Monoclonal antibodies for malaria prevention

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Monoclonal antibodies are highly specific proteins that are cloned from a single B cell and bind to a single epitope on a pathogen. These laboratory-made molecules can serve as prophylactics or therapeutics for infectious diseases and have an impressive capacity to modulate the progression of disease, as demonstrated for the first time on a large scale during the COVID-19 pandemic. The high specificity and natural starting point of monoclonal antibodies afford an encouraging safety profile, yet the high cost of production remains a major limitation to their widespread use. While a monoclonal antibody approach to abrogating malaria infection is not yet available, the unique life cycle of the malaria parasite affords many opportunities for such proteins to act, and preliminary research into the efficacy of monoclonal antibodies in preventing malaria infection, disease, and transmission is encouraging. This review examines the current status and future outlook for monoclonal antibodies against malaria in the context of the complex life cycle and varied antigenic targets expressed in the human and mosquito hosts, and provides insight into the strengths and limitations of this approach to curtailing one of humanity's oldest and deadliest diseases.

INTRODUCTION

Monoclonal antibodies (mAbs), defined as a single antibody (Ab) cloned from a single B cell, have been in use for decades as immune modulators for transplantation, autoimmune diseases, and cancer.[1] Most clinical uses take advantage of the high specificity of Abs which can safely target specific proteins to deplete cells or block receptor-ligand interactions. This specificity and the fact that Abs are naturally occurring proteins rather than foreign molecules make for an excellent clinical safety profile. It is curious, however, that although Abs were first discovered and used in the context of infectious disease, mAbs are only recently seeing a resurgence in their use for this purpose.[2,3] This review will focus on mAb development for one of the oldest and deadliest infectious diseases that remains without an effective long-term vaccine or chemoprophylactic: malaria.

CONSIDERATIONS FOR MONOCLONAL ANTIBODY DEVELOPMENT FOR INFECTIOUS DISEASES

mAbs are currently being adopted for numerous infectious diseases including respiratory syncytial virus,[4] anthrax,[5] HIV,[6–8] and Ebola.[9] They have recently been approved for use against Ebola and COVID-19, with the latter proving that mAbs can be a rapid and highly effective means of responding to emerging pathogens. However, as highlighted by the COVID-19 pandemic, developing mAbs for infectious diseases is not amenable to a one-size-fits-all approach. Special considerations must be taken to consider the host-pathogen immunobiology and epidemiology of each disease as well as the market environment for novel interventions. For example, mAbs can be used as a prophylactic, therapeutic, or both. Which approach is best depends on a number of factors including the likelihood of the mAb in preventing infection or disease, the utility of the mAb at the individual and population level, the underlying cause of disease following infection, and the intended recipient population. In addition, it must be determined whether preventing infection, disease, or transmission is the priority. Finally, mAbs must be considered in the context of available or emerging drugs and/or vaccines which will compete on a public health and market level.

In the context of drugs and vaccines for infectious diseases, mAbs offer a number of potential benefits. They have an excellent safety profile with minimal off-target effects and can be used in combination with little to no interference.[10] They can be delivered at effective doses in a single, directly observed injection or infusion and can persist at effective concentrations in the blood for longer than 1 year when using long-lasting variants.[6,11,12] Unlike vaccines, mAbs do not depend on the host immune system for production and therefore should have less variability across populations in terms of immediate serum Ab concentration. However, genetic mutations in the Fc receptor can influence the downstream effector mechanisms[13,14] and half-life[15] of anti-cancer mAbs. How such variations contribute to mAb efficacy in infectious diseases has not been well defined and will be specific for each target pathogen. Finally, unlike vaccines that often require multiple doses and at least weeks to have an effect, mAbs are effective almost immediately upon administration.

Still, the road ahead for mAbs against infectious diseases contains several hurdles. The first and foremost concern is cost. The cost of a course of mAbs depends greatly on the intended market,[16] but using COVID-19 as an example, the mAb therapy REGEN-COV is charged at ~$2,100/dose to the US Government.[17] This is small compared with the cost of even a short hospital stay but is orders of magnitude above the costs of COVID-19 vaccines. Thus, while COVID-19 has shown that mAbs can offer a cost-effective benefit in the absence of a vaccine, the cost of mAbs will need to decrease significantly before widespread

use against infectious diseases is possible, particularly in low- and middle-income countries (LMIC). Another consideration is that, typically, tens or hundreds of mAbs need to be screened for function before advancing clinical candidates. This is best achieved using predictive and high-throughput *in vitro* assays (e.g., neutralization assays), which exist for some pathogens such as SARS-CoV-2 but do not exist for other infectious agents such as tuberculosis and for most stages of infection for the parasite that causes malaria. Pathogens are also adept at evolving past even the complex immunity in populations and thus will likely be able to evolve around most individual mAbs and even cocktail combinations of mAbs. Therefore, careful work is required to select either immutable targets or combinations of targets to prevent resistance.[18] While a concern most relevant for dengue virus, the risk of Ab-dependent enhancement of disease due to the presence of non-neutralizing Abs must also be considered. Finally, even fully human monoclonals may be recognized as foreign by the recipient immune system, and "anti-drug antibodies" (ADAs) may form to either clear mAbs or reduce their efficacy. While ADA formation is dependent on many factors, experience with mAbs for infectious diseases have shown little evidence that ADAs will be an issue even following repeated injections.[7,8,19,20]

CONSIDERATIONS FOR MONOCLONAL ANTIBODY DEVELOPMENT FOR MALARIA

Malaria is the disease caused by infection with eukaryotic pathogens of the genus *Plasmodium*, which have a host range spanning from reptiles and birds to rodents, humans, and other mammals. There are multiple species of *Plasmodium* that infect humans, with the vast majority of disease caused by *Plasmodium falciparum* (*Pf*) common to Sub-Saharan Africa and *Plasmodium vivax* (*Pv*) which dominates in Southeast Asia and South America. *Plasmodium* parasites are transmitted via multiple species of Anopheline mosquitoes, and the mammalian portion of the parasite life cycle begins when an infected mosquito injects tens to hundreds of "sporozoite" forms of the parasite into the dermis. These sporozoites then actively migrate through the skin and into the blood where they will be carried to the liver. Here, the parasites replicate asymptomatically and asexually for ~7 days within a single hepatocyte, although at this stage *Pv* also forms dormant stages in the liver called "hypnozoites" that can persist and reactivate for years. At the end of the liver stage the parasites emerge as red blood cell-infectious "merozoites." These merozoites cyclically infect red blood cells, which rapidly expands the parasite burden and initiates the symptomatic stage of infection. Some parasites also undergo sexual replication to become male and female "gametocytes" which can then be picked up by a new mosquito vector. The invertebrate portion of the parasite life cycle starts in the mosquito midgut where the gametocytes will mate, forming a motile "ookinete" that invades the mosquito midgut, where new sporozoites will develop within the "oocyst" for approximately 2 weeks. These sporozoites will then emerge from the oocyst and migrate into the mosquito salivary gland where they can then be transmitted to a new host and complete the transmission cycle. In this review, we will divide this complex life cycle roughly into three phases: the skin-to-liver or "pre-erythrocytic" stage; the "erythrocytic" or blood stage; and the mosquito stage.

The *Plasmodium* life cycle is more complex than bacterial or viral infections, which, on the one hand, presents a challenge to mAb development but also serves as an opportunity, as Abs can function against each stage of infection (Figure 1). This susceptibility has driven the development of multiple prophylactic Ab-based vaccine candidates which have only recently achieved high levels of protection against disease.[21] However, protection against infection has been much more difficult to achieve and will be necessary to disrupt the transmission cycle and achieve malaria eradication. Such high levels of infection-blocking protection have been achieved in controlled human malaria infection (CHMI) studies in malaria-naive volunteers,[22,23] but this has not translated to field trials in endemic areas.[24–27] It is hypothesized that this is due in part to pre-existing malaria-specific immune modulation in previously infected persons. In this case, a long-acting prophylactic mAb would be ideal as it does not rely on the recipient immune system to produce Abs. However, parasite fitness and polymorphisms also play a pivotal role in vaccine efficacy,[28,29] which will need to be considered in developing anti-malarial mAbs.

Finally, it is important to consider that multiple effective and affordable drugs exist to prevent and treat malaria, and that vaccines can be made relatively cheaply if one is developed for malaria. Therefore, the current costs of mAbs would preclude large-scale administration campaigns similar to mass drug administration or mass vaccination. Even at current cost estimates, however, mAbs offer advantages over chemoprophylaxis for members of the military or travelers making multi-week or multi-month visits to endemic areas. This is because long-term chemoprophylaxis is still expensive[30] and suffers from low compliance (10%–50%) due to inconvenient schedules and side effects.[31–33] The high cost of mAbs is also lower than the costs of a medical evacuation and therefore could be cost-effective for visitors at high risk of malaria. Compared with the protracted regimens of vaccines and drugs and the lag time between vaccine administration and efficacy, mAbs would also offer the benefit of a simplified regimen given in a single directly observed administration that has immediate efficacy for unplanned or short-notice trips. While an exact "target product profile" for an anti-malaria mAb will depend on the user and goal, most models suggest that we will need >80% infection-blocking sterile protection for longer than a year to drive malaria toward elimination,[34–36] similar to what has been proposed for long-lasting injectable chemoprophylactics.[36] It is within this context that we will discuss the current state of mAbs for malaria as well as the short-term outlook for achieving the first competitive malaria mAb product capable of achieving high levels of infection-blocking protection. This will be discussed through the lens of the *Plasmodium* life cycle, given the profound impact of the distinct nature of each stage of infection on mAb development (see Figure 1 for summary of life cycle and potential mAb targets).

"PRE-ERYTHROCYTIC" STAGE TARGETED MONOCLONAL ANTIBODIES

As a bottleneck in the life cycle, the numbers of sporozoites injected by the mosquito at the skin-to-liver, or pre-erythrocytic (PE), stages are relatively small[37–39] and the time between injection, invasion of
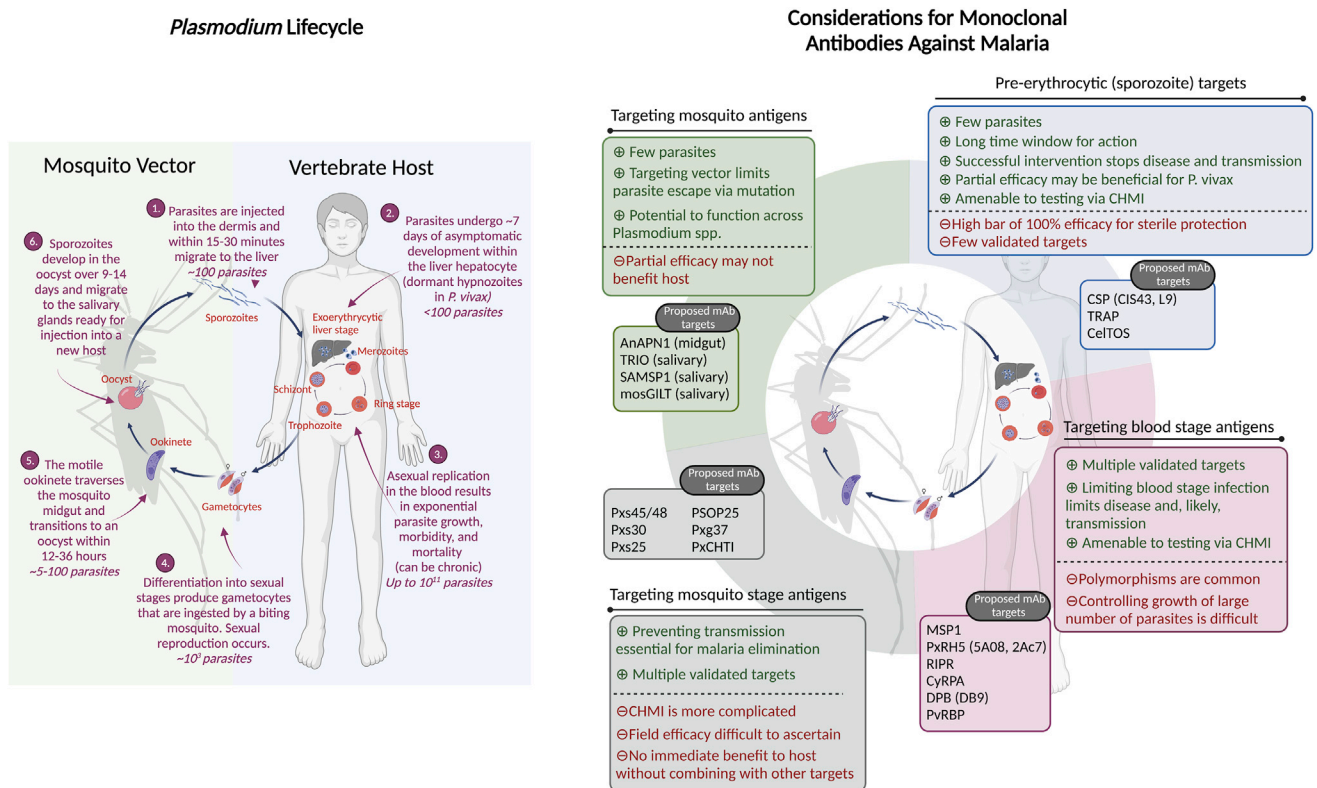
Figure 1. The *Plasmodium* parasite lifecycle and considerations for monoclonal antibodies against malaria
Left: schematic of the *Plasmodium* life cycle, including the approximate number of parasites present in, and duration of, each stage. Right: considerations for monoclonal antibodies against malaria, by life cycle stage. Ab targets that are discussed in this paper are listed by their abbreviation, with specific antibody names included in parentheses where relevant. When the antigen typically includes reference to a specific *Plasmodium* species, x is used instead of species designation. Full antigen names can be found in the text, where relevant. CHMI, controlled human malaria infection. Created with Biorender.com.

the vasculature, and transit to the liver is minutes to hours,[40–43] which provides a large window for infection-blocking Ab activity. The circumsporozoite protein (CSP) is the most abundant surface antigen present in the sporozoite stage of the *Plasmodium* parasite[44] and is critical to the normal development of sporozoites in the mosquito salivary gland[45] as well as their ability to invade and infect hepatocytes once inside the vertebrate host.[46] The two most successful and advanced malaria vaccines, RTS,S and R21, are both designed to elicit an immune response against CSP[47] and are thought to provide protection by neutralizing Abs. This is supported by studies showing the capacity of mAbs against *Pf* CSP (PfCSP) and *Plasmodium yoelii* CSP (PyCSP) to prevent hepatocyte invasion both *in vitro* and *in vivo*.[11,48–53] Using *Pv* infection of liver-humanized mice, Schäfer et al. recently demonstrated that an mAb against PvCSP could also reduce the overall liver burden and in turn relapse infection via reduction of the number of dormant hypnozoites.[54] This is critical, as the majority of *Pv* disease burden is driven by relapses from dormant liver hypnozoites.[55]

The mechanisms of anti-PfCSP mAbs have been well-studied. The CSP is composed of three domains: an N terminus, a C terminus,

and a central region characterized by a repeating amino acid sequences.[56] In *Pf*, this repeat region contains a major repeating sequence, NANP, and a minor repeat, NVDP. The CSP-specific mAb CIS43 was recently shown to be a "dual binder" in that it specifically binds to the central repeat region as well as a short junctional sequence that bridges the N terminus and the central repeat region,[11] and has been reported to provide sterile protection in two different mouse models following passive transfer.[48] This may be explained by the ability of CIS43 to bind multiple CSP epitopes, as such dual binding confers potent neutralizing capabilities to a number of other mAbs which target the major repeat and either the minor repeat or the junction sequence.[49,57] Of note, the introduction of the "LS" mutation into the Fc domain of CIS43 increased the serum half-life of the Ab while maintaining a high level of protection *in vivo*.[12] This modified CIS43LS has been taken into phase I clinical trials in malaria-naive adults, and this first-in-human study for malaria mAbs showed a very promising safety and pharmacokinetic profile after intravenous or subcutaneous administration.[58] Furthermore, participants underwent CHMI challenge and all nine were sterilely protected against infection.[58] However, the numbers of volunteers per dose was small and serum concentration at time of challenge

ranged from ~50 to 500 μg/mL, which is likely higher than what is feasible for long-term protection in the field due to cost. Still, the results from an ongoing field trial in Mali will be critical for understanding the potential for anti-malaria mAbs to perform against natural infection (ClinicalTrials.gov ID: NCT04329104). Encouragingly, a new dual-binding anti-PfCSP mAb, L9, outperformed six published neutralizing mAbs—including CIS43 and other dual binders—in mosquito bite challenges *in vivo* and therefore provides a path to improved potency should results with CIS43LS indicate the need for improvements.[53] In summary, mAbs against PfCSP are paving the way to become the first anti-malarial mAb to prevent infection. However, low levels of sterile protection achieved by CSP recombinant vaccines in the field despite high titers of anti-PfCSP Abs[21,25] and success in CHMI suggest that multiple avenues to improve mAb potency should continue to be pursued.

One such strategy is targeting additional PE antigens. Although no other PE Ab targets have been as well defined or as potent as CSP, the PE stages provide numerous points of possible intervention,[59] and non-CSP polyclonal Abs have recently been shown as potent inhibitors of parasite liver infection in humanized liver mice.[60] A leading target for PE mAb development is the thrombospondin-related adhesive protein (TRAP), a transmembrane protein essential for sporozoite motility and successful liver invasion.[61,62] High levels of anti-TRAP Abs have been correlated with higher protection against malaria in children,[63] suggesting that this protein may yield another promising mAb target. The idea of using TRAP in combination with CSP as an Ab target has been considered for decades, but studies have generated mixed results. One vaccine trial using TRAP in combination with RTS,S failed to show any significant protection, perhaps because of immune interference that reduced the anti-CSP Ab titers.[64] Another clinical trial combining RTS,S with viral-vectored TRAP showed no benefit to adding TRAP, yet interpretation of this is complicated by the combination of vaccine platforms.[65] This is in contrast to studies in mice where active vaccination with CSP and TRAP suggested the utility of adding TRAP,[66] and a TRAP/CSP fusion protein conferred sterile protection for 6 months in mice.[67] Concrete evidence of the utility of additional PE antigens would be best achieved by using passive transfer of polyclonal Abs or mAbs. A recent manuscript in preprint has shed some light on this and has demonstrated that anti-TRAP mAbs can significantly improve the protective efficacy of anti-CSP mAbs in both rodent models and liver-humanized mice to above 80% at low doses.[68] However, whether this enhancement is additive or synergistic was not addressed and will be critical for determining the utility of such combinations. Together, these data suggest that combinations of PE mAbs may be a pathway to achieving high levels of protection at serum concentrations achievable over extended periods.

A critical part of *Plasmodium* motility and infection at the PE stage is called traversal, where the parasite actively crosses through host cells as it migrates from the skin to the liver in the vertebrate host. Traversal is also utilized during the mosquito stages when the parasite invades the mosquito midgut and salivary glands. This active process involves several proteins including the cell traversal protein for ookinetes and sporozoites (CelTOS), which is required at multiple stages.[69] This antigen was first isolated in 2003, is highly immunogenic, and is highly conserved across *Plasmodium* species.[70,71] Polyclonal Abs against CelTOS are able to suppress parasite motility, inhibit hepatocyte invasion, and provide sterile protection in rodent models.[72] In addition, passive transfer of anti-CelTOS mAbs has been shown to reduce sporozoite infectivity in mice and decrease oocyst burden in mosquitoes.[73] This suggests that multiple functions of CelTOS can be targeted to disrupt multiple points of *Plasmodium* motility within each host, and this may be achievable with a single mAb.

In summary, with CSP leading the way, future studies of PE Ab targets will require improvement of mAbs against existing targets by way of similar detailed mechanistic studies as have been conducted for CSP. This should be supplemented with the identification of additional Ab targets, as we have only begun to explore the >30 sporozoite surface or secreted proteins potentially accessible to Ab binding.

BLOOD STAGE TARGETED MONOCLONAL ANTIBODIES

The gold standard for a prophylactic anti-malarial or vaccine is "sterile protection," defined as the prevention of blood stage infection. Achieving sterile protection by exclusively targeting the blood stages has proved extremely difficult, as most simply reduce but do not eliminate parasite replication and many blood stage proteins exhibit substantial antigenic polymorphism.[74] Sterile protection via vaccines has been rare and has been achieved in high proportions thus far only with vaccines and monoclonals targeting the PE stages. Given this difficulty in achieving sterile protection, a vaccine or mAb treatment that targets the asexual blood stages of the parasite could either more quickly alleviate or completely prevent symptomatic blood stage infection. Thus, antigens presented by the infective merozoites as well as those expressed on the red blood cells (RBCs) after infection offer appealing targets for mAbs either alone or in combination with PE targets.

The merozoite surface protein 1 (MSP1) complex is critical to the normal progression of the *Pf* life cycle and has been shown to be necessary for both RBC invasion and merozoite egress from infected erythrocytes.[75,76] MSP1 is the most abundant protein on the surface of the merozoite, making it a viable target for vaccine and mAb interventions.[77] It is proteolytically processed as the merozoite matures, resulting in a non-covalently linked complex of the fragments p83, p30, p38, and p42.[78] It has been shown that MSP1 mediates *Pf* merozoite interactions with human erythrocytes, and Abs targeting various fragments can disrupt parasite growth.[79,80] However, the prevalent polymorphisms within certain portions of MSP1 and relatively low levels of protection afforded by MSP1-based vaccines has likely limited enthusiasm for MSP1 as a mAb target, although detailed studies using mAbs targeting conserved epitopes are warranted.

A newer and more promising blood stage target with considerable mAb research is the *Pf* reticulocyte-binding protein homolog 5 (PfRH5). This protein binds the surface receptor basiginin on the erythrocyte membrane and has been shown to be essential to merozoite invasion.[81,82] During invasion, PfRh5 forms a complex with the cysteine-rich protective antigen (CyRPA) and PfRh5 interacting protein (PfRIPR).[83] Both CyRPA and PfRIPR are housed within parasite micronemes and are released during merozoite invasion to facilitate entrance into the erythrocyte via their assembly into a trimeric complex with PfRh5.[83,84] Given the essential nature of this protein to the parasite invasion of RBCs and the association of anti-PfRH5 with protection in field studies, as well as promising data as a vaccine target,[85,86] PfRh5 is leading the field as an mAb target at the blood stage. A variety of potent neutralizing mAbs targeting PfRH5 have been identified, three of which have been demonstrated to be capable of inhibiting merozoite invasion by >95% at low concentrations *in vitro*.[87] This work identified 5A08, an Ab that recognizes a highly immunogenic epitope on PfRh5, which has shed light on the mechanism of anti-PfRh5 mAb inhibition.[87] A more recent study showed that the anti-PfRH5 mAb 2Ac7 can provide sterilizing protection against stringent *Pf* blood stage challenge in non-human primates and also established the *in vitro* growth inhibition assay as predictive of protection *in vivo*.[88]

Still, the concentrations needed to provide protection are too high for direct clinical use, and the potency of anti-PfRh5 mAbs will need to be improved. One path to increasing potency is to disrupt the assembly of the PfRh5/RIPR/CyRPA complex rather than solely targeting PfRH5. This has been achieved using mAbs targeting both Rh5 and CyRPA that prevent the formation of the trimeric complex, and using Abs against PfCyRPA and PfRIPR that act synergistically to reduce merozoite invasion *in vitro*.[89] These results show the need for further research on these antigens and support mAbs targeting the entire complex as a viable path forward in improving the potency of blood stage mAbs.

Of particular concern in malaria-endemic areas is pregnancy-associated malaria (PAM), which threatens 125 million women per year and is a significant cause of maternal and infant mortality.[90] *Pf*-infected erythrocytes are known to sequester in the placenta owing to their ability to bind chondroitin sulfate A (CSA), and it has been shown that the parasite protein VAR2CSA, a member of the *Pf* erythrocyte membrane protein 1 (PfEMP1) family,[91] is upregulated in placental infected erythrocytes.[92,93] One study showed that women with high levels of anti-VAR2CSA immunoglobulin G gave birth to heavier infants and were at a significantly lower risk of delivering low-birth-weight children in comparison with mothers with low levels of circulating Ab.[92] Therefore, VAR2CSA is a logical vaccine target with the potential to protect pregnant women and their children. However, safety considerations have prevented pregnant women from receiving an experimental malaria immunization, let alone one targeted to preventing PAM.[94] Furthermore, antigenic variation in VAR2CSA complicates the development of a VAR2CSA vaccine, especially given the difficulty in developing

such a vaccine in a vulnerable population.[95] This provides an interesting case use for mAbs given their safety profile in pregnant women[96] with the possibility that a broadly neutralizing mAb could be administered during pregnancy, likely with only a single dose, in the absence of an effective vaccine with an anti-PAM component. Such an mAb could even be administered on top of a partially effective vaccine targeting other stages (e.g., RTS,S) to provide additional protection during pregnancy.

In *Pv*, the blood stage parasites are unique in that they infect immature reticulocytes rather than the mature erythrocytes targeted by *Pf*. Thus, there are unique invasion proteins to consider for *Pv* blood stage mAbs, including the erythrocyte-binding ligand family that is essential for *Pv* merozoite entry into the reticulocyte.[97] These proteins contain a cysteine-rich binding domain at the N-terminal region called the Duffy binding-like domain, which is the functional portion of the Duffy binding protein (DBP) ligand. This ligand must engage with the Duffy antigen receptor for chemokines (DARC) expressed on the host reticulocyte membrane surface in order for the parasite to begin invasion.[98] Natural exposure to malaria elicits DBP-specific Abs that inhibit the binding of the parasite[99] and are associated with clinical protection,[100] possibly due to the highly polymorphic capacity of the molecule that allows it to evade the host immune response.[101] Thus, DBP is a logical target for mAb development.

Moreover, an artificial DBPII immunogen consisting of the DARC-binding region II of the protein optimized for functional and non-polymorphic targets[102] was used to produce a panel of mAbs in BALB/c mice. A total of ten of these mAbs showed significant inhibition of parasite invasion *in vitro*.[103] Rawlinson et al. isolated mAbs from volunteers immunized with a PvDBPII vaccine candidate and found a promising mAb, DB9, that inhibits parasite invasion *in vitro* and prevents the binding of five variant alleles of PvDBPII to DARC.[100] Other groups have successfully isolated mAbs to PvDBPII from individuals with natural immunity to *Pv*, which may show enhanced inhibition and can transcend wild-type *Pv* strains.[104,105] Interestingly, mAbs that bind close to or at the DB9 epitope can provide additive inhibition while mAbs that bind different epitopes elsewhere in the PvDBPII molecule are antagonistic.

Pv reticulocyte invasion also requires the interaction between the *Pv* reticulocyte protein (PvRBP) and transferrin receptor on the host reticulocyte.[106,107] Four anti-PvRBP mAbs have been identified thus far that can prevent reticulocyte invasion *in vitro*, therefore providing another encouraging *Pv* blood stage target.[107] The ability of these mAbs to target unique proteins and invasion pathways to work in additivity or synergy, and the impact of any such mAb *in vivo*, will be critical data. However, this is difficult research to conduct given that blood stage culture of the *Pv* blood stages is limited to using fresh field isolates in short-term assays and that *Pv* blood stage challenge *in vivo* is only possible as a CHMI.[108–112] However, a manuscript in preprint at the time of this review suggests that a non-human primate model of *Pv* blood stage infection may be near and thus could fill a

significant gap in the preclinical assessment of *Pv* blood stage mAbs.[113]

MOSQUITO STAGE TARGETED MONOCLONAL ANTIBODIES

The transmission of parasites between the human host and mosquito vector is an appealing target for Abs, as these stages present another bottleneck in the parasite life cycle[37,38,114–117] and display minimal polymorphisms, likely due to the lack of evolutionary pressure by the human immune system,[118–121] and Abs against these stages are especially potent.[122–124] While Abs targeting solely the transmission of an established blood stage infection to mosquitoes offers no direct benefit to the individual, sufficient coverage of a local population with effective transmission-blocking Abs could have drastic effects on the burden of disease,[117,125,126] and preliminary data suggest that they may be readily accepted in affected communities.[127–129] Much of the data available on such transmission-blocking targets concern vaccine development, yet these data have clear applicability to the development of mAbs for passive immunization.

Abs against two proteins expressed on the transmissible gametocyte, Pfs48/45[121,130] and Pfs230,[121,131,132] have demonstrated substantial blocking of parasite development in the mosquito at concentrations as low as 1–3 μg/mL.[121] Notably, one such mAb, TB31F, is currently in a clinical trial (ClinicalTrials.gov ID: NCT04238689) aimed to test the safety and pharmacokinetics of intravenous and subcutaneous administration, down to 0.1 mg/kg. Importantly, antigens on the gametocyte may act synergistically as dual-antigen Ab targets that neutralize the gametocyte prior to fertilization in the mosquito midgut.[132,133] Once the parasite has begun to transition into a zygote and then ookinete, additional proteins, notably Pfs/Pvs25 and Pfs230, are expressed on the surface and can be targeted to prevent subsequent invasion and development within the midgut. These proteins—Pfs25, Pfs230, and Pfs48/45—are the only parasite antigens currently in clinical trials as a transmission-blocking vaccine candidate[125] (and ClinicalTrials.gov ID: NCT04862416). Additional targets of the parasite at early stages of mosquito development include PSOP25, Pbg37, and PfCHT1, which have yet to be validated for human parasite species, but suggest that the list of potential transmission-blocking candidates may be more extensive than those currently being developed as vaccine candidates.[134–137]

In addition to targeting the parasite, an intriguing strategy is to target mosquito proteins involved in parasite transmission. While in its infancy, targeting mosquito proteins is an especially appealing avenue because Abs against these proteins could disrupt transmission in a manner that transcends malaria species and is more resistant to evolutionary circumvention by the parasite itself. To this end, the mosquito midgut protein, AnAPN1, shows considerable promise as a nanoparticle vaccine in animal models that functions by blocking ookinete invasion of the mosquito midgut.[138–140] Another interesting approach targets the other side of mosquito transmission: the saliva proteins that are injected with the parasite during probing. These proteins have a myriad of functions, including immunomodulation during normal probing feeding.[141] Abs against the *Anopheles gambiae* TRIO salivary gland protein can provide partial protection against mosquito bite challenge with multiple *Plasmodium* species and have the potential to work in tandem with anti-sporozoite Abs.[142] Other components of the mosquito saliva, including SAMSP1 and mosGILT, have been shown to affect sporozoite motility to either aid or hinder the progress of the sporozoite,[143,144] suggesting that Abs raised to novel mosquito saliva proteins may be promising avenues for research. This approach is not unique to malaria control efforts, and ideas can be borrowed from strategies being pursued for arboviruses. For example, Abs to proteins in the *Aedes aegypti* saliva may prevent successful infection by flaviviruses[145] and one construct, AGS-v, has recently been shown to be safe and immunogenic in clinical trials.[146] In summary, mAb approaches to malaria need not be limited to classic parasite antigens, and a combination of both "traditional" and novel targets should be pursued to achieve high levels of protection and eradication.

FUTURE OUTLOOK

As with mAbs for many infectious diseases, mAbs for malaria are poised to become a paradigm-shifting intervention. The numerous lines of research in the preceding discussion indicate that they are indeed a promising avenue for clinical intervention at a number of stages across the parasite life cycle, and the small first-in-human trial is encouraging. Yet for malaria and other diseases that overwhelmingly affect people in LMIC, low investment in research paired with the need for a low cost of goods will be a major impediment. The latter is a technological barrier that is likely easier to overcome than the former, which is an impediment of will and interest by wealthy nations and funders. Optimistically, the COVID-19 pandemic has proved that mAbs can be developed faster, are better tolerated, are as efficacious, and are at least as adaptable compared with vaccines and drugs when it comes to battling infectious diseases. The high demand for and apparent profitability of mAbs in the COVID-19 pandemic will hopefully usher in a new wave of interest in improving the production of mAbs at scale and at lower costs for other diseases. With any luck, these accelerated technological advances to reduce cost will coincide with improved mAb efficacy for malaria that increases potency and reduces the dose required to achieve the high threshold of protection needed.

Such improvements in efficacy are likely to come in both detailed and iterative investigations of structure-function biology as has been performed for PfCSP and PfRh5. Yet it is perhaps too optimistic to assume that a single mAb targeting a single epitope will achieve sufficient efficacy to warrant stand-alone use as a prophylactic or therapeutic. Furthermore, it is likely unwise to use such a single-antigen approach given concerns over resistance and breakthrough infection. Therefore, combinations of mAbs that target multiple epitopes and multiple proteins will likely be needed. Yet the utility of such a combinatorial approach has lacked extensive evidence. Indeed, the blood stage anti-RIPR complex Abs have demonstrated efficacy and even synergy *in vitro*,[147,148] and combining PE targets such as CSP and TRAP may hold promise despite mixed results. However, it

remains to be seen whether additional gains can be made from combining mAbs targeting different stages. A major hurdle in developing such multi-stage approaches is that the infection cycle spanning the mosquito, PE, and blood stages is impossible to replicate *in vitro*, let alone in a high-throughput manner. Therefore, each mAb targeting each stage will need to be vetted individually in their respective assays and combined for final assessment *in vivo*. The preclinical model that is best poised to assess multiple stages—mosquito bite challenge of humanized liver mice repopulated with exogenous RBCs[54,149,150]—is tractable but expensive, lacks a gametocyte-transmission component, and is not high throughput. Even CHMI of actively or passively immunized volunteers has yet to be developed for such a multi-stage approach although the PE, blood stages, and transmission can be assessed independently.[151] Even with these limitations, whether the existing *in vitro* and *in vivo* models predict clinical success will require clinical testing of both optimal and suboptimal mAb regimens. This will stretch already limited funds which to date have been reserved for only the safest and most highly promising interventions.

In summary, the path to malaria elimination will require a long-lasting, effective, and simple intervention that can prevent infection in a high proportion of people. If this intervention is based on mAbs, it will require: (1) iterative improvements of mAbs against existing targets that can function at lower doses; (2) identification of novel targets and mechanisms that can be incorporated into next-generation mAb regimens; (3) the identification of additive or synergistic combinations of mAbs that improve efficacy and guard against resistance; (4) the improvement of preclinical assays to assess multi-stage interventions; and (5) simultaneous changes in the production of mAbs to make them affordable for global health use. Thus, while the challenges remain large, the components of a path to the first protective mAb product for malaria has been made clear by the impressive work reviewed here and can become a reality with the addition of sufficient interest, financial investment, and time.

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