

## Cancer and Artemisinin Research Literatures

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**Abstract:** Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the cancer and artemisinin related studies.

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### 1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

The following introduces recent reports as references in the related studies.

Alcantara, D. D., H. F. Ribeiro, et al. "In vitro evaluation of the cytotoxic and genotoxic effects of artemether, an antimalarial drug, in a gastric cancer cell line (PG100)." *J Appl Toxicol*. 2013 Feb;33(2):151-6. doi: 10.1002/jat.1734. Epub 2011 Sep 23.

Artemisinin is a sesquiterpene lactone endoperoxide, obtained from *Artemisia annua*, and extensively used as an antimalarial drug. Many studies have reported the genotoxic and cytotoxic effects of artemisinins; however, there are no studies that compare such effects between cancer cell lines and normal human cells after treatment with artemether, an artemisinin derivative. Gastric cancer is the fourth most frequent type of cancer and the second highest cause of cancer mortality worldwide. Thus, the aim of this study was to evaluate the in vitro genotoxic and cytotoxic effects induced by artemether in gastric cancer cell line (PG100) and compare them with the results obtained in human lymphocytes exposed to the same conditions. We used MTT (3-(4,5-methylthiazol-2-yl)-2, 5-diphenyl-tetrazolium

bromide) assay, comet assay and ethidium bromide/acridine orange viability staining to evaluate the cytotoxic and genotoxic effects of artemether in PG100. MTT assay showed a decrease in the survival percentages for both cell types treated with different concentrations of artemether ( $P < 0.05$ ). PG100 also showed a significant dose-dependent increase in DNA damage index at concentrations of 119.4 and 238.8 microg ml<sup>-1</sup> ( $P < 0.05$ ). Our results showed that artemether induced necrosis in PG100 at concentrations of 238.8 and 477.6 microg ml<sup>-1</sup>, for all the tested harvest times ( $P < 0.05$ ). In lymphocytes, artemether induced both apoptosis and necrosis at concentrations of 238.8 and 477.6 microg ml<sup>-1</sup>, for all the tested harvest times ( $P < 0.05$ ). In conclusion, human lymphocytes were more sensitive to the cytotoxic effects of the antimalarial drug than the gastric cancer cell line PG100.

Amorim, M. H., R. M. Gil da Costa, et al. "Sesquiterpene lactones: adverse health effects and toxicity mechanisms." *Crit Rev Toxicol*. 2013 Aug;43(7):559-79. doi: 10.3109/10408444.2013.813905.

Sesquiterpene lactones (STLs) present a wide range of biological activities, mostly based on their alkylating capabilities, which underlie their therapeutic potential. These compounds are the active constituents of a variety of plants, frequently used as herbal remedies. STLs such as artemisinin and its derivatives are in use as first-line antimalarials while others, such as parthenolide, have recently reached cancer clinical trials. However, the toxicological profile of these compounds must be thoroughly characterized, since the same properties that make STL useful medicines can also cause severe toxicity. STL-containing plants have long been known to induce a contact dermatitis in exposed farm workers,

and also to cause several toxic syndromes in farm animals. More recently, concerns are being raised regarding the genotoxic potential of these compounds and the embryotoxicity of artemisinins. A growing number of STLs are being reported to be mutagenic in different *in vitro* and *in vivo* assays. As yet no systematic studies have been published, but the genotoxicity of STLs seems to depend not so much on direct DNA alkylation as on oxidative DNA damage and other partially elucidated mechanisms. As the medicinal use of these compounds increases, further studies of their toxic potential are needed, especially those focusing on the structural determinants of genotoxicity and embryotoxicity.

Artimovich, E., K. Schneider, et al. "Persistence of Sulfadoxine-Pyrimethamine Resistance Despite Reduction of Drug Pressure in Malawi." *J Infect Dis.* 2015 Sep 1;212(5):694-701. doi: [10.1093/infdis/jiv078](https://doi.org/10.1093/infdis/jiv078). Epub 2015 Feb 11.

**BACKGROUND:** In 2007, Malawi replaced sulfadoxine-pyrimethamine (SP) with an artemisinin-based combination therapy as the first-line treatment for uncomplicated *Plasmodium falciparum* malaria in response to failing SP efficacy. Here we estimate the effect of reduced SP pressure on the prevalence of SP-resistant parasites and the characteristics of the associated selective sweeps flanking the resistance loci. **METHODS:** Samples obtained from individuals with clinical malaria during a period of high SP use (1999-2001), a transitional period (2007-2008), and a period of low SP use (2012) were genotyped for resistance markers at *pfdhfr*-ts codons 51, 59, and 108 and *pfdhps* codons 437, 540, and 581. Expected heterozygosity was estimated to evaluate the genetic diversity flanking *pfdhfr*-ts and *pfdhps*. **RESULTS:** An increase in the prevalence of the resistance haplotypes DHFR 511/59R/108N and DHPS 437G/540E occurred under sustained drug pressure, with no change in haplotype prevalence 5 years after reduction in SP pressure. The DHPS 437G/540E/581G haplotype was observed in 2007 and increased in prevalence during a period of reduced SP pressure. Changes to the sweep characteristics flanking *pfdhfr*-ts and *pfdhps* were minimal. **CONCLUSIONS:** In contrast to the rapid and complete return of chloroquine-susceptible *falciparum* malaria after chloroquine was withdrawn from Malawi, a reemergence of SP efficacy is unlikely in the near future.

Azimi Mohamadabadi, M., Z. M. Hassan, et al. "Arteether exerts antitumor activity and reduces CD4+CD25+FOXP3+ T-reg cells *in vivo*." *Iran J Immunol.* 2013 Sep;10(3):139-49. doi: [IJiv10i3A2](https://doi.org/10.1013A2).

**BACKGROUND:** Chemo-immunotherapy is one of the new achievements for treatment of cancer, by which the success of anti-cancer therapy can be increased. *In vitro* studies have been shown that Arteether (ARE) induces apoptosis in tumor cells, but not in normal cells. **OBJECTIVE:** To investigate the cytotoxic and immunomodulatory properties of Arteether *in-vivo* and *in-vitro*. **METHODS:** In this study, we used MTT assay for evaluation of cytotoxicity of Arteether on tumor cell line and Peripheral Blood Mononuclear Cells (PBMCs) from healthy individuals. Balb/c mice were subcutaneously transplanted with tumor tissue taken from Spontaneous Mouse Mammary Tumor (SMMT) bearing female mice. Arteether was administered to breast tumor-bearing Balb/c mice at a dose of 6mg/kg/day intraperitoneally. Tumor sizes, lymphocyte proliferation, cytokines production, and the percentage of splenic T-reg cells were measured. **RESULTS:** We observed that ARE could reduce the cell growth of 4T1 cell line in a dose-dependent manner but it had no cytotoxic effect on the growth of peripheral blood lymphocytes. ARE administered intraperitoneally to tumor-bearing Balb/c mice could reduce the tumor growth rate and splenic T-reg cells. No difference in the IFN-gamma, IL-10 and IL-4 production was observed between tumor antigen-stimulated splenocytes of mice treated with ARE and control mice. **CONCLUSION:** These results underscore antitumor properties of Arteether that may aid in development of more effective antitumor agents.

Berdelle, N., T. Nikolova, et al. "Artesunate induces oxidative DNA damage, sustained DNA double-strand breaks, and the ATM/ATR damage response in cancer cells." *Mol Cancer Ther.* 2011 Dec;10(12):2224-33. doi: [10.1158/1535-7163.MCT-11-0534](https://doi.org/10.1158/1535-7163.MCT-11-0534). Epub 2011 Oct 13.

Artesunate, the active agent from *Artemisia annua* L. used in the traditional Chinese medicine, is being applied as a first-line drug for malaria treatment, and trials are ongoing that include this drug in cancer therapy. Despite increasing interest in its therapeutic application, the mode of cell killing provoked by artesunate in human cells is unknown. Here, we show that artesunate is a powerful inducer of oxidative DNA damage, giving rise to formamidopyrimidine DNA glycosylase-sensitive sites and the formation of 8-oxoguanine and 1,N<sup>6</sup>-ethenoadenine. Oxidative DNA damage was induced in LN-229 human glioblastoma cells dose dependently and was paralleled by cell death executed by apoptosis and necrosis, which could be attenuated by radical scavengers such as N-acetyl cysteine. Oxidative DNA damage resulted in DNA double-strand breaks (DSB)

as determined by gammaH2AX foci that colocalized with 53BP1. Upon chronic treatment with artesunate, the level of DSB continuously increased over the treatment period up to a steady-state level, which is in contrast to ionizing radiation that induced a burst of DSB followed by a decline due to their repair. Knockdown of Rad51 by short interfering RNA and inactivation of DNA-PK strongly sensitized glioma cells to artesunate. These data indicate that both homologous recombination and nonhomologous end joining are involved in the repair of artesunate-induced DSB. Artesunate provoked a DNA damage response (DDR) with phosphorylation of ATM, ATR, Chk1, and Chk2. Overall, these data revealed that artesunate induces oxidative DNA lesions and DSB that continuously increase during the treatment period and accumulate until they trigger DDR and finally tumor cell death.

Blazquez, A. G., M. Fernandez-Dolon, et al. "Novel artemisinin derivatives with potential usefulness against liver/colon cancer and viral hepatitis." Bioorg Med Chem. 2013 Jul 15;21(14):4432-41. doi: 10.1016/j.bmc.2013.04.059. Epub 2013 Apr 29.

Antitumor and antiviral properties of the antimalaria drug artemisinin from *Artemisia annua* have been reported. Novel artemisinin derivatives (AD1-AD8) have been synthesized and evaluated using in vitro models of liver/colon cancer and viral hepatitis B and C. Cell viability assays after treating human cell lines from hepatoblastoma (HepG2), hepatocarcinoma (SK-HEP-1), and colon adenocarcinoma (LS174T) with AD1-AD8 for a short (6h) and long (72h) period revealed that AD5 combined low acute toxicity together with high antiproliferative effect (IC<sub>50</sub>=1-5μM). Since iron-mediated activation of peroxide bond is involved in artemisinin antimalarial activity, the effect of iron(II)-glycine sulfate (ferrosanol) and iron(III)-containing protoporphyrin IX (hemin) was investigated. Ferrosanol, but not hemin, enhanced antiproliferative activity of AD5 if the cells were preloaded with AD5, but not if both compounds were added together. Five derivatives (AD1>AD2>AD7>AD3>AD8) were able to inhibit the cytopathic effect of bovine viral diarrhoea virus (BVDV), a surrogate in vitro model of hepatitis C virus (HCV), used here to evaluate the anti-Flaviviridae activity. Moreover, AD1 and AD2 inhibited the release of BVDV-RNA to the culture medium. Co-treatment with hemin or ferrosanol resulted in enhanced anti-Flaviviridae activity of AD1. In HepG2 cells permanently infected with hepatitis B virus (HBV), AD1 and AD4, at non-toxic concentrations for the host cells were able to reduce the release of HBV-DNA to the medium. In conclusion, high pharmacological interest deserving

further evaluation in animal models has been identified for novel artemisinin-related drugs potentially useful for the treatment of liver cancer and viral hepatitis B and C.

Chikazawa, S., Y. Kitahara, et al. "Erythrophagocytosis enhances heme-dependent cytotoxicity of antimalarial drugs in canine histiocytic sarcoma cell line DH82." J Vet Med Sci. 2014 Mar 1;76(2):249-53. Epub 2013 Sep 20.

Antimalarial drugs, dihydroartemisinin (DHA) and artesunate (ATS), exhibit iron-dependent cytotoxicity in tumor cells. We hypothesized that erythrophagocytic uptake of heme-iron enhances the cytotoxicity of DHA and ATS. Erythrophagocytic (EP) treatment of the canine histiocytic sarcoma cell line DH82 markedly increased the cytotoxicity of DHA and ATS compared to controls. Succinyl acetone, an inhibitor of intracellular heme synthesis, decreased the cytotoxicity of DHA and ATS in normal cells, but this change was not observed in EP cells. These results suggest that exogenous heme derived from erythrocytes can enhance the cytotoxicity of DHA and ATS. Furthermore, our study suggests that heme could be a novel component of tumor treatment in veterinary medicine.

Crespo-Ortiz, M. P. and M. Q. Wei "Antitumor activity of artemisinin and its derivatives: from a well-known antimalarial agent to a potential anticancer drug." J Biomed Biotechnol. 2012;2012:247597. doi: 10.1155/2012/247597. Epub 2011 Nov 22.

Improvement of quality of life and survival of cancer patients will be greatly enhanced by the development of highly effective drugs to selectively kill malignant cells. Artemisinin and its analogs are naturally occurring antimalarials which have shown potent anticancer activity. In primary cancer cultures and cell lines, their antitumor actions were by inhibiting cancer proliferation, metastasis, and angiogenesis. In xenograft models, exposure to artemisinins substantially reduces tumor volume and progression. However, the rationale for the use of artemisinins in anticancer therapy must be addressed by a greater understanding of the underlying mechanisms involved in their cytotoxic effects. The primary targets for artemisinin and the chemical base for its preferential effects on heterologous tumor cells need yet to be elucidated. The aim of this paper is to provide an overview of the recent advances and new development of this class of drugs as potential anticancer agents.

Dadgar, N., S. E. Alavi, et al. "Study of toxicity effect of pegylated nanoliposomal artemisinin on breast cancer cell line." Indian J Clin Biochem. 2013

Oct;28(4):410-2. doi: 10.1007/s12291-013-0306-3. Epub 2013 Feb 10.

Nano carriers have greatly revolutionized the treatment of most diseases recently. One of these nano carriers, liposomes, has got particular significance. On the other hand, Artemisinin which is used as an effective anticancer drug has some side effects. To reduce such side effects, liposomes can be employed. In order to prepare pegylated nanoliposomal artemisinin, particular proportions of phosphatidylcholine, polyethylene glycol 2000 and artemisinin were combined. As a result, the mean diameter of nano liposomes is 455 nm. Besides, the encapsulation efficiency and the drug release from pegylated nanoliposomes for pegylated nanoliposomal artemisinin are respectively 91.62 +/- 3.5 and 5.17 %. The results also show that IC50 of the produced formulation is less than that of the standard drug. This study reveals that the amount of artemisinin cytotoxicity compared to standard drug is increased by pegylated nanoliposomal formulation.

Dadgar, N., M. Koohi Moftakhari Esfahani, et al. "Effects of nanoliposomal and pegylated nanoliposomal artemisinin in treatment of breast cancer." Indian J Clin Biochem. 2014 Oct;29(4):501-4. doi: 10.1007/s12291-013-0389-x. Epub 2013 Oct 1.

This study is aimed to investigate the nanoliposomal artemisinin preparation, and its implementation on breast cancer cells. Side effects have been one of the common challenges of drug usage, as well as cancer treatment. In order to reduce such effects, nanotechnology has been a great help. Nanoliposomes are provided through reverse phase evaporation. In this method, certain proportions of phosphatidylcholine, cholesterol and artemisinin were mixed together. Besides, the obtained formulation was pegylated by using polyethylene glycol 2000 in order to increase its stability and solubility. The mean diameter of non-pegylated and pegylated liposomal artemisinin was determined by Zeta sizer system. The percent of drug released from liposome was performed by dialysis. The encapsulation efficiency of both formulations was estimated by spectrophotometry method. As a result, encapsulation and drug release of nanoliposomal formulation were more than the pegylation of the same formulation. In addition, this study indicated that cytotoxicity effect of pegylated nanoliposomal artemisinin was more, in comparison with nanoliposomal artemisinin.

D'Alessandro, S., N. Basilico, et al. "Hypoxia modulates the effect of dihydroartemisinin on endothelial cells." Biochem Pharmacol. 2011 Sep 1;82(5):476-84. doi: 10.1016/j.bcp.2011.06.002. Epub 2011 Jun 13.

Artemisinin derivatives, the current cornerstone of malaria treatment, possess also anti-angiogenic and anti-tumor activity. Hypoxia plays a crucial role both in severe malaria (as a consequence of the cytoadherence of infected erythrocytes to the microvasculature) and in cancer (due to the restricted blood supply in the growing tumor mass). However, the consequences of hypoxia onto the effects of artemisinins is under-researched. This study aimed at assessing how the inhibition of microvascular endothelial cell (HMEC-1) growth induced by dihydroartemisinin (DHA, an antimalarial drug and the active metabolite of currently in-use artemisinins) is affected by oxygen tension. Low doses of DHA (achieved in the patients' plasma when treating malaria) were more inhibitory in hypoxia, whereas high doses (required for anti-angiogenic or anti-tumor activity) were more effective in normoxia. The peroxide bridge is essential for cellular toxicity (deoxyDHA was inactive). High doses of DHA caused HMEC-1 apoptosis and G2 cell cycle arrest. Effects were mediated by the generation of oxidative stress as demonstrated by DCF-DA fluorescence and membrane lipid peroxidation analysis. Overall, these results suggest that DHA inhibition of endothelial cell growth is related to the level of tissue oxygenation and drug concentration. This should be considered when studying both the effects of artemisinin derivatives as antimalarials and the potential therapeutic applications of these drugs as anti-tumor agents.

Das, A. K. "Anticancer Effect of AntiMalarial Artemisinin Compounds." Ann Med Health Sci Res. 2015 Mar-Apr;5(2):93-102. doi: 10.4103/2141-9248.153609.

The anti-malarial drug artemisinin has shown anticancer activity in vitro and animal experiments, but experience in human cancer is scarce. However, the ability of artemisinins to kill cancer cells through a variety of molecular mechanisms has been explored. A PubMed search of about 127 papers on anti-cancer effects of antimalarials has revealed that this class of drug, including other antimalarials, have several biological characteristics that include anticancer properties. Experimental evidences suggest that artemisinin compounds may be a therapeutic alternative in highly aggressive cancers with rapid dissemination, without developing drug resistance. They also exhibit synergism with other anticancer drugs with no increased toxicity toward normal cells. It has been found that semisynthetic artemisinin derivatives have much higher antitumor activity than their monomeric counterparts via mechanisms like apoptosis, arrest of cell cycle at G0/G1, and oxidative stress. The exact mechanism of activation and molecular basis of these anticancer effects are not

fully elucidated. Artemisinins seem to regulate key factors such as nuclear factor-kappa B, survivin, NOXA, hypoxia-inducible factor-1alpha, and BMI-1, involving multiple pathways that may affect drug response, drug interactions, drug resistance, and associated parameters upon normal cells. Newer synthetic artemisinins have been developed showing substantial antineoplastic activity, but there is still limited information regarding the mode of action of these synthetic compounds. In view of the emerging data, specific interactions with established chemotherapy need to be further investigated in different cancer cells and their phenotypes and validated further using different semisynthetic and synthetic artemisinin derivatives.

Dwivedi, A., A. Mazumder, et al. "In vitro anti-cancer effects of artemisone nano-vesicular formulations on melanoma cells." *Nanomedicine*. 2015 Aug 15. pii: S1549-9634(15)00153-7. doi: 10.1016/j.nano.2015.07.010.

Artemisone is a 10-amino-artemisinin derivative that is markedly superior in vitro and in vivo to current artemisinins against malaria and also possesses antitumor activity. In seeking to capitalise on the last property, we have examined the encapsulation of artemisone in nano-vesicular niosomes and solid lipid nanoparticles (SLNs), and have evaluated efficacies of the free and encapsulated artemisone against human melanoma A-375 cells and effects on human keratinocytes (HaCaT). Artemisone is successfully encapsulated into the nano-vesicles with encapsulation efficiencies of 67+/-6% and 79+/-5%, and with average particle sizes being 211+/-10nm and 295+/-18nm respectively. The formulations displayed highly selective cytotoxicity towards the melanoma cells with negligible toxicity towards the normal skin cells. The artemisone-loaded nano-vesicles almost completely inhibited the melanoma cells compared to the free drug. The results overall suggest a potentially more useful therapeutic strategy that needs to be evaluated for the treatment of melanoma and other cancers.

Efferth, T., F. Herrmann, et al. "Cytotoxic activity of secondary metabolites derived from *Artemisia annua* L. towards cancer cells in comparison to its designated active constituent artemisinin." *Phytomedicine*. 2011 Aug 15;18(11):959-69. doi: 10.1016/j.phymed.2011.06.008. Epub 2011 Aug 9.

*Artemisia annua* L. (sweet wormwood, qin hao) has traditionally been used in Chinese medicine. The isolation of artemisinin from *Artemisia annua* and its worldwide accepted application in malaria therapy is one of the showcase success stories of phytomedicine during the past decades.

Artemisinin-type compounds are also active towards other protozoal or viral diseases as well as cancer cells in vitro and in vivo. Nowadays, *Artemisia annua* tea is used as a self-reliant treatment in developing countries. The unsupervised use of *Artemisia annua* tea has been criticized to foster the development of artemisinin resistance in malaria and cancer due to insufficient artemisinin amounts in the plant as compared to standardized tablets with isolated artemisinin or semisynthetic artemisinin derivatives. However, artemisinin is not the only bioactive compound in *Artemisia annua*. In the present investigation, we analyzed different *Artemisia annua* extracts. Dichloromethane extracts were more cytotoxic (range of IC(50): 1.8-14.4 mug/ml) than methanol extracts towards *Trypanosoma b. brucei* (TC221 cells). The range of IC(50) values for HeLa cancer cells was 54.1-275.5 mug/ml for dichloromethane extracts and 276.3-1540.8 mug/ml for methanol extracts. Cancer and trypanosomal cells did not reveal cross-resistance among other compounds of *Artemisia annua*, namely the artemisinin-related artemisitene and arteanuin B as well as the unrelated compounds, scopoletin and 1,8-cineole. This indicates that cells resistant to one compound retained sensitivity to another one. These results were also supported by microarray-based mRNA expression profiling showing that molecular determinants of sensitivity and resistance were different between artemisinin and the other phytochemicals investigated.

Eichhorn, T., S. Schloissnig, et al. "Bioinformatic and experimental fishing for artemisinin-interacting proteins from human nasopharyngeal cancer cells." *Mol Biosyst*. 2012 Apr;8(4):1311-8. doi: 10.1039/c2mb05437j. Epub 2012 Feb 6.

Determining interacting cellular partners of drugs by chemical proteomic techniques is complex and tedious. Most approaches rely on activity-based probe profiling and compound-centric chemical proteomics. The anti-malarial artemisinin also exerts profound anti-cancer activity, but the mechanisms of action are incompletely understood. In the present investigation, we present a novel approach to identify artemisinin-interacting target proteins. Our approach overcomes usual problems in traditional fishing procedures, because the drug was attached to a surface without further chemical modification. The proteins identified effect among others, cell cycle arrest, apoptosis, inhibition of angiogenesis, disruption of cell migration, and modulation of nuclear receptor responsiveness. Furthermore, a bioinformatic approach confirmed experimentally identified proteins and suggested a large number of other interacting proteins. Theoretically predicted interaction partners

may serve as a starting point to complete the whole set of proteins binding artemisinin.

Eltayeb, S. E., Z. Su, et al. "Preparation and optimization of transferrin-modified-artemether lipid nanospheres based on the orthogonal design of emulsion formulation and physically electrostatic adsorption." *Int J Pharm.* 2013 Aug 16;452(1-2):321-32. doi: 10.1016/j.ijpharm.2013.05.019. Epub 2013 May 18.

Artemether has been used for a long time in the treatment of malaria as safe and non expensive drug. It possesses potent anticancer effects in cancer cell lines. Our aim was to develop transferrin-modified-artemether lipid nanospheres as targeted anticancer drug delivery system. In this study, artemether intravenous delivery system was prepared by emulsifying method as lipid nanospheres containing mixture of soya oil and crodamol as the core and soya lecithin and Tween 80 as coating layer. According to the physicochemical characterization, the process and formulation variables were optimized by orthogonal design and ANOVA analysis. Based on the electrostatic interaction, transferrin (TR) was physically adsorbed onto the coating layer; the effect of medium pH and the charge of the nanocarriers on the adsorption were investigated. The in vitro characterizations were carried out including, the zeta potential, AFM, TEM, FTIR, (1)H NMR and gel filtration. ART-LNSs with high entrapment efficiency, small size of about 50 nm and monodispersity were formulated. Optimized and stable TR-LNSs, a lipoprotein like structure and size, were produced. We showed a method by which TR can be bound to lipid nanospheres without the need for chemical modification as a base for the development of safe, effective and non expensive anticancer drug delivery system.

Fafowora, M. V., F. Atanu, et al. "Effect of oral coadministration of artesunate with ferrous sulfate on rat liver mitochondrial membrane permeability transition." *Drug Chem Toxicol.* 2011 Jul;34(3):318-23. doi: 10.3109/01480545.2010.547498.

The recent resurgence of interest in the study of mitochondria has been fuelled in large part by the recognition that genetic and/or metabolic alterations in this organelle are causative or contributing factors in a variety of human diseases including cancer. This study hypothesizes that co-administration of artesunate and ferrous sulfate could induce apoptosis which can be targeted on cancerous cells in such a manner, thus providing a novel, viable and perhaps inexpensive way of dealing with the cancer scourge. Artesunate and Ferrous sulfate were co-administered to rats at various doses for seven days. At the end of the

treatment, the rats were fasted overnight and sacrificed by cervical dislocation. Low ionic strength mitochondria were isolated from hepatic cells of the rats and assayed for protein content; changes in the absorbance of the liver mitochondria; and mitochondrial swelling. Co-administration of artesunate and ferrous sulfate resulted in a significant increase ( $P < 0.05$ ) in pore opening. The difference in pore opening was found to be statistically significant ( $P < 0.05$ ) when the artesunate and ferrous iron-treated groups were compared with the artesunate only treated group. Results from this study show that co-administration of artesunate and ferrous sulfate can cause an opening in the mitochondrial membrane transition pore. A combined dose of ferrous sulfate and artesunate may prove to be a more potent therapy for targeting cancerous cells.

Farsam, V., Z. M. Hassan, et al. "Antitumor and immunomodulatory properties of artemether and its ability to reduce CD4+ CD25+ FoxP3+ T reg cells in vivo." *Int Immunopharmacol.* 2011 Nov;11(11):1802-8. doi: 10.1016/j.intimp.2011.07.008. Epub 2011 Aug 6.

**BACKGROUND:** Development of agents that specifically kill cancer cells and simultaneously elicit antitumor immune response is a step forward in cancer therapy. In the present study, we investigated whether the administration of artemether contributes to the augmentation of antitumor immunity and the regression of tumor tissues in a mouse model of breast cancer. **METHODS:** An optimal immunostimulatory dose of artemether (ART) was defined by DTH reaction and antibody production in sRBC-challenged mice. Subsequent experiments were carried out on tumor-bearing BALB/c mice. In the first group of tumor-bearing mice, the dose of 10 mg/kg/day of artemether were intraperitoneally administered to each animal for six times. The second group was treated with 20 mg/kg/day of cyclophosphamide as a positive control, and the last group (negative control) received the ART diluents. Tumor size was measured during the 10-day experiment; on the last day, mice were sacrificed and their splenocytes and tumor infiltrating lymphocytes were harvested. The concentration of IL-4 and IFN-gamma cytokines (using ELISA assay) and the percentage of splenic and tumor Treg cells (using Flowcytometry analysis) were measured. **RESULTS:** Artemether could increase both DTH reaction and the production of hemagglutinating antibody in normal mice. Administration of ART profoundly suppressed the progression of tumor tissues. As well, it was significantly effective in the depletion of splenic CD4+ CD25+ Foxp3+ Treg cells ( $p$ -value $>0.05$ ). ART also increased the production of IL-4 ( $p$ -value $<0.05$ ) and IFN-gamma ( $p$ -value $>0.05$ ). As a

conclusion, the cytotoxic and immunomodulatory properties of artemether were acknowledged in vivo.

Gharib, A., Z. Faezizadeh, et al. "Experimental treatment of breast cancer-bearing BALB/c mice by artemisinin and transferrin-loaded magnetic nanoliposomes." Pharmacogn Mag. 2015 May;11(Suppl 1):S117-22. doi: 10.4103/0973-1296.157710.

**BACKGROUND:** The combination of artemisinin and transferrin exhibits versatile anticancer activities. In previous, we successfully prepared artemisinin and transferrin-loaded magnetic nanoliposomes and evaluated their anti-proliferative activity against MCF-7 and MDA-MB-231 cell lines in vitro. In this study, we investigate the in vivo anti-breast cancer activity of artemisinin and transferrin-loaded magnetic nanoliposome against breast transplanted tumors in BALB/c mice model. **MATERIALS AND METHODS:** Artemisinin and transferrin-loaded magnetic nanoliposomes were prepared and characterized for some physicochemical properties. Pieces of tumor tissue from the breast cancer-bearing BALB/c mice were transplanted subcutaneously to the syngeneic female BALB/c mice. In the presence of the external magnet that placed at the breast tumor site, the tissue distribution and tumor-suppressing effects of prepared nanoliposomes on tumor growth was evaluated. **RESULTS:** The prepared nanoliposomes have fine spherical shape, rough surface, nano-sized diameter and magnetic properties. At 2 h after treatment, the intravenous administration of artemisinin and transferrin-loaded magnetic nanoliposomes followed using the magnetic field approximately produced 10- and 5.5-fold higher levels of artemisinin and transferrin in the tumors, respectively, compared with free artemisinin and transferrin. Moreover, in the presence of an external magnetic field, the prepared nanoliposomes could significantly induce apoptosis in the mice breast cancer cells as well as could reduce tumor volume in tumorized mice at 15 days after treatment. **CONCLUSION:** The data suggested that the artemisinin and transferrin-loaded magnetic nanoliposomes would be a good choice for the breast tumor-targeted therapy, due to its high targeting efficiency.

Hargraves, K. G., L. He, et al. "Phytochemical regulation of the tumor suppressive microRNA, miR-34a, by p53-dependent and independent responses in human breast cancer cells." Mol Carcinog. 2015 Mar 19. doi: 10.1002/mc.22296.

The tumor suppressive microRNA miR-34a is transcriptionally regulated by p53 and shown to inhibit breast cancer cell proliferation as well as being

a marker of increased disease free survival. Indole-3-carbinol (I3C) derived from cruciferous vegetables, artemisinin, extracted from the sweet wormwood plant, and artesunate, a semi-synthetic derivative of artemisinin, are phytochemicals with anti-tumorigenic properties however, little is known about the role of microRNAs in their mechanism of action. Human breast cancer cells expressing wild-type (MCF-7) or mutant p53 (T47D) were treated with a concentration range and time course of each phytochemical under conditions of cell cycle arrest as detected by flow cytometry to examine the potential connection between miR-34a expression and their anti-proliferative responses. Real-time PCR and western blot analysis of extracted RNA and total protein revealed artemisinin and artesunate increased miR-34a expression in a dose-dependent manner correlating with down-regulation of the miR-34a target gene, CDK4. I3C stimulation of miR-34a expression required functional p53, whereas, both artemisinin and artesunate up-regulated miR-34a expression regardless of p53 mutational status or in the presence of dominant negative p53. Phytochemical treatments inhibited the luciferase activity of a construct containing the wild-type 3'UTR of CDK4, but not those with a mutated miR-34a binding site, whereas, transfection of miR-34a inhibitors ablated the phytochemical mediated down-regulation of CDK4 and induction of cell cycle arrest. Our results suggest that miR-34a is an essential component of the anti-proliferative activities of I3C, artemisinin, and artesunate and demonstrate that both wild-type p53 dependent and independent pathways are responsible for miR-34a induction. (c) 2015 Wiley Periodicals, Inc.

Holien, T., O. E. Olsen, et al. "Lymphoma and myeloma cells are highly sensitive to growth arrest and apoptosis induced by artesunate." Eur J Haematol. 2013 Oct;91(4):339-46. doi: 10.1111/ejh.12176. Epub 2013 Aug 20.

**OBJECTIVES:** The use of new drugs has improved the treatment of multiple myeloma and diffuse large B-cell lymphoma (DLBCL). Nevertheless, over time many patients relapse and develop resistance to treatment, and efforts are needed to overcome drug resistance. The widely used malaria drug artesunate has been reported to have antitumor activity, and we aimed to test the effects of artesunate on a panel of myeloma and lymphoma cells. **METHODS:** Myeloma and DLBCL cell lines were treated with artesunate in vitro. The effects of artesunate treatment were evaluated using ATP content measurements for proliferation and annexin V/propidium iodide labeling for apoptosis. Western blotting was used to look for artesunate-induced

protein changes. In addition, we measured artesunate effects on patient myeloma cells in the presence of bone marrow stromal cells. **RESULTS:** Artesunate treatment efficiently inhibited cell growth and induced apoptosis in cell lines. Apoptosis was induced concomitantly with downregulation of MYC and anti-apoptotic Bcl-2 family proteins, as well as with cleavage of caspase-3. The IC<sub>50</sub> values of artesunate in cell lines varied between 0.3 and 16.6  $\mu\text{m}$ . Furthermore, some primary myeloma cells were also sensitive to artesunate at doses around 10  $\mu\text{m}$ . Concentrations of this order are pharmacologically relevant as they can be obtained in plasma after intravenous administration of artesunate for malaria treatment. **CONCLUSION:** Our findings indicate that artesunate is a potential drug for treatment of multiple myeloma and DLBCL at doses of the same order as currently in use for treatment of malaria without serious adverse effects.

Jansen, F. H., I. Adoubi, et al. "First study of oral Artemimol-R in advanced cervical cancer: clinical benefit, tolerability and tumor markers." *Anticancer Res.* 2011 Dec;31(12):4417-22.

**BACKGROUND/AIM:** Artemimol-R is cytotoxic in transformed cervical cells and safety in humans is yet to be established. The present study investigates the clinical benefits, safety and the tumor marker effect of orally administered Artemimol-R in patients with advanced cervix carcinoma. **PATIENTS AND METHODS:** Ten patients were treated with Artemimol-R for 28 days. Clinical symptoms, vaginal discharge and pain were followed-up. Adverse events were recorded. Biopsy samples were analyzed by immunohistochemistry for the expression of relevant tumor markers. **RESULTS:** Artemimol-R treatment induced clinical remission with a median time for the disappearance of the symptoms being 7 days. No adverse events of grade 3 or 4 occurred. The expression of p53, Epidermal growth factor receptor (EGFR), and antigen Ki-67 as a cellular marker of proliferation, as well as the number of blood vessels stained by the CD31 antibody decreased, whereas the expression of transferrin receptor protein 1 (CD71) increased. **CONCLUSION:** The current pilot study provides evidence on the improvement of the clinical symptoms and the good tolerability of Artemimol-R in patients with advanced carcinoma of the cervix uteri. A survival trial with Artemimol-R in advanced patients is warranted.

Joubert, J. P., F. J. Smit, et al. "Synthesis and in vitro biological evaluation of aminoacridines and artemisinin-acridine hybrids." *Eur J Pharm Sci.* 2014 Jun 2;56:16-27. doi: 10.1016/j.ejps.2014.01.014. Epub 2014 Feb 18.

During this study, 9-aminoacridine and artemisinin-acridine hybrid compounds were synthesized and the in vitro for antimalarial activity against both the chloroquine sensitive but also gametocytocidal strain (NF54), and chloroquine resistant (Dd2) strains of *Plasmodium falciparum* was determined. In vitro cytotoxicity against CHO cells, apoptosis of HepG2 and SH-SY5Y as well as anticancer activity against HeLa cell lines were assessed. The hybrids were synthesized, using a microwave-assisted radiation method by covalently linking artemisinin and acridine pharmacophores by means of a liable, aminoethyl ether linker. The synthesized compounds were found active against both the *Plasmodium* strains and displayed superior selective toxicity towards the parasitic cells. Hybrid 7, however, containing ethylenediamine linker, proved the most active of all of the synthesized compounds. It had seven-fold higher antigametocytocidal activity compared to chloroquine and was also found to be seven-fold more potent than chloroquine against the Dd2 strain, with highly selective action towards the parasitic cells. This hybrid also showed favourable anti-cancer activity against the HeLa cells, three- and eight-fold higher than those of chloroquine and melphalan, respectively. This hybrid may therefore stand as drug candidate for further investigation in the search for new and effective drugs against malaria and cervical cancer.

Kast, R. E., G. Karpel-Massler, et al. "CUSP9\* treatment protocol for recurrent glioblastoma: aprepitant, artesunate, auranofin, captopril, celecoxib, disulfiram, itraconazole, ritonavir, sertraline augmenting continuous low dose temozolomide." *Oncotarget.* 2014 Sep 30;5(18):8052-82.

CUSP9 treatment protocol for recurrent glioblastoma was published one year ago. We now present a slight modification, designated CUSP9\*. CUSP9\* drugs--aprepitant, artesunate, auranofin, captopril, celecoxib, disulfiram, itraconazole, sertraline, ritonavir, are all widely approved by regulatory authorities, marketed for non-cancer indications. Each drug inhibits one or more important growth-enhancing pathways used by glioblastoma. By blocking survival paths, the aim is to render temozolomide, the current standard cytotoxic drug used in primary glioblastoma treatment, more effective. Although esthetically unpleasing to use so many drugs at once, the closely similar drugs of the original CUSP9 used together have been well-tolerated when given on a compassionate-use basis in the cases that have come to our attention so far. We expect similarly good tolerability for CUSP9\*. The combined action of this suite of drugs blocks signaling at, or the activity of, AKT phosphorylation, aldehyde

dehydrogenase, angiotensin converting enzyme, carbonic anhydrase -2, -9, -12, cyclooxygenase-1 and -2, cathepsin B, Hedgehog, interleukin-6, 5-lipoxygenase, matrix metalloproteinase -2 and -9, mammalian target of rapamycin, neurokinin-1, p-gp efflux pump, thioredoxin reductase, tissue factor, 20 kDa translationally controlled tumor protein, and vascular endothelial growth factor. We believe that given the current prognosis after a glioblastoma has recurred, a trial of CUSP9\* is warranted.

Khan, S., A. Ali, et al. "Affordable and rapid HPTLC method for the simultaneous analysis of artemisinin and its metabolite artemisinic acid in *Artemisia annua* L." Biomed Chromatogr. 2015 Oct;29(10):1594-603. doi: 10.1002/bmc.3465. Epub 2015 Mar 31.

Artemisinin (AN) and artemisinic acid (AA), valuable phyto-pharmaceutical molecules, are well known anti-malarials, but their activities against diseases like cancer, schistosomiasis, HIV, hepatitis-B and leishmaniasis are also being reported. For the simultaneous estimation of AN and AA in the callus and leaf extracts of *A. annua* L. plants, we embarked upon a simple, rapid, selective, reliable and fairly economical high performance thin layer chromatography (HPTLC) method. Experimental conditions such as band size, chamber saturation time, migration of solvent front and slit width were critically studied and the optimum conditions were selected. The separations were achieved using toluene-ethyl acetate, 9:1 (v/v) as mobile phase on pre-coated silica gel plates, G 60F254. Good resolution was achieved with R<sub>f</sub> values of 0.35 +/- 0.02 and 0.26 +/- 0.02 at 536 nm for AN and 626 nm for AA, respectively, in absorption-reflectance mode. The method displayed a linear relationship with r(2) value 0.992 and 0.994 for AN and AA, respectively, in the concentration range of 300-1500 ng for AN and 200-1000 ng for AA. The method was validated for specificity by obtaining in-situ UV overlay spectra and sensitivity by estimating limit of detection (30 ng for AN and 15 ng for AA) and limit of quantitation (80 ng for AN and 45 ng for AA) values. The accuracy was checked by the recovery studies conducted at three different levels with the known concentrations and the average percentage recovery was 101.99% for AN and 103.84% for AA. The precision was analyzed by interday and intraday precision and was 1.09 and 1.00% RSD for AN and 1.22 and 6.05% RSD for AA. The analysis of statistical data substantiates that this HPTLC method can be used for the simultaneous estimation of AN and AA in biological samples. Copyright (c) 2015 John Wiley & Sons, Ltd.

Krishna, S., S. Pulcini, et al. "Pumped up: reflections on PfATP6 as the target for artemisinins." Trends Pharmacol Sci. 2014 Jan;35(1):4-11. doi: 10.1016/j.tips.2013.10.007. Epub 2013 Nov 21.

Sarco/endoplasmic reticulum Ca<sup>2+</sup>-ATPases (SERCAs) are increasingly being studied for therapeutic interventions in the fields of cancer, heart disease, and infection. Our suggestion a decade ago that artemisinins (the most important antimalarial class) act by inhibiting parasite SERCAs (PfATP6 and orthologues) expressed in *Xenopus* oocytes stimulated new directions for research away from conventional site-of-action studies of the food vacuole of the parasite. There is, however, still no consensus on how artemisinins act. We have continued to explore the hypothesis that PfATP6 is a key target by confirming that artemisinins inhibit *Plasmodium falciparum* PfATP6 when it is expressed in yeast and that it is essential for survival of pathogenic asexual-stage parasites. These advances are discussed with their implications for our understanding of how parasites regulate calcium in different stages of asexual development and for the global challenge posed by artemisinin resistance.

Mannan, A., I. Ahmed, et al. "Effects of vegetative and flowering stages on the biosynthesis of artemisinin in *Artemisia* species." Arch Pharm Res. 2011 Oct;34(10):1657-61. doi: 10.1007/s12272-011-1010-6. Epub 2011 Nov 12.

Artemisinin is an endoperoxide sesquiterpene lactone, and has been proven to be very effective in treating drug resistant cases of malaria, cancer, etc. The compound is obtained from *Artemisia* species. In the current study, the effects of vegetative and flowering stages on artemisinin production were studied, to determine the proper harvesting time of naturally growing *Artemisia* species with the highest levels of artemisinin. Eight *Artemisia* species along with two varieties were selected for this analytical work. The results showed that artemisinin content was high in the leaves of *Artemisia indica*, *A. sieversiana*, *A. roxburghiana* var. *roxburghiana*, *A. roxburghiana* var. *gratae*, and *A. parviflora* at the flowering stage. The highest artemisinin content was measured in the leaves of *A. dracunculus* var. *dracunculus*. Upon comparisons of artemisinin content among the individual plant species, the highest amount of artemisinin was again in *A. dracunculus* var. *dracunculus* followed by *A. sieversiana* when harvested at the flowering stage. In overall comparisons, the plants at the flowering stage showed high levels of artemisinin, which is deemed the optimum harvesting time of *Artemisia* species in Pakistan for maximum artemisinin content.

Nguyen, K. T., P. R. Arsenault, et al. "Trichomes + roots + ROS = artemisinin: regulating artemisinin biosynthesis in *Artemisia annua* L." *In Vitro Cell Dev Biol Plant*. 2011 Jun;47(3):329-338.

Artemisinin is a highly effective sesquiterpene lactone therapeutic produced in the plant, *Artemisia annua*. Despite its efficacy against malaria and many other infectious diseases and neoplasms, the drug is in short supply mainly because the plant produces low levels of the compound. This review updates the current understanding of artemisinin biosynthesis with a special focus on the emerging knowledge of how biosynthesis of the compound is regulated in planta.

Noori, S. and Z. M. Hassan "Dihydroartemisinin shift the immune response towards Th1, inhibit the tumor growth in vitro and in vivo." *Cell Immunol*. 2011;271(1):67-72. doi: 10.1016/j.cellimm.2011.06.008. Epub 2011 Jul 12.

Some investigators have been found that Artemisinin and its derivatives have inhibitory effect on growth of cancer cells. Among these derivatives, Dihydroartemisinin (DHA) is well known as a semi-synthetic one. In addition, T cells are proved to be essential for the destruction of cancer cells. In this research, we assessed the effects of DHA on tumor cell growth inhibition in vitro by MTT assay and in vivo by intra tumor injection of DHA against breast cancer. The results showed that the IC(50) values of DHA for RIN pancreatic tumor cell line were 30  $\mu$ M and significant decrease in the tumor size in vivo. Also we evaluate the effect of DHA on the modulation of immune response in tumor bearing animals; these include the splenocyte proliferation using the BrdU kit; measurement of cytokine profile by ELISA, and evaluate the percentage of T regulatory cells in the spleen by flowcytometry. Our results demonstrated that a significant decrease in the level of IL-4 in the animals treated with DHA and significant decreased in the level of splenic CD4(+)CD25(+) Foxp3(+) T regulatory cells.

Ooko, E., M. E. Saeed, et al. "Artemisinin derivatives induce iron-dependent cell death (ferroptosis) in tumor cells." *Phytomedicine*. 2015 Oct 15;22(11):1045-54. doi: 10.1016/j.phymed.2015.08.002. Epub 2015 Aug 18.

**BACKGROUND:** Apoptosis and other forms of cell death have been intensively investigated in the past years to explain the mode of action of synthetic anticancer drugs and natural products. Recently, a new form of cell death emerged, which was termed ferroptosis, because it depends on intracellular iron. Here, the role of genes involved in iron metabolism and homeostasis for the cytotoxicity of ten artemisinin

derivatives have been systematically investigated. **MATERIAL AND METHODS:** Log10IC50 values of 10 artemisinin derivatives (artesunate, artemether, arteether, arteminol, artemisitene, arteanuin B, another monomeric artemisinin derivative and three artemisinin dimer molecules) were correlated to the microarray-based mRNA expression of 30 iron-related genes in 60 cell lines of the National Cancer Institute (NCI, USA) as determined in 218 different microarray hybridization experiments. The effect of desferoxamine and ferrostatin-1 on the cytotoxicity of arteminol of CCRF-CEM cells was determined by resazurin assays. The mRNA expression of TFRC was exemplarily validated by immunohistochemical detection of transferrin receptor protein expression. **RESULTS:** The mRNA expression of 20 genes represented by 59 different cDNA clones significantly correlated to the log10IC50 values for the artemisinins, including genes encoding transferrin (TF), transferrin receptors 1 and 2 (TFRC, TFR2), ceruloplasmin (CP), lactoferrin (LTF) and others. The ferroptosis inhibitor ferrostatin-1 and the iron chelator desferoxamine led to a significantly reduced cytotoxicity of arteminol, indicating ferroptosis as cell death mode. **CONCLUSION:** The numerous iron-related genes, whose expression correlated with the response to artemisinin derivatives speak in factor for the relevance of iron for the cytotoxic activity of these compounds. Treatment with ferroptosis-inducing agents such as artemisinin derivatives represents an attractive strategy for cancer therapy. Pre-therapeutic determination of iron-related genes may indicate tumor sensitivity to artemisinins. Ferroptosis induced by artemisinin-type drugs deserve further investigation for individualized tumor therapy.

Owumi, S. E., M. A. Gbadegesin, et al. "Toxicity associated with repeated administration of artemether-lumefantrine in rats." *Environ Toxicol*. 2015 Mar;30(3):301-7. doi: 10.1002/tox.21907. Epub 2013 Sep 25.

Chemotherapy remains an important approach in the fight against malaria. Artemether-lumefantrine combination is widely in use due to its effectiveness against *Plasmodium falciparum*. Misuse in the form of multiple repeated doses of this anti-malaria drug is rampant in Nigeria. This study was designed to assess the hepatotoxic and clastogenic potential of extreme misuse of artemether-lumefantrine in rats. Graded doses of artemether-lumefantrine (1-5 mg/kg body weight) were administered by oral gavage for 6 weeks, twice daily, for 3 consecutive days per week. Artemether-lumefantrine, at all doses, did not have significant effects on the body and relative liver weight of treated group compared to the negative control group. The

mean gamma-glutamyltransferase, alanine, and aspartate aminotransaminase activity in groups of artemether-lumefantrine treated rats were significantly higher ( $p < 0.05$ ) than that of the negative control group indicating that repeated administration of artemether-lumefantrine may be hepatotoxic. Findings from histological analyses of liver cross-section support the enzyme pattern of hepatotoxicity. In addition, the drug, at all experimental doses, significantly induced ( $p < 0.05$ ) formation of micronucleated polychromatic erythrocytes in the bone marrow cells of the treated rats compared with the negative control indicating clastogenic potential of the drug when misused.

Papanikolaou, X., S. Johnson, et al. "Artesunate overcomes drug resistance in multiple myeloma by inducing mitochondrial stress and non-caspase apoptosis." *Oncotarget*. 2014 Jun 30;5(12):4118-28.

Although novel drugs have contributed immensely to improving outcomes of patients with multiple myeloma (MM), many patients develop drug resistance and ultimately succumb to MM. Here, we show that artesunate, an anti-malarial drug, reliably induces cell death in vitro in naive as well as drug-resistant MM cells at concentrations shown to be safe in humans. Artesunate induced apoptosis predominantly through the non-caspase mediated pathway by primarily targeting mitochondria and causing outer mitochondrial membrane permeabilization that led to cytosolic and subsequent nuclear translocation of mitochondrial proteins apoptosis inducing factor (AIF) and endonuclease G (EndoG). Nuclear translocation of AIF and EndoG was accompanied by low levels of reactive oxygen species (ROS) and increased mitochondrial production of superoxide. These effects were present before apoptosis was evident and were related to intracellular levels of bivalent iron (Fe<sup>2+</sup>). Artesunate's unique mechanism probably was at least partially responsible for, its ability to act synergistically with multiple anti-myeloma agents. Our findings suggest that artesunate acts through iron to affect the mitochondria and induce low ROS and non-caspase-mediated apoptosis. Its potency, toxicity profile, and synergism with other drugs make it an intriguing new candidate for MM treatment.

Park, J., H. C. Lai, et al. "DNA damage in dihydroartemisinin-resistant Molt-4 cells." *Anticancer Res*. 2015 Mar;35(3):1339-43.

Artemisinin generates carbon-based free radicals when it reacts with iron, and induces molecular damage and apoptosis. Its toxicity is more selective toward cancer cells because cancer cells contain a higher level of intracellular free iron.

Dihydroartemisinin (DHA), an analog of artemisinin, has selective cytotoxicity toward Molt-4 human lymphoblastoid cells. A major concern is whether cancer cells could develop resistance to DHA, thus limiting its therapeutic efficacy. We have developed a DHA-resistant Molt-4 cell line (RTN) and found out that these cells exhibited resistance to DHA but no significant cross-resistance to artemisinin-tagged holotransferrin (ART-TF), a synthetic artemisinin compound. In the present study, we investigated DNA damage induced by DHA and ART-TF in both Molt-4 and RTN cells using the comet assay. RTN cells exhibited a significantly lower level of basal and X-ray-induced DNA damage compared to Molt-4 cells. Both DHA and ART-TF induced DNA damage in Molt-4 cells, whereas DNA damage was induced in RTN cells by ART-TF, and not DHA. The result of this study shows that by the cell selection method, it is possible to generate a Molt-4 cell line which is not sensitive to DHA, but sensitive to ART-TF, as measured by DNA damage.

Radwan, M., A. Hanora, et al. "Manzamines: a potential for novel cures." *Cell Cycle*. 2012 May 1;11(9):1765-72. doi: 10.4161/cc.20135. Epub 2012 May 1.

Manzamines are a unique class of beta-carboline marine alkaloids with an unusual tetra- or pentacyclic system. These alkaloids have shown a variety of bioactivities against infectious diseases, cancer and inflammatory diseases. The greatest potential for the manzamine alkaloids appears to be against malaria, with improved potency relative to chloroquine and artemisinin. Over 80 manzamine-related alkaloids have been isolated from more than 16 species of marine sponges belonging to five families distributed from the Red Sea to Indonesia, which suggests a possible microbial origin for manzamine alkaloids. The current review summarizes marine literature, focusing on the biological activities of manzamines, the possible microbial origin of this class of compounds and the Red Sea as a possible source of manzamines from biosynthetic gene clusters of Red Sea microbes.

Reichert, S., V. Reinboldt, et al. "A radiosensitizing effect of artesunate in glioblastoma cells is associated with a diminished expression of the inhibitor of apoptosis protein survivin." *Radiother Oncol*. 2012 Jun;103(3):394-401. doi: 10.1016/j.radonc.2012.03.018. Epub 2012 May 3.

BACKGROUND AND PURPOSE: Novel strategies to overcome an irradiation resistant phenotype may help to increase therapeutic efficacy in glioblastoma multiforme. The present study aimed to elucidate radiation sensitizing properties of artesunate,

a semi synthetic derivate of artemisinin and to assess factors involved in this effect. MATERIALS AND METHODS: LN229 and U87MG cells were treated with various concentrations of artesunate and radiation response was determined by a colony forming assay. Cell numbers, apoptosis induction, cell cycle distribution, and DNA repair following combined modality treatment were monitored by MTT-, caspase 3/7 assay, cytofluorometry, and gamma-H2AX foci formation. Expression of survivin, survivin-GFP fusion protein, XIAP, cellular (c)IAP1 and cIAP2 was monitored by Western immunoblotting. RESULTS: Treatment of glioma cells with artesunate and irradiation resulted in an increased apoptotic fraction, pronounced G2/M arrest and increased DNA damage as demonstrated by an elevated amount of gamma-H2AX foci/nucleus. Incubation with artesunate lowers survivin expression in a time and dose-dependent manner, whereas expression of XIAP, cIAP1 and cIAP2 was not affected. In clonogenic assays, treatment with artesunate revealed a significantly reduced surviving fraction, whereas stable over expression of a survivin-GFP protein reversed artesunate-mediated radiosensitization. CONCLUSION: Artesunate selectively down regulates survivin that contributes to a radiosensitization of glioma cells by an increased induction of apoptosis, cell cycle arrest, and a hampered DNA damage response.

Righeschi, C., M. Coronello, et al. "Strategy to provide a useful solution to effective delivery of dihydroartemisinin: development, characterization and in vitro studies of liposomal formulations." Colloids Surf B Biointerfaces. 2014 Apr 1;116:121-7. doi: 10.1016/j.colsurfb.2013.12.019. Epub 2014 Jan 6.

Dihydroartemisinin is one of the most potent anticancer artemisinin-like compounds, able to induce cancer cell death by apoptotic pathways. Besides its effectiveness, it is a poorly water soluble drug with low bioavailability and low half-life (34-90 min), therefore, the development of new formulations of dihydroartemisinin to increase bioavailability is in great need. Conventional (P90G and cholesterol) and stealth liposomes (P90G; cholesterol and PE 18:0/18:0 PEG 2000) to deliver dihydroartemisinin to cancer cells were developed for the first time. Both developed formulations show physical characteristics as drug carrier for parental administration and good values of encapsulation efficiency (71% conventional liposomes and 69% stealth liposomes). Physical and chemical stabilities were evaluated under storage condition and in presence of albumin. Cellular uptake efficiency of liposomes was determined by flow cytometry. Higher internalization occurred in the conventional liposomes rather than in the stealth

liposomes suggesting that hydrophilic steric barrier of PEG molecules can reduce cellular uptake. Flow cytometry analysis was also used as an alternative technique for rapid size determination of liposomes. Cytotoxicity studies in the MCF-7 cell line confirmed the absence of toxicity in blank formulations suggesting liposomes may be a suitable carrier for delivery of DHA avoiding the use of organic solvents. Cytotoxicity of DHA and of both liposomal formulations was evaluated in the same cell line, confirming a modified release of DHA from vesicles after cellular uptake.

Rijpma, S. R., J. J. van den Heuvel, et al. "Atovaquone and quinine anti-malarials inhibit ATP binding cassette transporter activity." Malar J. 2014 Sep 13;13:359. doi: 10.1186/1475-2875-13-359.

BACKGROUND: Therapeutic blood plasma concentrations of anti-malarial drugs are essential for successful treatment. Pharmacokinetics of pharmaceutical compounds are dependent of adsorption, distribution, metabolism, and excretion. ATP binding cassette (ABC) transport proteins are particularly involved in drug deposition, as they are located at membranes of many uptake and excretory organs and at protective barriers, where they export endogenous and xenobiotic compounds, including pharmaceuticals. In this study, a panel of well-established anti-malarial drugs which may affect drug plasma concentrations was tested for interactions with human ABC transport proteins. METHODS: The interaction of chloroquine, quinine, artemisinin, mefloquine, lumefantrine, atovaquone, dihydroartemisinin and proguanil, with transport activity of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), bile salt export pump (BSEP) and multidrug resistance-associated proteins (MRP) 1-4 were analysed. The effect of the anti-malarials on the ATP-dependent uptake of radio-labelled substrates was measured in membrane vesicles isolated from HEK293 cells overexpressing the ABC transport proteins. RESULTS: A strong and previously undescribed inhibition of BCRP-mediated transport by atovaquone with a 50% inhibitory concentration (IC50) of 0.23 µM (95% CI 0.17-0.29 µM) and inhibition of P-gp-mediated transport by quinine with an IC50 of 6.8 µM (95% CI 5.9-7.8 µM) was observed. Furthermore, chloroquine and mefloquine were found to significantly inhibit P-gp-mediated transport. BCRP transport activity was significantly inhibited by all anti-malarials tested, whereas BSEP-mediated transport was not inhibited by any of the compounds. Both MRP1- and MRP3-mediated transport were significantly inhibited by mefloquine. CONCLUSIONS: Atovaquone and quinine significantly inhibit BCRP- and P-gp-

mediated transport at concentrations within the clinically relevant prophylactic and therapeutic range. Co-administration of these established anti-malarials with drugs that are BCRP or P-gp substrates may potentially lead to drug-drug interactions.

Rutteman, G. R., S. A. Erich, et al. "Safety and efficacy field study of artesunate for dogs with non-resectable tumours." Anticancer Res. 2013 May;33(5):1819-27.

The anti-malarial drug artesunate has shown anticancer activity in vitro and in preliminary animal experiments, but experience in patients with cancer is very limited. Pre-clinical studies in dogs indicated morbidity at high dosage levels. This study evaluated the effects of artesunate in canine cancer cell lines and in canine cancer patients. Four canine cell lines were tested in vitro for sensitivity towards artesunate and dihydroartemisinin (DHA; active metabolite of artesunate). The half-maximal inhibitory concentration (IC<sub>50</sub>) values for artesunate or DHA were 2-60 μM in three cell lines, while one cell line was much less sensitive to artesunate (IC<sub>50</sub> 337 μM) than to DHA (IC<sub>50</sub> 50 μM). A safety/efficacy field study with artesunate was conducted in 23 dogs with non-resectable tumours. Artesunate was administered for 7-385 days at a dosage of 651-1178 (median 922) mg/m<sup>2</sup>. No neurological or cardiac toxicity was observed and seven dogs exhibited no adverse effects at all. Fever and haematological/gastrointestinal toxicity, mostly transient, occurred in 16 dogs. One dog died from pneumonia. Plasma artesunate and DHA levels fell below the limit of detection within 8-12 h after artesunate administration, while levels after two hours were close to 1 μM. Artesunate produced a long-lasting complete remission in one case of cancer and short-term stabilization of another seven cases.

Saeed, M. E., O. Kadioglu, et al. "Quantitative structure-activity relationship and molecular docking of artemisinin derivatives to vascular endothelial growth factor receptor 1." Anticancer Res. 2015 Apr;35(4):1929-34.

**BACKGROUND/AIM:** The antimalarial drug artemisinin has been shown to exert anticancer activity through anti-angiogenic effects. For further drug development, it may be useful to have derivatives with improved anti-angiogenic properties. **MATERIAL AND METHODS:** We performed molecular docking of 52 artemisinin derivatives to vascular endothelial growth factor receptors (VEGFR1, VEGFR2), and VEGFA ligand using Autodock4 and AutodockTools-1.5.7.rc1 using the Lamarckian genetic algorithm. Quantitative structure-activity relationship (QSAR) analyses of the

compounds prepared by Corina Molecular Networks were performed using the Molecular Operating Environment MOE 2012.10. **RESULTS:** A statistically significant inverse relationship was obtained between in silico binding energies to VEGFR1 and anti-angiogenic activity in vivo of a test-set of artemisinin derivatives (R=-0.843; p=0.035). This served as a control experiment to validate molecular docking predicting anti-angiogenic effects. Furthermore, 52 artemisinin derivatives were docked to VEGFR1 and in selected examples also to VEGFR2 and VEGFA. Higher binding affinities were calculated for receptors than for the ligand. The best binding affinities to VEGFR1 were found for an artemisinin dimer, 10-dihydroartemisininyl-2-propylpentanoate, and dihydroartemisinin alpha-hemisuccinate sodium salt. QSAR analyses revealed significant relationships between VEGFR1 binding energies and defined molecular descriptors of 35 artemisinins assigned to the training set (R=0.0848, p<0.0001) and 17 derivatives assigned to the test set (R=0.761, p<0.001). **CONCLUSION:** Molecular docking and QSAR calculations can be used to identify novel artemisinin derivatives with anti-angiogenic effects.

Singh, N. P., H. C. Lai, et al. "Effects of artemisinin dimers on rat breast cancer cells in vitro and in vivo." Anticancer Res. 2011 Dec;31(12):4111-4.

Artemisinin has been shown to be an effective antimalarial and anticancer compound. Dimers of artemisinin have been synthesized and shown to be potent antimalarials compared with monomers. In the present study, we investigated the effect of two artemisinin dimers (dimer-alcohol and dimer-hydrazone) on rat mammary adenocarcinoma cells (MTLn3) in vitro and in vivo compared with that of the artemisinin monomer dihydroartemisinin (DHA). We found that the dimers are considerably more potent than DHA in killing MTLn3 cells in vitro and suppressing the growth of MTLn3 breast tumors in vivo.

Smit, F. J., R. A. van Biljon, et al. "Synthesis and in vitro biological evaluation of dihydroartemisinin-chalcone esters." Eur J Med Chem. 2015 Jan 27;90:33-44. doi: 10.1016/j.ejmech.2014.11.016. Epub 2014 Nov 7.

A series of dihydroartemisinin-chalcone esters were synthesized through esterification of chalcones with dihydroartemisinin (DHA). The hybrids were screened against chloroquine (CQ) sensitive (3D7) and CQ resistant (W2) strains of intraerythrocytic Plasmodium falciparum parasites, and were all found to be active, with IC<sub>50</sub> values ranging between 1.5 and 11 nM against both strains,

with SI values over 5800. The esters featuring oxygenated aryl rings (7, 10 and 11), were found to be equipotent to DHA, but were 2-3 times more active than artesunate against the 3D7 and W2 strains of the malaria parasites. They were also screened in vitro against a panel of three cancer cell lines consisting of TK-10, UACC-62 and MCF-7. Compound 7, bearing a furan ring, displayed the most potent overall antitumor activity against all three cancer cell lines. TGA revealed that the targeted hybrids were all thermally more stable than DHA, which may be beneficial to the high temperature storage conditions that prevail in malaria endemic countries. During this study, ester 7 was identified as the best candidate for further investigation as a potential drug in search for new, safe and effective antimalarial drugs.

Srivastava, V. and H. Lee "Chloroquine-based hybrid molecules as promising novel chemotherapeutic agents." *Eur J Pharmacol.* 2015 Sep 5;762:472-86. doi: 10.1016/j.ejphar.2015.04.048. Epub 2015 May 8.

Chloroquine (CQ) has a broad spectrum of pharmacological activities including anticancer and anti-inflammatory, in addition to its well-known antimalarial activity. This very useful property of CQ may be rendered through a variety of different molecular and cellular mechanisms, including the induction of apoptosis, necrosis and lysosomal dysfunction. CQ alone may not be as effective as many well-known anticancer drugs; however, it often shows synergistic effects when combined with other anticancer agents, without causing substantial ill-effects. To increase its pharmacological activity, scientists synthesized many different chloroquine derivatives by a repositioning approach, some of which show higher activities than the parental CQ. To further improve anticancer activity, medicinal chemists have recently been focusing on generating CQ hybrid molecules by joining, directly or through a linker, 4-aminoquinoline and other pharmacologically active pharmacophore(s). Indeed, some CQ hybrid molecules substantially improved anticancer activity while maintaining desirable CQ property, providing an excellent opportunity of developing effective and safe novel anticancer agents. Since the approach of developing CQ hybrid molecules has advanced much more in the antimalarial drug research, it can provide an excellent template for anticancer drug development.

Suberu, J. O., I. Romero-Canelon, et al. "Comparative cytotoxicity of artemisinin and cisplatin and their interactions with chlorogenic acids in MCF7 breast cancer cells." *ChemMedChem.* 2014 Dec;9(12):2791-7. doi: 10.1002/cmde.201402285. Epub 2014 Sep 10.

In parts of Africa and Asia, self-medication with a hot water infusion of *Artemisia annua* (*Artemisia* tea) is a common practice for a number of ailments including malaria and cancer. In our earlier work, such an extract showed better potency than artemisinin alone against both chloroquine-sensitive and -resistant parasites. In this study, in vitro tests of the infusion in MCF7 cells showed high IC50 values (>200 µM). The combination of artemisinin and 3-caffeoylquinic acid (3CA), two major components in the extract, was strongly antagonistic and gave a near total loss of cytotoxicity for artemisinin. We observed that the interaction of 3CAs with another cytotoxic compound, cisplatin, showed potentiation of activity by 2.5-fold. The chelation of cellular iron by 3CA is hypothesized as a possible explanation for the loss of artemisinin activity.

Tilaoui, M., H. A. Mouse, et al. "Differential effect of artemisinin against cancer cell lines." *Nat Prod Bioprospect.* 2014 Jun;4(3):189-96. doi: 10.1007/s13659-014-0024-4. Epub 2014 Jun 5.

The present study aims at defining the differential cytotoxicity effect of artemisinin toward P815 (murine mastocytoma) and BSR (kidney adenocarcinoma of hamster) cell lines. Cytotoxicity was measured by the growth inhibition using MTT assay. These in vitro cytotoxicity studies were complemented by the determination of apoptotic DNA fragmentation and Annexin V- streptavidin-FITC assay. Furthermore, we examined the in vitro synergism between artemisinin and the chemotherapeutic drug, vincristin. The in vivo study was investigated using the DBA2/P815 (H2d) mouse model. While artemisinin acted on both tumor cell lines, P815 was much more sensitive to this drug than BSR cells, as revealed by the respective IC50 values (12 µM for P815 and 52 µM for BSR cells). On another hand, and interestingly, apoptosis was induced in P815 but not induced in BSR. These data, reveal an interesting differential cytotoxic effect, suggesting the existence of different molecular interactions between artemisinin and the studied cell lines. In vivo, our results clearly showed that the oral administration of artemisinin inhibited solid tumor development. Our study demonstrates that artemisinin caused differential cytotoxic effects depending not only on the concentration and time of exposure but also on the target cells.

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