Review

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Recent Advances in the Treatment of Malaria

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Malaria is an infectious disease caused by protozoan parasites belonging to the *Plasmodium* species. The disease has been a major cause of mortality and morbidity, especially in populations of African and South-East Asian countries. A well-developed treatment regimen including the artemisinins as a potent antimalarial and other safety preventive measures have played a major role in reducing global burden of malaria over the years. However, recent reports of drug resistance against the artemisinins should be a wakeup call, for the artemisinins have been the mainstay towards the treatment of the disease in recent past. There is a need for newer antimalarials that can be active on more than one stage of the parasite life cycle. These may be complementary to the artemisinins and may also help in keeping a check on the menace of drug resistance. The current review focuses on clinical drug candidates with activity against more than one stages of the malarial parasite life cycle.

INTRODUCTION

Malaria is an ancient disease that has been decimating humans since ages. Malaria kills around 600,000 people each year, mostly children from sub-Saharan Africa. Modern treatment and insect control programs have been implemented in an attempt to control the disease. As a result, the number of malaria cases globally has decreased from an estimated 262 million in 2000 to 214 million in 2015, a decline of 18% whereas the number of malaria deaths has decreased from an estimated 839,000 in 2000 to 438,000 in 2015, a decline of 48%. According to WHO, most deaths in 2015 were in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%). It is estimated that a cumulative 1.2 billion fewer malaria cases and 6.2 million fewer malaria deaths occurred globally between 2001 and 2015 than would have been the case had incidence and mortality rates remained unchanged since 2000 (WHO, 2015a). In the last few years, the cases of malaria have dwindled; as many countries have

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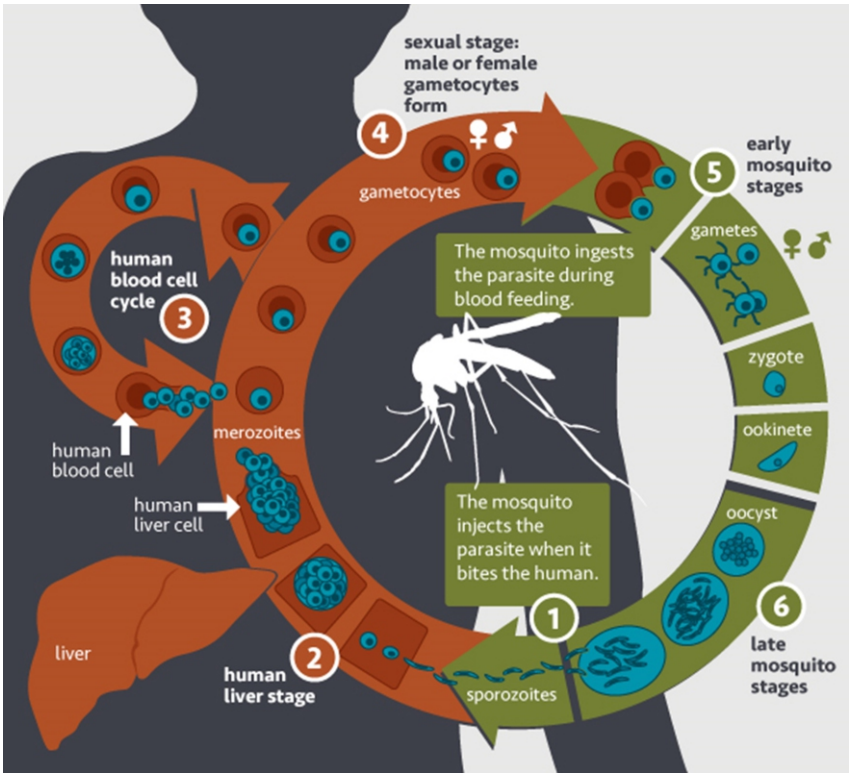

Figure 1: Different stages in the malarial parasite life cycle (NIAID, 2015).

updated their treatment protocol set up by WHO from monotherapy such as chloroquine, amodiaquine to the currently recommended ACT's (Artemisinin-based combination therapy) (WHO, 2015b). However, increasing resistance in *Plasmodium falciparum* and *P. vivax* parasites means current drugs may not remain effective for long.

The disease is most commonly transmitted by an infected female Anopheles mosquito. The parasite has a complicated life cycle; it develops different surface antigens during different stages of its life cycle enabling it to evade immune clearance in the host. The malarial parasite life cycle comprises of 4 stages and every stage has to be considered in order to eradicate the disease. Fig. 1 illustrates the different phases in the parasite life cycle. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood. The parasites travel to the liver where they mature and reproduce. Five species of *Plasmodium* can infect and be spread by humans. Most deaths are caused by *P. faliparum* because *P. vivax*, *P. ovale* and *P. malariae* generally cause a milder form of malaria. Recently, *P. knowlesi* has also been seen to infect humans, but such cases are rare.

Malarial parasites are continuously evolving and their ability to develop drug resistance forces us to develop newer and more effective drugs. Development of new antimalarials with novel mechanism of action i.e. active against novel targets are needed to fight this war. The idea of developing antimalarials with activity at more than one stage of the life cycle has always been advocated but was not considered practical till a few years ago. A drug candidate acting on both the liver and blood stages or killing the gametes could prove to be a magic bullet in the war against this debilitating disease. Drug research in malaria often focuses on blood stage parasites because they are responsible for the symptoms of the disease and are easier to manipulate in the laboratory. The lack of proper assay for the liver stage has been a major hurdle in developing drugs. The recent advances in phenotypic screening have allowed researchers to target the pre-erythrocytic (liver) stage of the parasite life cycle, which was previously a cumbersome task (Biamonte *et al*., 2013).

MMV (Medicines for Malaria Venture), a non-profit organization based in Geneva, Switzerland aims to develop, discover antimalarials at an affordable cost. MMV works in partnerships with NGO's, research institutions, Pharma companies and is financed with aid from these groups. The R&D portfolio managed by MMV is by far the largest one ever developed for the treatment of malaria (Hentschel and Meguni, 2003). The contribution of MMV in the antimalarial treatment can be easily gauged by looking at the large numbers of preclinical candidates in the global antimalarial drugs portfolio. This review will focus on the latest developments in the treatment of malaria that target more than one stage of the lifecycle of the malarial parasite.

ANTI-MALARIAL TREATMENT

Current Line of Therapy

Widespread resistance to most antimalarial drug classes has led to the global adoption of artemisinin-based combination (ACTs) as first-line therapies. ACT's are a combination of two drugs approved for the treatment of severe malaria. The most popular combinations currently in use are artemether + lumefantrine, artesunate + amodiaquine, artesunate + SP (sulfadoxine + pyrimrethamine) and dihydroartemisinin + piperaquine. The current regimen according to WHO guidelines is a 3-day course of artemisinin which helps in clearing out majority of the parasite with

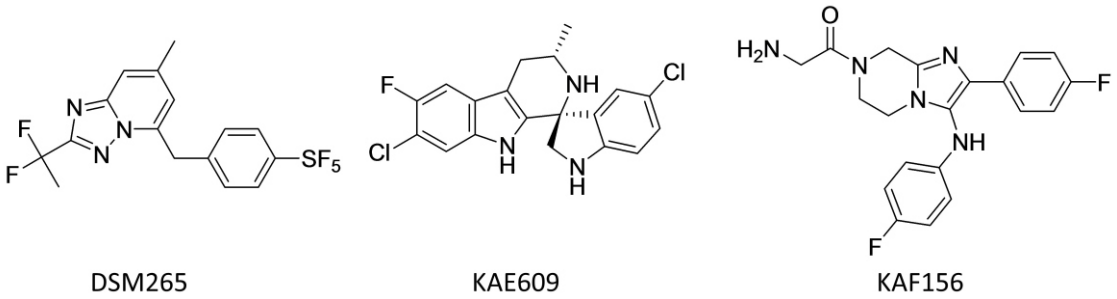

Figure 2: Drug candidates currently in Phase 2 clinical trials.

the remaining parasites are killed by the partner drug (lumefantrine/amodiaquine/ piperaquine) (WHO, 2015b). Artemisinin and its derivatives have rapid onset of action but is quickly cleared from the bloodstream, hence it becomes necessary to combine it with a drug which has a slow clearance rate. Primaquine has the unique distinction of acting on both the liver and blood stage of the malarial parasite. Primaquine, atovoquone and proguanil are used as prophylactics.

Move towards Eradication

Antimalarial drug discovery has always focused on targeting the erythrocytic (blood) stages of the parasite life cycle. The parasite can be easily studied in the blood stage whereas the pre-erythrocytic (liver) stage could be studied only by isolating parasites directly from the mosquito and infecting liver cells for developing an assay (Biamonte *et al*., 2013). The search for drugs acting on the pre-erythrocytic (liver) stage had been stagnant in the past due to lack of proper culture techniques and cumbersome animal models. The development of a phenotypic screening method (Meister *et al*., 2011) by the Novartis-GNF collaboration that targets the parasite lifecycle at the liver stage was a critical advance in the discovery of novel and newer leads. Currently research has focused on developing compounds which are active against both the liver as well as the blood stages of the malarial parasite; such an antimalarial would be extremely effective in eradicating the disease burden in poorer countries.

KAE609 (Fig. 2) is the first antimalarial drug candidate with a novel mechanism of action to achieve positive clinical proof-of-concept in over 20 years. A spirotetrahydro-β-carboline hit was discovered by the phenotypic screening of a Novartis library of 12,000 natural products and synthetic compounds against *P. falciparum*. The spirotetrahydro-β-carboline hit was optimized to improve

potency and oral bioavailability providing the clinical candidate KAE609. In vitro, KAE609 has potent activity against both the pre-erythrocytic (liver) and erythrocytic (blood) stages of the malaria parasite (Novartis, 2014). Spirotetrahydro-β-carbolines inhibit PfATP4, a parasite plasma membrane Na^+-ATPase that regulates sodium and osmotic homeostasis (Yeung *et al*., 2010). A single oral dose of KAE609 provided a cure in a *P. berghei* rodent model of blood-stage malaria. The entire work was carried out at the Novartis Institute for Tropical Diseases in Singapore in collaboration with the Genomics Institute of the Novartis Research Foundation (GNF), the Biomedical Primate Research Centre and the Swiss Tropical Institute. Currently, this compound has completed Phase 2a trials and is undergoing malaria challenge studies in healthy volunteers (controlled human induced blood stage activity) (MMV, 2016).

A Novartis-GNF collaboration identified the imidazolopiperazine scaffold as an attractive hit based on a screening program using a cell based proliferation assay (Nagle *et al*., 2012; Wells *et al*., 2015). Further optimization of these imidazolopiperazine scaffolds led to GNF19 and GNF156 (Fig. 2), of which GNF156 was found to be more promising (Nagle *et al*., 2012). KAF156 (GNF156) not only attacks the asexual but also the sexual stages of malarial parasite life cycle. The compound is currently undergoing Phase 2a clinical trials (MMV, 2016).

DSM265 is a triazolopyrimidine-based inhibitor of the enzyme dihydroorotate dehydrogenase (DHODH) (Phillips *et al*., 2015). It is the first DHODH inhibitor to reach clinical development for treatment of malaria. The compound was found to attack Plasmodium's ability to synthesize the nucleotide precursors required for the synthesis of DNA and RNA. DSM265 (Fig. 2), is a long-acting inhibitor for the treatment and prevention of malaria and which kills *P. falciparum* in blood and liver. DSM265 is a potential drug combination partner for either single-dose malaria treatment or once weekly doses for ongoing disease prevention (Coteron *et al*., 2011). Currently, the compound is undergoing Phase 2 clinical trials in patients affected with *P. falciparum* or *P. vivax* and is in Phase 1b tests where its efficacy against blood stage parasites in combination with OZ439 is undergoing trials (MMV, 2016).

Researchers from University of South

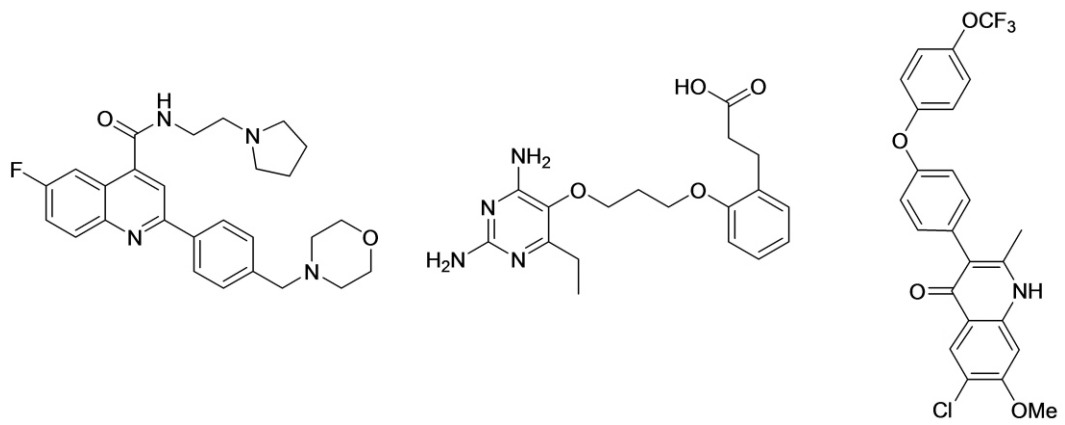

Figure 3: Compounds currently in preclinical stages.

Florida, Drexel University, Monash University, the Portland Veteran Affairs Medical Center, and the Oregon Health and Science University along with Medicines for Malaria Venture (MMV) have developed a new class of anti-malarials - quinolone-3-diarylethers (Broadwith, 2013). ELQ300 drew its inspiration from endochin and the first antimalarial pyridone based drug developed by GSK. The diaryl ether group, part of the pyridone based compound was found to improve its metabolic stability. ELQ300 (Fig. 3) was selected as a preclinical candidate since it targets the liver and blood stages of falciparum malaria, as well as the forms that are crucial to transmission of the disease namely the gametocytes, zygotes, and ookinetes. ELQ300 inhibits the mitochondrial cytochrome bc_1 complex, responsible for ATP and pyrimidine synthesis. It is believed that it would be difficult for the parasite to develop resistance compared to existing drugs targeting the same pathway (Nilsen *et al*., 2013). However, poor aqueous solubility and high crystallinity proved to be an obstacle in the clinical development of this compound. However, a bioreversible O-linked carbonate ester prodrug of the compound, named ELQ 337 (Miley *et al*., 2015), was found to deliver the active drug at concentrations sufficient for single dose cure.

Dundee University in collaboration with MMV developed DDD498 (Fig. 3), a new drug candidate which demonstrates the potential to address a variety of clinical needs, including single-dose treatment, blocking transmission and chemo-protection. DDD498 was developed from a screening programme against blood-stage malaria parasites. This drug targets

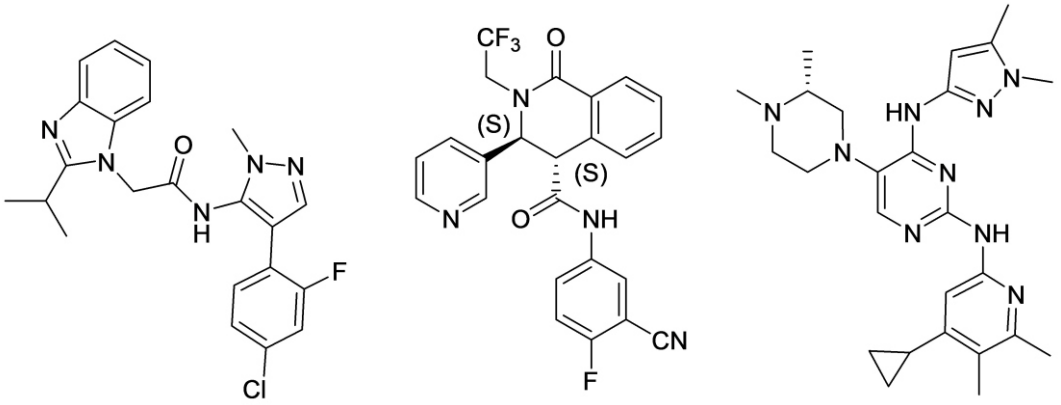

Figure 3.1: Compounds currently in preclinical stages.

the translation elongation factor 2 (eEF2), which is responsible for the GTP-dependent translocation of the ribosome along messenger RNA, and is essential for protein synthesis (Baragana *et al*., 2015). Merck Serono and MMV joined hands to develop this potential antimalarial therapy (MMV, 2015). DDD498 showed an EC_{50} < 1 nM against the liver schizont forms of *P. berghei* and *P. yoelii*. DDD498 potently inhibited both male and female gamete formation at similar concentrations. DDD498 blocked subsequent oocyst development in the mosquito after 7 days with an EC_{50} of 1.8 nM (Baragana *et al*., 2015). This compound is currently undergoing preclinical GLP toxicology studies (MMV, 2016).

BIOTEC (National Center for Genetic Engineering and Biotechnology, Thailand) together with the MMV, developed P218 (Fig. 3) a dihydrofolate reductase inhibitor. Mutations in PfDHFR lead to change in its geometry, thereby restricting the activity of pyrimethamine (Yuthavong *et al*., 2012). Using SBDD, the team designed P218 such that it shows irreversible inhibition. P218 shows excellent selectivity toward PfDHFR, thereby providing safety to humans. The clinical status of this candidate is not known at this time.

Small molecules numbering 500,000 were screened from the AZ (AstraZeneca) collection and TAPs (triamino-pyrimidines) were identified as promising lead series for further evaluation. The compounds have a novel mechanism of action involving inhibition of V-type H^+ ATPase. Medicinal chemistry optimization of TAPs resulted in selection of MMV253 (Fig. 3.1) as a candidate drug with ideal properties like novel chemical class, novel mechanism of action, fast kill

in-vitro and *in vivo*, predicted long half-life in humans and good safety margins in rats and guinea pigs (Hameed *et al*., 2015). TAPs offer the potential for single dose cure in combination with suitable partner drugs as the reported half-life in humans is 36 hours. It is active against multiple strains of *P. falciparum* including those resistant to current antimalarials as well as novel antimalarials in clinical development. The TAPs kill plasmodium parasites rapidly, and the emergence of spontaneous resistance under *in vitro* conditions to this chemical class is rare. The compound is expected to complete preclinical studies soon.

A team of scientists from Drexel University, University of Washington and GNF identified pyrazoleurea and pyrazoleamide derivatives as hits via structure based *in silico* screening of compound libraries. These molecules displayed good activity against both *P. falciparum* and *P. vivax* in animal studies. Optimization of the hits gave rise to 3 lead compounds with nanomolar activity. Of the three, PA92 (Fig. 3.1) was chosen as the drug candidate for further studies. Once inside the host, the parasite induces changes in the host cell membrane so that more nutrients are taken in, which triggers an increase in sodium concentration within red blood cells. The parasite keeps its own sodium levels low with the help of a protein (PfATP4), which pumps sodium out of the parasite. PA92 inhibits this pump causing increase in the Na^+ concentrations within the parasite. This results in excessive water intake, cell swelling and eventually, bursting of the parasite (Vaidya *et al*., 2014).

In search of compounds that inhibit proliferation of parasites, researchers from St. Jude Children's Research hospital in collaboration with MMV and other universities executed a whole-cell phenotypic HTS of more than 1.2 million compounds to identify novel chemicals that kill the malaria parasite (Jimenez-Diaz *et al*., 2014). Three high-priority lead series from this work were pursued: the dihydroisoquinolones (DHIQs), dihydropyridines (DHPs), and diamino-napthoquinones (DANQs). DHIQs was found to be the most promising series, further optimization of the lead led to the development of SJ773 (Fig. 3.1), a fast parasite clearing drug candidate approved for clinical studies by MMV. (+)-SJ733 acts on a cation-transporting ATPase which is responsible for maintaining low intracellular Na^+ levels in the parasite. Treatment of parasitized erythrocytes with (+)-SJ733 in vitro caused a rapid

perturbation of Na^+ homeostasis in the parasite. This disturbance in the level of Na^+ was followed by profound physical changes in the infected cells, including increased membrane rigidity and externalization of phosphatidylserine, consistent with eryptosis (erythrocyte suicide) or senescence (Jimenez-Diaz *et al*., 2014). The mechanism of action of SJ773 and PA92 are similar. Preclinical studies showed this compound as having high oral bioavailability, very good safety margin as well as transmission blocking activity. This compound is currently undergoing preclinical GLP toxicology studies (MMV, 2016).

The proteasome is a multi-component protease complex responsible for regulating key processes such as the cell cycle and antigen presentation (Li *et al*., 2016). Compounds that target the proteasome are potentially valuable tools for the treatment of pathogens that depend on proteasome function for survival and replication. Proteasome inhibitors have been known to inhibit all the stages of the malarial parasite life cycle. However, the major hurdle was lack of selectivity with the parasite over the host cells, making them toxic to humans. Researchers recently have reported a small molecule that can kill the parasite in mice with few side effects. The molecule works by inhibiting the proteasome, the cell's protein-degrading machine, in the parasites but to a much lesser extent in the host. Selective proteasome inhibitors are believed to complement current antimalarial drugs. Also, recent findings suggest proteasome inhibitors suppress artemisinin-resistant strains. Matthew Bogyo and his team at Stanford University School of Medicine first screened a library of peptides to determine sequences favored for degradation by parasite proteasomes but not human ones. They used that information to design selective inhibitors (Goldman, 2016).

They along with the team at the MRC Laboratory of Molecular Biology used cryoelectron microscopy to obtain a structure of the parasite proteasome bound to a designed inhibitor. This structure of the malarial proteasome at the inhibitor-binding site helped further optimization of the inhibitors. A parasite-selective inhibitor, a peptide like molecule called WLL-vs (Fig. 4), was developed that killed artemisinin-sensitive and -resistant malaria parasites. A single dose of WLL-vs substantially reduced parasite levels in mice without any apparent toxic effects. WLL-vs could be combined with artemisinin to decrease the spread of

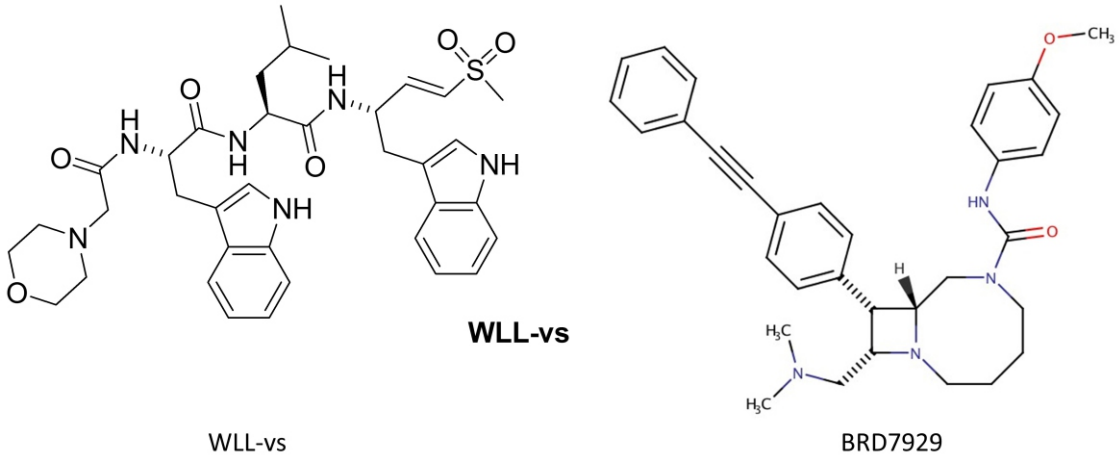

Figure 4: Structures of proteasome inhibitor WLL-vs and bicyclic azetidine BRD7929.

malarial drug resistance, if it can pass efficacy and toxicity trials.

Stuart Schreiber's group at Harvard and Broad Institute (Kato *et al*., 2016) have identified a bicyclic azetidine BRD7929 (Fig. 4) as novel agents that hit all three stages of the malarial lifecycle. They screened a 100,000-member synthetic library built using Diversity Oriented Synthesis that allowed them to access hitherto unknown chemical space. This molecule was capable of blocking transmission and had activity against both the liver and blood stages in multiple in-vivo models (*P. falciparum and P. berghei*). BRD 7929 inhibits the cytosolic Phenylalanyl tRNA synthetase of the parasite thus affecting protein synthesis. BRD 7929 needs further optimization before it can enter the clinic; however, the identification of Phenylalanyl tRNA synthetase as the target should allow researchers around the world to develop newer drugs that act via this mechanism.

FUTURE ASPECTS/CONSIDERATIONS

PfATP4 seems to be the hot target amongst researchers with as many as 3 drug candidates in the clinical trials. All the three drugs have transmission blocking activity in addition with blood stage activity. KAE609 and DDD498 appear to be the most promising of the lot with activity against more than one stage of the parasite life cycle. The current pipeline looks strong and promising with quite a few of them having novel mechanism of action which shows that newer targets have been explored namely eEF2, V type H^+-ATPase. The screening cascade and the hits identified by Stuart Schreiber's group warrants further investigation both in terms of the novel chemical matter and the biological pathways inhibited by them.

The finding of the structure of protein used by mosquito to infect the humans could help in the development of vaccine (Wilson, 2016). The early signs showed by CRISPR and proteasome inhibitors are promising and it is quite hopeful that they would be part of the treatment agenda in the future (Johnson, 2015). MMV has played a major role in the buildup of this pipeline of drugs. MMV's R&D portfolio also includes many drug combinations which are there in the later stages of clinical trials. Though the drugs which are there in the pipeline propose to be one-man army, it would be more logical for these drugs (if approved for human use) to be given in combination with artemisinin derivatives. Investments in R&D and collaboration with various other research organizations have proved to be a winning formula in speeding up the process of drug discovery in the malaria context. One may never know how many compounds synthesized across the world, because of lack of sufficient funding or unavailability of proper techniques/ technologies have seen its way into the bin. It's not surprising to see the amount of contribution of developed countries in R&D activities. So, it becomes imperative that the respective governments take these issues seriously.

A complete ideal package would be a molecule that can target the blood stage of the disease to alleviate the symptoms, the liver stage to prevent relapses, and the transmission stage to protect other humans. Of late researchers are cracking open the doors of genomics to seek an answer to this problem. A malaria vaccine hence is very much a possibility in the near future. Continued progress in combating malaria requires development of newer drugs with broad-ranging activity against all manifestations of the disease. Increased investment in the R&D, more collaborative efforts and disciplined follow ups of the protocols set up by WHO would play a big role towards eradication of malaria. Antimalarial strategies for prevention are ideally a balanced use of mosquito control, anti-Plasmodium treatments, and a general improvement of sanitation and awareness, strategies which the developed countries used to eradicate malaria. Expanding the existing robust pipeline, to create and enlarge the range of combination therapies against blood stage and other parasite stages can go a long way in helping reach the much awaited goal of elimination of malaria.

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