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Artemisinin, the Magic Drug Discovered from Traditional Chinese Medicine

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ABSTRACT

Artemisinin and its derivatives represent the most important and influential class of drugs in the fight against malaria. Since the discovery of artemisinin in the early 1970s, the global community has made great strides in characterizing and understanding this remarkable phytochemical and its unique chemical and pharmacological properties. Today, even as artemisinin continues to serve as the foundation for antimalarial therapy, numerous challenges have surfaced in the continued application and development of this family of drugs. These challenges include the emergence of delayed treatment responses to artemisinins in malaria and efforts to apply artemisinins for non-malarial indications. Here, we provide an overview of the story of artemisinin in terms of its past, present, and future. In particular, we comment on the current understanding of the mechanism of action (MOA) of artemisinins, and emphasize the importance of relating mechanistic studies to therapeutic outcomes, both in malarial and non-malarial contexts.

1. Introduction

Malaria has been a debilitating disease with global influence since ancient times and continues to be one of the most widespread and damaging infectious diseases today [1]. With the cause of disease having long been misattributed to "bad air," the transmissible and parasitic nature of malaria remained unknown until the works of Charles Louis Alphonse Laveran and Ronald Ross in the late 1800s. Their findings established that protozoa belonging to the genus *Plasmodium* caused malaria, and that *Anopheles* mosquitoes were the primary vectors of malarial infections. These observations made Laveran and Ross two of the earliest recipients of the Nobel Prize in Physiology or Medicine [2].

In the decades following their discoveries, ground-breaking progress has been made in the battle against the disease. The crusade launched by the Chinese government in the late 1960s to search for cures for malaria ultimately culminated in the discovery of artemisinin. Artemisinin (and its various derivatives, which we will refer to collectively as "artemisinin" unless otherwise specified) is a sesquiterpene lactone compound (Fig. 1) with a unique chemical structure derived from the sweet wormwood plant, *Artemisia annua* L. (Fig. 2). Since its discovery, it has become the most important and effective antimalarial drug [3].

In many ways, artemisinin is a truly fascinating drug. From the tumultuous process of its discovery, which was deeply tied to traditional Chinese medicine (TCM), to its remarkable potency and impact as an antimalarial drug, it is not surprising that artemisinin has captured a great deal of attention since its introduction to the world stage [1]. Over 40 years after its discovery, artemisinin remains our bulwark against malaria and is the foundation of all major antimalarial therapies [4]. Years of research spanning a range of disciplines have gone into the exploration and elucidation of the mechanisms of artemisinin in its antimalarial role [5]. Beyond that, efforts have been made to repurpose artemisinin for non-malarial applications, thereby raising considerable anticipation over the future development of this drug [6].

With that in mind, we feel that it is a good time to broadly review the timeline of this influential drug, spanning its past, present, and future. Beginning with a look back at the story of the discovery and development of artemisinin, we then review

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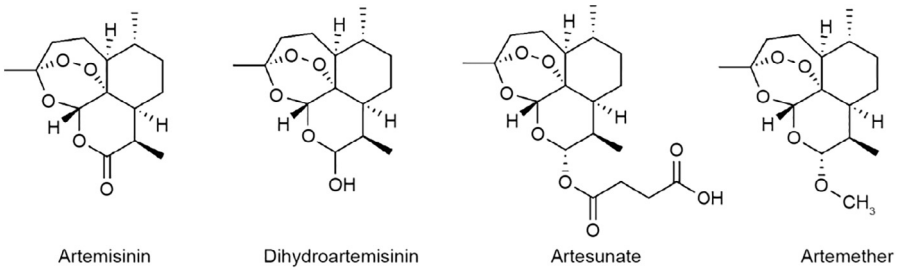

Fig. 1. Artemisinin and its clinically used derivatives.

Fig. 2. *Artemisia annua* L. in the field.

and discuss the contemporary understanding of the mechanism of action (MOA) of artemisinin in malaria. We conclude by looking ahead at current efforts to repurpose artemisinin for possible roles outside of malaria. We believe that this article will provide a well-rounded background of artemisinin, along with relevant insights into the salient topics surrounding this remarkable drug.

2. The journey of discovery

We begin with a brief tracing of the remarkable journey that led to the discovery and development of artemisinin. Records of malaria in TCM date back thousands of years, and the same is true for the usage of *Artemisia* (Qinghao) plants as medicinal herbs. First mentioned as a specific remedy for malarial symptoms in Ge Hong's *Zhouhou Beiji Fang* (*Handbook of Prescriptions for Emergency*) dating back to the Eastern Jin Dynasty (317–420 AD), the application of Qinghao and other techniques for malarial relief was subsequently noted in a series of historical Chinese medical writings that included the influential *Bencao Gangmu* (*Compendium of Materia Medica*) by Li Shizhen (Ming Dynasty, 1368–1644 AD). This wealth of ancient knowledge would later prove to be instrumental in the discovery and development of artemisinin.

In the years following World War II, the development and deployment of the potent insecticide dichloro-diphenyl-trichloro-ethane (DDT) and new antimalarial drugs such as chloroquine (CQ) resulted in great progress in combating malaria. However, the World Health Organization (WHO)'s campaign in the 1950s to combat and eradicate malaria around the world was eventually met with challenges related to resistance. The emergence of DDT-resistant vectors and drug-resistant parasites led to a rebound of the disease, especially in regions such as Southeast Asia and sub-Saharan Africa [7]. This setback prompted an urgent need for novel antimalarial drugs. Significant efforts had been made by the United States due to the Vietnam War and the prevalence of drug-resistant malaria in that region. The Chinese government also initiated efforts in malarial research around this time. In particular, a national project called Project 523 (named after its date of inauguration, 23 May 1967) was set up to consolidate malarial research on a national level [8].

In 1969, Professor Youyou Tu was selected to lead a research group within the project that focused on screening TCM for novel antimalarial drugs. This work took place at the Institute of Chinese Materia Medica of the China Academy of Chinese Medical Sciences. Drawing from a massive repository of TCM knowledge that included ancient literature, folklore, and oral interviews with practitioners, Tu and colleagues worked from a list of over 2000 herbal remedies, of which some 640 were deemed to be possible "hits." From this selection, over 380 extracts from approximately 200 herbs (including Qinghao/*Artemisia* extracts) were eventually collected and tested, mostly giving unsatisfactory results [1,9]. The Qinghao extract nevertheless drew particular interest starting around 1971, as it produced promising but inconsistent results [1]. This finding prompted a revisitation of the literature, and led to perhaps the most important breakthrough in the discovery process.

Returning to the earliest record of the use of Qinghao to treat malarial symptoms, which was in Ge Hong's *Zhouhou Beiji Fang* (*Handbook of Prescriptions for Emergency*), Tu noted that the instructions for the Qinghao prescription involved consuming the strained "juice" of the Qinghao plant immersed in water. It was notable that the instruction made no mention of heating the medicine—something that was otherwise common for prescriptions in TCM. Drawing from the literature and her own knowledge of TCM, Tu arrived at the idea to modify the extraction process to use low-temperature conditions. The extracts produced from this new procedure were further purified by separation of the acidic and neutral phases in order to retain active components while reducing the toxicity of the original extract. The resultant substance displayed a striking 100% effectiveness against rodent malaria in experiments carried out around October 1971. This remarkable result was then fully reproduced in monkey malaria experiments carried out in late December of the same year, thus establishing the efficacy of the Qinghao extract beyond doubt [1].

The breakthrough had been made, but the journey of drug development was by no means complete. Conditions in China at that time made it difficult to perform clinical trials of new drug candidates to ascertain their safety for humans. In an attempt to accelerate the process due to the seasonal and time-sensitive nature of malarial research, Tu and colleagues decided to volunteer themselves as the first human subjects for toxicity and dose-finding tests [8]. This act established the safety profile of the Qinghao extract and enabled clinical trials to be carried out immediately, in the latter half of 1972. The trials (which were carried out in Hainan Province and at the 302 Hospital PLA (now incorporated into the Fifth Medical Center of the Chinese PLA General Hospital) in Beijing) proved successful, and paved the way for Qinghao research to be pushed to the national level. A subsequent concerted effort on the part of the Chinese scientific community at large drove further research and development of Qinghao forward. The active component of the Qinghao extract, artemisinin (also known as Qinghaosu) itself, was isolated in November 1972 by Tu's team at the Institute of Chinese Materia Medica. The team would later go on to develop dihydroartemisinin (DHA), which remains one of the most pharmacologically relevant derivatives today. In collaboration with other institutes across China, further groundwork in drug development, including the determination of the stereo-structure of artemisinin and further derivatization of artemisinin, was carried out in the following decade [10,11]. These efforts, among others, culminated in the fourth meeting of the Scientific Working Group on the Chemotherapy of Malaria held in Beijing in 1981, where the findings were presented by Tu for the first time. The results were published in 1982 as a series of papers under the name "China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials" [12,13]; thus the gift from Chinese medicine was delivered to the rest of the world.

In the subsequent years of the 1980s, artemisinin and its derivatives were successfully employed in China to treat thousands of malaria patients [1]. As the problem of drug-resistant malaria continued to worsen elsewhere, it was not long before the commencement of clinical studies with artemisinin in other endemic regions in Asia [14–19]. Consistent and encouraging results led to the expansion of such studies, particularly toward Africa [19–24]. The evidence was clear that artemisinin-based therapy, especially in combination with a slower-acting antimalarial such as mefloquine or piperaquine, led to significant improvements in parasite clearance and a rapid diminishing of symptoms for both uncomplicated and severe *Plasmodium falciparum* malaria infection. At the same time, its tolerability was shown to be excellent, as reports of toxicity and safety concerns remained minimal [25]. Across more than a decade's worth of independent randomized clinical studies and meta-analyses, the outstanding efficacy and safety of artemisinin-based therapy became increasingly clear. Finally, in 2006, the WHO announced an alteration of its strategy to fully employ artemisinin combination therapies (ACTs) as the first-line treatment against malaria [26]. ACTs remain the most effective and recommended antimalarial therapies today [4].

3. The search for a mechanism of action

It has been more than a decade since the implementation of ACTs as the official first-line treatment for malaria and over three decades since the discovery of artemisinin. In this time, the clinical and pharmacological characteristics of artemisinin therapy have been extensively scrutinized and reported [27–30]. Although the specifics of various derivatives can differ, artemisinin drugs are characterized by rapid action and potency, low toxicity, and a short half-life, which makes combination therapy with longer-acting antimalarial drugs ideal and recommended [30]. Apart from its pharmacological properties, elucidating the MOA of a drug is important for optimizing treatment regimens. Dosages, drug combinations, and even considerations of drug resistance are closely related to the molecular basis of a drug's activity. It is thus somewhat surprising that despite decades of widespread application, our understanding of the MOA of artemisinin remains fairly incomplete. Here, we provide a brief overview of the prevailing understanding as well as more recent developments in mechanistic studies of artemisinin [31,32]. In general, the outstanding therapeutic properties of artemisinin can be thought of as a result of two major processes: its unique mechanism of activation, and its downstream activity and drug targets. These mechanisms combine to yield a highly potent, yet highly specific, drug.

3.1. Drug activation

Artemisinin and its derivatives are sesquiterpene lactones that bear the 1,2,4-trioxane moiety as the pharmacophore [33]. In particular, the endoperoxide bridge within this group is well understood to be essential for the pharmacological activity of artemisinin [13,34,35]. Artemisinins are prodrugs in two senses: first, many derivatives are rapidly converted to DHA *in vivo*, and second, their MOA depends on activation by cleavage of the endoperoxide bridge. The mechanism of this cleavage remains an issue in active research [36]. Malarial parasites are characterized by extensive hemoglobin uptake and digestion during the erythrocytic stage of their life cycle [37,38]. This releases copious amounts of free redox-active heme and free ferrous iron (Fe^{2+}), which are thought to underlie the parasite specificity of artemisinin. Indeed, hemoglobin digestion has been strongly linked with artemisinin susceptibility in parasites [38,39]. Multiple models have been proposed with regard to the mechanism of endoperoxide cleavage by either free redox-active heme or free ferrous iron, and the downstream molecular events that follow cleavage [36,40–48]. These proposals differ in terms of the nature of the cleavage and the identity of the reactive intermediates produced by drug activation. In general terms, however, they explain the parasite-specific drug activation through which reactive species are produced, leading to cellular damage and parasite killing. Recent evidence suggests that free redox-active heme may play a predominant role in drug activation [49,50]. A 2008 study provided *in vitro* data that indicated that ferrous heme may be a stronger activator of artemisinin than other iron-containing species, including hemin, free ferrous iron, and undigested hemoglobin [49]. Similar observations were made in live parasites, in which artemisinin activation was blocked by inhibiting hemoglobin digestion but not by the chelation of free ferrous iron [47]. Thus, the process of hemoglobin digestion in infected erythrocytes, which is required for parasite growth, is the key to the specificity of artemisinin activation [38].

Interestingly, in studies using yeast cells as a proxy for malaria parasites [51,52], it was found that mitochondria were directly involved in both the activation and action of artemisinin, thus further linking artemisinin action to reactive oxidative species (ROS) production and oxidative damage. It is also plausible that multiple redundant activation pathways may exist in different environments or localities, where the conditions and magnitude of activation can differ [53]. Looking ahead, it will be crucial to consider the pivotal role of drug activation in the activity of artemisinin and to further elucidate its mechanisms under different conditions.

3.2. Downstream mechanism

The crucial step in elucidating a drug's MOA is to identify its cellular targets. In the conventional understanding of drug design and mechanisms, a drug modifies one or more specific cellular targets, such as proteins, in order to effect downstream changes. However,

the exceedingly fast-acting and potent nature of artemisinin activity, taken together with its ability to alkylate targets, may be due to quite a different mechanism.

First of all, heme releases from hemoglobin digestion functions that lie beyond drug activation, as previously outlined. Excess heme is converted in infected erythrocytes to hematin, which is toxic to the parasite via oxidative damage and direct lysis of cell membranes [54]. Malarial parasites have therefore evolved a detoxifying mechanism that converts hematin to the nontoxic and inert crystallized hemozoin via a biocrystallization process [55]. Activated artemisinin has been reported to prevent the formation of hemozoin by alkylating heme; therefore, it functions in a similar capacity to other antimalarial drugs that act on hemozoin formation, such as CQ [45,56–58]. Thus, free heme from hemoglobin digestion serves as both the activator and the target of artemisinin [45].

Given that activated artemisinin is thought to generate ROS, it is unsurprising that artemisinin has also been reported to directly alkylate protein targets [59,60]. The translationally controlled tumor protein (TCTP) and the *Plasmodium* sarco/endoplasmic reticulum Ca^{2+}-ATPase PfATP6 were among the first targets of interest that were identified as interacting partners of artemisinin [61–63]. Consideration of the role of single targets in the activity of artemisinin has now evolved into MOAs that may depend on multiple targets, as later studies have shown [64–67]. Using unbiased proteomics methods, it has been observed that artemisinin targeting may be promiscuous rather than monotarget-specific. In the first study that systematically reported artemisinin binding targets, over 100 proteins were identified in live parasite strains [47]. An independent study carried out by Ismail et al. [68] led to consistent findings. These results support a promiscuous mechanism of artemisinin targeting in which activated artemisinin alkylates and damages many cellular proteins, thereby disrupting multiple key biological functions and resulting in toxicity and lethality in parasites [47,48,50]. Interestingly, PfATP6 and other key transporters such as PfCRT and Pfmdr1 are consistently labeled in these types of experiments. These findings are consistent with PfATP6 being an important target for artemisinins [47,68]. As an independent line of evidence, the mapped binding sites of artemisinin to TCTP further support a heme-activated promiscuous mechanism in which modification sites are proximity-based and essentially random [50].

Our current knowledge of artemisinin paints a picture of a drug with a unique and elegant mechanism. Artemisinin and its derivatives are prodrugs that absolutely require endoperoxide group cleavage for drug activation and subsequent anti-parasite activity. Artemisinin activation is dependent on a heme-rich environment, which is specific to infected erythrocytes as well as being an unavoidable outcome of parasite metabolism. The heme-rich environment itself is then exploited by the activated drug to achieve efficient parasite killing. This mechanism essentially links infection and parasite growth to drug activation, thus ensuring both the outstanding specificity and the tolerability of artemisinin therapy. At the same time, activated artemisinin indiscriminately damages proximal proteins and cellular structures. Rather than targeting a single protein or cellular function, like the majority of conventional drugs (including most antimalarials), artemisinin acts like a less-discriminative "bomb" that detonates upon activation to cause widespread damage. The specificity of artemisinin may therefore be seen to be based on its activation rather than on its targets. These unique properties of artemisinin make it almost the ideal weapon against malaria, especially in combination with other drugs that act via distinct mechanisms and complement the pharmacological profile of artemisinin. An obvious advantage of a promiscuously targeting drug is also worth noting here: The development of drug resistance is much more difficult when mutation in one or a few specific targets is not sufficient to seriously impact drug activity. This advantage could well explain why artemisinin has remained generally efficacious despite its ubiquitous use over decades.

Nevertheless, recent trends have signaled the incidence and rise of malaria that is being cleared more slowly by ACTs, especially in the Asian endemic regions [69]. This topic has been comprehensively covered from various angles by recent reviews and commentaries [69–75]. Regardless of the controversies about the exact definition of "artemisinin resistance" in the field, the threat is undoubtedly real, given the place that artemisinin occupies in the control of malaria [76,77]. To resolve this burning issue, two major challenges must be overcome: ① A full understanding of the MOA of artemisinin must be achieved; and ② the genetic and physiological features of the newly emerged artemisinin-resistant strains must be defined. Even though the MOA of artemisinin has been largely demystified in the past few years, the molecular characterization of artemisinin-resistant malaria is far from clear. Continued efforts are required to achieve a complete picture of how artemisinin resistance relates to its mode of action. Based on this new knowledge, new therapeutic strategies can then be developed and tested.

4. Repurposing artemisinin

Artemisinin therapy is characterized by its outstanding tolerability and relative affordability. This combination of proven safety and accessibility make artemisinin a drug of exceptional interest for repurposing studies. Indeed, interest in non-malarial applications of artemisinin has increased steadily over time since artemisinin was first made known to the world [78]. While malaria remains the only disease for which artemisinin is an approved treatment, the potential applications of artemisinin in anti-cancer, anti-inflammatory, anti-parasitic (outside of malaria) and anti-viral roles, among others, have been explored in earnest over the years [78–82]. Here, we briefly comment on some promising research in artemisinin repurposing, especially in the field of cancer treatment, as a window into future drug development.

The efficacy of artemisinin in cancer cultures was first reported in 1993, and has since been expanded on and extensively characterized [83–85]. It is now well-reported that artemisinin and its derivatives display selective cytotoxicity against a range of cancer types in both *in vitro* and *in vivo* studies [86]. Forays into clinical testing have been generally promising, if limited in number and scale [87–89]. More than two decades of research on the basis of artemisinin action in cancer has uncovered a plethora of implicated targets and mechanisms. Artemisinin has been reported to induce mitochondrial apoptosis and other forms of cell death such as necroptosis, inhibit cancer angiogenesis and metastasis, and arrest the cancer cell cycle [90–97]. These outcomes are reportedly mediated by a combination of oxidative damage, DNA damage, alteration of gene expression, and interactions with a wide array of signaling pathways including mammalian target of rapamycin (mTOR), NF-κB, mitogen-activated protein (MAP) kinases, and Wnt/β-catenin, among many others [82,98–102]. These pathways and mechanisms have been extensively reviewed in recent publications [79–82].

While pathway validation is an important aspect of mechanistic study, it is also necessary to consider the big picture in terms of unifying drug activation and downstream activity in a manner similar to what was done in malaria studies. As is the case with malarial parasites, the activation mechanism of artemisinin in cancer cells is likely to be heavily linked to its specificity of action. Thus, the role of free ferrous iron versus free redox-active heme is once again being put under scrutiny, especially considering that iron is intimately linked to artemisinin-induced cytotoxicity in cancer [103,104]. Recent studies have once again shed light on

the role of heme in artemisinin activation in cancer cells, thereby drawing parallels with the case in malaria. In particular, a range of methodologies have been used to demonstrate that modulation of heme synthesis and availability clearly correlates with cytotoxicity [105–108]. It is also important to note that cancer cells have been reported to possess enhanced levels of heme metabolism and synthesis, and that this could underpin the cancer specificity of artemisinin in a similar manner to the case in malaria [109–111]. Specific targeting of artemisinin to mitochondria (the site of mammalian cell heme synthesis) or enhancement of heme levels by treatment with the heme precursor aminolevulinic acid (ALA) both improved anti-cancer activity [112–114]. A heme-centric mechanism of activation and an iron-dependent mechanism of downstream cytotoxicity could possibly be a point of reconciliation between the roles of those two species in the anti-cancer activity of artemisinin [115]. Further work to fully understand the basis of artemisinin specificity in cancer will be critical for future therapeutic applications.

At the same time, it is necessary to consider the appropriate direction when moving forward in terms of validating artemisinin MOAs in cancer. Consider the case in malaria, where artemisinin is proposed to indiscriminately attack adjacent targets upon activation. If artemisinin is activated in a similar manner in cancer cells, it is plausible that the same promiscuous multi-target mechanism would take place. This would explain the remarkable range of cellular effects and implicated pathways that have already been reported, as multiple targets and functional pathways are likely to be simultaneously affected by such a mechanism. Indeed, recent unbiased studies of artemisinin cancer targets using proteomics approaches have revealed a similar multi-target MOA by artemisinin in cancer cells [48,113,114]. The mechanism of cytotoxicity itself is also a matter of great interest, especially with regard to non-apoptotic forms of cell death. Recent work has closely linked artemisinin-induced cytotoxicity to oxidative damage and lysosomal function, with a focus on the role of iron in contributing to the iron-dependent form of cell death known as ferroptosis [116–118]. In particular, lysosome-mediated degradation of ferritin under autophagy conditions (termed ferritinophagy) releases free ferrous iron, which in turn contributes to both ferroptosis and iron-mediated generation of ROS [93,119]. Autophagy itself is a cellular process that is reportedly activated by artemisinin, but has ambiguous effects on cancer cell survival and the cytotoxicity of artemisinin [115,119]. It is clear that the relationship between autophagy, lysosomal activity, free ferrous iron, and iron-dependent ferroptotic cell death following artemisinin exposure represents a major area of uncertainty in the anti-cancer mechanism of artemisinin. However, efforts in unveiling novel, cancer-specific targets and mechanisms are steadily ongoing and continue to contribute to a grand view of artemisinin as an anti-cancer drug. Artemisinin-mediated effects on cancer stem cells, immunomodulation, cancer metastasis, cancer metabolism including the regulation of glycolysis, and a plethora of signaling pathways including signal transducer and activator of transcription 3 (STAT3), NF-κB, mTOR, and CREBP signaling are among recent reports, and indicate novel directions for further validation [115,120,121]. In particular, the potential ability of artemisinin to serve as an immunomodulator in cancer by regulating regulatory T cell (Treg) activity and the production of pro-cancer-survival immunosuppressive cytokines such as prostaglandin E2 (PGE_2) is noteworthy, given the complex role of immunomodulatory drugs in cancer therapy [122–125]. Finally, efforts to improve the formulation and delivery of artemisinin-based drugs have shown promise in delivering enhanced efficacy and reduced susceptibility to drug resistance. These results include novel synthetic dimers, trimers, and drug conjugates (especially transferrin-conjugated systems), in addition to combination therapies; they represent an exciting ongoing area of research that has been reviewed comprehensively in recent publications [126–135].

In addition to the possible applications of artemisinin in cancer treatment, active research is taking place on its potential roles in addressing a range of other diseases. In particular, anti-inflammatory effects against autoimmune diseases and allergic asthma, among other conditions, have been reported in a range of disease models [78]. Some of these results correlate with observations of immunosuppression in patients undergoing artemisinin therapy for malaria [136]. Strong anti-viral effects of artemisinin have also been reported in herpes and in hepatitis B and C viruses, and other parasitic diseases including schistosomiasis have also been shown to respond to artemisinin treatment [137–141]. Recent findings have even identified a remarkable—if controversial—role of artemisinin in diabetes through inducing transdifferentiation of pancreatic α cells to generate β cells [142,143]. The MOA for these alternative applications is frequently discussed in terms of the canonical model of ROS generation and oxidative damage induction upon endoperoxide cleavage; however, non-canonical (including endoperoxide-independent) mechanisms have also been proposed, especially in the case of immunomodulation [78,144]. It will be essential to pursue a clear view of how drug mechanisms and functions may differ under varying applications and conditions, while considering the importance of the conditions of drug activation. It is also worth noting that repurposing research might be best carried out in patients and regions that are not burdened with or at risk of malaria, in order to avoid possible interference or complications. Every care must be taken to ensure that the full potential of artemisinin can be realized without compromising its current applications.

5. Conclusion

The artemisinins are a class of remarkable drugs that have redefined the landscape of antimalarial therapy. A combination of outstanding potency, safety, and accessibility has put artemisinin at the forefront of the ongoing battle against the malaria scourge, where it has already impacted millions of lives. Since its discovery, a concerted effort by the global community has assembled a picture of a drug with a unique set of properties that makes it almost the ideal antimalarial drug. Active research in other fields has also revealed a broad spectrum of promising applications for artemisinin outside of malaria. We believe that it is only logical to seek to maximize the utility of this drug in a range of capacities. In the context of malaria, doing so means to continue to clarify the mechanisms of activation and action of artemisinin, while working to further improve its pharmacological properties both alone and in combination [145]. Combined with a firm grasp of the principles of artemisinin activity, this could be the key to clearing the uncertainties of artemisinin resistance. Such efforts would ensure that the drug can continue to perform in a similar or even greater capacity within the role that it has served for so long. Looking ahead, repurposing studies driven by a robust understanding of differential MOAs in different diseases and systems will also be instrumental in defining the future of artemisinin. Ultimately, it is our sincere hope that this gift from Chinese medicine can continue to serve the pursuit of health for people all around the world, for many years to come.

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Compliance with ethics guidelines

Jigang Wang, Chengchao Xu, Yin Kwan Wong, Yujie Li, Fulong Liao, Tingliang Jiang, and Youyou Tu declare that they have no conflict of interest or financial conflicts to disclose.

References

[1] Tu Y. Artemisinin—a gift from traditional Chinese medicine to the world (Nobel lecture). Angew Chem Int Ed 2016;55(35):10210–26.
[2] Cox FE. History of the discovery of the malaria parasites and their vectors. Parasit Vectors 2010;3(1):5.
[3] Krishna S, Bustamante L, Haynes RK, Staines HM. Artemisinins: their growing importance in medicine. Trends Pharmacol Sci 2008;29(10):520–7.
[4] World Health Organization. World malaria report 2017. Geneva: World Health Organization; 2017.
[5] Ding XC, Beck HP, Raso G. Plasmodium sensitivity to artemisinins: magic bullets hit elusive targets. Trends Parasitol 2011;27(2):73–81.
[6] Efferth T, Romero MR, Bilia AR, Osman AG, Sohly ME, et al. Expanding the therapeutic spectrum of artemisinin: activity against infectious diseases beyond malaria and novel pharmaceutical developments. World J Tradit Chin Med 2016;2(2):1–23.
[7] D'Alessandro U, Buttiëns H. History and importance of antimalarial drug resistance. Trop Med Int Health 2001;6(11):845–8.
[8] Tu Y. The discovery of artemisinin (Qinghaosu) and gifts from Chinese medicine. Nat Med 2011;17(10):1217–20.
[9] Liao F. Discovery of artemisinin (Qinghaosu). Molecules 2009;14(12):5362–6.
[10] Collaboration Research Group for Qinghaosu. A new sesquiterpene lactone—Qinghaosu. Chin Sci Bull 1997;3:142. Chinese.
[11] Wang MY. Publication process involving the discovery of artemisinin (Qinghaosu) before 1985. Asian Pac J Trop Biomed 2016;6(6):461–7.
[12] China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials. Chemical studies on Qinghaosu (artemisinine). J Tradit Chin Med 1982;2(1):3–8. Chinese.
[13] Klayman DL. Qinghaosu (artemisinin): an antimalarial drug from China. Science 1985;228(4703):1049–55.
[14] Jiang JB, Li GQ, Guo XB, Kong YC, Arnold K. Antimalarial activity of mefloquine and Qinghaosu. Lancet 1982;2(8293):285–8.
[15] Looareesuwan S, Viravan C, Vanijanonta S, Wilairatana P, Suntharasamai P, Charoenlarp P, et al. Randomised trial of artesunate and mefloquine alone and in sequence for acute uncomplicated falciparum malaria. Lancet 1992;339(8797):821–4.
[16] Nosten F, Luxemburger C, ter Kuile FO, Woodrow C, Eh JP, Chongsuphajaisiddhi T, et al. Treatment of multidrug-resistant *Plasmodium falciparum* malaria with 3-day artesunate-mefloquine combination. J Infect Dis 1994;170(4):971–7.
[17] Tran TH, Day NP, Nguyen HP, Nguyen TH, Tran TH, Pham PL, et al. A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. N Engl J Med 1996;335(2):76–83.
[18] Looareesuwan S, Wilairatana P, Viravan C, Vanijanonta S, Pitisuttithum P, Kyle DE. Open randomized trial of oral artemether alone and a sequential combination with mefloquine for acute uncomplicated falciparum malaria. Am J Trop Med Hyg 1997;56(6):613–7.
[19] Adjuik M, Babiker A, Garner P, Olliaro P, Taylor W, White N, et al; International Artemisinin Study Group. Artesunate combinations for treatment of malaria: meta-analysis. Lancet 2004;363(9402):9–17.
[20] Von Seidlein L, Jaffar S, Pinder M, Haywood M, Snounou G, Gemperli B, et al. Treatment of African children with uncomplicated falciparum malaria with a new antimalarial drug, CGP 56697. J Infect Dis 1997;176(4):1113–6.
[21] Von Seidlein L, Bojang K, Jones P, Jaffar S, Pinder M, Obaro S, et al. A randomized controlled trial of artemether/benflumetol, a new antimalarial and pyrimethamine/sulfadoxine in the treatment of uncomplicated falciparum malaria in African children. Am J Trop Med Hyg 1998;58(5):638–44.
[22] Doherty JF, Sadiq AD, Bayo L, Alloueche A, Olliaro P, Milligan P, et al. A randomized safety and tolerability trial of artesunate plus sulfadoxine—pyrimethamine versus sulfadoxine-pyrimethamine alone for the treatment of uncomplicated malaria in Gambian children. Trans R Soc Trop Med Hyg 1999;93(5):543–6.
[23] Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, Chhaganlal KD, et al; AQUAMAT Group. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. Lancet 2010;376(9753):1647–57.
[24] WWARN Artemisinin based Combination Therapy (ACT) Africa Baseline Study Group. Clinical determinants of early parasitological response to ACTs in African patients with uncomplicated falciparum malaria: a literature review and meta-analysis of individual patient data. BMC Med 2015;13:212.
[25] Efferth T, Kaina B. Toxicity of the antimalarial artemisinin and its derivatives. Crit Rev Toxicol 2010;40(5):405–21.
[26] World Health Organization. Guidelines for the treatment of malaria. Geneva: World Health Organization; 2006.
[27] Luo XD, Shen CC. The chemistry, pharmacology, and clinical applications of Qinghaosu (artemisinin) and its derivatives. Med Res Rev 1987;7(1):29–52.
[28] White NJ. Clinical pharmacokinetics and pharmacodynamics of artemisinin and derivatives. Trans R Soc Trop Med Hyg 1994;88(Suppl 1):S41–3.
[29] De Vries PJ, Dien TK. Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. Drugs 1996;52(6):818–36.
[30] German PI, Aweeka FT. Clinical pharmacology of artemisinin-based combination therapies. Clin Pharmacokinet 2008;47(2):91–102.
[31] Robert A, Dechy-Cabaret O, Cazelles J, Meunier B. From mechanistic studies on artemisinin derivatives to new modular antimalarial drugs. Acc Chem Res 2002;35(3):167–74.
[32] Li J, Zhou B. Biological actions of artemisinin: insights from medicinal chemistry studies. Molecules 2010;15(8):1378–97.
[33] Van Agtmael MA, Eggelte TA, van Boxtel CJ. Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication. Trends Pharmacol Sci 1999;20(5):199–205.
[34] Meshnick SR, Taylor TE, Kamchonwongpaisan S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. Microbiol Rev 1996;60(2):301–15.
[35] Posner GH, O'Neill PM. Knowledge of the proposed chemical mechanism of action and cytochrome p450 metabolism of antimalarial trioxanes like artemisinin allows rational design of new antimalarial peroxides. Acc Chem Res 2004;37(6):397–404.
[36] O'Neill PM, Barton VE, Ward SA. The molecular mechanism of action of artemisinin—the debate continues. Molecules 2010;15(3):1705–21.
[37] Lew VL, Tiffert T, Ginsburg H. Excess hemoglobin digestion and the osmotic stability of *Plasmodium falciparum*-infected red blood cells. Blood 2003;101(10):4189–94.
[38] Klonis N, Crespo-Ortiz MP, Bottova I, Abu-Bakar N, Kenny S, Rosenthal PJ, et al. Artemisinin activity against *Plasmodium falciparum* requires hemoglobin uptake and digestion. Proc Natl Acad Sci USA 2011;108(28):11405–10.
[39] Xie SC, Dogovski C, Hanssen E, Chiu F, Yang T, Crespo MP, et al. Haemoglobin degradation underpins the sensitivity of early ring stage *Plasmodium falciparum* to artemisinins. J Cell Sci 2016;129(2):406–16.
[40] Zhang F, Gosser DK Jr, Meshnick SR. Hemin-catalyzed decomposition of artemisinin (Qinghaosu). Biochem Pharmacol 1992;43(8):1805–9.
[41] Posner GH, Oh CH, Wang D, Gerena L, Milhous WK, Meshnick SR, et al. Mechanism-based design, synthesis, and *in vitro* antimalarial testing of new 4-methylated trioxanes structurally related to artemisinin: the importance of a carbon-centered radical for antimalarial activity. J Med Chem 1994;37(9):1256–8.
[42] Wu WM, Wu Y, Wu YL, Yao ZJ, Zhou CM, Li Y, et al. Unified mechanistic framework for the Fe(II)-induced cleavage of Qinghaosu and derivatives/analogues. The first spin-trapping evidence for the previously postulated secondary C-4 radical. J Am Chem Soc 1998;120(14):3316–25.
[43] Stocks PA, Bray PG, Barton VE, Al-Helal M, Jones M, Araujo NC, et al. Evidence for a common non-heme chelatable-iron-dependent activation mechanism for semisynthetic and synthetic endoperoxide antimalarial drugs. Angew Chem Int Ed 2007;46(33):6278–83.
[44] Haynes RK, Chan WC, Lung CM, Uhlemann AC, Eckstein U, Taramelli D, et al. The Fe^{2+}-mediated decomposition, PfATP6 binding, and antimalarial activities of artemisone and other artemisinins: the unlikelihood of C-centered radicals as bioactive intermediates. ChemMedChem 2007;2(10):1480–97.
[45] Meunier B, Robert A. Heme as trigger and target for trioxane-containing antimalarial drugs. Acc Chem Res 2010;43(11):1444–51.
[46] Haynes RK, Cheu KW, N'Da D, Coghi P, Monti D. Considerations on the mechanism of action of artemisinin antimalarials: part 1—the 'carbon radical' and 'heme' hypotheses. Infect Disord Drug Targets 2013;13(4):217–77.
[47] Wang J, Zhang CJ, Chia WN, Loh CC, Li Z, Lee YM, et al. Haem-activated promiscuous targeting of artemisinin in *Plasmodium falciparum*. Nat Commun 2015;6:10111.
[48] Zhou Y, Li W, Xiao Y. Profiling of multiple targets of artemisinin activated by hemin in cancer cell proteome. ACS Chem Biol 2016;11(4):882–8.
[49] Zhang S, Gerhard GS. Heme activates artemisinin more efficiently than hemin, inorganic iron, or hemoglobin. Bioorg Med Chem 2008;16(16):7853–61.
[50] Li W, Zhou Y, Tang G, Xiao Y. Characterization of the artemisinin binding site for translationally controlled tumor protein (TCTP) by bioorthogonal click chemistry. Bioconjug Chem 2016;27(12):2828–33.
[51] Li W, Mo W, Shen D, Sun L, Wang J, Lu S, et al. Yeast model uncovers dual roles of mitochondria in action of artemisinin. PLoS Genet 2005;1(3):e36.
[52] Wang J, Huang L, Li J, Fan Q, Long Y, Li Y, et al. Artemisinin directly targets malarial mitochondria through its specific mitochondrial activation. PLoS One 2010;5(3):e9582.
[53] Sun C, Li J, Cao Y, Long G, Zhou B. Two distinct and competitive pathways confer the cellcidal actions of artemisinins. Microb Cell 2015;2(1):14–25.

[54] Fitch CD, Chevli R, Kanjananggulpan P, Dutta P, Chevli K, Chou AC. Intracellular ferriprotoporphyrin IX is a lytic agent. Blood 1983;62(6):1165–8.
[55] Egan TJ. Recent advances in understanding the mechanism of hemozoin (malaria pigment) formation. J Inorg Biochem 2008;102(5–6):1288–99.
[56] Cazelles J, Robert A, Meunier B. Alkylation of heme by artemisinin, an antimalarial drug. Acad Sci Chem 2001;4(2):85–9.
[57] Robert A, Benoit-Vical F, Claparols C, Meunier B. The antimalarial drug artemisinin alkylates heme in infected mice. Proc Natl Acad Sci USA 2005;102 (38):13676–80.
[58] Loup C, Lelièvre J, Benoit-Vical F, Meunier B. Trioxaquines and heme-artemisinin adducts inhibit the *in vitro* formation of hemozoin better than chloroquine. Antimicrob Agents Chemother 2007;51(10):3768–70.
[59] Yang YZ, Little B. Meshnick SR. Alkylation of proteins by artemisinin. Effects of heme, pH, and drug structure. Biochem Pharmacol 1994;48(3):569–73.
[60] Meshnick SR. Artemisinin: mechanisms of action, resistance and toxicity. Int J Parasitol 2002;32(13):1655–60.
[61] Bhisutthibhan J, Pan XQ, Hossler PA, Walker DJ, Yowell CA, Carlton J, et al. The *Plasmodium falciparum* translationally controlled tumor protein homolog and its reaction with the antimalarial drug artemisinin. J Biol Chem 1998;273 (26):16192–8.
[62] Eckstein-Ludwig U, Webb RJ, Van Goethem ID, East JM, Lee AG, Kimura M, et al. Artemisinins target the SERCA of *Plasmodium falciparum*. Nature 2003;424(9651):957–61.
[63] Uhlemann AC, Cameron A, Eckstein-Ludwig U, Fischbarg J, Iserovich P, Zuniga FA, et al. A single amino acid residue can determine the sensitivity of SERCAs to artemisinins. Nat Struct Mol Biol 2005;12(7):628–9.
[64] Krishna S, Pulcini S, Fatih F, Staines H. Artemisinins and the biological basis for the PfATP6/SERCA hypothesis. Trends Parasitol 2010;26(11):517–23.
[65] Arnou B, Montigny C, Morth JP, Nissen P, Jaxel C, Møller JV, et al. The *Plasmodium falciparum* Ca^{2+}-ATPase PfATP6: insensitive to artemisinin, but a potential drug target. Biochem Soc Trans 2011;39(3):823–31.
[66] Cui L, Wang Z, Jiang H, Parker D, Wang H, Su XZ, et al. Lack of association of the S769N mutation in *Plasmodium falciparum* SERCA (PfATP6) with resistance to artemisinins. Antimicrob Agents Chemother 2012;56 (5):2546–52.
[67] Krishna S, Pulcini S, Moore CM, Teo BH, Staines HM. Pumped up: reflections on PfATP6 as the target for artemisinins. Trends Pharmacol Sci 2014;35(1):4–11.
[68] Ismail HM, Barton V, Phanchana M, Charoensutthivarakul S, Wong MH, Hemingway J, et al. Artemisinin activity-based probes identify multiple molecular targets within the asexual stage of the malaria parasites *Plasmodium falciparum* 3D7. Proc Natl Acad Sci USA 2016;113(8):2080–5.
[69] Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al; Tracking Resistance to Artemisinin Collaboration (TRAC). Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Eng J Med 2014;371(5):411–23.
[70] Dondorp AM, Yeung S, White L, Nguon C, Day NP, Socheat D, et al. Artemisinin resistance: current status and scenarios for containment. Nat Rev Microbiol 2010;8(4):272–80.
[71] Paloque L, Ramadani AP, Mercereau-Puijalon O, Augereau JM, Benoit-Vical F. *Plasmodium falciparum*: multifaceted resistance to artemisinins. Malar J 2016;15:149.
[72] Tilley L, Straimer J, Gnädig NF, Ralph SA, Fidock DA. Artemisinin action and resistance in *Plasmodium falciparum*. Trends Parasitol 2016;32(9):682–96.
[73] Wang J, Xu C, Lun ZR, Meshnick SR. Unpacking 'artemisinin resistance'. Trends Pharmacol Sci 2017;38(6):506–11.
[74] Gil JP, Krishna S. pfmdr1 (*Plasmodium falciparum* multidrug drug resistance gene 1): a pivotal factor in malaria resistance to artemisinin combination therapies. Expert Rev Anti Infect Ther 2017;15(6):527–43.
[75] Hanscheid T, Hardisty DW. How "resistant" is artemisinin resistant malaria?—The risks of ambiguity using the term "resistant" malaria. Travel Med Infect Dis 2018;24:23–4.
[76] Meshnick S. Perspective: artemisinin-resistant malaria and the wolf. Am J Trop Med Hyg 2012;87(5):783–4.
[77] World Health Organization. Artemisinin and artemisinin-based combination therapy resistance. Report. Geneva: World Health Organization; 2016 Apr.
[78] Ho WE, Peh HY, Chan TK, Wong WS. Artemisinins: pharmacological actions beyond anti-malarial. Pharmacol Ther 2014;142(1):126–39.
[79] Firestone GL, Sundar SN. Anticancer activities of artemisinin and its bioactive derivatives. Expert Rev Mol Med 2009;11:e32.
[80] Crespo-Ortiz MP, Wei MQ. Antitumor activity of artemisinin and its derivatives: from a well-known antimalarial agent to a potential anticancer drug. J Biomed Biotechnol 2012;2012:247597.
[81] Lai HC, Singh NP, Sasaki T. Development of artemisinin compounds for cancer treatment. Invest New Drugs 2013;31(1):230–46.
[82] Efferth T. Artemisinin—second career as anticancer drug? World J Tradit Chin Med 2015;1(4):2–25.
[83] Woerdenbag HJ, Moskal TA, Pras N, Malingré TM, el-Feraly FS, Kampinga HH, et al. Cytotoxicity of artemisinin-related endoperoxides to Ehrlich ascites tumor cells. J Nat Prod 1993;56(6):849–56.
[84] Lai H, Singh NP. Selective cancer cell cytotoxicity from exposure to dihydroartemisinin and holotransferrin. Cancer Lett 1995;91(1):41–6.
[85] Efferth T, Dunstan H, Sauerbrey A, Miyachi H, Chitambar CR. The anti-malarial artesunate is also active against cancer. Int J Oncol 2001;18(4):767–73.
[86] Efferth T, Sauerbrey A, Olbrich A, Gebhart E, Rauch P, Weber HO, et al. Molecular modes of action of artesunate in tumor cell lines. Mol Pharmacol 2003;64(2):382–94.
[87] Zhang ZY, Yu SQ, Miao LY, Huang XY, Zhang XP, Zhu YP, et al. Artesunate combined with vinorelbine plus cisplatin in treatment of advanced non-small cell lung cancer: a randomized controlled trial. J Chin Integr Med 2008;6 (2):134–8.
[88] Jansen FH, Adoubi I, JC KC, DE Cnodder T, Jansen N, Tschulakow A, et al. First study of oral Artenimol-R in advanced cervical cancer: clinical benefit, tolerability and tumor markers. Anticancer Res 2011;31(12):4417–22.
[89] Krishna S, Ganapathi S, Ster IC, Saeed ME, Cowan M, Finlayson C, et al. A randomised, double blind, placebo-controlled pilot study of oral artesunate therapy for colorectal cancer. EBioMedicine 2014;2(1):82–90.
[90] Efferth T, Rücker G, Falkenberg M, Manns D, Olbrich A, Fabry U, et al. Detection of apoptosis in KG-1a leukemic cells treated with investigational drugs. Arzneimittelforschung 1996;46(2):196–200.
[91] Willoughby JA Sr, Sundar SN, Cheung M, Tin AS, Modiano J, Firestone GL. Artemisinin blocks prostate cancer growth and cell cycle progression by disrupting Sp1 interactions with the cyclin-dependent kinase-4 (CDK4) promoter and inhibiting CDK4 gene expression. J Biol Chem 2009;284 (4):2203–13.
[92] Steinbrück L, Pereira G, Efferth T. Effects of artesunate on cytokinesis and G_2/M cell cycle progression of tumour cells and budding yeast. Cancer Genomics Proteomics 2010;7(6):337–46.
[93] Hamacher-Brady A, Stein HA, Turschner S, Toegel I, Mora R, Jennewein N, et al. Artesunate activates mitochondrial apoptosis in breast cancer cells via iron-catalyzed lysosomal reactive oxygen species production. J Biol Chem 2011;286(8):6587–601.
[94] Anfosso L, Efferth T, Albini A, Pfeffer U. Microarray expression profiles of angiogenesis-related genes predict tumor cell response to artemisinins. Pharmacogenomics J 2006;6(4):269–78.
[95] Button RW, Lin F, Ercolano E, Vincent JH, Hu B, Hanemann CO, et al. Artesunate induces necrotic cell death in schwannoma cells. Cell Death Dis 2014;5:e1466.
[96] Buommino E, Baroni A, Canozo N, Petrazzuolo M, Nicoletti R, Vozza A, et al. Artemisinin reduces human melanoma cell migration by down-regulating αVβ3 integrin and reducing metalloproteinase 2 production. Invest New Drugs 2009;27(5):412–8.
[97] Chen T, Li M, Zhang R, Wang H. Dihydroartemisinin induces apoptosis and sensitizes human ovarian cancer cells to carboplatin therapy. J Cell Mol Med 2009;13(7):1358–70.
[98] Li PC, Lam E, Roos WP, Zdzienicka MZ, Kaina B, Efferth T. Artesunate derived from traditional Chinese medicine induces DNA damage and repair. Cancer Res 2008;68(11):4347–51.
[99] Hou J, Wang D, Zhang R, Wang H. Experimental therapy of hepatoma with artemisinin and its derivatives: *in vitro* and *in vivo* activity, chemosensitization, and mechanisms of action. Clin Cancer Res 2008;14(17):5519–30.
[100] Berdelle N, Nikolova T, Quiros S, Efferth T, Kaina B. Artesunate induces oxidative DNA damage, sustained DNA double-strand breaks, and the ATM/ATR damage response in cancer cells. Mol Cancer Ther 2011;10(12):2224–33.
[101] Huang C, Ba Q, Yue Q, Li J, Li J, Chu R, et al. Artemisinin rewires the protein interaction network in cancer cells: network analysis, pathway identification, and target prediction. Mol Biosyst 2013;9(12):3091–100.
[102] Li X, Ba Q, Liu Y, Yue Q, Chen P, Li J, et al. Dihydroartemisinin selectively inhibits PDGFR α-positive ovarian cancer growth and metastasis through inducing degradation of PDGFR α protein. Cell Discov 2017;3:17042.
[103] Efferth T, Benakis A, Romero MR, Tomicic M, Rauh R, Steinbach D, et al. Enhancement of cytotoxicity of artemisinins toward cancer cells by ferrous iron. Free Radic Biol Med 2004;37(7):998–1009.
[104] Ba Q, Zhou N, Duan J, Chen T, Hao M, Yang X, et al. Dihydroartemisinin exerts its anticancer activity through depleting cellular iron via transferrin receptor-1. PLoS One 2012;7(8):e42703.
[105] Zhang S, Gerhard GS. Heme mediates cytotoxicity from artemisinin and serves as a general anti-proliferation target. PLoS One 2009;4(10):e7472.
[106] Stockwin LH, Han B, Yu SX, Hollingshead MG, ElSohly MA, Gul W, et al. Artemisinin dimer anticancer activity correlates with heme-catalyzed reactive oxygen species generation and endoplasmic reticulum stress induction. Int J Cancer 2009;125(6):1266–75.
[107] Zhang S, Chen H, Gerhard GS. Heme synthesis increases artemisinin-induced radical formation and cytotoxicity that can be suppressed by superoxide scavengers. Chem Biol Interact 2010;186(1):30–5.
[108] Mercer AE, Copple IM, Maggs JL, O'Neill PM, Park BK. The role of heme and the mitochondrion in the chemical and molecular mechanisms of mammalian cell death induced by the artemisinin antimalarials. J Biol Chem 2011;286 (2):987–96.
[109] Hooda J, Cadinu D, Alam MM, Shah A, Cao TM, Sullivan LA, et al. Enhanced heme function and mitochondrial respiration promote the progression of lung cancer cells. PLoS One 2013;8(5):e63402.
[110] Hooda J, Shah A, Zhang L. Heme, an essential nutrient from dietary proteins, critically impacts diverse physiological and pathological processes. Nutrients 2014;6(3):1080–102.
[111] Hooda J, Alam M, Zhang L. Measurement of heme synthesis levels in mammalian cells. J Vis Exp 2015;101:e51579.
[112] Zhang X, Ba Q, Gu Z, Guo D, Zhou Y, Xu Y, et al. Fluorescent coumarin-artemisinin conjugates as mitochondria-targeting theranostic probes for enhanced anticancer activities. Chemistry 2015;21(48):17415–21.
[113] Zhang CJ, Wang J, Zhang J, Lee YM, Feng G, Lim TK, et al. Mechanism-guided design and synthesis of a mitochondria-targeting artemisinin analogue with enhanced anticancer activity. Angew Chem Int Ed 2016;55(44):13770–4.

[114] Wang J, Zhang J, Shi Y, Xu C, Zhang C, Wong YK, et al. Mechanistic investigation of the specific anticancer property of artemisinin and its combination with aminolevulinic acid for enhanced anticolorectal cancer activity. ACS Cent Sci 2017;3(7):743–50.
[115] Wong YK, Xu C, Kalesh KA, He Y, Lin Q, Wong WSF, et al. Artemisinin as an anticancer drug: recent advances in target profiling and mechanisms of action. Med Res Rev 2017;37(6):1492–517.
[116] Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 2012;149(5):1060–72.
[117] Eling N, Reuter L, Hazin J, Hamacher-Brady A, Brady NR. Identification of artesunate as a specific activator of ferroptosis in pancreatic cancer cells. Oncoscience 2015;2(5):517–32.
[118] Ooko E, Saeed ME, Kadioglu O, Sarvi S, Colak M, Elmasaoudi K, et al. Artemisinin derivatives induce iron-dependent cell death (ferroptosis) in tumor cells. Phytomedicine 2015;22(11):1045–54.
[119] Yang ND, Tan SH, Ng S, Shi Y, Zhou J, Tan KS, et al. Artesunate induces cell death in human cancer cells via enhancing lysosomal function and lysosomal degradation of ferritin. J Biol Chem 2014;289(48):33425–41.
[120] Efferth T. From ancient herb to modern drug: *Artemisia annua* and artemisinin for cancer therapy. Semin Cancer Biol 2017;46:65–83.
[121] Abba ML, Patil N, Leupold JH, Saeed MEM, Efferth T, Allgayer H. Prevention of carcinogenesis and metastasis by artemisinin-type drugs. Cancer Lett 2018;429:11–8.
[122] Noori S, Hassan ZM. Dihydroartemisinin shift the immune response towards Th1, inhibit the tumor growth *in vitro* and *in vivo*. Cell Immunol 2011;271 (1):67–72.
[123] Farsam V, Hassan ZM, Zavaran-Hosseini A, Noori S, Mahdavi M, Ranjbar M. Antitumor and immunomodulatory properties of artemether and its ability to reduce $CD4^+CD25^+FoxP3^+$ T reg cells *in vivo*. Int Immunopharmacol 2011;11(11):1802–8.
[124] Zhang LX, Liu ZN, Ye J, Sha M, Qian H, Bu XH, et al. Artesunate exerts an anti-immunosuppressive effect on cervical cancer by inhibiting PGE_2 production and Foxp3 expression. Cell Biol Int 2014;38(5):639–46.
[125] Cui C, Feng H, Shi X, Wang Y, Feng Z, Liu J, et al. Artesunate down-regulates immunosuppression from colorectal cancer Colon26 and RKO cells *in vitro* by decreasing transforming growth factor β1 and interleukin-10. Int Immunopharmacol 2015;27(1):110–21.
[126] Reiter C, Fröhlich T, Gruber L, Hutterer C, Marschall M, Voigtländer C, et al. Highly potent artemisinin-derived dimers and trimers: synthesis and evaluation of their antimalarial, antileukemia and antiviral activities. Bioorg Med Chem 2015;23(17):5452–8.
[127] Fröhlich T, Çapcı Karagöz A, Reiter C, Tsogoeva SB. Artemisinin-derived dimers: potent antimalarial and anticancer agents. J Med Chem 2016;59 (16):7360–88.
[128] Fröhlich T, Ndreshkjana B, Muenzner JK, Reiter C, Hofmeister E, Mederer S, et al. Synthesis of novel hybrids of thymoquinone and artemisinin with high activity and selectivity against colon cancer. ChemMedChem 2017;12 (3):226–34.
[129] Efferth T. Cancer combination therapies with artemisinin-type drugs. Biochem Pharmacol 2017;139:56–70.
[130] Fröhlich T, Hahn F, Belmudes L, Leidenberger M, Friedrich O, Kappes B, et al. Synthesis of artemisinin-derived dimers, trimers and dendrimers: investigation of their antimalarial and antiviral activities including putative mechanisms of action. Chemistry 2018;24(32):8103–13.
[131] Horwedel C, Tsogoeva SB, Wei S, Efferth T. Cytotoxicity of artesunic acid homo- and heterodimer molecules toward sensitive and multidrug-resistant CCRF-CEM leukemia cells. J Med Chem 2010;53(13):4842–8.
[132] Reiter C, Herrmann A, Çapci A, Efferth T, Tsogoeva SB. New artesunic acid homodimers: potent reversal agents of multidrug resistance in leukemia cells. Bioorg Med Chem 2012;20(18):5637–41.
[133] Reiter C, Capcı Karagöz A, Fröhlich T, Klein V, Zeino M, Viertel K, et al. Synthesis and study of cytotoxic activity of 1,2,4-trioxane- and egonol-derived hybrid molecules against *Plasmodium falciparum* and multidrug-resistant human leukemia cells. Eur J Med Chem 2014;75:403–12.
[134] Reiter C, Fröhlich T, Zeino M, Marschall M, Bahsi H, Leidenberger M, et al. New efficient artemisinin derived agents against human leukemia cells, human cytomegalovirus and *Plasmodium falciparum*: 2nd generation 1,2,4-trioxane-ferrocene hybrids. Eur J Med Chem 2015;97:164–72.
[135] Leto I, Coronnello M, Righeschi C, Bergonzi MC, Mini E, Bilia AR. Enhanced efficacy of artemisinin loaded in transferrin-conjugated liposomes versus stealth liposomes against HCT-8 colon cancer cells. ChemMedChem 2016;11 (16):1745–51.
[136] Bunnag D, Viravan C, Looareesuwan S, Karbwang J, Harinasuta T. Clinical trial of artesunate and artemether on multidrug resistant falciparum malaria in Thailand. A preliminary report. Southeast Asian J Trop Med Public Health 1991;22(3):380–5.
[137] Efferth T, Romero MR, Wolf DG, Stamminger T, Marin JJ, Marschall M. The antiviral activities of artemisinin and artesunate. Clin Infect Dis 2008;47 (6):804–11.
[138] Keiser J, Utzinger J. Artemisinins and synthetic trioxolanes in the treatment of helminth infections. Curr Opin Infect Dis 2007;20(6):605–12.
[139] Saeed MEM, Krishna S, Greten HJ, Kremsner PG, Efferth T. Antischistosomal activity of artemisinin derivatives *in vivo* and in patients. Pharmacol Res 2016;110:216–26.
[140] Lam NS, Long X, Su XZ, Lu F. Artemisinin and its derivatives in treating helminthic infections beyond schistosomiasis. Pharmacol Res 2018;133:77–100.
[141] Efferth T. Beyond malaria: the inhibition of viruses by artemisinin-type compounds. Biotechnol Adv 2018;36(6):1730–7.
[142] Li J, Casteels T, Frogne T, Ingvorsen C, Honoré C, Courtney M, et al. Artemisinins target GABAA receptor signaling and impair α cell identity. Cell 2017;168(1–2):86–100.e15.
[143] Van der Meulen T, Lee S, Noordeloos E, Donaldson CJ, Adams MW, Noguchi GM, et al. Artemether does not turn α cells into β cells. Cell Metab 2018;27 (1). 218 25.e4.
[144] Ho WE, Cheng C, Peh HY, Xu F, Tannenbaum SR, Ong CN, et al. Anti-malarial drug artesunate ameliorates oxidative lung damage in experimental allergic asthma. Free Radic Biol Med 2012;53(3):498–507.
[145] Isacchi B, Arrigucci S, la Marca G, Bergonzi MC, Vannucchi MG, Novelli A, et al. Conventional and long-circulating liposomes of artemisinin: preparation, characterization, and pharmacokinetic profile in mice. J Liposome Res 2011;21(3):237–44.