



Al Qaraghuli, Mohammed M. and Obeid, Mohammad A. and Aldulaimi, Omar and Ferro, Valerie A. (2017) Control of malaria by bio-therapeutics and drug delivery systems. Journal of Medical Microbiology & Diagnosis. (In Press) ,

This version is available at <https://strathprints.strath.ac.uk/61900/>

Strathprints is designed to allow users to access the research output of the University of Strathclyde. Unless otherwise explicitly stated on the manuscript, Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Please check the manuscript for details of any other licences that may have been applied. You may not engage in further distribution of the material for any profitmaking activities or any commercial gain. You may freely distribute both the url (<https://strathprints.strath.ac.uk/>) and the content of this paper for research or private study, educational, or not-for-profit purposes without prior permission or charge.

Any correspondence concerning this service should be sent to the Strathprints administrator: strathprints@strath.ac.uk

Control of malaria by bio-therapeutics and drug delivery systems

Authors

Mohammed M. Al Qaraghuli^{1, 2, *}, Mohammad A. Obeid^{1, 3}, Omar Aldulaimi^{4,5}, Valerie A. Ferro¹

Affiliations

¹ Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, UK.

² Department of Chemical & Process Engineering, University of Strathclyde, 75 Montrose Street, Glasgow, G1 1XJ, UK.

³ Faculty of Pharmacy, Yarmouk University, Irbid, Jordan.

⁴ College of Pharmacy, Al-Mustansiriyah University, Baghdad, Iraq

⁵ Institute for Science and Technology in Medicine, Keele University, Staffordshire ST5 5BG, United Kingdom

* Corresponding author: Email address: mohammed.al-qaraghuli@strath.ac.uk. Phone: +44 (0)141 548 2176

Abstract

Malaria is an ubiquitous disease that can affect more than 40% of the world's population who live with some risk of contracting this disease. The World Health Organization (WHO) has recently highlighted the high spread of this disease in Sub-Saharan Africa. Despite the considerable fall in mortality rate over the past decade, the development of resistance against main treatment strategies still exists. This problem has provoked scientific efforts to develop various treatment strategies including use of vaccines, drug delivery systems, and bio-therapeutics approaches.

A vaccination strategy is being implemented to trigger direct clearance of the causative parasites from the human host. However, the complex life-cycle of *Plasmodium* parasites with continuous antigenic mutations has partly hindered this approach so far. The application of different types of drug delivery systems for the delivery of anti-malarial drugs is also being considered in order to improve the efficacy, pharmacokinetics, tolerability, and reduce toxicity of existing anti-malarial drugs. A third approach has emerged from the high success of antibodies to treat complex diseases like cancer and autoimmune diseases. Various antibody engineering methods and formats have been proposed to tackle the notable sophisticated life-cycle of malaria.

Within the malaria research field, the characteristics of these diverse treatment strategies, individually, are broadly acknowledged. This review article considers the current status of these approaches and the future outlook.

Key words

Immuno-conjugates, Antibodies, Drug Delivery, Vaccines, Malaria

Introduction

Malaria is an infectious disease that is caused by the parasite *Plasmodium*. This transmittable disease affects around 200 million annually, killing about 650,000 people per year, especially children less than 5 years old living in sub-Saharan Africa [1]. The WHO 2015 Fact Sheet reported that over 15 years (2000-2015), there was a global reduction in malaria incidence rates and mortality by 37% and 60%, respectively. However, the subsequent Fact Sheet in 2016 confirmed the emergence of parasite resistance to antimalarial medicines and mosquito resistance to insecticides, which could trigger a rise in global malaria mortality if ignored.

The five main parasite species in this respect are *P. vivax*, *P. knowlesi*, *P. ovale*, *P. malariae*, and *P. falciparum*; the latter represents the most lethal [2]. The parasite life cycle in humans typically begins by injection of sporozoites *via* the skin, which can then migrate to hepatocytes in less than one hour [3], where they replicate and generate merozoites. These merozoites complete the journey to erythrocytes of the patient (clinical stage), and then differentiate into gametocytes that eventually reach the parasite holder (the mosquito) through infected human blood [4].

Various reports have indicated the growth in malaria mortality rate, due to emergence and spread of multidrug-resistant *P. falciparum* against established antimalarial compounds [5,6]. Moreover, therapeutic failure of some anti-malarial medications has been attributed to their toxic side effects as well as their inconvenient dosing schedules. Therefore, there is an urgent requirement to identify new treatment strategies against malaria [7]. These approaches have been directed towards enhancing the characterisation of natural products, adaptation of effective vaccine and drug delivery strategies, and the development of specific bio-therapeutic agents [8–13]. The main objective of this article is to review the anti-malarial role of bio-

therapeutic formulations, and to evaluate their potential as effective treatments to malaria in the future.

Vaccines and immune-conjugates

Significant efforts have been dedicated over the past decades to develop vaccines that can protect humans against malaria parasites. Vaccine development has been directed to different infection stages including transmission blocking vaccines, pre-erythrocytic vaccines, and blood-stage vaccines; these have been reviewed comprehensively for both *P. falciparum* and *P. Vivax* [14–16]. Generally, vaccines have either been subunits of well-defined and conserved parasite antigens, or whole attenuated sporozoites. The most advanced malaria vaccine (RTS,S: MosquitixTM) is currently in Phase III clinical trial, and contains the conserved central repeat and C-terminal regions of the *P. falciparum* circumsporozoite protein (CSP) that is expressed on sporozoites in early liver stages [17,18]. Despite this advancement, vaccine development against malaria has been dishearteningly hindered by the complex life cycle of the parasites, which results in several morphological changes and displays antigenic variations.

Immuno-conjugation refers to the use of a delivery system to deliver a conjugated drug to facilitate its delivery into a target tissue. An example of this strategy is the delivery of Angiopep-2 conjugated paclitaxel through the use of the low-density lipoprotein receptor-related protein (LRP) as a carrier. This contrasts with the concept of drug delivery systems that can be used with either conjugated or unconjugated drugs [19]. Immuno-conjugation strategies can be used as "Trojan-horses" for specific delivery of antimalarial drugs, to reduce the emergence of resistant strains, and curtail the adverse drug reactions and toxicity of these medicines. This approach is broadly implemented in various medical applications, especially to target cancer cells [19–22]. Generally, anti-malarial conjugates can be ferried to the infected

host cells by parenteral routes through either passive or active targeting [23]. Passive targeting has been accomplished by conventional nano-carriers such as micelles, liposomes and polymerosomes [24–27]. Whilst, active targeting can be achieved by functionalisation of the nano-carriers with specific biomolecules such as antibodies, proteins, or peptides [23].

Considering the peculiarities of erythrocytes, liposomal nanocarriers are premeditated as a promising approach for the targeted delivery of antimalarial drugs [28]. For instance, artemether and lumefantrine were co-loaded into nanostructured lipid carriers, and their antiplasmodial effect was evaluated [29]. Similarly, curcuminoid-loaded liposomes in combination with arteether has prevented the recrudescence of malaria in mice [30]. An advancement to liposomal research was actualised through the introduction of nanomimics based on polymersomes for blocking invasion, and causing augmented exposure of the infective form of *P. falciparum* to the immune system [31]. Moreover, advanced drug delivery systems based on conjugation of, for example, artesunate to nanoerythrocytes have shown controlled delivery to evade drug leakage, improve stability, and reduce cost and toxicity [32]. Passive targeting could also be achieved by surface modification of the nano-carrier with poly(ethyleneglycol) (PEG) to delay phagocytosis, thus prolonging the plasma half-life of the drug, resulting in alteration in the pharmacokinetic profile of the drug [33]. Another conceptualisation has involved the iron uptake systems of microorganisms to deliver siderophore–drug complexes, which are recognised by specific siderophore receptors, and is thereupon actively transported across the outer bacterial membrane [34], and could be useful against malaria [35]. Conjugation of desferrioxamine B to methyl anthranilic acid or nalidixic acid have, for instance, evinced significant effects against multidrug resistant *P. falciparum* [36].

The essential role of cysteine proteases in the malaria parasite is widely appreciated, and both small inhibitors, like leupeptin and vinyl sulphones, and macromolecular inhibitors, such as falstatin expressed in *P. falciparum*, were analysed [37,38]. These promising macromolecule inhibitors are mostly competitive, and utilise loop-like structures to interact with malarial cysteine proteases [39]. A recent example has implemented computational approaches to better understand falcipains structure and ligand binding [40]. It is also essential when new drugs are established to concurrently study resistance processes in order to avoid a seemingly inevitable outcome [41]. The new approach of targeting "hot-spot" protein-protein interactions of macromolecular inhibitor-enzyme complexes is less liable to drug resistance point mutation, and represents a promising field in drug development. These hot spots can also include potential targetable steps in the protein export pathway that are essential for parasite survival [42]. Drug repurposing is another possibility to find approved drugs that could have efficacy against malaria parasites. A recent example is illustrated by the development of the protein farnesyltransferase inhibitors (PFTIs), that block the transfer of a farnesyl group as a post-translational modification onto specific proteins [43]. A panel of PFTIs was tested to inhibit *in vitro* growth of *P. falciparum* parasites, and a series of tetrahydroquinoline (THQ) PFTIs was identified with excellent potency [44].

Delivery systems for anti-malarial drugs

Since the initial conceptualisation of the "magic bullet" principle by Paul Ehrlich, which was based on specifically destroying foreign microbes without harming the human body itself, the drug delivery field has evolved noticeably. Drug delivery is based on using a delivery carrier to carry and release a therapeutic agent to a particular site in the body at a specific rate [45]. Different types of drug delivery systems can be used for this purpose including liposomes, niosomes, lipid nano-emulsions, poly(lactide-co-glycolide) (PLGA), and natural polymers such

as collagen and chitosan [46–48]. The most commonly used delivery systems for the delivery of anti-malarial agents are summarised in Table 1.

Liposomes are the most extensively studied system for the delivery of different therapeutic agents. As lipid based nanoparticles, they are formed by the self-assembly of their lipid components into bilayer structures encapsulating an aqueous moiety. This results in a versatile structure in which hydrophilic drugs can be encapsulated in the inner aqueous core while hydrophobic agents will be embedded in the lipid bilayer structure [49]. Several research groups have investigated the use of liposomal formulations for the delivery of different anti-malarial agents in order to improve their pharmacokinetics or therapeutic index. Gabriels *et al.* (2003) developed a formulation that can improve patient compliance towards artesunate, which is an anti-malarial agent that requires frequent administration due to its rapid elimination, through the use of liposomes [50]. They developed a slow release preparation by encapsulating artesunate into liposomes containing egg-phosphatidylcholine/cholesterol in a molar ratio of 4:3 [50].

Chloroquine (CQ) is an effective anti-malarial drug against all five species of parasites. The activity of CQ is thought to take place in the parasite's acidic digestive vacuole (DV) against the intraerythrocytic stage of the human malaria parasite [51]. However, inside the acidic DV, CQ becomes protonated and less membrane-permeable leading to its accumulation in the DV with subsequent efflux out of the DV, away from its primary site of accumulation and action, and reduction in the anti-malarial activity of CQ [52]. In order to reduce the efflux of CQ from DV, chitosan–tripolyphosphate (CS–TPP) nanoparticles (NPs) were conjugated to CQ and examined in Swiss mice infected with attenuated *P. berghei* [11]. These NPs were demonstrated to act as an effective formulation, eliminating parasites, while protecting lymphocytes, serum and red blood cells against *P. berghei* infection at a dose of 250 mg/kg

body weight for 15 days treatment. Another approach was adopted using galactose coated poly-l-lysine dendrimers loaded with CQ, and haemolytic toxicity was drastically reduced by at least 50% through a sustained drug release behaviour compared to free CQ both *in vitro* and *in vivo* [53].

Primaquine (PQ) is another anti-malarial drug which exerts a broad spectrum activity against various stages of parasitic malaria. PQ targets latent liver stage of malaria infection caused by different plasmodia such as *P. vivax* and *P. ovale* [54]. Moreover, PQ is also prescribed for terminal prophylaxis to prevent infection by *P. falciparum* and *P. vivax*. However, PQ can cause severe tissue toxicity including haematological and gastrointestinal related side effects [55]. PQ targeting of the liver, would possibly help to reduce therapeutic dose and subsequently its dose related toxic effects. Encapsulation of PQ in different delivery systems such as liposomes was initially designed, and shown to significantly increase the LD₅₀ and LD₉₀ in mice, as a result of changing the distribution pattern of PQ after encapsulation [56]. In an attempt to target PQ to hepatocytes, Dierling *et al.* (2005) encapsulated PQ into chylomicron emulsion, with an average particle size of 180 nm, which led to significantly enhanced accumulation of PQ in the liver compared to free PQ [10]. Whilst the *in vitro* anti-leishmanial activity of PQ-loaded polyisohexylcyanoacrylate (PIHCA) NPs showed a 21-fold increase in ED₅₀ compared with free PQ [57]. Moreover, when PQ was incorporated into an oral lipid nanoemulsion, PQ exhibited improved oral bioavailability, and was taken up preferentially by the liver with a drug concentration 45% higher than the free PQ. This resulted in a 25% lower dose required to achieve effective antimalarial activity against a *P. bergheii* infection in Swiss albino mice compared to free oral doses of PQ [58]. Other systems investigated for PQ delivery include dendrimeric NPs [59], poly(lactide) NPs [60], and the use of gum arabic microspheres [61].

Anti-malarial antibodies

Alternatively, the active targeting of malaria parasites can be achieved using antibodies, which has high proven efficacy against cancer and several other autoimmune diseases [62–65]. The antimalarial drug CQ showed improved efficacy when delivered inside immunoliposomes targeted with the pRBC-specific monoclonal antibody BM1234 [28]. Likewise, CQ-loaded MAb F10-liposomes were able to clear not only CQ-susceptible, but also CQ-resistant parasites in mice [66]. Antibodies are glycoproteins belonging to the immunoglobulin (Ig) superfamily, and have been widely used in different biomedical applications. The antibody molecule is structurally composed of two heavy and two light polypeptide chains, linked together by disulphide bonds [67]. One light chain type (λ or κ) can be linked to one heavy chain (μ , δ , γ_{1-4} , α_{1-2} , or ϵ) to create any of the nine antibody subclasses in humans (IgM, IgD, IgG₁₋₄, IgA₁₋₂, or IgE) [68–72]. Functionally, an antibody consists of three fragments: a fragment crystallisable region (Fc) that represents the stem of the "Y" shaped molecule, and two fragment antigen-binding (Fab) regions (Figure 1A). While the Fab fragments are responsible for antigen binding, the Fc fragment interacts with other elements of the immune system including Fc-receptors (FcRs), pattern recognition receptors (PRR), and components of the complement cascade, to promote removal of the antigen [73,74]. Within the Fab region, each of the variable heavy (V_H) or light (V_L) chains consist of three complementarity determining regions (CDRs), which are accountable for antigen recognition [75].

Antibodies are prominent immune modulators that bridge innate and acquired immunity, and therefore, can be effective against micro-organisms, if they do not mediate a direct biological effect within the infection process [76]. This perception has sustained their candidacy to combat malaria by, for instance, curtailing the damage associated with any inappropriate host inflammatory responses [77]. The role of antibodies in malaria protection can also be attributed

to inhibition of merozoite invasion of erythrocytes [78], antibody-mediated phagocytosis through FcR and complement pathways [79], and antibody-dependent cellular inhibition [80,81]. Both autoantibodies and antibody immune complexes can drive B-cell responses, through the PRR toll-like receptor-9, and support their potential in malaria [82]. Several years of repeated infections are, however, required to develop protective responses to malaria [83], in defiance of the critical importance of humoral immunity in the development of acquired immunity to malaria [84,85]. Variation of surface antigens and antigenic diversity facilitates the development of recurrent infections over the years, as new infections seem to exploit gaps in the repertoire of variant-specific antibodies [84,86]. *P. falciparum* expressed antigens on erythrocyte surfaces, for instance, appear to be highly polymorphic and undergo clonal antigenic diversity, and antibodies against these antigens typically inaugurate a high degree of strain specificity [87,88].

Previous studies have acknowledged the fact that upon exposure to a new malaria infection, parasite-specific antibody levels rise noticeably within 1-2 weeks [89,90]. The boosted antibodies then reduce quickly after the infection is controlled, and accordingly signify that protective memory for a specific antibody response is either not provoked or is being debilitated [91]. Passive transfer of IgG from immune African adults to African children was observed to be highly effective against malaria parasites [80,92]. Furthermore, transfer of serum from partially immune individuals to non-immune persons induces significant anti-malarial activity [92,93]. This anti-malarial response was verified to be associated with malaria specific antibodies [94,95]. Nevertheless, serum therapy is notoriously correlated with high difficulty of finding a sufficient number of donors, possibility of transferring other infectious diseases, and the impracticality of dealing with human blood products. In addition, sera normally consists of polyclonal antibodies, which might contain numerous nonspecific antibodies [96,97]. Consequently, serum treatment is associated with several limitations, and

adoption of a bespoke antibody engineering approach is essential to match the sophisticated life cycle of this parasite and the scale of this ubiquitous disease.

Amongst the four IgG subclasses, anti-malarial protective antibodies are restricted to a panel of IgG1 and IgG3 subclasses [81]. The IgG2 subclass can compete with IgG1 and IgG3, and interfere with their protection effectiveness [98], although others have suggested IgG2 antibodies participate in protection if individuals possess a rare mutated allele encoding an Fc gamma receptor-type IIA (Fc γ RIIA) that can bind IgG2, IgG3, and IgG1 subclasses [99]. On the other hand, IgG4 antibodies are considered as completely non-protective [98,100–102]. Subsequently, the IgG3 subclass is epitomised as the prevailing isotype of antibody responses incarnated with protection against malaria [101–103]. The propagated antibodies were primarily of the IgG2a and IgG3 subclasses [104,105]. In addition, immunisation with an antigen preparation derived from *P. falciparum* merozoite surface protein (MSP)-1 has induced a shift to IgG2b [106], even though most protein antigens in a murine model are expected to induce IgG1 antibodies. Interestingly, mouse IgG2b is to a certain degree the equivalent of human IgG3 [107], and has a shorter half-life than other mouse IgG subclasses [108]. Consequently, a human vaccine aimed at eliciting antibody protection against blood-stage *P. falciparum* would preferentially generate IgG1 and/or IgG3 antibody responses against the selected candidate antigens, and downregulate a concomitant IgG4 and IgG2 antibody response. Therefore, an anti-malarial vaccine should ideally be administered in combination with an adjuvant that stimulates the production of cytokines, such as interleukin (IL)-10 and/or transforming growth factor (TGF)- β [109,110], in target cells to switch Ig responses to IgG1 and IgG3.

Along with IgG class, other Ig classes were explored to envisage whether infection with *Plasmodium* parasites can be preferentially inhibited. The therapeutic inappropriateness of IgE

antibodies to treat malaria was commonly suggested, due to their observed role in malaria pathogenesis [111,112]. Nevertheless, a reduced risk of subsequent malaria infection was also linked to the existence of high levels of parasite-specific IgE antibodies [113]. Pentameric IgM antibodies were additionally implemented as an adjuvant for malaria vaccine development, through their ability to stimulate the development of acquired T-cell immunity [114]. Whilst the ability to steer IgA antibodies to target Fc α R have shown remarkable potential in eliminating serum pathogens [115]. Re-appraisal of the role of IgA in malarial infections is necessary, since *Plasmodium*-specific IgA antibodies were detected at high levels in humans breast milk [116,117] and serum [118].

Different antibody formats can be accoutred to neutralise *Plasmodium* parasites, ranging from a full monoclonal antibody (mAb) to smaller fragments including Fab, a single chain antibody (scFv), or even a single domain antibody (sdAb) (Figures 1 and 2). Whole mAbs are time-honoured bio-therapeutic molecules, through their ability to maximise the benefits of activating the cellular response by Fc regions [119]. In the murine malaria model, the recruitment of effector cells by Fc is vital, as the passive transfer of specific antibodies to malarial MSP1 could not impede death in FcR-deficient and immunodeficient models [81,120]. However, the utilisation of mAbs in malaria might be inappropriate *per se*, especially if these antibodies interact with the incongruously inhibitory FcRs [115]. Moreover, high concentrations of anti-malarial mAbs are requisite to compete for FcRs binding with infection induced low-affinity polyclonal antibodies [121]. These low-affinity antibodies were developed against short highly repetitive amino acid sequences, cross-reactive with several malarial antigens, and might be generated from a process of immune evasion [122].

In order to develop a “magic bullet” that would specifically neutralise and eradicate invading microbes, like malaria parasites, various antibody engineering approaches and formats have

been investigated. This includes bispecific antibodies (BsAbs) that were developed to recognise both *P. yoelii* MSP1 and human Fc γ R1 [9]. Another bispecific scFv combination, linked by a flexible peptide linker (Gly₄-Ser)₃, has been developed to target *P. falciparum* blood-stage malaria parasites, by linking CD3 antigen of human T-cells and MSP1 [123]. Even a trispecific antibody has been developed in the malaria field, as previously involved in cancer treatment development, to link two potential targets of malaria [merozoite surface protein 1 (MSP1) and malarial Apical Membrane Antigen-1 (AMA1)] with FCR [9,124]. An alternative antibody format, which has been extensively used in malarial research, is the binding “arm” Fab fragment. The comprehensive search for anti-malarial antibodies in the Protein Data Bank (PDB) has retrieved eleven mouse Fabs that were developed against different malaria targets (Table 2). The smallest binding domains, camelids (VHH) and shark (VNAR) sdAbs (Figure 1 C and D), can also be used to neutralise malaria parasites since they are highly acclaimed to bind cryptic epitopes [125–127]. These cryptic cavities and clefts are secluded to full mAbs due to steric hindrance, and therefore, can be conveniently accessed by smaller sdAbs (Figure 2). The selection and affinity maturation of two shark VNARs (PDB ID: 1VES and 1VER) targeting *P. falciparum* AMA1 were developed for diagnostic applications [128], as summarised in Table 2. Unusually, CDR3 of the 1VES sdAb has displayed an extended-hairpin structure (Figure 2), which has indulged this sdAb with a distinct selective advantage in accessing cryptic epitopes [129]. To achieve a comparable objective, camel VHH sdAb (PDB ID: 4GFT) was generated to target MyoA-binding domain (D3) of *P. falciparum* myosin tail interaction protein (MTIP) [130]. This sdAb binds favourably to an area that is slightly overlapping with the MyoA binding groove, and impedes MyoA binding by MTIP. Antibodies have been thoroughly investigated in targeting specific malarial antigens and antimalarial drugs for both therapeutic [131–137] and diagnostic [138–141] purposes. Moreover, antibodies possess high potential to deliver anti-malarial drugs directly to parasites, thus reducing the risk

of adverse drug reactions. However, the exploitation of antibodies with respect to this concept remains not fully explored, and requires further pursuance in the future.

Future perspectives

Malaria is a highly infectious disease that has diminished the lives of millions around the globe. Treatment strategies to date are based on either natural/synthetic small molecules, or macromolecules such as vaccines and antibodies. Most treatment approaches have been hindered by the complex life-cycle of the parasite that has continuously caused the emergence of drug-resistant species. Despite this unprecedented difficulty, several promising drug delivery approaches, vaccines, and antibody formats have been developed to tackle this fatal disease. Future research should be directed to find new antimalarial candidates with either new mechanisms of action, resistance modifying actions or target novel metabolic pathways that are essential for parasite survival and applying new tools for designing these drugs. In addition, more novel combinations of small molecules or micro-macro complexes should be implemented as combination strategies or antibody-small molecule drug conjugates to synergise the treatment effect. In order to achieve this objective, additional funding is required to support the drug discovery process academically, and to attract pharmaceutical companies to invest within this highly pandemic, but not very commercially-attractive field.

References

- [1] Patarroyo ME, Patarroyo MA, Pabón L, Curtidor H, Poloche LA. Immune protection-inducing protein structures (IMPIPS) against malaria: the weapons needed for beating Odysseus. *Vaccine* 2015;33:7525–37. doi:10.1016/j.vaccine.2015.09.109.
- [2] Grimberg BT, Mehlotra RK. Expanding the Antimalarial Drug Arsenal-Now, But How? *Pharm Basel Switz* 2011;4:681–712. doi:10.3390/ph4050681.
- [3] Biamonte MA, Wanner J, Le Roch KG. Recent advances in malaria drug discovery. *Bioorg Med Chem Lett* 2013;23:2829–43. doi:10.1016/j.bmcl.2013.03.067.
- [4] Batista R, Silva A de J, de Oliveira AB. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. *Mol Basel Switz* 2009;14:3037–72. doi:10.3390/molecules14083037.
- [5] Guantai E, Chibale K. How can natural products serve as a viable source of lead compounds for the development of new/novel anti-malarials? *Malar J* 2011;10 Suppl 1:S2. doi:10.1186/1475-2875-10-S1-S2.
- [6] Ginsburg H, Deharo E. A call for using natural compounds in the development of new antimalarial treatments – an introduction. *Malar J* 2011;10:S1. doi:10.1186/1475-2875-10-S1-S1.
- [7] Sinha S, Medhi B, Sehgal R. Challenges of drug-resistant malaria. *Parasite Paris Fr* 2014;21:61. doi:10.1051/parasite/2014059.
- [8] Garraud O, Mahanty S, Perraut R. Malaria-specific antibody subclasses in immune individuals: a key source of information for vaccine design. *Trends Immunol* 2003;24:30–5.
- [9] Pleass RJ, Holder AA. Antibody-based therapies for malaria. *Nat Rev Microbiol* 2005;3:893–9. doi:10.1038/nrmicro1267.
- [10] Dierling AM, Cui Z. Targeting primaquine into liver using chylomicron emulsions for potential vivax malaria therapy. *Int J Pharm* 2005;303:143–52. doi:10.1016/j.ijpharm.2005.07.015.
- [11] Tripathy S, Das S, Chakraborty SP, Sahu SK, Pramanik P, Roy S. Synthesis, characterization of chitosan–tripolyphosphate conjugated chloroquine nanoparticle and its in vivo anti-malarial efficacy against rodent parasite: A dose and duration dependent approach. *Int J Pharm* 2012;434:292–305. doi:10.1016/j.ijpharm.2012.05.064.
- [12] Aldulaimi O, Uche FI, Hameed H, Mbye H, Ullah I, Drijfhout F, et al. A characterization of the antimalarial activity of the bark of *Cylicodiscus gabunensis* Harms. *J Ethnopharmacol* 2017. doi:10.1016/j.jep.2017.01.014.
- [13] Aldulaimi O, Li W, Li W, Li W. Fingerprint Of Tiger Balm® By Thermal Desorption Gas Chromatography Mass Spectroscopy. *Pharmacogn J* 2016;8:230–3. doi:10.5530/pj.2016.3.9.

- [14] Mueller I, Shakri AR, Chitnis CE. Development of vaccines for *Plasmodium vivax* malaria. *Vaccine* 2015;33:7489–95. doi:10.1016/j.vaccine.2015.09.060.
- [15] Long CA, Zavala F. Malaria vaccines and human immune responses. *Curr Opin Microbiol* 2016;32:96–102. doi:10.1016/j.mib.2016.04.006.
- [16] Arama C, Troye-Blomberg M. The path of malaria vaccine development: challenges and perspectives. *J Intern Med* 2014;275:456–66. doi:10.1111/joim.12223.
- [17] Agnandji ST, Lell B, Soulanoudjingar SS, Fernandes JF, Abossolo BP, Conzelmann C, et al. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med* 2011;365:1863–75. doi:10.1056/NEJMoa1102287.
- [18] 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. *The Lancet* 2015;386:31–45. doi:10.1016/S0140-6736(15)60721-8.
- [19] Mazza M, Uchegbu IF, Schätzlein AG. Cancer and the blood–brain barrier: ‘Trojan horses’ for courses?’ *Br J Pharmacol* 2008;155:149–51. doi:10.1038/bjp.2008.274.
- [20] Ben-Jacob E. Engineering Trojan-horse bacteria to fight cancer. *Blood* 2013;122:619–20. doi:10.1182/blood-2013-06-508481.
- [21] Tazzyman S, Muthana M, Harrison J, Lewis C, Conner J, Chantry A. Development of a Trojan horse oncolytic virus for treatment of myeloma. *ASCO Meet Abstr* 2015;33:e14035.
- [22] Fenollosa R, Garcia-Rico E, Alvarez S, Alvarez R, Yu X, Rodriguez I, et al. Silicon particles as trojan horses for potential cancer therapy. *J Nanobiotechnology* 2014;12:35. doi:10.1186/s12951-014-0035-7.
- [23] Thakkar M, S B. Combating malaria with nanotechnology-based targeted and combinatorial drug delivery strategies. *Drug Deliv Transl Res* 2016;6:414–25. doi:10.1007/s13346-016-0290-2.
- [24] Vauthier C, Couvreur P. Nanomedicines: A New Approach for the Treatment of Serious Diseases. *J Biomed Nanotechnol* 2007;3:223–34. doi:10.1166/jbn.2007.038.
- [25] Barratt G. Colloidal drug carriers: achievements and perspectives. *Cell Mol Life Sci CMLS* 2003;60:21–37.
- [26] Immordino ML, Dosio F, Cattel L. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. *Int J Nanomedicine* 2006;1:297–315.
- [27] Gref R, Domb A, Quellec P, Blunk T, Müller RH, Verbavatz JM, et al. The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. *Adv Drug Deliv Rev* 2012;64, Supplement:316–26. doi:10.1016/j.addr.2012.09.008.
- [28] Urbán P, Estelrich J, Adeva A, Cortés A, Fernández-Busquets X. Study of the efficacy of antimalarial drugs delivered inside targeted immunoliposomal nanovectors. *Nanoscale Res Lett* 2011;6:620. doi:10.1186/1556-276X-6-620.

- [29] Parashar D, P AN, R MRS. Development of artemether and lumefantrine co-loaded nanostructured lipid carriers: physicochemical characterization and in vivo antimalarial activity. *Drug Deliv* 2016;23:123–9. doi:10.3109/10717544.2014.905883.
- [30] Aditya NP, Chimote G, Gunalan K, Banerjee R, Patankar S, Madhusudhan B. Curcuminoids-loaded liposomes in combination with arteether protects against *Plasmodium berghei* infection in mice. *Exp Parasitol* 2012;131:292–9. doi:10.1016/j.exppara.2012.04.010.
- [31] Najer A, Wu D, Bieri A, Brand F, Palivan CG, Beck H-P, et al. Nanomimics of host cell membranes block invasion and expose invasive malaria parasites. *ACS Nano* 2014;8:12560–71. doi:10.1021/nn5054206.
- [32] Agnihotri J, Saraf S, Singh S, Bigoniya P. Development and evaluation of anti-malarial bio-conjugates: artesunate-loaded nanoerythrocytes. *Drug Deliv Transl Res* 2015;5:489–97. doi:10.1007/s13346-015-0246-y.
- [33] Torchilin VP. Multifunctional nanocarriers. *Adv Drug Deliv Rev* 2012;64, Supplement:302–15. doi:10.1016/j.addr.2012.09.031.
- [34] Górska A, Sloderbach A, Marszał MP. Siderophore–drug complexes: potential medicinal applications of the ‘Trojan horse’ strategy. *Trends Pharmacol Sci* 2014;35:442–9. doi:10.1016/j.tips.2014.06.007.
- [35] Smith HJ, Meremikwu M. Iron chelating agents for treating malaria. *Cochrane Database Syst Rev* 2003:CD001474. doi:10.1002/14651858.CD001474.
- [36] Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P. Microbial siderophores and their potential applications: a review. *Environ Sci Pollut Res Int* 2016;23:3984–99. doi:10.1007/s11356-015-4294-0.
- [37] Pandey KC, Singh N, Arastu-Kapur S, Bogyo M, Rosenthal PJ. Falstatin, a cysteine protease inhibitor of *Plasmodium falciparum*, facilitates erythrocyte invasion. *PLoS Pathog* 2006;2:e117. doi:10.1371/journal.ppat.0020117.
- [38] Moura PA, Dame JB, Fidock DA. Role of *Plasmodium falciparum* Digestive Vacuole Plasmepsins in the Specificity and Antimalarial Mode of Action of Cysteine and Aspartic Protease Inhibitors. *Antimicrob Agents Chemother* 2009;53:4968–78. doi:10.1128/AAC.00882-09.
- [39] Pandey KC. Macromolecular inhibitors of malarial cysteine proteases —An invited review. *J Biomed Sci Eng* 2013;2013. doi:10.4236/jbise.2013.69108.
- [40] Boris D, Bekono FN-K. Targeting Cysteine Proteases from *Plasmodium falciparum*: A General Overview, Rational Drug Design and Computational Approaches for Drug Discovery. [Http://WwwEurekaselectCom](http://WwwEurekaselectCom) n.d. <http://www.eurekaselect.com/148601/article> (accessed March 12, 2017).
- [41] Duru V, Witkowski B, Ménard D. *Plasmodium falciparum* Resistance to Artemisinin Derivatives and Piperaquine: A Major Challenge for Malaria Elimination in Cambodia. *Am J Trop Med Hyg* 2016;95:1228–38. doi:10.4269/ajtmh.16-0234.

- [42] Gilson PR, Chisholm SA, Crabb BS, de Koning-Ward TF. Host cell remodelling in malaria parasites: a new pool of potential drug targets. *Int J Parasitol* 2017;47:119–27. doi:10.1016/j.ijpara.2016.06.001.
- [43] Klug DM, Gelb MH, Pollastri MP. Repurposing strategies for tropical disease drug discovery. *Bioorg Med Chem Lett* 2016;26:2569–76. doi:10.1016/j.bmcl.2016.03.103.
- [44] Nallan L, Bauer KD, Bendale P, Rivas K, Yokoyama K, Hornéy CP, et al. Protein Farnesyltransferase Inhibitors Exhibit Potent Antimalarial Activity. *J Med Chem* 2005;48:3704–13. doi:10.1021/jm0491039.
- [45] Obeid MA, Gebril AM, Tate RJ, Mullen AB, Ferro VA. Comparison of the Physical Characteristics of Monodisperse Non-ionic Surfactant Vesicles (NISV) Prepared Using Different Manufacturing Methods. *Int J Pharm* 2017. doi:10.1016/j.ijpharm.2017.02.007.
- [46] Al Qaraghuli M, Alzahrani A, Niwasabutra K, Obeid M, Ferro V. Where Traditional Drug Discovery Meets Modern Technology in the Quest for New Drugs. *Ann Pharmacol Pharm* 2017;11:1061.
- [47] Obeid MA, Khadra I, Mullen AB, Tate RJ, Ferro VA. The effects of hydration media on the characteristics of non-ionic surfactant vesicles (NISV) prepared by microfluidics. *Int J Pharm* 2017;516:52–60. doi:10.1016/j.ijpharm.2016.11.015.
- [48] Obeid MA, Elburi A, Young LC, Mullen AB, Tate RJ, Ferro VA. Formulation of Nonionic Surfactant Vesicles (NISV) Prepared by Microfluidics for Therapeutic Delivery of siRNA into Cancer Cells. *Mol Pharm* 2017;14:2450–8. doi:10.1021/acs.molpharmaceut.7b00352.
- [49] Gregoriadis G. The carrier potential of liposomes in biology and medicine (second of two parts). *N Engl J Med* 1976;295:765–70. doi:10.1056/NEJM197609302951406.
- [50] Gabriëls M, Plaizier-Vercammen J. Physical and chemical evaluation of liposomes, containing artesunate. *J Pharm Biomed Anal* 2003;31:655–67.
- [51] Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, et al. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol Cell* 2000;6:861–71.
- [52] Lehane AM, Kirk K. Efflux of a range of antimalarial drugs and “chloroquine resistance reversers” from the digestive vacuole in malaria parasites with mutant PfCRT. *Mol Microbiol* 2010;77:1039–51. doi:10.1111/j.1365-2958.2010.07272.x.
- [53] Agrawal P, Gupta U, Jain NK. Glycoconjugated peptide dendrimers-based nanoparticulate system for the delivery of chloroquine phosphate. *Biomaterials* 2007;28:3349–59. doi:10.1016/j.biomaterials.2007.04.004.
- [54] Baird JK, Hoffman SL. Primaquine Therapy for Malaria. *Clin Infect Dis* 2004;39:1336–45. doi:10.1086/424663.
- [55] Baird JK, Rieckmann KH. Can primaquine therapy for vivax malaria be improved? *Trends Parasitol* 2003;19:115–20.

- [56] Pirson P, Steiger RF, Trouet A, Gillet J, Herman F. Primaquine liposomes in the chemotherapy of experimental murine malaria. *Ann Trop Med Parasitol* 1980;74:383–91.
- [57] Gaspar R, Opperdoes FR, Pr at V, Roland M. Drug targeting with polyalkylcyanoacrylate nanoparticles: in vitro activity of primaquine-loaded nanoparticles against intracellular *Leishmania donovani*. *Ann Trop Med Parasitol* 1992;86:41–9.
- [58] Singh KK, Vingkar SK. Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine. *Int J Pharm* 2008;347:136–43. doi:10.1016/j.ijpharm.2007.06.035.
- [59] Bhadra D, Yadav AK, Bhadra S, Jain NK. Glycodendrimeric nanoparticulate carriers of primaquine phosphate for liver targeting. *Int J Pharm* 2005;295:221–33. doi:10.1016/j.ijpharm.2005.01.026.
- [60] Rodrigues JM, Fessi H, Bories C, Puisieux F, Devissaguet J -ph. Primaquine-loaded poly(lactide) nanoparticles: physicochemical study and acute tolerance in mice. *Int J Pharm* 1995;126:253–60. doi:10.1016/0378-5173(95)04135-4.
- [61] Nishi KK, Jayakrishnan A. Preparation and in vitro evaluation of primaquine-conjugated gum arabic microspheres. *Biomacromolecules* 2004;5:1489–95. doi:10.1021/bm0499435.
- [62] Sela M. Immunomodulatory vaccines against autoimmune diseases. *Rejuvenation Res* 2006;9:126–33. doi:10.1089/rej.2006.9.126.
- [63] Anderson RP, Jabri B. Vaccine against autoimmune disease: antigen-specific immunotherapy. *Curr Opin Immunol* 2013;25:410–7. doi:10.1016/j.coi.2013.02.004.
- [64] Melief CJM, Hall T van, Arens R, Ossendorp F, Burg SH van der. Therapeutic cancer vaccines. *J Clin Invest* 2015;125:3401–12. doi:10.1172/JCI80009.
- [65] Guo C, Manjili MH, Subjeck JR, Sarkar D, Fisher PB, Wang X-Y. Therapeutic Cancer Vaccines: Past, Present and Future. *Adv Cancer Res* 2013;119:421–75. doi:10.1016/B978-0-12-407190-2.00007-1.
- [66] Owais M, Varshney GC, Choudhury A, Chandra S, Gupta CM. Chloroquine encapsulated in malaria-infected erythrocyte-specific antibody-bearing liposomes effectively controls chloroquine-resistant *Plasmodium berghei* infections in mice. *Antimicrob Agents Chemother* 1995;39:180–4.
- [67] Edelman GM. Dissociation of γ -globulin. *J Am Chem Soc* 1959;81:3155–6. doi:10.1021/ja01521a071.
- [68] Woof JM, Burton DR. Human antibody-Fc receptor interactions illuminated by crystal structures. *Nat Rev Immunol* 2004;4:89–99. doi:10.1038/nri1266.
- [69] Preud'homme JL, Petit I, Barra A, Morel F, Lecron JC, Leli vre E. Structural and functional properties of membrane and secreted IgD. *Mol Immunol* 2000;37:871–87.

- [70] Monteiro RC, Van De Winkel JGJ. IgA Fc receptors. *Annu Rev Immunol* 2003;21:177–204. doi:10.1146/annurev.immunol.21.120601.141011.
- [71] Gould HJ, Sutton BJ, Bevil AJ, Bevil RL, McCloskey N, Coker HA, et al. The biology of IGE and the basis of allergic disease. *Annu Rev Immunol* 2003;21:579–628. doi:10.1146/annurev.immunol.21.120601.141103.
- [72] Boes M. Role of natural and immune IgM antibodies in immune responses. *Mol Immunol* 2000;37:1141–9.
- [73] Porter RR. The hydrolysis of rabbit γ -globulin and antibodies with crystalline papain. *Biochem J* 1959;73:119–26.
- [74] Ryle AP, Porter RR. Parapepsins: two proteolytic enzymes associated with porcine pepsin. *Biochem J* 1959;73:75–86.
- [75] Wu TT, Kabat EA. An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity. *J Exp Med* 1970;132:211–50.
- [76] Casadevall A, Pirofski L. Antibody-mediated regulation of cellular immunity and the inflammatory response. *Trends Immunol* 2003;24:474–8.
- [77] Samuelsson A, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* 2001;291:484–6. doi:10.1126/science.291.5503.484.
- [78] Quinn TC, Wyler DJ. Mechanisms of action of hyperimmune serum in mediating protective immunity to rodent malaria (*Plasmodium berghei*). *J Immunol Baltim Md* 1950 1979;123:2245–9.
- [79] Pleass RJ, Ogun SA, McGuinness DH, van de Winkel JGJ, Holder AA, Woof JM. Novel antimalarial antibodies highlight the importance of the antibody Fc region in mediating protection. *Blood* 2003;102:4424–30. doi:10.1182/blood-2003-02-0583.
- [80] Bouharoun-Tayoun H, Attanath P, Sabchareon A, Chongsuphajaisiddhi T, Druilhe P. Antibodies that protect humans against *Plasmodium falciparum* blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes. *J Exp Med* 1990;172:1633–41.
- [81] Bouharoun-Tayoun H, Oeuvray C, Lunel F, Druilhe P. Mechanisms underlying the monocyte-mediated antibody-dependent killing of *Plasmodium falciparum* asexual blood stages. *J Exp Med* 1995;182:409–18.
- [82] Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin–IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 2002;416:603–7. doi:10.1038/416603a.
- [83] Garnham PCC. Malarial immunity in Africans; effects in infancy and early childhood. *Ann Trop Med Parasitol* 1949;43:47–61.

- [84] Bull PC, Marsh K. The role of antibodies to *Plasmodium falciparum*-infected-erythrocyte surface antigens in naturally acquired immunity to malaria. *Trends Microbiol* 2002;10:55–8.
- [85] Osier FHA, Fegan G, Polley SD, Murungi L, Verra F, Tetteh KKA, et al. Breadth and magnitude of antibody responses to multiple *Plasmodium falciparum* merozoite antigens are associated with protection from clinical malaria. *Infect Immun* 2008;76:2240–8. doi:10.1128/IAI.01585-07.
- [86] Marsh K, Howard RJ. Antigens induced on erythrocytes by *P. falciparum*: expression of diverse and conserved determinants. *Science* 1986;231:150–3.
- [87] Scherf A, Lopez-Rubio JJ, Riviere L. Antigenic variation in *Plasmodium falciparum*. *Annu Rev Microbiol* 2008;62:445–70. doi:10.1146/annurev.micro.61.080706.093134.
- [88] Guizetti J, Scherf A. Silence, activate, poise and switch! Mechanisms of antigenic variation in *Plasmodium falciparum*. *Cell Microbiol* 2013;15:718–26. doi:10.1111/cmi.12115.
- [89] Kinyanjui SM, Bull P, Newbold CI, Marsh K. Kinetics of antibody responses to *Plasmodium falciparum*-infected erythrocyte variant surface antigens. *J Infect Dis* 2003;187:667–74. doi:10.1086/373994.
- [90] Ofori MF, Dodoo D, Staalsoe T, Kurtzhals JAL, Koram K, Theander TG, et al. Malaria-induced acquisition of antibodies to *Plasmodium falciparum* variant surface antigens. *Infect Immun* 2002;70:2982–8.
- [91] Soe S, Theisen M, Roussilhon C, Aye K-S, Druilhe P. Association between protection against clinical malaria and antibodies to merozoite surface antigens in an area of hyperendemicity in Myanmar: complementarity between responses to merozoite surface protein 3 and the 220-kilodalton glutamate-rich protein. *Infect Immun* 2004;72:247–52.
- [92] Cohen S, McGREGOR IA, Carrington S. Gamma-Globulin and Acquired Immunity to Human Malaria. *Nature* 1961;192:733–7. doi:10.1038/192733a0.
- [93] Sabchareon A, Burnouf T, Ouattara D, Attanath P, Bouharoun-Tayoun H, Chantavanich P, et al. Parasitologic and clinical human response to immunoglobulin administration in *falciparum* malaria. *Am J Trop Med Hyg* 1991;45:297–308.
- [94] Roussilhon C, Oeuvray C, Müller-Graf C, Tall A, Rogier C, Trape J-F, et al. Long-Term Clinical Protection from *Falciparum* Malaria Is Strongly Associated with IgG3 Antibodies to Merozoite Surface Protein 3. *PLoS Med* 2007;4. doi:10.1371/journal.pmed.0040320.
- [95] Perraut R, Marrama L, Diouf B, Sokhna C, Tall A, Nabeth P, et al. Antibodies to the conserved C-terminal domain of the *Plasmodium falciparum* merozoite surface protein 1 and to the merozoite extract and their relationship with in vitro inhibitory antibodies and protection against clinical malaria in a Senegalese village. *J Infect Dis* 2005;191:264–71. doi:10.1086/426398.
- [96] Uthaipibull C, Aufiero B, Syed SE, Hansen B, Guevara Patiño JA, Angov E, et al. Inhibitory and blocking monoclonal antibody epitopes on merozoite surface protein 1 of

- the malaria parasite *Plasmodium falciparum*. *J Mol Biol* 2001;307:1381–94. doi:10.1006/jmbi.2001.4574.
- [97] Guevara Patiño JA, Holder AA, McBride JS, Blackman MJ. Antibodies that inhibit malaria merozoite surface protein-1 processing and erythrocyte invasion are blocked by naturally acquired human antibodies. *J Exp Med* 1997;186:1689–99.
- [98] Bouharoun-Tayoun H, Druilhe P. *Plasmodium falciparum* malaria: evidence for an isotype imbalance which may be responsible for delayed acquisition of protective immunity. *Infect Immun* 1992;60:1473–81.
- [99] Aucan C, Traoré Y, Tall F, Nacro B, Traoré-Leroux T, Fumoux F, et al. High Immunoglobulin G2 (IgG2) and Low IgG4 Levels Are Associated with Human Resistance to *Plasmodium falciparum* Malaria. *Infect Immun* 2000;68:1252–8.
- [100] Rzepczyk CM, Hale K, Woodroffe N, Bobogare A, Csurhes P, Ishii A, et al. Humoral immune responses of Solomon Islanders to the merozoite surface antigen 2 of *Plasmodium falciparum* show pronounced skewing towards antibodies of the immunoglobulin G3 subclass. *Infect Immun* 1997;65:1098–1100.
- [101] Taylor RR, Allen SJ, Greenwood BM, Riley EM. IgG3 antibodies to *Plasmodium falciparum* merozoite surface protein 2 (MSP2): increasing prevalence with age and association with clinical immunity to malaria. *Am J Trop Med Hyg* 1998;58:406–13.
- [102] Oeuvray C, Bouharoun-Tayoun H, Gras-Masse H, Bottius E, Kaidoh T, Aikawa M, et al. Merozoite surface protein-3: a malaria protein inducing antibodies that promote *Plasmodium falciparum* killing by cooperation with blood monocytes. *Blood* 1994;84:1594–602.
- [103] Morell A, Terry WD, Waldmann TA. Metabolic properties of IgG subclasses in man. *J Clin Invest* 1970;49:673–80.
- [104] Rotman HL, Daly TM, Clynes R, Long CA. Fc receptors are not required for antibody-mediated protection against lethal malaria challenge in a mouse model. *J Immunol Baltim Md 1950* 1998;161:1908–12.
- [105] Cavinato RA, Bastos KR, Sardinha LR, Elias RM, Alvarez JM, d'Império Lima MR. Susceptibility of the different developmental stages of the asexual (schizogonic) erythrocyte cycle of *Plasmodium chabaudi chabaudi* to hyperimmune serum, immunoglobulin (Ig)G1, IgG2a and F(ab')₂ fragments. *Parasite Immunol* 2001;23:587–97.
- [106] Ahlborg N, Ling IT, Holder AA, Riley EM. Linkage of exogenous T-cell epitopes to the 19-kilodalton region of *Plasmodium yoelii* merozoite surface protein 1 (MSP1(19)) can enhance protective immunity against malaria and modulate the immunoglobulin subclass response to MSP1(19). *Infect Immun* 2000;68:2102–9.
- [107] Clark MR. IgG effector mechanisms. *Chem Immunol* 1997;65:88–110.
- [108] Vieira P, Rajewsky K. The half-lives of serum immunoglobulins in adult mice. *Eur J Immunol* 1988;18:313–6. doi:10.1002/eji.1830180221.

- [109] Garraud O, Nutman TB. The role of cytokines in human B-cell differentiation into immunoglobulin-secreting cells. *Bull Inst Pasteur* 1996;94:285–309. doi:10.1016/S0020-2452(97)87084-4.
- [110] Garraud O, Diouf A, Holm I, Perraut R, Longacre S. Immune responses to *Plasmodium falciparum*–merozoite surface protein 1 (MSP1) antigen, II. Induction of parasite-specific immunoglobulin G in unsensitized human B cells after in vitro T-cell priming with MSP119. *Immunology* 1999;97:497–505. doi:10.1046/j.1365-2567.1999.00804.x.
- [111] Perlmann P, Perlmann H, Flyg BW, Hagstedt M, Elghazali G, Worku S, et al. Immunoglobulin E, a pathogenic factor in *Plasmodium falciparum* malaria. *Infect Immun* 1997;65:116–21.
- [112] Troye-Blomberg M, Perlmann P, Mincheva Nilsson L, Perlmann H. Immune regulation of protection and pathogenesis in *Plasmodium falciparum* malaria. *Parassitologia* 1999;41:131–8.
- [113] Berezcky S, Montgomery SM, Troye-Blomberg M, Rooth I, Shaw M-A, Färnert A. Elevated anti-malarial IgE in asymptomatic individuals is associated with reduced risk for subsequent clinical malaria. *Int J Parasitol* 2004;34:935–42. doi:10.1016/j.ijpara.2004.04.007.
- [114] Harte PG, Cooke A, Playfair JH. Specific monoclonal IgM is a potent adjuvant in murine malaria vaccination. *Nature* 1983;302:256–8.
- [115] Shi J, McIntosh RS, Pleass RJ. Antibody- and Fc-receptor-based therapeutics for malaria. *Clin Sci Lond Engl* 1979 2006;110:11–9. doi:10.1042/CS20050136.
- [116] Leke RG, Ndansi R, Southerland NJ, Quakyi IA, Taylor DW. Identification of anti-*Plasmodium falciparum* antibodies in human breast milk. *Scand J Immunol Suppl* 1992;11:17–22.
- [117] Kassim OO, Ako-Anai KA, Torimiro SE, Hollowell GP, Okoye VC, Martin SK. Inhibitory factors in breastmilk, maternal and infant sera against in vitro growth of *Plasmodium falciparum* malaria parasite. *J Trop Pediatr* 2000;46:92–6.
- [118] Biswas S, Saxena QB, Roy A, Kabilan L. Naturally occurring plasmodium-specific IgA antibody in humans from a malaria endemic area. *J Biosci* 1995;20:453–60. doi:10.1007/BF02703849.
- [119] Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol* 2014;5:520. doi:10.3389/fimmu.2014.00520.
- [120] Good MF. Towards a blood-stage vaccine for malaria: are we following all the leads? *Nat Rev Immunol* 2001;1:117–25. doi:10.1038/35100540.
- [121] Abele DC, Tobie JE, Hill GJ, Contacos PG, Evans CB. Alterations in serum proteins and 19s antibody production during the course of induced malarial infections in man. *Am J Trop Med Hyg* 1965;14:191–7.

- [122] Anders RF, Coppel RL, Brown GV, Kemp DJ. Antigens with Repeated Amino Acid Sequences from the Asexual Blood Stages of *Plasmodium falciparum*. In: Perlmann P, Wigzell H, editors. *Chem. Immunol. Allergy*, vol. 41, S. Karger AG; 1988, p. 148–72.
- [123] Yoshida S, Kobayashi T, Matsuoka H, Seki C, Gosnell WL, Chang SP, et al. T-cell activation and cytokine production via a bispecific single-chain antibody fragment targeted to blood-stage malaria parasites. *Blood* 2003;101:2300–6. doi:10.1182/blood-2002-03-0831.
- [124] Ruf P, Lindhofer H. Induction of a long-lasting antitumor immunity by a trifunctional bispecific antibody. *Blood* 2001;98:2526–34.
- [125] Nguyen VK, Desmyter A, Muyldermans S. Functional heavy-chain antibodies in Camelidae. *Adv Immunol* 2001;79:261–96.
- [126] Stanfield RL, Dooley H, Flajnik MF, Wilson IA. Crystal structure of a shark single-domain antibody V region in complex with lysozyme. *Science* 2004;305:1770–3. doi:10.1126/science.1101148.
- [127] Al Qaraghuli MM, Ferro VA. Analysis of the binding loops configuration and surface adaptation of different crystallized single-domain antibodies in response to various antigens. *J Mol Recognit* 2016:n/a-n/a. doi:10.1002/jmr.2592.
- [128] Nuttall SD, Humberstone KS, Krishnan UV, Carmichael JA, Doughty L, Hattarki M, et al. Selection and affinity maturation of IgNAR variable domains targeting *Plasmodium falciparum* AMA1. *Proteins* 2004;55:187–97. doi:10.1002/prot.20005.
- [129] Streltsov VA, Varghese JN, Carmichael JA, Irving RA, Hudson PJ, Nuttall SD. Structural evidence for evolution of shark Ig new antigen receptor variable domain antibodies from a cell-surface receptor. *Proc Natl Acad Sci U S A* 2004;101:12444–9. doi:10.1073/pnas.0403509101.
- [130] Khamrui S, Turley S, Pardon E, Steyaert J, Fan E, Verlinde CLMJ, et al. The structure of the D3 domain of *Plasmodium falciparum* myosin tail interacting protein MTIP in complex with a nanobody. *Mol Biochem Parasitol* 2013;190:87–91. doi:10.1016/j.molbiopara.2013.06.003.
- [131] Wright KE, Hjerrild KA, Bartlett J, Douglas AD, Jin J, Brown RE, et al. Structure of malaria invasion protein RH5 with erythrocyte basigin and blocking antibodies. *Nature* 2014;515:427–30. doi:10.1038/nature13715.
- [132] Coley AM, Gupta A, Murphy VJ, Bai T, Kim H, Anders RF, et al. Structure of the Malaria Antigen AMA1 in Complex with a Growth-Inhibitory Antibody. *PLOS Pathog* 2007;3:e138. doi:10.1371/journal.ppat.0030138.
- [133] Saxena AK. Structure of Fab fragment of malaria transmission blocking antibody 2A8 against *P. vivax* P25 protein. *Int J Biol Macromol* 2012;50:153–6. doi:10.1016/j.ijbiomac.2011.10.012.
- [134] Chen E, Paing MM, Salinas N, Sim BKL, Tolia NH. Structural and Functional Basis for Inhibition of Erythrocyte Invasion by Antibodies that Target *Plasmodium falciparum* EBA-175. *PLoS Pathog* 2013;9. doi:10.1371/journal.ppat.1003390.

- [135] Igonet S, Vulliez-Le Normand B, Faure G, Riottot M-M, Kocken CHM, Thomas AW, et al. Cross-reactivity studies of an anti-Plasmodium vivax apical membrane antigen 1 monoclonal antibody: binding and structural characterisation. *J Mol Biol* 2007;366:1523–37. doi:10.1016/j.jmb.2006.12.028.
- [136] Pizarro JC, Chitarra V, Verger D, Holm I, Pêtres S, Dartevelle S, et al. Crystal structure of a Fab complex formed with PfMSP1-19, the C-terminal fragment of merozoite surface protein 1 from Plasmodium falciparum: a malaria vaccine candidate. *J Mol Biol* 2003;328:1091–103.
- [137] Tsubata S, Ebe K, Kawamura T, Ishimoto Y, Tomiyama-Miyaji C, Watanabe H, et al. Protection against malaria by anti-erythropoietin antibody due to suppression of erythropoiesis in the liver and at other sites. *Immunol Cell Biol* 2005;83:638–42. doi:10.1111/j.1440-1711.2005.01385.x.
- [138] Trisirivanich S, Laothavorn J, Na-Bangchang K, Khusmith S. Characterization of specific monoclonal antibodies for detection of mefloquine in body fluids. *Southeast Asian J Trop Med Public Health* 2000;31:439–43.
- [139] Christie G, Breckenridge AM, Park BK. Drug-protein conjugates--XVIII. Detection of antibodies towards the antimalarial amodiaquine and its quinone imine metabolite in man and the rat. *Biochem Pharmacol* 1989;38:1451–8.
- [140] Cho SJ, Lee J, Lee HJ, Jo H-Y, Sinniah M, Kim H-Y, et al. A Novel Malaria Pf/Pv Ab Rapid Diagnostic Test Using a Differential Diagnostic Marker Identified by Network Biology. *Int J Biol Sci* 2016;12:824–35. doi:10.7150/ijbs.14408.
- [141] Jourdan J, Matile H, Reift E, Biehlmaier O, Dong Y, Wang X, et al. Monoclonal Antibodies That Recognize the Alkylation Signature of Antimalarial Ozonides OZ277 (Arterolane) and OZ439 (Artefenomel). *ACS Infect Dis* 2016;2:54–61. doi:10.1021/acsinfecdis.5b00090.
- [142] Bhadra D, Bhadra S, Jain NK. PEGylated peptide dendrimeric carriers for the delivery of antimalarial drug chloroquine phosphate. *Pharm Res* 2006;23:623–33. doi:10.1007/s11095-005-9396-9.
- [143] Ibrahim S, Tagami T, Ozeki T. Effective-Loading of Platinum-Chloroquine into PEGylated Neutral and Cationic Liposomes as a Drug Delivery System for Resistant Malaria Parasites. *Biol Pharm Bull* 2017;40:815–23. doi:10.1248/bpb.b16-00914.
- [144] Munjeri O, Hodza P, Osim EE, Musabayane CT. An investigation into the suitability of amidated pectin hydrogel beads as a delivery matrix for chloroquine. *J Pharm Sci* 1998;87:905–8. doi:10.1021/js9801283.
- [145] Movellan J, Urbán P, Moles E, de la Fuente JM, Sierra T, Serrano JL, et al. Amphiphilic dendritic derivatives as nanocarriers for the targeted delivery of antimalarial drugs. *Biomaterials* 2014;35:7940–50. doi:10.1016/j.biomaterials.2014.05.061.
- [146] Urbán P, Valle-Delgado JJ, Mauro N, Marques J, Manfredi A, Rottmann M, et al. Use of poly(amidoamine) drug conjugates for the delivery of antimalarials to Plasmodium. *J Control Release Off J Control Release Soc* 2014;177:84–95. doi:10.1016/j.jconrel.2013.12.032.

- [147] Rajendran V, Rohra S, Raza M, Hasan GM, Dutt S, Ghosh PC. Stearylamine Liposomal Delivery of Monensin in Combination with Free Artemisinin Eliminates Blood Stages of *Plasmodium falciparum* in Culture and *P. berghei* Infection in Murine Malaria. *Antimicrob Agents Chemother* 2015;60:1304–18. doi:10.1128/AAC.01796-15.
- [148] Saxena AK, Singh K, Su H-P, Klein MM, Stowers AW, Saul AJ, et al. The essential mosquito-stage P25 and P28 proteins from *Plasmodium* form tile-like triangular prisms. *Nat Struct Mol Biol* 2006;13:90–1. doi:10.1038/nsmb1024.

1 **Figure legends**

2 **Figure 1: Antibody structure and alternative formats**

3 The refined structures of **A)** IgG_{2a} mAb (PDB ID: 1IGT), and **B)** scFv formats of the same
4 antibody for illustration. The antibody domains were colour coded as follow; VL: red, VH:
5 blue, CL: green, CH1: yellow, CH2: magentas/orange, CH3: cyan/grey and, and Linker: light
6 grey. The IgG mAb is composed of two Fab and one FC regions. **C)** VNAR sdAb (PDB ID:
7 1VES), and **D)** VHH sdAb (PDB ID: 4GFT). The atoms of **C)** and **D)** were coloured as carbon:
8 green; Oxygen: red; nitrogen: blue. Structures were viewed and coloured by PyMOL 1.3
9 (academic version).

10 **Figure 2: Binding site topography and CDRs orientation**

11 CDRs orientation of Fab (PDB ID: 2J5L), VHH (PDB ID: 4GFT), and VNAR (PDB ID: 1VES)
12 domains were examined as top (T) and side (S) views. The CDR regions were colour coded for
13 CDR1: red, CDR2: green, CDR3: blue, HV2 (1VES VNAR): yellow, and HV4 (1VES VNAR):
14 magenta, CDRL1 (2J5L): cyan, CDRL2 (2J5L): orange, CDRL3 (2J5L): violet. The PDB
15 entries of these crystal structures are depicted at the lower corner of each picture. Structures
16 were viewed by PyMOL 1.3 (academic version).

17

Table 1: Outline of the anti-malarial drug delivery systems

Anti-malarial drugs	Delivery system used	Purpose	Reference
artesunate	liposomes	Improve patient compliance for multiple administrations	[50]
chloroquine	chitosan–tripolyphosphate nanoparticles	Treatment of chloroquine resistant malaria parasites	[11]
chloroquine	dendrimers	Reduce chloroquine toxicity	[53]
primaquine	liposomes	Reduce primaquine toxicity	[56]
primaquine	chylomicron emulsion	Target primaquine to hepatocytes	[10]
primaquine	polyisohexylcyanoacrylate (PIHCA) nanoparticles	Reduce primaquine toxicity	[57]
primaquine	oral lipid nanoemulsion	Improved primaquine oral bioavailability and liver targeting	[58]
chloroquine	PEGylated poly-L-lysine-based dendrimers	To induce controlled and sustained delivery	[142]
chloroquine	PEGylated Neutral and Cationic Liposomes	Treatment of chloroquine resistant malaria parasites	[143]

chloroquine	amidated pectin hydrogel beads	Delay the release of oral chloroquine to distal parts of the gastrointestinal tract	[144]
chloroquine and primaquine	dendritic derivatives	Reduce the toxicity of the used anti-malarial drugs	[145]
chloroquine	poly(amidoamines) drug conjugates	Selectively deliver chloroquine to <i>Plasmodium</i> -infected red blood cells	[146]
monensin	Liposomes	Improving the anti-malarial activity of monensin	[147]

Table 2: Summary of the crystal structures retrieved from the PDB

PfRH5: *P. falciparum* reticulocyte-binding protein homologue 5; Pvs25: *P. vivax* P25 protein; AMA-1: Malarial Apical Membrane Antigen-1; PfEBA-175: *P. falciparum* EBA-175; MSP1: Merozoite surface protein 1; MyoA- MTIP: myosin A- myosin tail interaction protein; Fab: fragment antigen-binding; VNAR: single variable new antigen receptor domain antibody fragments; VHH: variable domain of heavy chain antibodies.

	PDB entry	Resolution (Å)	Species	Format	Target	Reference
1	4U1G	3.1	<i>Mus musculus</i>	Fab	PfRH5	[131]
2	4U0R	2.3	<i>Mus musculus</i>	Fab	PfRH5	[131]
3	1Z3G	3.3	<i>Mus musculus</i>	Fab	Pvs25	[148]
4	2Q8B	2.3	<i>Mus musculus</i>	Fab	AMA1	[132]
5	2Q8A	2.4	<i>Mus musculus</i>	Fab	AMA1	[132]
6	3S62	4.01	<i>Mus musculus</i>	Fab	Pvs25	[133]
7	4QEX	4.5	<i>Mus musculus</i>	Fab	PfEBA-175 RII	[134]
8	4K2U	2.45	<i>Mus musculus</i>	Fab	PfEBA-175 F1	[134]
9	2J5L	2.9	<i>Mus musculus</i>	Fab	AMA1	[135]
10	2J4W	2.5	<i>Mus musculus</i>	Fab	AMA1	[135]
11	1OB1	2.9	<i>Mus musculus</i>	Fab	MSP1-19	[136]
12	1VER	2.82	<i>Orectolobus maculatus</i>	VNAR	AMA1	[129]
13	1VES	2.18	<i>Orectolobus maculatus</i>	VNAR	AMA1	[129]
14	4GFT	1.6	<i>Lama glama</i>	VHH	MyoA-MTIP	[130]

