African Journal of Biochemistry Research Vol. 6(2), pp. 20-26, 30 January, 2012
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 ©2012 Academic Journals
DOI: 10.5897/AJBR11.109

Review

The significance of artemisinin in roll back malaria partnership programmes and cancer therapy

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Accepted 13 December, 2011

The role of the natural product, artemisinin, is discussed, with a view to elucidating its importance as an antimalarial, essential to sustainable global development, especially in the health and welfare industry. An estimated 243 million cases of malaria led to an estimated 863,000 deaths in 2008. The Roll Back Malaria (RBM) Partnership is a global network for co-ordinated action against malaria, launched in 1998 by the World Health Organization (WHO), United Nations International Children Emergency Fund (UNICEF), United Nations Development Programme (UNDP), and World Bank. It promotes consensus among key actors in malaria control, harmonizes action, and mobilizes resources to fight malaria in endemic countries. Large scale use of antimalarial monotherapies such as quinoline compounds (for example, chloroquine) and antifolate drugs (for example, sulfonamides), have culminated in cross-resistance, especially of *Plasmodium falciparum,* to these conventional antimalarial drugs. Artemisinin, (a sesquiterpene lactone), isolated from the plant *Artemisia annua*, is a drug used to treat multi-drug resistant strains of falciparum malaria. *Saccharomyces cerevisiae* microbes can produce the precursor artemisinic acid, by a technique of synthetic biology. The total synthesis of artemisinin can also be performed using the organic reagents, isopulegol. The iron-porphyrin complex-moiety, produced during plasmodium infection of the red blood cells, reacts with artemisinin, a potent inhibitor of hemozoin formation, to produce reactive oxygen radicals which damage the parasite leading to its death. Artemisinin acts on the electron transport chain, by causing the depolarization of the parasite's mitochondrial membrane, and kills the asexual forms of plasmodium at the erythrocytic stage. Artemisinin selectively inhibits the production of estrogen receptor-alpha gene and thus, arrests the growth of estrogen responsive breast cancer cells. Artemesinin is not used for malaria prophylaxis because of it's extremely short activity. Artemisinin is fast-acting and poorly bioavailable. The use of semi-synthetic derivatives and analogues of artemisinin, such as artesunate, and artemether, in the production of Artemisinin-based combination therapies (ACTs), for example, lumefantrine-artesunate, increase the therapeutic efficiency of artemisinin to more than 90%, and prevents recrudescence.

Key words: Artemisinin, monotherapies, plasmodium, artemisinin-based combination therapies.

INTRODUCTION

Recent estimates of the global malaria burden have shown increasing levels of malaria morbidity and mortality, reflecting the deterioration of the malaria situation in Africa during the 1990s. Half of the world's population is at risk of malaria. An estimated 243 million cases led to an estimated 863,000 deaths in 2008. Administration of fake malarial drugs has been implicated as a major causative factor of proliferation of drug-resistant malarial parasites leading to large scale deaths, due to malarial infections (Basco, 2004). About 90% of malaria deaths occur in Africa south of the Sahara, and the great majority of them, are children under five (Acton and Roth, 1992). The RBM partnership is a global network for co-ordinated action against malaria, launched in 1998 by WHO, UNICEF, UNDP, and World Bank. It promotes consensus among key actors in malaria

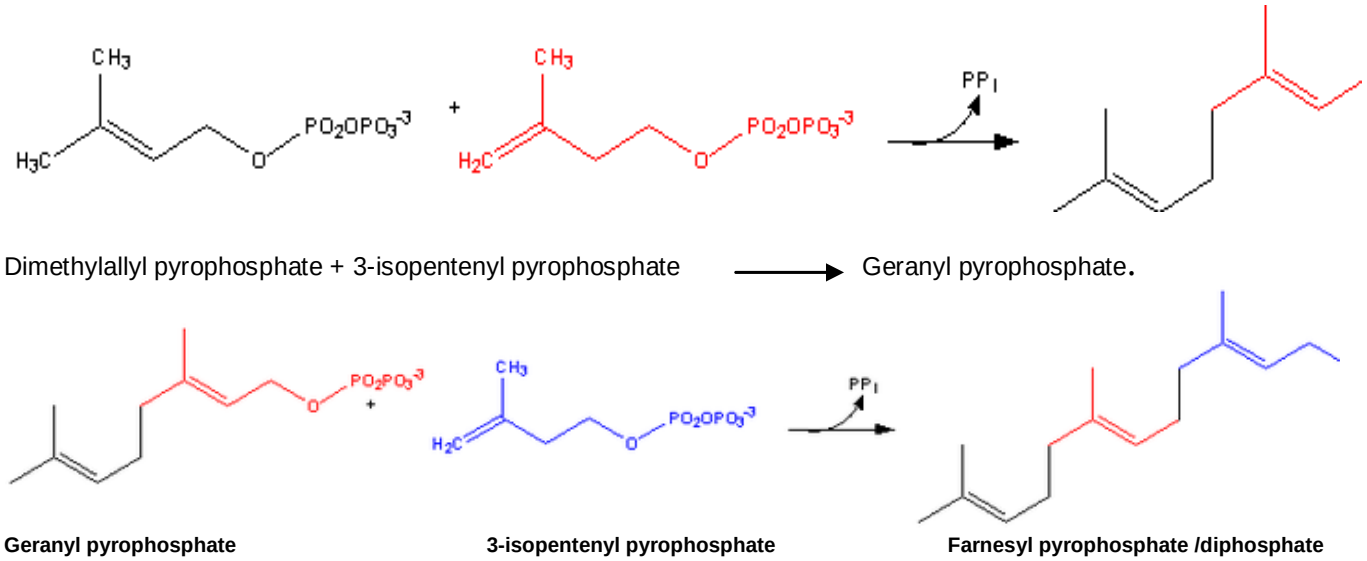

Figure 1. Biosynthesis of farnesyl pyrophosphate (Wikipedia, 2011).

control, harmonizes action, and mobilizes resources to fight malaria in endemic countries. The use of long-lasting insecticidal nets; enlightenment, encouraging indoor spraying of insecticides coupled with artemisinin-based combination therapies has engendered large-scale global malaria control. Antimalarial drugs were deployed on a large scale, always as monotherapies, introduced in sequence, and were generally poorly managed, in that, their use was continued despite unacceptably high levels of resistance.

In addition, there has been over-reliance on both quinoline compounds (that is, quinine, chloroquine, amodiaquine, mefloquine and primaquine) and antifolate drugs (that is, sulfonamides, pyrimethamine, proguanil and chlorproguanil), with consequent encouragement of cross-resistance among these compounds (Mutabingwa et al., 2005). This abuse of antimalarial drugs during the past century, resulting in the widespread resistance of *Plasmodium falciparum* to conventional antimalarial drugs, such as chloroquine, sulfadoxine–pyrimethamine (SP) and amodiaquine, has contributed to the increasing malaria mortality and morbidity (Rowen, 2009). Artemisinin, (a sesquiterpene lactone), is derived from a herb, *Artemisia annua*, and is used as a drug to treat multi-drug resistant strains of falciparum malaria (Parker et al., 1999). Artemisinin is used in Chinese traditional medicine, though it is usually chemically modified and combined with other medications (Acton and Roth, 1992). Physical and chemical data on artemisinin include: Systematic (IUPAC) name: (3R,5aS,6R,8aS,9R,12S,12aR)-octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10(3H)-one; Formula: $C_{15}H_{22}O_5$; Mol. mass: 282.332 g/mol.; Density: 1.24 ± 0.1 g/cm^3; Melt. Point: 152 to 157°C (306 to 315°F); Routes of administration: Oral, rectal, intramuscular, or intravenous uses (Acton and Roth, 1992).

BIOSYNTHESIS OF ARTEMISININ

Saccharomyces cerevisiae microbes can produce the precursor artemisinic acid, by a technique of synthetic biology, via the mevalonate pathway, a pathway for the production of terpenes and terpene products such as cholesterol and plant sterols. The metabolism of the microbe is engineered to produce artemisinic acid, a precursor to artemisinin. Starting from acetyl-CoA (an abundant product of the central metabolism of many microbes), the microbes produce, in turn, mevalonate, farnesyl pyrophosphate (FPP) as shown in Figure 1. Amorphadiene, produced by the enzymatic catalysis of amorphadiene synthase on farnesyl pyrophosphate. A novel cytochrome P450 monooxygenase (hydroxylase) oxidizes artemisinic alcohol to artemisinic acid (Sarpong and Keasling, 2006). The artemisinic acid is released from the microbes, purified from the culture media, and chemically converted to artemisinin. Dihydroartemisinic acid, the final precursor to artemisinin, undergoes photoxidation to produce dihydroartemisinic acid hydroperoxide. Ring expansion by the cleavage of hydroperoxide and a second oxygen-mediated hydroperoxidation furnish the biosynthesis of artemisinin. A three-step oxidation of amorpha-4, 11-diene gives the resulting artemisinic acid (Martin et al., 2001). Once the artemisinin is produced, it must be further chemically

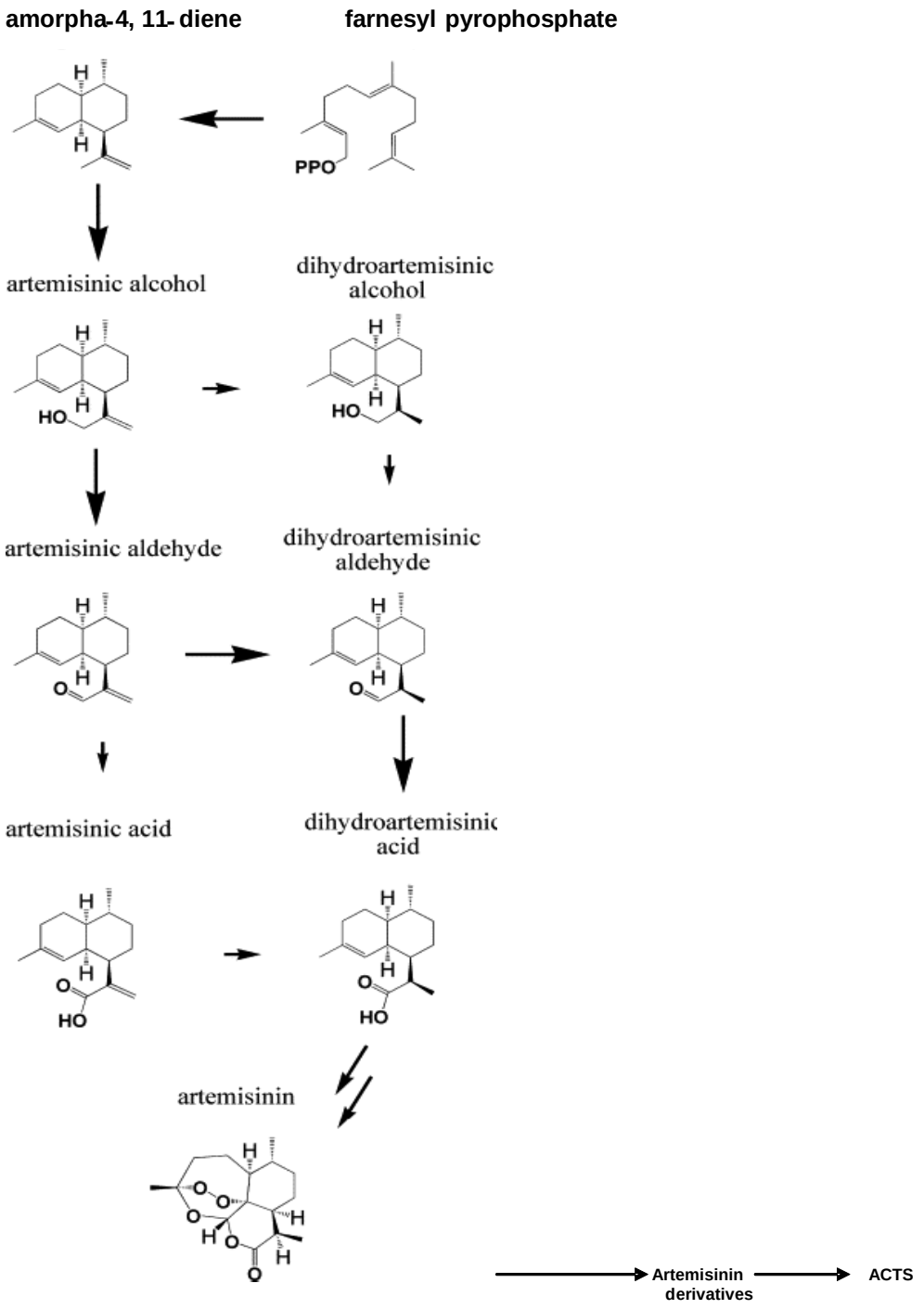

Figure 2. Technique of synthetic biology employed in the microbial synthesis of Artemisinin (Covello et al., 2007).

converted into a derivative such as artesunate or artemether, which are integrated into Artemisinin-based Combination Therapies (ACTs) for the treatment of malaria.

The scheme for the microbial synthesis of artemisinin is shown in Figure 2. Farnesyl pyrophosphate synthase (aprenyl transferase) catalyzes sequential condensation reactions of dimethylallyl pyrophosphate with 2 units of 3-

isopentenyl pyrophosphate to form farnesyl pyrophosphate. The total chemical synthesis of Artemisinin can also be performed using basic organic reagents. The starting material (Isopulegol) is converted to methoxymethyl ether. The ether is hydroborated and oxidized to yield a hydroxylated compound. The primary hydroxyl group is benzylated and the methoxymethyl ether is cleaved, protonated and treated with (E)-(3-iodo-1-methyl-1-propenyl)-trimethylsilane to yield a ketone compound. The resulting ketone is reacted with lithium methoxy(trimethylsily)methylide to obtain two diastereomeric alcohols, one (the l-stereoisomer) which is debenzylated using (Li, NH_3) to yield a lactone. The vinylsilane is oxidized to a ketone which is reacted with fluoride ion that causes it to undergo desilylation, enol ether formation, and carboxylic acid formation. A hydroperoxide function is introduced at C(3) of the resulting compound, photooxygenated and then treated with acid to produce artemisinin (Schmid and Hofheinz, 1983).

PHARMACOKINETICS AND PHARMACODYNAMICS OF ARTEMISININ

Artemisinin and artemisinin derivatives function to destroy plasmodium parasites by alkylation of host heme by carbon-centered free radicals, interference with proteins such as the sarcoplasmic/endoplasmic calcium ATPase (SERCA), as well as damaging of normal mitochondrial functions (Li and Zhou, 2010). The plasmodium parasite consumes hemoglobin and liberates free heme, an iron-porphyrin complex- moiety, during plasmodium infection of the red blood cells. The complex, produced, reacts with artemisinin to produce reactive oxygen radicals which damage the parasite leading to its death. Artemisinin is a potent inhibitor of hemozoin formation activity of malaria parasite. Artemisinin acts on the electron transport chain, generates local reactive oxygen species, and causes the depolarization of the parasite's mitochondrial membrane. Artemisinin kills the asexual forms of plasmodium at the erythrocytic stage (Miller et al., 2002). Artemisinins have also been shown to inhibit PfATP6, a SERCA-type enzyme (calcium transporter) (Jambou et al., 2005), and thus affects adversely, the calcium metabolism of malarial parasites.

USE OF ARTEMISININ IN CANCER THERAPY

The antimalarial artesunate exerts profound cytotoxicity toward tumor cells. The cytostatic and apoptotic effects of artesunate are not diminished by concomitant immunosuppression (Ramacher et al., 2009). Treatment of human breast cancer cells with artemisinin, disrupts estrogen responsiveness and stops cell growth. Artemisinin acts in breast cancer cells by inhibiting the production of the estrogen receptor-alpha (ERα) gene without altering the level of the related estrogen receptor-beta gene (ERβ). Artemisinin-regulated cellular pathways selectively inhibits the production of ERα and arrests the growth of estrogen responsive breast cancer cells by altering the function of nuclear cellular proteins (transcription factors) that are used by breast cancer cells to enhance the synthesis of the ERα gene (Firestone, 2006). Artemisinin selectively kills cancer cells which have more intracellular free iron than do normal cells. Combined HBO_2 and artemisinin exposure may be an effective anticancer chemotherapeutic strategy (Ohgami et al., 2010).

The pleiotropic response elicited in cancer cells by artemisinin and artemisinin derivatives include growth inhibition by cell cycle arrest, apoptosis, inhibition of angiogenesis, disruption of cell migration, and modulation of nuclear receptor responsiveness (Firestone and Sundar, 2009). The anti-cancer potential of artemisinin and artemisinin derivatives has been demonstrated in various cancer cells including those of leukemia and other cancer cells of breast, ovary, liver, lung, pancreas and colon (Lu et al., 2008). The anti-cancer mechanism of artemisinin and artemisinin derivatives is likely to be related to the cleavage of the iron- or heme-mediated peroxide bridge, followed by the generation of reactive oxygen species (ROS) (Efferth et al., 2003). Gao et al. (2011) showed that dihydroartemisinin (DHA)-induced apoptosis in human leukemia cells *in vitro* and exhibited an anti-leukemic activity *in vivo* through a process that involves of mitogen-activated protein kinase (MEK)/ extracellular signal-regulated protein kdve4ycinase (ERK) inactivation, Induced myeloid leukemia cell differentiation protein (Mcl-1) down-regulation, culminating in cytochrome c release and caspase activation.

Dosing and contra-indications

The WHO approved adult dose of co-artemether (artemether-lumefantrine) for malaria is 4 tablets at 0, 8, 24, 36, 48 and 60 h (six doses). This has been proven to be superior to regimens based on amodiaquine Vugt et al. (1999); Lefevre et al. (2001), Sutherland et al. (2005) and Jansen (2006). Artemesinin are not used for malaria prophylaxis (prevention) because of the extremely short activity of the drug. The adverse side effects from Artemsinin are similar to the symptoms of malaria: nausea, vomiting, anorexia, and dizziness. The combination drugs may have additional side effects. The drug should not be prescribed for pregnant women less than 3 months, except in the event of cerebral or pernicious malaria. Use of artemisinin by itself as a monotherapy is explicitly discouraged by the WHO as there have been signs that malarial parasites are developing resistance to the drug (WHO, 2001a, b, 2008) (Table 1).

Table 1. Adoption of artemisinin combination therapies (ACTs) by countries of the world (Bosman and Mendis, 2007).

Parameter	Number of countries	
	Africa	Rest of the world
Changed treatment policy to ACT[a]	14[b]	14[c]
Changed treatment policy to CT	3	1
In process of treatment policy review	11	5
Studying efficacy of ACT options	4	1

[a]Adoption does not immediately translate into implementation: In Africa only 5 out of the 14, and outside Africa 10 out of 14 countries which have adopted ACTs are deploying these drugs in the public sector. [b]Burundi, Cameroon, Comoros, Cote d'Ivoire, Eq. Guinea, Gabon, Ghana, Kenya, Mozambique, Sao Tome and Principe, Senegal, South Africa, Zambia, Zanzibar; [c]Bhutan, Bolivia, Cambodia, Ecuador, Guyana, Indonesia, Lao PDR, Myanmar, Papua New Guinea, Peru, Philippines, Surinam, Thailand, Vietnam (WHO, 2008).

Table 2. How access to ACTs is being ensured.

Purpose	Effort
Quality assurance, prequalification and sourcing project	Establishment by WHO in collaboration with other United Nations agencies, of an international mechanism to pre-qualify manufacturers of artemisinin compounds and ACTs [for example, the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM)], on the basis of compliance with internationally recommended standards of manufacturing and quality
WHO-UNICEF call for tenders of ACTS	WHO and UNICEF have called for tenders of co-blistered combinations of the following products for which there are not yet pre-qualified manufacturers: (i) artesunate plus amodiaquine; (ii) artesunate plus sulfadoxine/pyrimethamine; iii) artesunate plus mefloquine; and iv) amodiaquine plus sulfadoxine/pyrimethamine
Negotiated prices and centralized procurement artemether/lumefantrine (Coartem®)	WHO and Novartis, the manufacturer of artemether–lumefantrine (Coartem®), have entered into a special pricing agreement: Novartis provides the drug at cost price (US$ 0.9 and 2.4 per child and adult treatment course, respectively)
Financing of ACTs Global fund expenditure on ACTs	GFATM, established in 2002, is now the largest funder of ACTs in countries, and have requested that countries apply for the most effective treatments to roll back malaria. The artemisinin Project is a program by Sanofi-Aventis, Amyris Biotechnologies, the Institute for OneWorld Health, and Jay Keasling, a researcher from the University of California, to combat malaria by producing artemisinin at low cost (Hamm, 2009)
Propagation of the plant *Artemisia annua*	Plans to have the plant *Artemisia annua* grow in other areas of the world outside Vietnam and China (Kenya, Tanzania and Madagascar) (Ro et al., 2006)

World Bank (2003) and WHO (2008).

Artemisinin- based combination therapies (ACTs)

Artemisinin itself has physical properties such as poor bioavailability that limit its effectiveness. Semi- synthetic synthetic derivatives and analogues of artemisinin, such as Artesunate, Artemether, Artelinic acid, Artenimol, and Artemotil, with more efficient bio-availabity have been developed. Artemisinin and its derivatives are fast-acting, but other drugs are often required to clear the body of all parasites and prevent recrudescence. For this reason,

artemisinin is administered together with other antimalarial drugs, unrelated to the artemisinin family, in what is known as ACTs, which are the preferred treatment for malaria and are both effective and well tolerated in patients. The artemisinin derivative, Artemether, is typically administered, in simultaneous combination with lumefantrine (also known as benflumetol) to treat uncomplicated falciparum malaria. Lumefantrine has a half-life of about 3 to 6 days and prevents the disease from returning. Other examples of ACTs are artesunate-mefloquine, and artesunate-amodiaquine. ACTs are more than 90% efficient (Eline Korenromp et al., 2005; Bloland, 2001). Since 2001, 32 countries have adopted one of the aforementioned five combination therapies, several as first-line treatment and a few as second-line. A multi-artemisinin combination therapy is Artesunate-(Sulphadoxine-Pyrimethamine). The therapy is an initial administration of sulphadoxine-pyrimethamine combination with a subsequent administration of artesunate, approximately, 24hours after the initial administration (Elamin et al., 2005). Many others are in the process of policy change. WHO has provided continuous technical cooperation to ministries of health on all aspects of national treatment policy change monitoring the therapeutic efficacy of medicines, and updating and implementing ACT-based treatment policies and (Jambou et al., 2005; Grupper, 2005; Olumese, 2006). Information on how to access ACTs is given in Table 2.

CONCLUSION

Artemisinin, a sesquiterpene lactone, isolated from the plant *A. annua*, used as a drug, to treat multi-drug resistant strains of falciparum malaria, can be produced by a technique of synthetic microbiology, and also in the laboratory from basic organic reagents. In addition, artemisinin is curative of breast, ovary, liver, lung, pancreas and colon cancers. Artemisinin is a potent inhibitor of hemozoin formation activity of malaria parasite. Artemisinin have also been shown to inhibit the calcium transporter enzyme, thus posing toxicity to micro-organisms. Drug efficacy of artemisinin is optimized in ACTs.

ACKNOWLEDGEMENT

The author acknowledges the technical support given by Dr. Seb. Obasi and the resource contribution of Engr. Abraham Enyichukwu Obimba.

REFERENCES

Acton N, Roth RJ (1992). On the conversion of dihydroartemisinic acid into artemisinin. J. Org. Chem., 57: 3610-3614.

Basco LK (2004). Molecular Epidemiology of Malaria in Cameroon. XIX. Quality of Antimalarial Drugs Used for Self-medication. Am. J. Trop. Med. Hyg., 70(3): 245-250.

Bloland P (2001). Drug Resistance in Malaria. A Background Document for the WHO Global Strategy for Containment of Antimicrobial Resistance. Geneva: World Health Organization.

Bosman A, Mendis KN (2007). A major Transition in Malarial Treatment: The adoption and deployment of Artemisinin-based combination Therapies. Am. J. Trop. Med. Hyg., 77(6): 193-197.

Covello PS, Teoh KH, Polichuk DR, Reed DW, Nowak G (2007). Functional genomics and the biosynthesis of artemisinin. Phytochem., 6814 : 1864-1871.

Efferth T, Sauerbrey A, Olbrich A, Gebhart E, Rauch P, Weber HO, Hengstler JG, Halatsch ME, Volm M, Tew KD, Ross DD, Funk JO (2003). Molecular modes of action of artesunate in tumor cell lines. Mol. Pharmacol., 64: 382-394.

Elamin SB, Malik EM, Abdelgadir T, Khamiss AH, Mohammed MM, Ahmed ES, Adam I (2005). Artesunate plus sulfadoxine-pyrimethamine for treatment of uncomplicated Plasmodium falciparum malaria in Sudan. Malaria J., 4: 41.

Eline KJM, Nahlen B, Wardlaw T, Young M (2005). World Malaria Report: World Health Organization (WHO), Roll Back Malaria (RBM) and United Nations Children's Fund (UNICEF). Geneva: World Health Organization.

Firestone G (2006). Artemisinin Disrupts Estrogen Receptor-Alpha and Cell Growth.Carlifornia Breast cancer Research Program. http://www.cbcrp.org/research/PageGrant.asp?grant_id=4768

Firestone GL, Sundar SN (2009). Anticancer activities of artemisinin and its bioactive derivatives. Expert. Rev. Mol. Med., 11:e32.

Gao N, Budhraja A, Cheng S, Liu EH, Huang C, Chen J, Yang Z, Chen D, Zhang Z, Shi X (2011). Interruption of the MEK/ERK signaling cascade promotes dihydroartemisinin-induced apoptosis *in vitro* and *in vivo*. Apoptosis. 16(5): 511-523.

Grupper M (2005). Meeting on the Production of Artemisinin and ACTs. Arusha, Tanzania: DFID Health Resource Centre, Roll Back Malaria, World Health Organization.

Hamm S (2009). Crative Capitalism' versus Malaria. Business Week. 083.

Jambou R, Legrand E, Niang M, Khim N, Lim P, Volney B, Ekala MT, Bouchier C, Esterre P, Fandeur T, Mercereau-Puijalon O (2005). Resistance of *Plasmodium falciparum* field isolates to *in-vitro* artemether and point mutations of the SERCA-type PfATPase6. Lancet, 366: 1960-1963.

Jansen FH (2006). The herbal tea approach for artemesinin as a therapy for malaria? Trans R Soc Trop. Med. Hyg., 100(3): 285-286.

Lefevre G, Looareesuwan S, Treeprasertsuk S (2001). A clinical and pharmacokinetic trial of six doses of artemether-lumefantrine for multidrug-resistant *Plasmodium falciparum* malaria in Thailand. Am. J. Trop. Med. Hyg., 64(5-6): 247-256.

Li J, Zhou B (2010). Biological Actions of Artemisinin: Insights from Medicinal Chemistry Studies. Molecule, 15(3): 1378-1397.

Lu JJ, Meng LH, Cai YJ, Chen Q, Tong LJ, Lin LP, Ding J (2008). Dihydroartemisinin induces apoptosis in HL-60 leukemia cells dependent of iron and p38 mitogen-activated protein kinase activation but independent of reactive oxygen species. Cancer Biol. Ther., 7: 1017-1023.

Martin VJ, Yoshikuni Y Keasling JD (2001). The *in vivo* synthesis of plant sesquiterpenes by *Escherichia coli*. Biotech. Bioeng., 7: 497-503.

Miller LH, Baruch DI, Marsh K, Doumbo OK (2002). The pathogenic basis of malaria. Nature 415: 673-679.

Mutabingwa TK, Anthony D, Heller A (2005). Amodiaquine alone, amodiaquine+sulfadoxine-pyrimethamine, amodiaquine+artesunate, and artemether-lumefantrine for outpatient treatment of malaria in Tanzanian children: a four-arm randomised effectiveness trial. Lancet 365(9469): 1474-1480.

Ohgami Y, Elstad CA, Chung E, Shirachi DY, Quock RM, Lai HC (2010). Effect of Hyperbaric Oxygen on the Anticancer Effect of

Artemisinin on Molt-4 Human Leukemia Cells. Intl. J. Cancer Res. Treatment. 30(11): 4467-4470.
Olumese P (2006). Guidelines for the Treatment of Malaria. Geneva: World Health Organization.
Parker MH, Northrop J, Elias J, Ploypradith S, Xie P, Shapiro S, Theresa A (1999). Orally Active, Hydrolytically Stable, Semisynthetic, Antimalarial Trioxanes in the Artemisinin Family. J. Med. Chem., 42 (2): 300-304.
Ramacher M, Umansky V, Efferth T (2009). Effect of artesunate on immune cells in ret-transgenic mouse melanoma model. Anti-Cancer Drugs. 20(10): 910-917.
Ro DK, Paradise EM, Ouellet M, Fisher KJ, Newman KL, Ndungu JM, Ho KA, Eachus RA, Ham TS, Kirby J, Chang MC, Withers ST, Shiba Y, Sarpong R, Keasling JD (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440: 940-943.
Rowen RJ (2009). Artemisinin: From Malaria to Cancer Treatment. Wikipedia encyclopaedia. http://en.wikipedia.org/wiki/Artemisinin. (3-12-2011).
Sarpong R, Keasling JD (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast Nature. 440: 940-943.
Schmid G, Hofheinz W. (1983). Total Synthesis of qinghaosu J. Am. Chem. Soc., 105(3): 624-625.
Sutherland CJ, Ord R, Dunyo S, Jawara M, Drakeley CJ, Alexander N, Coleman R, Pinder M, Walraven G, Targett GA (2005). Reduction of malaria transmission to Anopheles mosquitoes with a six-dose regimen of co-artemether. PLoS Med., 2: e92.
Vugt MV, Wilairatana P, Gemperli B (1999). Efficacy of six doses of artemether-lumefantrine (benflumetol) in multidrug-resistant Plasmodium falciparum malaria. Am. J. Trop. Med. Hyg., 60(6): 936-942.
Wikipedia (2011). Isopentenyl pyrophosphate. http://en.wikipedia.org/wiki/Isopentenyl_pyrophosphate. (3-12-2011).
World Bank (2003). Report of the Expert Consultation on the Procurement & Financing of Antimalarial Drugs . Washington D.C.
World Health Organization. (2001a). The use of antimalarial drugs. Report of a WHO Informal Consultation. Geneva, World Health Organization. (WHO/CDS/RBM/2001.33).
World Health Organization. (2001b). Antimalarial drug combination therapy: Report of WHO technical consultation . Geneva, World Health Organization (WHO/CDS/RBM/2001.35).
World Health Organization. (2003). Procurement, quality and sourcing project: access to artemisinin-based combination antimalarial drugs of acceptable quality. Geneva, World Health Organization.
World Health Organization (2008). WHO ultimatum on artemisinin monotherapy is showing results. http://www.bmj.com/cgi/content/full/332/7551/1176-b. (3-12-2011).