in French, Dutch, and German-speaking schools in Belgium. By the end of September, we expect to see the results of

"...the model for the future is to identify and bring together expertise, both within and outside the country, rather than duplicate each other's efforts."

this trial, and our goal is to present these findings to the Minister of Education and propose implementing this program in all schools nationwide.

Secondly, a significant part of my work is dedicated to improving and accelerating the conduct of vaccine trials, both within our country and internationally. We are seeing a range of novel vaccines emerging from various pipelines, and it is crucial to understand what is in development and how to address these vaccines, so they can be registered rapidly. This is particularly important for preparing for future pandemics, especially if we can accelerate and simplify the whole process.

I believe the model for the future is to identify and bring together expertise, both within and outside the country, rather than duplicate each other's efforts. This is one of the key lessons from the COVID-19 pandemic—in order to succeed, we must foster collaboration, which includes combining regional, national, and European/global expertise and nurturing extensive communication, trust, and transparency.

This approach has been put into practice in a project called MusiCC, which involves collaboration of Belgian teams with the UK, USA, Singapore, and the Netherlands. MusiCC is a CEPI/Health Emergency Preparedness and Response Authority consortium focused on mucosal immunity in human coronavirus infections. The goal of this project is to support the development of the next-generation nasal or inhalation COVID-19 vaccines, specifically by examining how vaccines can block transmission via mucosal immunity.

We will use controlled human infection models (CHIMs), leveraging facilities in the UK, USA, Belgium, the Netherlands, and Singapore. The project will operate in parallel across these locations to increase the capacity and accelerate assessments. We will vaccinate a population with these vaccine candidates and also include a placebo group. Both groups will then be exposed to a challenge agent to determine if the vaccine not only provides protection against disease but also interferes with the transmission. This approach aims to control upper respiratory tract infections on a larger scale, which will hopefully have a major impact on future pandemics. For example, the next generation of vaccine candidates for flu and RSV will hopefully focus not just on individual protection but also on preventing transmission in communities.

Finally, we have our clinical trial facility, Vaccinopolis, supported by both the Belgian and Flemish governments. This relatively new campus facility allows us to conduct vaccine trials from Phase 1 through Phase 4, as well as CHIM studies. During the pandemic, we also received support to collaborate with the Université Libre de Bruxelles and Arnaud Marchant to establish the virtual European Plotkin Institute for Vaccinology that connects two research teams from different universities. While we handle the conduct of trials, the other research team focuses on immunoassays and other experimental immunological studies in their laboratory.

"Researchers who are accustomed to conducting therapeutic trials may assume they can easily transition to vaccine trials, but vaccine trials require specialized laboratories to analyze immunological readouts."

Q The VACCELERATE Consortium held a workshop last year on adaptive platform trial methodology—how could this concept be implemented for vaccine clinical trials going forward?

PVD: During the VACCELERATE Consortium workshop [2], we aimed to unite expertise in vaccine trial sites and vaccine assessment. Coordinating this during the COVID-19 pandemic was challenging, but we learned many lessons from this project and discussed several key challenges.

Firstly, vaccine trials are very different from therapeutic trials. Researchers who are accustomed to conducting therapeutic trials may assume they can easily transition to vaccine trials, but vaccine trials require specialized laboratories to analyze immunological readouts. This necessitates standardization, harmonization, and validation over different laboratories, which is challenging, but so needed.

Secondly, the study participants of vaccine trials are mostly healthy volunteers, and would ideally be immunologically naïve, which is especially challenging for widespread conditions such as COVID-19.

Another key discussion point was how to accelerate the conduct of vaccine trials by utilizing adaptive design, common in therapeutic trials. Adaptive design allows for the activation or design of multiple arms within a protocol. These arms are approved in advance, enabling informed decisions about whether to continue certain arms, adjust sample sizes, add new trial arms, or include specific populations later in the vaccine trial. For example, the elderly and pregnant women are often excluded from vaccine trials. Utilizing adaptive design allows for adding new trial arms for these specific groups if initial Phase 1 and Phase 2 data on safety are promising.

On a similar vein, an important strategy for improving pandemic preparedness is to develop template protocols during inter-pandemic periods. These protocols could be pre-approved by the European agency and ethics committees. Since many aspects, such as vaccine composition or platforms, are likely to remain consistent, this approach could significantly accelerate the entire process. In addition, in the interpandemic period companies and research institutions should be able to explore research questions, such as vaccine combinations and co-administration, different schedules, and methods of administration, in greater detail than may be possible when facing an urgent pandemic threat.

Q How is the role of CHIM trials evolving?

PVD: So far, only a limited number of vaccines have been approved through CHIM studies, with the cholera vaccine being one example. However, we have seen advances in the utilization of CHIM, especially in the UK during the COVID-19 pandemic. While these trials did not necessarily lead to immediate clinical applications, they have greatly enhanced our understanding of infectious diseases and could accelerate the selection of prototype vaccines in the future.

Instead of recruiting tens of thousands of participants to a randomized controlled trial that might end up proving the vaccine ineffective, a much smaller CHIM-based trial could help to determine whether a vaccine candidate shows promise early on, saving time and money.

Another advantage of a CHIM study is its ability to provide better documentation for vaccines against various pathogens. Field trials for pan-coronavirus or pan-influenza vaccines are challenging because the circulating strains are often unpredictable. However, CHIM trials allow for exposing volunteers to specific challenge agents, such as H2N3, H5N1, and H1N1, and evaluate whether the vaccine offers protection against these variants.

CHIM trials can also be used to study transmission by mimicking a household setting within a contained environment. These controlled settings minimize the risks to the environment and volunteers, allowing researchers to assess whether a vaccine effectively blocks transmission. Ethics committees closely assess these practices, but the concept is to create an 'artificial epidemic' within a fully contained facility to document individual protection and transmission blocking potential. This approach will allow researchers to explore new endpoints related to mucosal immunity and test them to determine what correlates best with protection.

Overall, adaptive design trials and prototype vaccine documentation open numerous new opportunities. The next step is to ensure that the EMA and Ethics Committees is also aligned with this evolution of CHIM trials, so we will have many meetings and constant communication with the regulators to clarify the risks involved, how they are mitigated, and the data we aim to generate. It is important that these data can also be used in approval, registration, and license packages by companies or research institutions.

Q What challenges arise in the availability, source, and origin of pathogen strains for CHIM studies?

PVD: First and foremost, the number of currently available challenge agents/pathogen strains in the public domain is limited. Additionally, some challenge agents are restricted to private domains, making them more expensive. Therefore, it is crucial to set up a library of challenge agents, and we are currently working on this alongside colleagues. The goal is to create a publicly available library of challenge agents for researchers, ensuring that these agents

are standardized in terms of dose, characteristics, preparation, and GMP. This standardization is essential for comparing results across different CHIM studies.

We are currently in an inter-pandemic period—an ideal time to build a bank of challenge agents and seek support from governments, organizations such as CEPI and Bill & Melinda Gates Foundation, and potentially private companies.

A further goal is to conduct modeling studies to determine the appropriate concentrations and dosages of challenge agents that trigger an infection, and sufficient attack rate without causing severe disease.

Q What challenges have been raised by the new European Clinical Trial Regulation and how can the EU retain a robust clinical trial ecosystem?

PVD: The new Clinical Trial Regulation aims to harmonize and centralize clinical trial procedures across EU countries. This is a rational and important goal, and this initiative is fantastic. However, in practice, it has led to a tripling in the time needed to get the approval to conduct a clinical trial. This means that many companies are now choosing not to do trials in EU countries and instead go in search of faster processes elsewhere. While Europe offers competitive quality and price, the speed of the process is just as important.

It is essential to maintain and enhance the European vaccines ecosystem, which encompasses manufacturing, small and medium-sized biotech companies, diagnostics, and academic partners. This unique ecosystem could lose some of these key players if we do not find a way to work more efficiently with the European Commission and streamline the approval mechanisms.

This could be improved by centralizing the information system between regulatory, ethics and research bodies. Sometimes questions can quickly be answered by the principal investigator or the research team, but the distance between decision-makers and those who are drafting the questions is much longer than it used to be in the past, when local relationships with investigators and familiarity with facilities helped expedite processes. Now, everything needs to be checked, which is important but time-consuming. Therefore, finding ways to shorten these communication lines is one of the priorities. Moreover, vaccine trials often have unique demands, such as recruiting a larger number of healthy volunteers and spanning multiple countries and sites, which adds more complexity.

Q What is the most important recent innovation in the vaccines development field, and why?

PVD: The most essential development, in my view, is on the one hand the growing role CHIM studies will play in the future in the assessment of prototype vaccines and monoclonal

antibodies, and on the other hand the increasing focus on mucosal immunity, which was often under-examined and under-assessed due to other priorities. While it is amazing to witness the launch of successful pertussis and influenza vaccines, we could pay more attention to how the next-generation vaccines could block transmission. Some progress is already being made in the development of mucosal immunity-based vaccines, especially in the pertussis and hopefully COVID-19 field.

The focus on mucosal immunity opens up new possibilities for public health. By understanding and harnessing mucosal immunity instead of just humoral or cellular immunity, we can potentially develop vaccines that not only protect individuals but also reduce transmission rates, which would also offer broader community protection. Consequently, correlates of protection will probably have to be redefined, taking mucosal immunity biomarkers into account.

Finally, the evolution of new ways of administering vaccines, intradermally as well as mucosal, are important innovations in the vaccine field. We should not forget that it is not the vaccine that saves lives but the vaccination!

Q What's next for your work?

PVD: The main focus for my whole team and I in the next 5–10 years will be, the conduct of Phase 1 and 2 adaptive design vaccinology trials and CHIM trials in the Vaccinopolis infrastructure at the University of Antwerp, to help accelerate the development of novel vaccines.

My second focus will be underlining the importance of vaccinology in (para)medical education.

And finally, transferring my duties, knowledge and expertise to my successor and team members, will be my third focus.

REFERENCES

1. De Waele A, Hendrickx G, Valckx S, *et al.* The Vaccine Training Barometer: assessing healthcare providers' confidence to answer vaccine-related questions and their training needs. *Vaccine* 2024; 42(9), 2421–2428.
2. Bethe U, Pana ZD, Drosten C, *et al.* Innovative approaches for vaccine trials as a key component of pandemic preparedness—a white paper. *Infection* 2024; doi: 10.1007/s15010-024-02347-1.

BIOGRAPHY

PIERRE VAN DAMME obtained his PhD in Epidemiology and Public Health in 1994. He has been active for more than 35 years in vaccine trial and infectious disease research, and conducted more than 500 vaccine trials as PI or co-PI. Since 2000, he has been full Professor of Vaccinology at the University of Antwerp, Antwerp, Belgium and teaches vaccinology in

national and international courses. Pierre Van Damme heads the Centre for the Evaluation of Vaccination (CEV, University of Antwerp), which he founded in 1994, and is director of Vaccinopolis, a unique facility in Europe for vaccine trials and human challenge studies. With Arnaud Marchant, he set up the recently founded European Plotkin Institute for Vaccinology, an initiative to accelerate the evaluation of vaccines for pandemic and endemic pathogens. The CEV is a WHO Collaborating Centre for the WHO European Region for the control and prevention of infectious diseases. Pierre Van Damme has been a regular advisor for national and international organizations for more than 25 years, including the Belgian National Immunization Technical Advisory Group, and the World Health Organization (European Regional Office and Headquarters). He has been appointed as chairman of the European Technical Advisory Group of Experts on communicable diseases and vaccines for the WHO European Region (ETAGE; 2005–2015). Pierre Van Damme has received several awards: the Research Award of the University of Antwerp, the Belgian Social Medicine Award 'Jean Van Beneden' for his work on the introduction of universal hepatitis B immunization programs; the prestigious Bill Marshall award of the ESPID society (2014); the ACRP (Association of Clinical Research Professionals) with the European Outstanding Leadership Award (2017); the prestigious Paul Harris Fellowship by the Rotary Foundation 2017); the Balmis Distinction Award (Almeria, Spain, 2017), the AHA Antwerp Innovation award (2019) and the science communication award UAntwerpen (2021). He has been a member of the Belgian Royal Academy of Medicine since 2008.

AFFILIATIONS

Pierre Van Damme MD PhD
Full Professor, Faculty of Medicine and Health Sciences,
University of Antwerp
Antwerp, Belgium
and
Director, Centre for the Evaluation of Vaccination (UAntwerp),
WHOCC,
Antwerp, Belgium
and
Director Vaccinopolis (UAntwerp),
University of Antwerp

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author is PI or co-PI of vaccine trials for which the university obtains grants.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: Invited.

Interview conducted: Jul 31, 2024; **Revised manuscript received:** Sep 4, 2024;

Publication date: Sep 12, 2024.

VACCINE INSIGHTS

PRECLINICAL AND CLINICAL RESEARCH

SPOTLIGHT

Large animal virus challenge models in vaccine development: a One Health approach

Sara Louise Cosby
Agri-food and Biosciences Institute

"Research on human infections that are not zoonotic is more difficult. However, large animal hosts often have viral infections that are closely related to virus species in humans..."

VIEWPOINT

Vaccine Insights 2024; 3(5), 167–170

DOI: 10.18609/vac.2024.028

Information obtained from virus challenge models is necessary to inform vaccine design, development, and eventual vaccine trials. Large animals may offer a more relevant model of either zoonotic or equivalent virus infection than rodent species.

Animal models of virus infections are highly important for understanding disease parameters, including clinical signs, pathology, and immune response, as well as virus–host pathogen dynamics. Information obtained from suitable challenge models is necessary to inform vaccine design, development and eventual vaccine trials. Many human and veterinary virus vaccines are initially tested in rodents [1], which in most cases do not have an equivalent virus infection to the natural host.

Studying virus infection and vaccination in the natural species is feasible for livestock such as cattle, sheep, and pigs and therefore the preferred choice as directly relevant data is obtained. For veterinary vaccines for livestock, it may be much more appropriate to go straight to trial in the natural host rather than use rodents where the immune response may differ significantly, and other factors cannot be examined. For example, many small animal models have been used for foot and mouth disease viruses, but aspects such as virus carrier status and virus shedding can only be realistically examined in the natural host [2]. It may not be possible to use the challenge pathogen in another animal species due to non-permissive factors, such as lack of the virus receptor, or immunological factors that limit the infection. In such cases transgenic rodent models have been used, where human virus receptors are knocked in, or cytokine genes or receptors knocked out to allow infection, but this may not give a valid presentation of the disease. This is exemplified by transgenic models for SARS-CoV-2 [3].

For human infections, animal models should mimic the pathogenesis of the disease as closely as possible. If the disease is zoonotic, the most accurate information about the infection and efficacy of a vaccine may be obtained by using the natural animal host. This is a valid approach for many of the new and emerging zoonotic pathogens on the WHO list of priority virus diseases, e.g., Rift Valley Fever virus, Crimean-Congo hemorrhagic fever virus, Nipah virus, and Zika virus. This strategy also allows a One Health approach to designing and producing vaccines, preferably on the same technology platform, for both the animal and human host.

Research on human infections that are not zoonotic is more difficult. However, large animal hosts often have viral infections that are closely related to virus species in humans, e.g., bovine respiratory syncytial virus (bRSV), bovine coronavirus (BCoV), influenza virus, parainfluenza viruses, and porcine rotavirus. Clinical presentation, immune response and pathology may be similar to infection in humans with the equivalent human pathogen, e.g., BCoV in cattle is closely related to human coronavirus OC43. BCoV infects the respiratory and digestive organs of cattle and causes neonatal calf diarrhea, bloody diarrhea in adult cattle, and respiratory symptoms [4]. Approximately 30% of animals with BCoV infection can be asymptomatic but act as reservoirs of infection. Affected cattle have a range of clinical signs from mild respiratory symptoms to severe pneumonia and/or diarrhea [4,5]. This has strong correlates to SARS-CoV-2, which also has a wide diversity in clinical respiratory signs and up to 28% of patients developing gastrointestinal symptoms with or without other symptoms [6].

bRSV also closely parallels the clinical picture for human RSV (hRSV). This includes nasal discharge, abnormal lung sounds, dyspnea, fever, hypoxia, and cough with severe infection, interstitial pneumonia, and bronchiolitis. Studies in human infants are restrictive due to both ethical and technical issues. As a natural pathogen of cattle, bRSV mimics hRSV pathogenesis more closely than experimental infection of semi-permissive laboratory animals and is a useful model for vaccine trials [7]. Bovine and human parainfluenza viruses are also closely related [8], which would allow similar studies with this group of respiratory viruses.

Developers may be reluctant to undertake trials in large animals as they are more

expensive than rodent studies. However, the extra cost may be justified due to the relevance of the model and the wider set of data obtained as measurement of a larger number of parameters is usually possible. The large size of calves, pigs, and sheep, even in neonates, allows for frequent collection of large volumes of blood and mucosal secretions (the latter to measure virus shedding and IgA). The large blood volumes collected also allow peripheral blood mononuclear cell preparation and sera collection to facilitate multiple tests. Multiple sites in tissues can be sampled for diverse testing, from omics to immunohistology. As a non-invasive process, ultrasound can be carried out throughout the study, particularly to monitor lesion development in lung infections. In some cases, biopsies are also possible at several time points. These large sample preparations and other procedures would be impossible or limited in small animal models. Even if a virus challenge is not possible, an immunogenicity trial for a human vaccine may be feasible in a large animal with the advantages of sampling outlined above.

In the Agri-food and Biosciences Institute we are developing (with Queen's University Belfast) and trialing recombinant vaccines using new and established large animal virus challenge models. Current models include bRSV, bovine herpes virus 1, and parainfluenza 3 (under development). This has allowed genomic studies identifying the genes regulated in the early response to the viral infection [9–11], which can be compared to the equivalent human infections. This approach is also being used to look at the early immune response to vaccines. Studies are also underway with collaborators to examine the effect on both the respiratory and gastro-intestinal microbiome of vaccination and virus challenge.

REFERENCES

1. Meeusen ENT, Walker J, Peters A, Pastoret PP, Jungersen G. Current status of veterinary vaccines. *Clin. Microbiol. Rev.* 2007; 20(3), 489–510.

2. Jiao J, Wu P. A meta-analysis on the potency of foot-and-mouth disease vaccines in different animal models. *Sci. Rep.* 2024; 14(1), 8931.

3. Jarnagin K, Alvarez O, Shresta S, Webb DR. Animal models for SARS-Cov2/Covid19 research—a commentary. *Biochem. Pharmacol.* 2021; 188, 114543.

4. Workman AM, Kuehn LA, McDaneld TG, Clawson ML, Loy JD. Longitudinal study of humoral immunity to bovine coronavirus, virus shedding, and treatment for bovine respiratory disease in pre-weaned beef calves. *BMC Vet. Res.* 2019; 15(1), 161.

5. Tråvén M, Näslund K, Linde N, *et al.* Experimental reproduction of winter dysentery in lactating cows using BCV—comparison with BCV infection in milk-fed calves. *Vet. Microbiol.* 2001; 81(2), 127–151.

6. Kawasuji H, Takegoshi Y, Kaneda M, *et al.* Transmissibility of COVID-19 depends on the viral load around onset in adult and symptomatic patients. *PLoS One* 2020; 15(12), e0243597.

7. Guerra-Maupome M, Palmer MV, McGill JL, Sacco RE. Utility of the neonatal calf model for testing vaccines and intervention strategies for use against human RSV infection. *Vaccines (Basel)* 2019; 7(1), 7.

8. Neill JD, Ridpath JF, Valayudhan BT. Identification and genome characterization of genotype B and genotype C bovine parainfluenza type 3 viruses isolated in the United States. *BMC Vet. Res.* 2015; 11, 112.

9. Johnston D, Earley B, McCabe MS, *et al.* Messenger RNA biomarkers of bovine respiratory syncytial virus infection in the whole blood of dairy calves. *Sci. Rep.* 2021; 11(1), 9392.

10. Johnston D, Kim J, Taylor JF, *et al.* ATAC-Seq identifies regions of open chromatin in the bronchial lymph nodes of dairy calves experimentally challenged with bovine respiratory syncytial virus. *BMC Genomics* 2021; 22(1), 14.

11. O'Donoghue S, Earley B, Johnston D, *et al.* Whole blood transcriptome analysis in dairy calves experimentally challenged with bovine herpesvirus 1 (BoHV-1) and comparison to a bovine respiratory syncytial virus (BRSV) challenge. *Front. Genet*. 2023; 14, 1092877.

BIOGRAPHY

SARA LOUISE COSBY is Head of Virology at the Agri-food and Biosciences Institute, Belfast, UK and an Emeritus/Honorary Professor in the Wellcome Wolfson Institute, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, UK.

AFFILIATION

Sara Louise Cosby
Head of Virology,
Agri-food and Biosciences Institute,
Belfast, UK

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has no conflicts of interest.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Revised manuscript received: Sep 4, 2024; **Publication date:** Sep 12, 2024.

VACCINE INSIGHTS

PRECLINICAL AND CLINICAL RESEARCH

SPOTLIGHT

INTERVIEW

One Health approaches to vaccine development in the post-pandemic era

Trina Racine, Director of Vaccine Development at the Vaccine and Infectious Disease Organization (VIDO), joins **Charlotte Barker**, Commissioning Editor, *Vaccine Insights*, to discuss the benefits of conducting animal and human vaccine research within one organization, prospects for a broadly protective coronavirus vaccine, and the importance of considering manufacturing needs right from the first phases of development.

Vaccine Insights 2024; 3(5), 181–188

DOI: 10.18609/vac.2024.030

Q What led you to focus your work on vaccine development?

TR: I completed my PhD in Molecular Virology, focusing on virus genetics. During that time, I saw numerous discoveries in my own and neighboring labs that had the potential to advance into clinical testing and eventually benefit people. However, the scientists overseeing these projects were primarily academic researchers, and unfamiliar with the processes required to move these discoveries forward. I wanted to help move scientific discoveries from the lab to the clinic.

As a post doc, I had the opportunity run a Phase 1 clinical trial for a DNA-based vaccine. This experience served as the foundation for my current work, where I focus on understanding the development process and assisting early-stage academic discovery projects in progressing toward clinical development, and, hopefully, commercialization.

Q What makes VIDO unique?

TR: The Vaccine and Infectious Disease Organization (VIDO) has been in existence for almost 50 years and originally started as the Veterinary Infectious Disease Organization, a spinoff from the University of Saskatchewan's Western College of Veterinary Medicine. It was established to fill the need for a dedicated organization to develop vaccines for the livestock industry, in addition to studying the pathogenesis of circulating livestock pathogens.

It was in the early 2000s that the name of the organization was updated to reflect the expansion of our research to human pathogens and the development of vaccines and therapeutics for them. For over 20 years, we have adopted a One Health approach to our research, focusing on the intersection of animal health, human health, and the environment, recognizing how deeply intertwined they are.

Over 70% of human pathogens have a zoonotic origin, so gaining a deeper understanding of animal health and developing animal vaccines could prove critical for human health. This unique approach, which VIDO adopted more than two decades ago, has recently gained broader recognition in the vaccine development space.

Q What are the benefits for researchers of combining human and animal research in one center?

TR: The dual knowledge of both animal and human health significantly enhances vaccine development efforts at VIDO. Insights can be drawn from both fields and applied cross-species. Additionally, we are equipped with substantial containment infrastructure, operating Canada's largest bio-containment facility. This allows for the development of a wide variety of animal models.

Our capability to develop animal models enables the testing of veterinary vaccines in the intended species, and human products in the most relevant animal models. While non-human primates are often the go-to species, they are not always the optimal choice, depending on the pathogen.

Before the pandemic, one of our scientists, Dr Darryl Falzarano, was working on a vaccine for camels to combat Middle East Respiratory Syndrome coronavirus (MERS-CoV), which has been circulating since 2012. Camels are known to be the reservoir for MERS, and while spillover events to humans are relatively rare, they are highly lethal, with a mortality rate of

"If we can contain tuberculosis in animal species that are natural carriers, we might also help mitigate human tuberculosis, as they are likely spillover events that go unnoticed."

33%. The goal was to develop a vaccine for camels, which could reduce or eliminate the need for a human vaccine by preventing zoonotic transmission. Our high-containment facilities allow us to work with such high-risk viruses.

Dr Falzarano's work on MERS-CoV provided him with extensive knowledge of coronaviruses. When SARS-CoV-2 emerged, we were the first facility in Canada to isolate the virus due to our pre-existing experience and expertise. Building on the MERS vaccine design, we adapted it to create a SARS-CoV-2 vaccine which we then advanced into clinical trials. This pre-existing knowledge of a virus circulating in animals was instrumental in our ability to contribute to Canada's response to the pandemic.

Q What projects are you excited about currently?

TR: Building on our work with the COVID-19 vaccine we brought to clinical trials, we are now developing a second-generation vaccine—a broadly protective coronavirus vaccine—with initial funding provided by CEPI.

Progress on this project has been promising. We have identified a lead candidate that has demonstrated the ability to protect against SARS-CoV-2 and SARS-CoV-1, as well as generate neutralizing antibodies against various other sarbecoviruses.

We also have several projects on the animal health side. For example, we are developing vaccines for bovine tuberculosis (TB) and bison TB. In Canada, we have reintroduced bison into our national parks. However, these bison are susceptible to TB and can potentially transmit the disease to nearby cattle. From an industry perspective, exporting TB-infected cattle would be highly detrimental as it could lead to trade restrictions and significant economic losses for Canada. If we can contain TB in animal species that are natural carriers, we might also help mitigate human TB, as they are likely spillover events that go unnoticed. By controlling the virus in its animal hosts, the risk of transmission to humans could be reduced.

Another project that we are working on is a vaccine for African swine fever, which affects pigs. Thankfully, it is not yet circulating in North America, but it has caused significant devastation in Asia and Africa and has even been reported in Haiti. If it were to spread to North America, it could have serious economic consequences, as pork production and exports in Canada are worth billions of dollars annually. Moreover, we have a population of wild pigs that could become carriers of the virus, making containment extremely difficult. In the event of an outbreak, targeting infected animals in the wild is far more challenging than in a controlled farm environment. Proactive measures are being taken to prevent potential outbreaks.

"...an important focus should be on developing combination vaccines that protect against multiple viruses, rather than having a separate vaccine for each virus."

Q What are the biggest threats from zoonotic pathogens?

TR: Respiratory viruses are particularly concerning. For instance, highly pathogenic avian influenza has recently spread to dairy cattle in the USA and has subsequently been transmitted to several humans. With the upcoming fall migration season for birds, this situation is something to monitor closely. Due to our large containment facilities, we can study the pathogenesis of this virus in cattle and potentially develop a vaccine against it—something that many facilities cannot do. We have been collaborating with partners in the USA on this research, which has been very productive.

Coronaviruses are another concern, given the number of different coronaviruses circulating in bat species and pangolins. There is always a risk of a new coronavirus emerging.

Nipah virus, a level 4 pathogen, is another threat. It circulates in bats and has recently caused fatalities after zoonotic transmission to humans. Although transmission events are rare, they tend to be highly lethal when they do occur. As human populations grow and urbanization encroaches on forest areas, such spillover events are likely to increase. We must develop vaccines and therapeutics now to prepare for these emerging threats.

Q Looking at the vaccine development field more broadly, what are the biggest priorities right now?

TR: Vaccine schedules are increasingly crowded. WHO currently recommends vaccination against 13 different pathogens, with plans to increase this to 17. With that in mind, I believe an important focus should be on developing combination vaccines that protect against multiple viruses, rather than having a separate vaccine for each virus.

Of course, there are already some combination vaccines available, such as DPT. However, people are experiencing fatigue with the increasing number of vaccinations. While immunization was traditionally focused on children, it is now extending into adulthood, with vaccines for influenza, COVID-19, RSV, and more. Developing combination vaccines could increase uptake by making it more convenient.

However, creating combination vaccines is challenging, especially when combining vaccines that have not already been individually approved. In such cases, it is necessary to demonstrate

the safety, efficacy, and protective effect of each component as well as the combination. Additionally, it is important to show that the combination does not diminish the effectiveness of any individual vaccines or raise new safety concerns.

If the vaccines to be combined are already approved individually, the process is somewhat easier. In this case, only bridging studies may be required to demonstrate that the immune response to the combination is consistent with previously demonstrated protective levels. However, for new pathogens, the bar is set much higher. From a regulatory perspective, the level of investment required—in terms of time and money—may discourage the industry from pursuing this approach.

Q What are the most important or controversial developments in the field recently?

TR: I believe that advances in AI and machine learning are particularly impactful, especially in the design of antigens. These technologies are becoming increasingly important, and I expect their significance will only grow as our understanding deepens and the software systems continue to improve.

I believe they will have special value in the development of broadly protective vaccines. If we aim to develop a vaccine that protects against a range of related viruses, the challenge becomes much more complex than targeting a single pathogen. How do we create an antigen that can offer protection across various viruses that are only slightly related to each other? AI and machine learning can assist us in solving this problem more effectively than we could on our own.

A controversial question in the field is whether it is feasible to develop a broadly protective vaccine for viruses that are not currently circulating in the human population. Some scientists believe in the importance of this work for pandemic preparedness, arguing that we should be proactive in creating these vaccines. On the other hand, those on the regulatory side often raise the question of how to prove the efficacy of such vaccines against viruses that have not yet appeared. It may be possible to generate immunological, B-cell, or antibody data, but how do we know if these correlate with true protection? Additionally, how do we define the indication for use of such a vaccine if its efficacy cannot be demonstrated in a traditional Phase 3 clinical trial?

I believe this is an important discussion. I'm in favor of developing broadly protective vaccines, especially from a pandemic preparedness standpoint. However, I understand the regulatory perspective that it may be challenging to 'prove' efficacy in the traditional sense. Therefore, there needs to be ongoing work to ensure alignment between the scientific community's objectives in developing these vaccines and the data requirements that regulatory agencies find acceptable.

Q Post-pandemic, how can we seize the opportunity to enhance the clinical trial ecosystem?

TR: The pandemic provided an interesting perspective on the clinical trial process. During that time, we saw the pharmaceutical industry take unprecedented risks by conducting trials in an overlapping manner—progressing to the next phase without waiting for the complete data from the previous phase. Regulatory agencies also allowed this, accepting a certain level of risk. However, this created a somewhat misleading impression of how quickly clinical trials can typically progress.

There were expedited reviews by ethics boards and regulatory agencies, as well as faster contracting between study sponsors and clinical trial sites. All of these processes were accelerated compared to the usual timelines. Unfortunately, this is no longer the standard practice, and legal processes and other formalities often slow things down.

To build on the lessons from the pandemic and improve the clinical trial system, one approach could be to establish more networks of clinical trial sites. While some networks already exist, expanding their availability and standardizing protocols could reduce the back-and-forth with regulatory agencies. If everyone agrees upfront on the required data for specific types of vaccines—whether it's for a respiratory virus or an RNA vaccine—the process could be more streamlined.

Furthermore, increasing the number of clinical researchers and nurses qualified to run clinical trial sites is crucial. Finding a site with the necessary expertise is not always easy, so expanding access to trained professionals would be beneficial. Strengthening this infrastructure would significantly improve the efficiency and effectiveness of clinical trials.

Q Finally, what's next for your work?

TR: For me, the immediate goal is to advance the broadly protective coronavirus vaccine that I mentioned earlier into the manufacturing stage and then into clinical testing. This will be the first proof of concept for the vaccine platform we've developed, demonstrating that it is indeed possible to design vaccines with broad protective capabilities. This effort will involve close collaboration with regulatory agencies to discuss their perspective on this approach, as there are still some concerns to address.

Looking ahead, we are also focusing on utilizing our new manufacturing facilities at VIDO. This infrastructure, along with the expertise of our team, will significantly advance the science we conduct and the products we develop. Having manufacturing capabilities in-house allows our scientists to gain a deeper understanding of the requirements for moving a discovery product into manufacturing. For example, they need to be aware that certain elements, such as ampicillin-resistant genes, cannot be present in plasmids used in our manufacturing process. This knowledge can prevent the need to re-clone and redo studies later on.

Moreover, there are other critical considerations, such as intellectual property rights and licensing agreements for adjuvants added to vaccines. If these elements make commercialization too expensive, the vaccine may no longer be viable, especially in the veterinary field, where price points are very low. Early awareness of these factors will help minimize the need for repeated experiments and adjustments later in the development process. The cost of materials, such as media used in production, must also be considered—using a $1,000/liter medium isn't feasible when scaling up to 1,000 liters.

I am excited for our organization as our scientists and students will begin to integrate this knowledge into their discovery programs, ultimately enhancing the efficiency and viability of our vaccine development efforts.

BIOGRAPHY

TRINA RACINE has a PhD in Microbiology and Immunology from Dalhousie University, Halifax, Nova Scotia, Canada and is currently the Director of Vaccine Development at the Vaccine and Infectious Disease Organization (VIDO), a research center part of the University of Saskatchewan, Saskatoon, Canada. Upon completion of her PhD, Dr Racine joined the Special Pathogens Program at the National Microbiology Laboratory (NML), part of the Public Health Agency of Canada. While at the NML, Dr Racine worked on the development of vaccines and therapeutics for various emerging and re-emerging infectious diseases, including Ebola, Zika and MERS. Dr Racine coordinated clinical trials and provided diagnostic support to the Ebola outbreak in West Africa in 2014–2016. Prior to joining VIDO, Dr Racine was a Scientific and Regulatory Affairs Consultant for GeneOne Life Science, Inc., a South Korean-based biopharmaceutical company. As Director of Vaccine Development at VIDO, Dr Racine is responsible for guiding the development, manufacturing, and clinical/field testing of VIDO's internal products using a Stage Gate process. Dr Racine is also responsible for VIDO's Vaccine Development Centre (VDC), a pilot scale, Containment Level 3 capable, GMP biomanufacturing facility capable of producing veterinary vaccines to North American licensure and human vaccines to Phase 2 clinical trials.

AFFILIATION

Trina Racine
Director of Vaccine Development,
Vaccine and Infectious Disease Organization,
Saskatoon, Canada

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Trina Racine is an employee of the Vaccine and Infectious Disease Organization (VIDO), part of the University of Saskatchewan. VIDO receives operational funding from the Canada Foundation for Innovation through the Major Science Initiatives, the Government of Saskatchewan through Innovation Saskatchewan, and the Ministry of Agriculture. VIDO research projects may also be funded through Canadian and international funding agencies.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Interview conducted: Aug 8, 2024; **Revised manuscript received:** Sep 4, 2024;

Publication date: Sep 13, 2024.

VACCINE INSIGHTS

PRECLINICAL & CLINICAL RESEARCH

SPOTLIGHT

INTERVIEW

Vaccinating cattle against H5N1: challenges and benefits

Alan Young, Professor of Veterinary and Biomedical Science, South Dakota State University, and Chief Technology Officer, Medgene Labs LLC, speaks with **Charlotte Barker**, Commissioning Editor, *Vaccine Insights*, about the current outbreak of H5N1 avian influenza in US dairy herds and the prospects for a vaccine. He also shares insights into regulatory pathways for veterinary vaccines and working at the intersection of academia and industry.

Vaccine Insights 2024; 3(5), 189–193

DOI: 10.18609/vac.2024.031

Q How did you get involved in veterinary vaccine development?

AY: I grew up in Ontario, Canada, in a small dairy farming community. I went to the University of Toronto for my undergraduate and postgraduate studies, just when immunology was growing as a science. I became fascinated with the immune system of animals.

When I graduated in the mid-1990s, I took a position at the Basel Institute for Immunology in Switzerland as part of the ruminant immunology group and spent the next 6 years working on developmental aspects of B cells.

In 2001, I landed at South Dakota State University as a faculty member in veterinary biomedical sciences. I worked on animal diseases such as mad cow disease and chronic wasting disease, focusing on the immune responses in different species—if it's got feet, fins, or feathers, I've probably worked with it over my career!

As an immunologist, working on vaccines is a natural progression. In 2009, I became a founding member of the Center of Excellence and Zoonotic Animal Disease in Kansas, working on vaccines for foreign animal diseases, starting with Rift Valley Fever.

Around that time, I was approached by a group interested bringing my research to the greater animal health world and Medgene Labs was born with a mission to produce animal vaccines. Though I'm still a working professor, I'm enjoying my active role in vaccine technology on a commercial level.

Q What types of pathogens does Medgene target?

AY: We use a well-established baculovirus-based protein platform to make vaccines for a variety of animal diseases. Although the first product we worked on was Rift Valley Fever vaccine, we really got our start in studying commercially relevant emerging diseases, specifically the porcine epidemic diarrhea virus of 2013–14.

Q How did that transition to Medgene's current status in prescription platform vaccine technology?

AY: Traditionally, it takes around 5 years and USD$5 million plus to bring a veterinary vaccine to market. In an emergency situation, or when dealing with a rapidly mutating virus, it became clear that a faster route is needed.

In response, the USDA brought in Veterinary Services Memorandum 800.213, which recognized platform technologies. For example, if you already have an initial license for an influenza vaccine based on a hemagglutinin protein, you can substitute a new hemagglutinin into the process and get a conditional license much faster and with less expense.

In fact, the USDA went further and agreed that, if you are using a non-replicating killed system like baculovirus, you can swap out the gene of interest from that platform and market those vaccines in the field, provided you demonstrate safety in the species of interest at the maximum antigen level to be used in the field.

The USDA forbids companies from making efficacy claims for these products but allows them to send the vaccine formulation out to any veterinarian that requests it across the USA. That has allowed Medgene to create and supply vaccines very quickly, specifically in rapidly mutating diseases (e.g., influenzas) or viruses that cannot be grown in the lab (e.g., Rotavirus C), and so are not amenable to traditional development routes. While we cannot claim efficacy, several of our vaccines have become standard in livestock across the USA.

Another target for Medgene is in minor species. The market for some species is not large enough to warrant a $5 million investment but by getting a species extension, we have been able to develop vaccines for species farmed on a small scale, such as white-tailed deer.

We have a library of baculovirus constructs and extensive bioinformatics to ensure we are targeting the correct antigenic sites and allow us to act fast to respond to emerging threats. We now have vaccines for a host of species, including cattle, swine, sheep, goats, poultry, and rabbits.

Sometimes the bar set for introducing solutions to problems in animal health is very high. We occupy a niche where we can put products out into the field (after ensuring they are safe)

"We have over half a million pre-orders from dairy farmers because of the economic loss caused by H5N1. Dairies are effectively shut down if any virus is detected in the bulk milk tank, and lose significant revenue."

and test efficacy in the real world. Ultimately, farmers will not pay for products that do not deliver results, so the market quickly weeds out ineffective products.

Q What is the current status of Medgene's bovine H5N1 vaccine?

AY: We actually formed a construct on H5N1 when it started showing up in mammals. The jump to cattle was as much a surprise to us as it was to everybody else, but because we already have our prescription platform license in cattle, it was relatively straightforward to develop a vaccine, which we have now done. However, vaccinating livestock for H5N1 is not allowed in the US due to trade ramifications, so we are not able to release the vaccine. However, on August 28, 2024, the USDA released CVB Notice 24-13 permitting field studies with non-viable, non-replicating vaccines targeting H5N1 which specifically describes our baculovirus prescription product. As a result of this, we are now actively developing the vaccine for conditional licensing in preparation for potential distribution.

The concern that limits use is that vaccination could risk exporting poultry or cattle infected at a subclinical level, and affect the ability to test for the pathogen. Many vaccines, including ours, allow differentiating infected from vaccinated animals; however, some countries have already been prevented from exporting poultry after instituting vaccination programs, and this is a huge concern for the industry and USDA.

Q What are the arguments for vaccinating cattle?

AY: Our concern as a veterinary vaccine company is predominantly to provide our clients with what they need. We have over half a million pre-orders from dairy farmers because of the economic loss caused by H5N1. Dairies are effectively shut down if any virus is detected in the bulk milk tank, and lose significant revenue. In addition, milk yields drop 10–15% during infection, and in some cases never recovers.

From a human health point of view, it is also a concern to have H5N1 spreading in cattle and infecting farm workers, as it may increase the risk of the virus adapting to human hosts. And while pasteurization kills the virus, there is a population in the US who like to drink unpasteurized milk, believing it has health benefits.

It is frustrating as a vaccine maker to have a potential solution for our customers but be unable to provide it, although we absolutely understand the economic and political concerns.

Q Having viewed vaccine development from both academic and commercial perspectives, what insights can you share from either side?

AY: In academia, everybody gets very excited about the latest high-end technology. But working in industry has taught me that demonstrating efficacy is the easiest part of the entire process. At millions of dollars for veterinary vaccines and hundreds of millions for human vaccines, the cost (and risk) of bringing a vaccine to market is immense.

In the animal health industry, we have to produce vaccines that cost pennies per dose so efficient scale up is essential. No matter how scientifically elegant your vaccine is, if you can't produce large volumes quickly and cheaply, you are unlikely to ever reach the livestock market. Plus, a significant proportion of your costs have nothing to do with making the vaccine, but are spent on boxing, labeling, testing, and regulatory approvals.

Another simple but important lesson from industry is to talk to your customers about what they want. I once heard a colleague discuss a vaccine that was effective but must be given to pigs at 14 days old. What they hadn't realized is that large farms will only touch the pigs at certain points (birth, processing, weaning), so your vaccine needs to fit with their schedule – they will not be willing to go to the farrowing barn and vaccinate all the pigs on day 14 with your one vaccine.

My message for academia is that if you want to see your vaccine being used in the field, you must understand the farming process and try to tailor your product to fit with that.

Q What's next for your research and the company?

AY: The researcher part of me still likes the idea of emerging diseases and foreign animal disease vaccines, to prevent epidemics amongst livestock and zoonotic infection of humans. We often hear about the need for vaccines against foreign animal diseases that could threaten US agriculture like foot and mouth disease, African swine fever, or Rift Valley disease. However, it is extremely difficult to get a license for these vaccines and it is not commercially viable for companies to invest millions of dollars into a product they may never be able to sell.

Since our model allows much faster and less expensive development, we are able to develop potential vaccines to emerging threats, ready to deploy if needed.

Above all, vaccines need to make practical sense for farmers. As Medgene grows and expands its influence around the world, we're digging deeper into the ideas of cost, practicality and benefit to farmer, marketplace, and greater animal health.

But, one particular aspect of vaccine science that's got me excited is vector-borne disease. Our application of platform technology has proven itself with vaccines against tick-born illnesses. The attention has been really positive—it's another example where it would be difficult to get vaccine licensed through traditional routes, since we are vaccinating against the tick itself rather than the diseases it carries.

Speaking for the entire team, we're all excited that the hard work we've invested in vaccine R&D has gone beyond the pioneering stage and now making a positive impact in many species.

Our communications department came up with a slogan that says it well—it's a new day for animal health care. We're all glad to be with the veterinarians and farmers and making a strong difference in how animal health is practiced.

BIOGRAPHY

ALAN YOUNG is a comparative immunologist focused on the diversity and conservation of the immune response in animals and humans. Young received his BSc and PhD in Immunology from the University of Toronto, Ontario, Canada focusing on large animal models of lymphocyte function and recirculation. Young then became a scientific member and eventual head of the Ruminant Immunology laboratory at the Basel Institute for Immunology, and later faculty of the Department of Surgery, Harvard Medical School. In 2001, he moved to the Department of Veterinary and Biomedical Sciences at South Dakota State University, Brookings, SD, USA, where he continues to teach Immunology and serve as Assistant Director of the Veterinary Education curriculum. His training as an immunologist focuses much of his work on the response of domestic animals to emerging and zoonotic diseases, and the application of that model to creation of new vaccines. Since 2004, Young has been an active participant in translational technology development, and in 2011, Young co-founded Medgene, Brookings, SD, USA, to develop new vaccine and diagnostic tools for human and veterinary medicine. Medgene is a fully-USDA licensed vaccine provider, and has leveraged platform-based technologies in the development, licensing, and deployment of both standard, commercial targets to serve the needs of the animal agriculture industry, as well as developed and deployed emergency response vaccines against foreign animal diseases.

AFFILIATION

Alan Young PhD,
Professor of Veterinary
and Biomedical Science,
South Dakota State University,
Brookings, SD, USA

and
Chief Technology Officer,
Medgene Labs LLC,
Brookings, SD, USA

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Young A has exchanged consulting fees, has support for attending meetings/travel, and holds stock/stock options in VST LLC, dba Megagene. Young A has patents planned/issued/pending with US Prov. Ser. No. 63/648, 832. Young A holds a leadership/fiduciary role in the USAHA Committee on Biologics and Biotechnology as Chair (unpaid).

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; this article is based on an interview.

Interview held: Sep 13, 2024; **Revised manuscript received:** Sep 11, 2024;

Publication date: Sep 26, 2024.

VACCINE INSIGHTS

PRECLINICAL AND CLINICAL RESEARCH

SPOTLIGHT

Innovations in passive immunization in the fight against serious infectious diseases

Tonya Villafana
AstraZeneca

"We need to continue learning from past experiences with passive immunization and adapting the strategies we are using to address today's problems."

VIEWPOINT

Vaccine Insights 2024; 3(5), 163–166

DOI: 10.18609/vac.2024.027

Vaccines are undeniably considered one of the most important advancements in the history of modern medicine. While vaccines offer protection against serious infectious diseases, that protection is contingent upon the individual's ability to mount a robust immune response. The most vulnerable among our society, like the immunocompromised, the very young, and the elderly, may have a suboptimal response to vaccination. This leaves them insufficiently protected and facing a much higher chance of serious outcomes from infectious diseases—inspiring the pursuit of alternative solutions for protection. Passive immunization with monoclonal antibodies (mAbs), can offer rapid protection for vulnerable groups who may have an inadequate response to vaccination or need additional protection from infectious diseases.

AN APPROACH TO PROTECT THE MOST VULNERABLE

Vulnerable populations with underdeveloped or weakened immune systems may be significantly more susceptible to severe viral or bacterial infections, compared to healthy populations. People living with chronic respiratory or cardiovascular conditions may be at higher risk of more severe outcomes from viral or bacterial infections. Groups such as the immunocompromised, including those living with cancer are at disproportionate risk of hospitalization due to infections such as COVID-19. The elderly and other populations with weakened immune systems who contract a *Clostridium difficile* infection are at higher risk for recurrent infections, which could drive repeat hospitalizations and multiple treatments with powerful antibiotics over the course of their lifetime.

Over the past century, various passive immunization strategies have been used to successfully prevent diphtheria infections, hepatitis B infections in newborns, respiratory syncytial virus (RSV) infections in high-risk infants, and COVID-19 infections in the immunocompromised. More recently, we have seen a clear impact on public health with the rapid uptake of passive immunization strategies to protect the broad infant population against RSV. Beyfortus™ (nirsevimab-alip) is the first mAb approved for the prevention of RSV lower respiratory tract disease across the infant population, from preterm or immunocompromised infants to those born full-term and healthy. Nirsevimab was the first mAb to be recommended by the US Advisory Committee on Immunization Practices for broad infant use and was included in the Vaccines for Children program supporting equitable access in the US.

And the impact on public health is becoming clear, with early data from the USCDC showing that in its first season (2023/4), nirsevimab was associated with a 90% reduction in RSV-associated hospitalization among infants, with similar results replicated in the Galicia region of Spain, where nirsevimab demonstrated effectiveness of 89% in severe RSV-related lower respiratory tract infection among infants.

More mAbs are being investigated for the prevention of infectious disease, including for Ebola, malaria, *Staphylococcus aureus*, *C. difficile* and Zika. Providing the opportunity for this important platform to be utilized and have broader impact on prevention of infectious diseases.

At AstraZeneca, our scientists' efforts include novel engineered antibodies with the capability to provide an extended duration of protection compared to traditional mAbs.

We are also working to make the development of mAbs as adaptable and efficient as possible to rapidly address viral and bacterial pathogens as they arise. This includes investigation of a range of innovative approaches that deliver medicines faster and in a cost-effective manner.

A CASE FOR ACCELERATING RESEARCH AND ACCESS

As scientific understanding and capabilities advance, so must the systems we put in

place to make these technologies accessible to patients. Innovations in passive immunization can be supported by flexible, tailored access frameworks and applied appropriately within clinical care standards. We need to create a sustainable mAb model in which innovation and adaptability access are championed.

A key challenge for infectious disease interventions is ensuring they can be developed and deployed quickly when needed. Traditional regulatory approval pathways need to be flexible to support this objective. Strategies such as immunobridging can allow for efficacy of a product to be inferred based on a correlate such as neutralizing antibody titers or other relevant measures. Recently this approach has been used by regulators to support timely assessment of mAbs but more systematic and consistent adoption could facilitate the development of more mAbs for protection against infectious diseases and enable more expedient access.

LOOKING TO THE FUTURE

Championing passive immunization through mAbs will continue to be my priority at AstraZeneca. We need to continue learning from past experiences with passive immunization and adapting the strategies we are using to address today's problems. I'm inspired by the innovations happening in our labs and others around the world and look forward to continued collaboration between scientists, clinicians, and health authorities to drive this forward.

BIOGRAPHY

TONYA VILLAFANA has dedicated her career to protecting people around the world from the most challenging infectious diseases. Dr Villafana has led the development of several vaccine and monoclonal antibody programs targeting areas of distinct unmet medical need, including influenza, malaria, HIV, Ebola, COVID-19, and RSV. Most notably, Dr Villafana led the development of nirsevimab, a novel monoclonal antibody which, for the first time, can offer protection against RSV disease in all infants entering their first RSV season. Dr Villafana was seconded to the World Bank and served as the International Federation of Pharmaceutical Manufacturers and Associations World Bank Fellow, where she supported the Global Medicines Regulatory Harmonization Initiative with a focus on strengthening regulatory systems in Africa and co-authored the Bank's position on Non-Communicable Diseases for the 2011 UN High Level Meeting on NCDs. In collaboration with Oxford University and AstraZeneca colleagues she received the Copley Award and in 2023 was named as one of Fierce Biopharma's Top 20 Women in R&D. Dr Villafana received a PhD in immunology from Weill Cornell University Graduate School of Medical Sciences, New York, NY, USA and an MPH from Harvard School of Public Health, Boston, MA, USA.

AFFILIATION

Tonya Villafana PhD
Global Franchise Head, mAbs
and
Head of Scientific Affairs,
AstraZeneca,
Bethesda, MD, USA

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author is an AstraZeneca employee and stock holder.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Revised manuscript received: Aug 20, 2024; **Publication date:** Sep 24, 2024.