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Bioidentical Hormones Natural Thyroid

Artemisinin our Ultimate Cancer Weapon a Gift from China

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by Jeffrey Dach MD

Beating Colon Cancer with Artemisinin

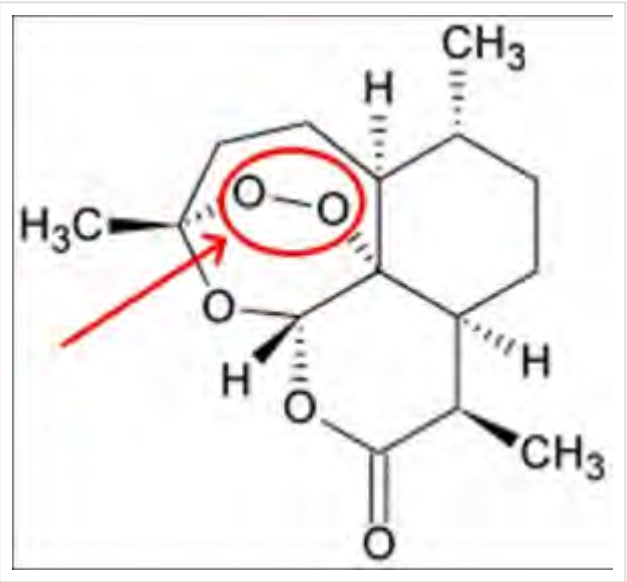
Susan, a 56 year old house wife, noticed abdominal pain and change in bowel habits. An X- Ray test revealed Susan has colon cancer (lower left image), with a typical apple core appearance on barium Xray of the colon. Susan was sent to a colon cancer surgeon who told her she needed an operation to remove the cancer from her colon. *Left image: Tu You You winner of 2015 Nobel Prize in Medicine Courtesy of China Daily News.* This article is part one, for part two click here.

Artemisinin Prospective Randomized Trial in Colon Cancer

Susan read in the newspaper about Dorothy Bradshaw, a colon cancer patient who participated in a clinical study with Dr. Sanjeev Krishna at St George University London in 2010.(71)

Dorothy took a new anti-cancer drug called Artesunate (an artemisinin derivative) for two weeks before her colon resection to remove the tumor. Five years later, she is alive and well. This randomized study was done with 20 colon cancer patients awaiting surgery. Half received the Artesunate pill for two weeks prior to surgery, and the other half a placebo. Five years later, there were 6 cancer recurrences in the placebo group, but only one in the Artesunate (Artemisinin) group. There were **no deaths** in the Artesenate group, compared to **three deaths** in the placebo group.(70-71) I was very impressed by this.

Susan called me to ask if Artemisinin could improve her chances of beating cancer recurrence after surgery. I said to Susan, ***"Artemisinin is our ultimate weapon against cancer, a gift from China"***



Left Image Artemisinin chemical structure. Active portion of molecule is the endo-peroxide bridge (red arrows) highly reactive oxygen bridge which reacts with the iron in malaria organisms, and the iron in cancer cells.

Ridiculing Chinese Herbs

Back in the old days when I was hospital based physician, we took lunch in the doctor's dining room where conversation included amazement and ridicule at the stories of patients who took Chinese herbs for their medical condition. The surgeons had a good laugh, expressing amazement that any intelligent American would choose to take a Chinese herb for a medical condition. *Below image: Dr. Tu YouYou wins **Nobel prize in Medicine 2015** for discovery of Artemisinin. Image Courtesy of IFLScience and China's Daily .*

Artemisinin – A Gift From The Chinese Government

China wins first Nobel Prize in medicine

Tu Youyou, 84, wins for her work in pharmacology

By **SHAN JUAN** and **CHENG YINGQI** in Beijing and **HEZI JIANG** in New York

China has its first Nobel Prize in Medicine. And 84-year-old Tu Youyou said she was not surprised to get it.

"I learned about it from the TV news," she told Qianjiang Evening News on Monday evening. "A little unexpected, but also not quite surprised. This is not my personal achievement, but an award to all Chinese scientists. We worked on this together for decades, so the prize shouldn't be a surprise."

Half of the 2015 Nobel Prize in Physiology or Medicine went to Tu for developing a drug that fights malaria, and the other half was awarded to to William Campbell of Ireland and Satoshi Omura of Japan for discovering therapies against infections caused by roundworm parasites. The winners will share an award of $960,000.

Tu discovered Artemisinin, a drug that has significantly reduced the death rate of malaria patients, and saved millions of lives across the globe, especially in the developing world, the Nobel Assembly at Karolinska Institute, which awards the Nobel Prize in physiology or medicine, said on Monday.

The Assembly said that discoveries that help fight parasitic diseases are crucial because those diseases "affect the world's poorest populations and represent a huge barrier to improving human health".

Chinese pharmacologist Tu Youyou, pictured in 2011, jointly won the 2015 Nobel Prize for Physiology or Medicine along with Irish-born William Campbell and Japan's Satoshi Omura, the Nobel Assembly at Sweden's Karolinska Institute announced on Monday. Tu won part of the prize for discoveries related to a novel therapy against malaria. XINHUA / JIN LIWANG

> **This is not my personal achievement, but an award to all Chinese scientists. We worked on this together for decades, so the prize shouldn't be a surprise."**
>
> **Tu Youyou**, pharmacologist at the China Academy of Traditional Chinese Medicine

Chinese Premier Li Keqiang has sent a congratulatory letter.

"Tu's winning the prize signifies China's prosperity and progress in scientific and technological field, marks a great contribution of Traditional Chinese Medicine to the cause of human health, and showcases China's growing strengths and rising international standing," Li said.

Tu, born in 1930 in Ningbo, China, has been a pharmacologist at the China Academy of Traditional Chinese Medicine since 1965, now known as the China Academy of Chinese Medical Sciences.

In the 1960s, the main treatments for malaria were chloroquine and quinine, but they were proving increasingly ineffective. In 1969, Tu started to chair a government project aimed at eradicating malaria. She and her colleagues experimented with 380 extracts in 2,000 candidate recipes before they finally succeeded in obtaining the pure substance Qinghaosu, later known as Artemisinin, which became the standard regimen for malaria in the World Health Organization's catalog of essential medicines.

Juleen R. Zierath, chairman of the Nobel Committee for Physiology or Medicine, told Xinhua that Tu's "inspiration from traditional Chinese medicine" was important.

"But what was really critical was that Tu Youyou identified the active agent in that plant extract," said Zierath, adding "there was a lot of modern chemistry, bio-chemistry attached to this to bring forward this new drug."

In 2011, Tu was awarded the Lasker DeBakey Clinical Medical Research Award, popularly known as "America's Nobels", and many expected Tu to win the Nobel that year.

"More than 40 years after her findings the prize finally came," said Cao Hongxin, the head of the science and technology department of the State Administration of Traditional Chinese Medicine, and a former director of the China Academy of Traditional Chinese Medicine.

"It's an overdue honor for Tu and the world's recognition of Traditional Chinese Medicine. Now we have Tu winning the first Nobel Prize in Physiology or Medicine; We should be more confident that Chinese scientists will make more high-level breakthroughs in the future," he said.

Back in the 1960's during the Vietnam War, the North Vietnamese soldiers succumbed to malaria in great numbers. This military issue prompted the Chinese government to develop better and more effective anti-malarial drugs. China enlisted the help of a young medical student named, Dr Tu YouYou who began working. She uncovered an ancient Chinese medical text in an archaeological excavation written by Ge Hong (281–340 AC), which describes an herbal tea, sweet wormwood, to treat fevers and chills. This sweet wormwood tea was later mentioned in the 1596 Chinese Compendium by Li Shizen. Dr Tu Youyou ultimately isolated the active molecule in the tea, called artemisinin (figure 1 above), an effective anti-malaria drug, for which she received the 2015 Nobel prize in Medicine. Artemisinin and derivatives like artesunate are now life saving malaria drugs used by millions. Who's laughing at Chinese Herbs Now?

Antimalarial Drugs are Also Anti-Cancer Drugs

Left *Upper Image: Artemisinin reacting with Heme courtesy of O'neill, et al Molecular Mechanism of Action of Artemisinine Debate ONeill 2010. (131)*

Dr. Das reports in a 2015 article that Artemisinin (or derivatives) are effective against 55 cancer cell lines with inhibitory effects against pancreatic cancer, osteosarcoma, lung cancer, colon, melanoma, breast, ovarian, prostate, central nervous system, lymphoma, leukemia and renal cancer cells. (1)

The molecular mechanism by which artemisinin compounds serve as effective anti-cancer agents can be found in its molecular structure, the endo-peroxide bridge which reacts with the iron molecule. (*see figure 1 above*)

Cancer Cells Contain Massive Amounts of Iron (Fe)

We can exploit key metabolic differences between cancer cells and normal cells, to come up with ways to kill cancer cells, while leaving normal cells unharmed as discussed in a previous article, cancer as a metabolic disease. This is the targeted non-toxic approach to cancer treatment. One such key metabolic difference is the higher **Iron (Fe)** content of cancer cells.

*Left Image shows endo-peroxide bridge (oxygen) in Artemisinin **(1)** attaching to Heme iron (Fe) **(2)** courtesy of Robert et al, "Characterization of the alkylation product of heme by the antimalarial drug artemisinin." (132)*

Cancer Cells Contain Massive Amounts of Iron in Lysosomes

Cancer cells express high levels of the transferrin receptors for internalizing iron (Fe) at a tremendous rate.(21) This is a useful difference from normal cells. The cancer cells have a voracious appetite for Iron, and contain massive quantities of iron compared to normal cells. In addition, the transport mechanism for iron in cancer cells, called transferrin receptors are massively up-regulated.(18) In fact, a correlation between expression of transferrin membrane receptor and Ki-67 (a marker of tumor proliferation) has also been reported, (19) The more aggressive the tumor, the greater number of transferring receptors. This difference in iron content and iron transport is why artemisinin compounds kill cancer cells selectively while sparing normal cells.

Addition of Iron Enhances Killing Effect of Artemisinin

*Left image: Schematic of Hemoglobin Molecule with Central **Fe** (Red). This Fe is Iron in the center of Heme Molecule .Courtesy of wikimedia.*

Since the anti-cancer effect of artemisinin relies on presence of Iron in the cancer cell, its effects are up-regulated 1.5-10 fold by ingestion of iron supplements, as you might expect.(20)

There is also synergy of ART with ALA (Amino Levulinic Acid, the precursor molecule for heme synthesis). However, considering the high cost of GleoLan ($4,000) per vial), perhaps ingestion of liquid chlorophyll would provide similar precursors and increase heme synthesis at lower cost.(152-155) See: Xu, X. F., et al. "Effects of sodium ferrous chlorophyll treatment on anemia of hemodialysis patients and relevant biochemical parameters." Journal of biological regulators and homeostatic agents 30.1 (2016): 135-140.

Fenton Reaction in Lysosomes – Ferroptosis

What are lysosomes? Lysosomes are the cell organelles which contain acid used for digestion and degradation of unwanted intracellular debris and endocytosed bacteria and proteins. For example, our white cells, called neutrophils, kill bacteria in our blood stream by eating them. Once eaten, these unwanted proteins are digested in lysosomes, The acid in the lysosome is produced by a molecular machine called the "V-ATPase", a molecular pump for acid production. Lysosomes often accumulate large amounts of iron, especially in cancer cells, which may then react with oxygen, called Fenton reaction, causing release of hydroxyl radicals. This compromises the lysosomal membrane with release of acid contents freely into the cell cytosol initiating a form of cell death is called "ferroptosis" (53) (as opposed to apoptosis), as we will detail below. (5) In this scenario, Artemisinin enters the cancer cell lysosomes, which already contain iron as a degradation product from ferritin. The endoperoxide oxygen bridge in artemisinin reacts with iron , the Fenton Reaction, and hydroxyl radicals are produced. The diagram below illustrates the mode of action of Artemisinin in the cancer cell.

Mode of Action of Artemisinin Cancer Cell Death from Yang et al.(6)

Figure 9E from Yang et al. Artemisinin enters cancer cell along with iron loaded ferritin. Both enter the lysosome which then triggers mitochondria reactive oxygen species (ROS) and caspase 3 programmed cell death.(6)

Cancer Cell Killing Mechanism according to Yang et al:

Firstly Artemisinin (ART) accumulates in the lysosomes. Second, ART increases lysosomal acidification, cathepsin enzyme activity, and protein degradation via promoting lysosomal V-ATPase assembly. ART induces autophagy, based on the observations that ART increased autophagosomes formation and enhanced autophagic flux.

*"We believe that this **perinuclear clustering of lysosomes** is, in fact, an indication of autophagy induction. We observed increase of mitochondrial ROS implicating lysosomal iron as a critical mediator in ART-induced mitochondrial ROS production and cell death. It is possible that enhanced lysosomal degradation of ferritin induced by ART leads to the transient increase of cytosolic ferrous iron, which then affects the mitochondria, leading to enhanced mitochondrial ROS production."(6)*

BAF (bafilomycin A1), a lysosomal (V)-ATPase inhibitor, was able to effectively inhibit the cell death induced by ART . *"Ferritin delivery and degradation in lysosomes is required for the toxicity of ART. Therefore, lysosomal inhibitors significantly protect the cells from ART-induced cell death via blockage of ferritin degradation."(6)*

Chloroquin inhibits Ferritin Degradation and Cell Death induced by ART

"In fact, we also found that chloroquine inhibited the ferritin degradation and cell death induced by ART as efficiently as BAF (data not shown)."

BAF is a lysosomal inhibitor which prevents artemisinin cell death. DFO is iron chelator which inhibits cell death. NAC (N acetyl cysteine inhibits ROS)inhibits cell death. Images courtesy of Yang, Nai-Di, et al. (6) "Artesunate induces cell death in human cancer cells "

Lysosomal Clustering and Accumulation At Nucleus

When Artemisinin enters the cancer cell, it causes a peculiar re-arrangement of the lysosomes and mitochondria in a pattern typical for autophagy (the cell eats itself and dies) as reported by Dr Brady (see below). This peri-nuclear clumping has also been observed in experiments which knock out KIF5B, the only microtubule motor protein associated with the lysosomes. Therefore it has been speculated that the effect of Artemisinin in the lysosome is to impair the microtube motor proteins. This perinuclear clustering of organelles is a prelude to programmed cell death.

Left image: Top Row are Untreated Breast Cancer Cells showing lysosomes (dark stained particles) dispersed throughout cells.

Bottom Row, Breast cancer cells treated with Artemisinin. Yellow arrows point to peri-nucelar clustering of autophagosomes (lysosomes).

Images from Figure 3 courtesy of Hamacher-Brady, Anne, et al. (80) "Artesunate Activates Mitochondrial Apoptosis in Breast Cancer Cells via Iron-catalyzed Lysosomal Reactive Oxygen Species Production." J. Biol. Chem 2011.286 (2010): 6587-6601.

Left Image shows Breast Cancer Cells (Top row, yellow arrow) treated with artemisinin and holotransferrin demonstrating clustering of mitochondria (green) and lysosomes(red). Normal breast cells (lower row) show no clustering effects. Images from Figure 9 courtesy of Hamacher-Brady, Anne, et al.(80)

Dr Kundu reports in a 2015 article in Acta tropica that many anti-malarial drugs serve as anti-cancer drugs and vice versa (22). **Curcumin**, resveratrol, pterostilbene, allicin (from garlic) are among the many natural

substances that work together in synergy with Artemisinin. In addition, Artimisisin, and derivatives, enhance the anti-cancer activity of most conventional chemotherapy agents.(22)

Artemisinin Derivatives and Ferritin Tagging

Efficacy of Artimisinin may be impaired by poor absorption and relatively low blood level. How can the potency and efficacy of Artemisinin be increased ? Make a derivative such a artemether, artesenuate. Derivatives are modified artemisinin compounds possessing greater bio-availability and potency. Be careful here because greater bio-availablity and potency may be associated with increased toxicity which we will discuss below.

Transferrin Tagged Artemisinin

Another technique is to tag the Artemisin molecule with transferrin, the Iron carrier protein. This combination had greater anti-cancer potency see **Anticancer properties of artemisinin derivatives and their targeted delivery by transferrin conjugation.** Nakase. (83)

Other Anti-Cancer Treatments Work in Synergy

Firstly, intra-cellular iron is required for Artemisinin to kill the cancer cell. Having the patient take an Iron supplement (such as Iron bis-glycinate, Optferrin Pure Encapsulations) increases the effect of the Artemisinin. However one should be careful to take the iron supplement at a different time from the Artemisinin to avoid reacting directly with the iron in the GI tract. Most Artemisinin practitioners will use a schedule to allow “rest days”. The Artemisinin is taken 14 days, and then take 4 days off. This is thought to improve GI absorption.

Additional anti-cancer supplements are synergistic with artemisinin. These include Vitamin D, Iodine (iodoral) Berberine, Curcumin (lipospheric) (24-29), Resveratrol, Pterostilbene, Allicin, Melatonin, Sulphoraphane, Butyrate, and high dose IV Vitamin C (79), fresh juicing with organic carrots and organic beets, Lipo-Colostrum (Soveriegn Labs), a good multivitamin (One Multi Pure Encapsulation) . (79)

Eureka ! High Dose IV Vitamin C Synergy with Artemisinin (79)

The suppressed knowledge of IV vitamin as an effective anticancer treatment is finally coming to light with new research over the last decade. High dose IV vitamin C (ascorbate) 50-75 grams produces blood levels producing pro-oxidant effects acting in synergy with the oxidizing effects of Artemisinin, making both treatments more effective in killing cancer cells selectively without harming normal cells.(79) Since both high dose IV Vitamin C and Artemisinin are extremely safe, this highly effective synergy is considered the cornerstone of a successful anti-cancer protocol. This IV vitamin C bag may be followed by IV Alpha Lipoic Acid (300 mg IV) which shunts cancer cell metabolism towards aerobic respiration. The alpha lipoic acid heightens the Vitamin C effect as explained in my article on this topic. IV artesunate (avaliable as first line thereapy for severe malaria) is commonly given IV just before the IV vitamin C. This augments the pro-oxidative effects.

Artemisinin-Curcumin Synergy

In a 2006 study using malaria infected cultured RBC;s, the combination of artemisinin with curcumin was found to synergize.(56) This combination showed greater malaria killing activity than the individual compounds. The molecular pathways were not elucidated. One might anticipate a synergistic effect for this combination in killing cancer cells. However, to my knowledge this has not yet been studied, and would be a good topic for NIH funding for future research.(56)

Artemisinin – Allicin (Garlic) Synergy

Allicin,(diallyl thiosulfinate) the active ingredient in garlic, has known anti-cancer effects. Allicin induces caspase mediated apoptosis in lymphoma and other cancers. Dr Jiang (see Left Image) reported in 2013 the synergistic combination of Artesunate (artemisinin derivative) with allicin had a heightened anti-cancer effect on osteosarcoma cell lines in vitro and in vivo.(57) Left Image Fig 3B from Jiang showing synergistic effect of combining Artesunate (an Artemisin derivative) with Allicin. Bar Chart shows apoptosis levels after treating Osteosarcoma cells (in culture) with Artesunate (green arrow), Allicin (blue arrow) or combination of Artesunate and Allicin (Red Arrow). (57) Dr Jiang also reported additional mouse tumor xenograft studies were done, and these in-vivo studies also showed enhanced synergy for the combination of Allicin and Artesunate. (57)

Platelet Inhibition

Allicin,and thiosulfinates, are **potent platelet-aggregation inhibitors** which can aggravate bleeding, so caution is advised when used with other platelet inhibitors such as Feverfew, Aspirin, Vitamin E, Fish Oil or ibrutinib (Imbruvica – Bruton's-Kinase Inhibitor).

Synergy Artemisinin with Resveratrol

In 2014, Drs Li and Yang reported a synergistic effect of the combination of Resveratrol with Artemisinin in Hepatoma (HepG2) and Cervical Cancer (HeLa) cell lines. The combination significantly increased apoptosis and necrosis of the two cancer cell lines. (58) *Left Image Bar Chart from Li and Yang showing cancer cell death (apoptosis) after treatment with Artemisinin (green arrow), Resveratrol (blue arrow) and the Combination (red arrow) for two cancer cell lines (black and grey bars).(58)*

Although Pterostilbene was not used in this study, the Pterostilbene is a derivative of Resveratrol, chemically similar with better bioavailability, and I would therefore expect Pterostilebene to demonstrate an even greater synergy with Artemisinin compared to Resveratrol. A 2005 study by Dr Tolomeo showed the 3-Hydroxy Pterostilbene to be 50-100 times stronger than Resveratrol in killing multi-drug resistance leukemia cells. However, the plain pterostilbene was the least toxic to normal hematopoetic cells. (59)

Synergy of Artemisinin with Butyrate (76, 122-128)

Dr Lai reports in 2005 that the cancer cell killing effect of Arte
short chain fatty acid.(76) Their study used a lymphoblastic leukemia cell line, treated with a combination of dihydroartimisin and butyrate.

Left Image Bar Chart courtesy of Dr Lai 2005, Cell Count after treatment with Dihydroartemisinin(Green Arrow), after Butyrate (blue Arrow), After Combination (Red Arrow)

In a 2007 report, Dr Pajak outlined the molecular basis of anti-cancer effects of butyrate. (123) Butyrate, a small four-carbon chain fatty acid, is a normal product of large bowel microbial fermentation of dietary fiber. Butyrate inhibits (HDAC) histone deacetylase activity, allowing DNA binding of several transcription factors which increases expression of proapoptotic genes, and results in amplification of apoptotic pathways (programmed cell suicide) in the cancer cell, while sparing normal cells.

In a 2013 report, Dr JK Fauser studied the anticancer effects of butyrate and coconut oil (lauric acid) on a colon cancer cell line. Dr Fauser reported butyrate induces apoptosis by:" **inhibiting histone deacetylase activity, inducing cell cycle arrest, promoting differentiation, activating NF-κB, downregulating α 2 β 1 , modifying glucose availability, and inducing caspase activation in colon cancer cell." (125)**

Dr Choi reported in a 2006 study on the anti-cancer effects of butyrate on a leukemia cell line.(124) Dr Choi found that butyrate induced apoptosis of human leukemic cells by inhibition of telomerase activity. In addition, **butyrate served as a (HDAC) histone deacetylase inhibitor, resulting in** dose-dependent apoptosis associated with up-regulation in pro-apoptotic Bax expression, and down-regulation of anti-apoptotic Bcl-2 and Bcl-XL.(124)

In a 2006 report Dr Heider studied the effect of the HDAC, sodium butyrate, on three Mantle Cell Lymphoma types. Dr Heider reported butyrate induced potent programmed cell death (apoptosis) of all three Mantle Cell Lymphoma types in a dose-dependent manner. (136)

Buy Sodium Butyrate on Amazon. Link to a review. A probiotic named Clostridium butyricum is known to produce butyrate This is available from Japan in a product called "Miyarisan" available on Amazon. It is also contained in **Advanced Orthomolecular Research AOR Probiotic 3.**

Targeting (Progenitor) Cancer Stem Cells

Cytotoxic chemotherapy may be quite useful for controlling hematologic cancers such as lymphoma and leukemia. Although highly toxic, and frequently associated with adverse effects, these drugs may induce complete remission (CR), with disappearance of tumor masses and clearing of radionuclide activity on a follow up PET scans. In the more aggressive cell types, relapse with metastatic disease is inevitable. Relapse is thought caused by **cancer stem cells** (progenitor cells) which are dormant and not actively replicating. These cancer stem cells are insensitive (resistant) to cytotoxic chemotherapy which is not effective for non-replicating cells. (86-88) Are there natural non-toxic targeted therapies which target cancer stem cells?

Yes, and here they are a few of the many: **Berberine, Resveratrol, Vitamin D3, Curcumin, Feverfew, and Sulforaphane. (89-90)** These are natural substances that target cancer stem cells as discussed by Dr Yanyan Li in his 2011 article : which also includes soy isoflavone, epigallocatechin-3-gallate, lycopene, piperine, and vitamin D3. (134)

FeverFew – Targeting Cancer Stem Cells – Depleting Glutathione

The well known anti-migraine botanical , Feverfew (parthenolide) has been found to target the cancer stem cells. (74) The authors state:

> " *Parthenolide preferentially targets AML progenitor and stem cell populations….The molecular mechanism of Parthenolide- with inhibition of nuclear factor κ B (NF-κB), proapoptotic activation of p53, and* ***increased reactive oxygen species*** *(ROS). On the basis of these findings, we propose that the activity of Parthenolide triggers Cancer Stem Cell-specific apoptosis and as such represents a potentially important new class of drugs for Cancer Stem Cell-targeted therapy."(74)*

Feverfew (Parthenolide) is a widely used botanical which exerts potent anti-cancer effects by blocking nuclear activation of Nuclear Factor Kappa Beta (NFKB), and **depleting the cancer cell of glutathione**, rendering it sensitive to ROS (reactive oxygen species) which triggers apoptotic pathways. (92-108) Parthenolide shares some similarities in chemical structure with Artemisinin. One might speculate that the two agents might work synergistically together with enhanced cancer cell killing effects, and this would be a good topic for NIH funding and future study. (92-108)

Sulforaphane – Targeting Cancer Stem Cells and Depleting Glutathione

The active ingredient in broccoli is sulforaphane, a widely used nutritional supplement with no adverse effects. Sulforaphane has been widely studied as an effective anti-cancer agent which targets cancer stem cells and **depletes glutathione in the cancer cell,** thus rending it more sensitive to oxidative damage.(109-115)

Dr Yanyan Li, et al reported in 2010 that sulforaphane eliminated breast cancer stem cells in vivo in a mouse xenograft model. Tumor bearing primary mice were treated with sulforaphane. The tumor cells from the primary mice were then re-implanted into secondary mice, showing no growth of tumor cells, indicating the cancer stem cells has been eradicated. (112) I was impressed by this. A 2012 study by Dr Rodova showed Sulforaphane is effective anti cancer treatment for pancreatic cancer stem cells via blockade of hedgehog signalling.(135)

Other Re-Purposed Drugs that may Work with Artemisisin:

Sulfasalazine (Asulfadine)- an old anti-inflammatory drug widely used in rheumatology, inhibits the active transport of cystine into the cancer cell. (39-46) (116-121)

When the cancer cell is deficient in cystine, it cannot make glutathione, the intra-cellular anti-oxidant. Lack of anti-oxidant protection leads directly to "ferroptosis" in cancer cell studies.(see image at left).(39-46)

Left above image: schematic of sulfasalazine blocking cystine uptake by cancer cell, thereby inducing Ferroptosis. Courtesy of Dixon et al., "Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death ". Cell, vol 149, pp. 1060–1072 (2012).

Sulfasalazine has a long history of use for inflammatory bowel disease and rheumatoid arthritis. Benefits are thought to arise from its ability to suppress nuclear factor Kappa Beta.

Sulfasalazine is also a potent suppressor of lymphoma cells on the basis of suppression of cystine uptake which impairs antioxidant defense. Dr Gout found that sulfasalazine suppressed 90% of lymphoma xenografts in mice, a remarkable finding. (41) In addition, sulfasalazine inhibits growth of mantle cell lymphoma in a murine MCL model as reported by Dr Bebb in 2003 Blood.

Since "**Ferroptosis"** is also seen when cancer cells are treated with Artemisinin, one might logically assume the two drugs **Artemisinin and Sulfasalazine** would be synergistic, augmenting cancer cell death. Unfortunately, the appropriate cell culture studies (in-vitro or in-vivo) have not yet been done. This would be a good study for NIH funding of further research.(39-46)

Chloroquin

Hydroxychloroquine (Plaquenil) and Chloroquine (Avelan), are old anti-malarial drugs. They serve as lysosomal inhibitors. (47-52) Pretreatment with chloroquin augmented the cancer killing effect of artemisinin in a 2014 cancer cell study by Dr Ganguli et al.

However, a prior cell study by Dr Hamacher-Brady, found that concurrent chloroquin treatment (at the same time) as artemisinin impaired cancer cell killing activity.(80) Dr Nai-Di Yang also found that Chloroquin inhibited the effect of artemisinin in his study, similar to the activity of the lysosomal V-ATP-ase inhibitor, Baflinomycin. See: Yang, Nai-Di, et al. (6) Artesunate induces cell death in human cancer cells.

So, it is clear that simultaneous or concurrent use of chloroquin together with Artemisinin should be avoided, as this inhibits the cell killing effects. Rather, as suggested by Dr Ganguli et al., it might be appropriate to use the chloroquin drug as a pre-treatment. In other words, take the chloroquin during one of the OFF-DAYS when the patient is **not** taking the artemisinin. A typical artemisinin schedule is 14 days on and 4 days off, (although this may vary). So, taking the chloroquin during the days off would serve as a pre-treatment for the next Artemisinin cycle as suggested by Dr Ganguli et al.

Chloroquin and has a long track record of safety in treatment of rheumatoid arthritis at a dosage of 250 mg daily for years. The most worrisome toxicity relates to ocular and macular toxicity at high doses over long periods of time. The use of a small dose of 250 mg of chloroquin once a week prior to the start of a 5 day course of artemisinin is considerably less than usual chloroquin dosage for rheumatologic disease, and therefore considered safe. Usual chloroquin dosage for travelers prevention of malaria is one 500 mg tablet once a week starting one week before departure, and continuing for 4 weeks after returning. Although higher doses may have retinal toxicity, typical prophylactic doses are considered not harmful to the retina.

Sequential, and not concurrent, use of Chloroquin might ultimately prove to be effective for augmenting the anti-cancer effect of Artemisinin. I would like to see mouse xeno-graft tumor validation studies of this combination.

However, as far as I know, these types of in-vivo studies have not been done. This would be a good subject for NIH funding of future research.(47-52)

Mefloquin (Lariam)

Mefloquin was found to be superior to Chloroquin in a 2012 report by Dr Sharma who found Mefloquin caused cell death in breast cancer cell cultures. In a 1992 report by Dr Glaumann, Mefloquin caused expansion of lysosomes in rat livers starting at 24 hours after administration and lasting for 7 days. The lysosomes later harbored multi-lamellar bodies which disappeared after 7-10 days.

Mefloquin – Acute Myelogenous Leukemia (17)

Dr M Sukhai et al. reported in their 2013 article "Lysosomal disruption preferentially targets acute myeloid leukemia cells and progenitors. Dr Sukhai screened a library of 100 on and off patent drugs for activity against AML (acute luekemia). At the top of the list was Ivermectin (see below), and the second most acive was the anti-malarial drug mefloquin which selectively killed a panel of leukemia cells and leukemia stem cells in mice.(17)

Mefloquin – Malaria Prophylaxis for Travelers

Mefloquin is currently recommended for malaria prophylaxis in travelers. Dosage is usually on 250 mg tablet per week for a few weeks prior to the trip. Toxicity: There may be neuro-psychiatric symptoms of toxicity reported by some. Artemisinin has been used in combination with Chloroquin or Mefloquin for decades, however l**ong term use can result in neurotoxicity,** so caution is advised.

Artemisinin Synergy with Chemotherapy – Rituximab

Left Image: Lymphoma Cell viability after (Black Arrow) Rituxumab , (Green Arrow) Artesenuate, (Red Arrow) combination of two. Synergy of Rituximab with Artesenuate from Sieber, Sebastian, et al. 2009 Combination Rituximab and Artesunate..

The anti-CD-20 monoclonal antibody, rituximab, has revolutionized the treatment of lymphoma, by targeting the CD20 protein on B cell lymphoma membranes, and increasing response and survival rates. Artemisinin has been given with rituximab with good synergism and increased anticancer activity. Dr Seiber says: "*both agents act synergistically by activating at least partially converging signaling pathways."* See: Sieber, Sebastian, et al. "Combination treatment of malignant B cells using the anti-CD20 antibody rituximab and the anti-malarial artesunate." International journal of oncology 35.1 (2009): 149-158. Combination treatment of malignant B cells Rituximab and Artesunate Sieber Sebastian Int j oncology 2009

Artemisinin Safety and Toxicity

A case report of elevated liver enzymes in an Artemisinin user was reported in 1999. Therefore, it is prudent to monitor liver enzymes in patients on long treatment. Neurotoxicity of the more potent artemisinin derivatives has been reported in studies using animals (dogs and mice.). Anecdotal reports of cerebellar toxicity have been

reported in humans. In spite of this, Artemisinin is remarkably safe as demonstrated in this study of 242 Vietnamese human subjects who showed no brain stem adverse effects after multiple courses of artemisinin (or its derivatives) for malaria treatment. Another study, looking at **post mortem neuropathology** also found no evidence of neurotoxic effects.

Reproductive Toxicity of Artesunate: In a 2011 study **by Dr Olumide and in a later 2014 study**, long term administration to mice of the more potent artemisinin derivative, artesunate, **induced reversible infertility**, with reduced sperm counts.

Cannabinoid Extracts Have Anti Cancer Activity – See my **previous article** on the use of cannabis extracts in treatment of cancer. Cannabis extracts have a long history of beneficial use in chemotherapy patients for relief of nausea, and increase appetite effects. There is also a pain relief effect from cannabis extracts. Another unexpected benefit is the anti-cancer effects of cannabis are substantial, especially in Leukemia and Lymphoma cells which are specifically sensitive to cannabis extracts as they express increased CB1 and CB2 cannabinoid receptors. Numerous research studies show that Cannabinoid extracts and ligands induce apoptosis (programmed cell death) in cancer cells via ceramide accumulation. (30-38)

Is there synergism and augmentation of anti-cancer effects with cannabis extracts and artemisinin compounds ? Taken in together combination, would cannabis extracts increase the anti-cancer effect of artemisinin ? This would be a good topic for NIH funding of future research. My best guess is that future research studies will demonstrate this is indeed the case.

Medicinal Use of Cannabis Extracts have been approved in 23 states so far. If you reside in one of these states, then you may qualify as a medicinal cannabis patient and may legally obtain medicinal cannabis according to your specific state law. A reputable source for high quality medical grade cannabis oil is Mara Gordon of Aunt Zelda's Dispensary in San Fransisco, California. (note: I have no financial relationship with Aunt Zeldas) . For more on information, see my book on Amazon: **Cannabis Extracts in Medicine**

Gleditsia sinensis – Korean thorn

This natural plant botanical has shown anticancer activity. Possible synergy with artemisinin might be explored. (60-61)

Ivermectin (62-68) (Stromectol for humans and Ivomec for Dogs)

The **2015 Nobel Prize in Medicine** was awarded to William C. Campbell and Satoshi Ōmura for discovery of Ivermectin, **the Wonder Drug From Japan**, a well known anti-parasitic agent used to treat **"billons"** of pets and livestock around the world. (62-68)

Left image : Ivermectin (HeartGuard) for dogs. Image Courtesy of All Four Paws .

In humans, Ivermectin is well known as treatment for Lice (Pediculosis) and Scabies (Mites). Ivermectin also treats parasites such as Nematodes, Onchocerciasis, Strongyloidiasis, Ascariasis, cutaneous larva migrans, filariases, Gnathostomiasis and Trichuriasis.

In 2010, Dr Sharmeen published his study. (62) He screened a long list of drugs looking for cytotoxicity to leukemia cells, and discovered **ivermectin** induced cell death at low micromolar concentrations in acute myeloid leukemia cell lines, while sparing normal cells. He then studied three mouse models of leukemia showing that tumor growth was delayed *"at drug concentrations that appear **pharmacologically achievable***"(62)

Dr Sharmeen reported ivermectin blocks the glutamate-gated chloride channels, increases intracellular chloride ion concentrations and cell size of leukemia cells. This causes plasma membrane hyperpolarization. Ivermectin also increased (ROS) reactive oxygen species in cancer cells functionally important for ivermectin-induced cell death. Finally, ivermectin synergized with conventional chemotherapy agents, ARA-C (cytarabine) and Adriamycin (anthracycline drug also known as Daunorubicin) to increase ROS, reactive oxygen species production. (62)

Above image: mechanism of Ivermectin induced caner cell death from Fig 7 Model of P2X4/P2X7/Pannexin-1-induced cancer cell death. Draganov 2015

Dr Dobrin Draganov reported in 2015 that Ivermectin "*kills mouse and human triple-negative breast cancer (TNBC) cells through augmented P2X7-dependent purinergic signaling associated with caspase-1 and caspase-3 activation.*" …"*also involved is the recruitment and activation of T cells, macrophages and dendritic cells, a form of immunomodulation and cancer immunotherapy*."

In 2014, Dr Alice Melotti reported that Ivermectin inhibits the WNT-TCF pathway in cancer cells. further elucidating the molecular mechanism of cancer cell death.(130) In a colon cancer cell model, Dr Melotti found Ivermectin effective at micromolar concentrations against both tumor bulk as well as cancer stem cells. The authors suggested ivermectin," *might be useful as a routine prophylactic agent, for instance, against colon cancer*

in familial polyposis, or to prevent nascent cancer in the general aging population." (130) I was astonished by this statement.

Ivermectin Effective Against Ovarian Cancer Cell Line

In 2009, Dr Hashimoto showed Ivermectin Effective against ovarian cancer cell lines. (152) "Ivermectin inactivates the kinase PAK1 and blocks the PAK1-
dependent growth of human ovarian cancer and NF2 tumor cell lines" 152.

Targeting WNT Eliminates Cancer Stem Cells

Some aggressive cancers have a high relapse rate due to cancer stem cells which are resistant to conventional chemotherapy. Targeting the WNT pathway successfully eliminated cancer stem cells in a Mantle Cell Lymphoma cell line.(133)

Cannabinoids also **inhibit cancer cells via the WNT-TCF signalling**, so there may be some overlap in similar mechanisms. Interestingly, Lithium stimulates hippocampal neurogeneisis through the WNT pathway.

Safety and Dosage of Ivermectin

About 200 million are currently taking Ivermectin as treatment/prevention of river blindness. According to Dr. **Crump**, Ivermectin is "astonishingly safe for human use." He says *"Indeed, it is such a safe drug, with minimal side effects, that it can be administered by non-medical staff and even illiterate individuals in remote rural communities,"*

Dr Guzzo reports in 2002, no indication of associated CNS toxicity for Ivermectin doses up to 10 times the highest FDA-approved dose of 200 microg/kg.(120 mg single dose is 10 times the 12 mg recommended dose). There was better absorption with higher plasma levels when the drug is taken with food.

Ivermectin *d*osage for treatment of head lice (pediculosis) is Two Tablets, each one given a week apart, tablet size is 200 mcg/kg (12 mg tablet for a 60kg male).(137) As much as 11 single dosages of 150 mcg/kg may be safely repeated every three months, however higher dosing (800 mcg/kg) every three months was associated with **opthalmological complaints.** (138)

Ivermectin Synergy with Artemisinin ?

Ivermectin and Artemesin have been safely used in combination **(ACT)** for treatment of malaria. Would Ivermectin combined with Artemisinin serve as anti-cancer treatment ? The two agents use differing mechanisms to accomplish the same thing, selectively increasing (ROS), reactive oxygen species, inside the cancer cell. The Artemsinin works via Fenton reaction, the oxidation reaction with iron, while the Ivermectin acts on Chloride Ion channels. Would the two agents enhance each others cell killing effects, working in synergy ? This would be an excellent topic for future study with NIH funding.

Artemisinin Synergy with Alpha Lipoic Acid ?

Alpha lipoic acid (ALA) and artemisinin infusions are commonly given together as part of a comprehensive cancer treatment. Alpha Lipoic acid may work in synergy with the artemisinin, causing oxidative damage to mitochondria

and inducing mitochondrial apoptoosis in the cancer cell.

Artemisinin Targets Cancer Stem cells

Two studies suggest artemisinin compounds down regulate the WNT pathway and targets cancer stem cells in the lung tumor and brain glioma cell models .(154-155) In a colorectal cancer cell study, Artemisinin was found to strongly inhibit the WNT/Beta Catenin pathway, suggesting utility as a stem cell agent. (157) Indeed, in 2016, Dr Amit Subedi and Dr Nishi reported "High-throughput screening identifies **artesunate** as selective inhibitor of cancer stemness: Involvement of mitochondrial metabolism." (163)

Artemisinin Downregulates Nuclear Factor-Kappa Beta and Inflammatory Cytokines- PI3 kinase/Akt signal pathway

A number of studies show striking downregulation and inhibition of the major inflammatory transcriptor, NF Kappa Beta and downstream signalling pathway such as c-Myc and Cyclin D1. There was striking synergy between lenalidamide and artemisinin when used together(158-160)(170-171)

Dr Xu showed artesunate inhibits TNF-α-induced production of proinflammatory cytokines via **inhibition of NF-κB and PI3 kinase/Akt signal pathway** in human rheumatoid arthritis fibroblast-like synoviocytes." (158) A number of PI3K inhibitors anti-cancer drugs in development such as buparlisib and Idelalisib target this same **PI3 kinase/Akt signal pathway** inhibited nicely by artesunate. (161)

In an endometrial cancer model, Dr Tran revealed the exact mechanism of NF-kB inhibition.(171) The p65 and p50 are subunits of the NF-kB protein residing in the cytosol. Artemisinin prevents p65 and p50 nuclear translocation by interacting with IκB-α, the NF-κB inhibitor, leading to a loss of CDK4 gene expression.(171)

Artesunate Striking Inhibition of WNT Pathway in SKM Cells

Below Image is Fig 5. from Xu (162). LSCM was used to detect E-cadherin and β-catenin subcellular localization. Cells were fixed after different concentration of ART treatment for 48h, and stained with A. E-cadherin and B. β-catenin. Pictures were taken by LSCM (scale bar = 35μM). a-d represents different concentrations of Artesunate (a=0μg/mL, b=12.5μg/mL, c=25μg/mL, d=50μg/mL). Blue Arrow indicates Nuclear location of E-cadherin (left panel) and β-catenin (right panel). Red arrows indicates membrane locations for both.

In 2015 Dr Na Xu studied the effect of Artesunate on SKM-1 cells in vitro, a model for Myelodysplastic Syndrome. (162) Dr Na Xu found ***"ART treatment inhibited Wnt/β-catenin downstream expression of targets such as c-myc and cyclinD1."*** *When* SKM-1 cells were treated with Artesunate, both β-catenin and E-cadherin translocated from the nucleus to the membrane, thereby forming the β-catenin/E-cadherin complex and **strengthening cell-cell adhesion**.(see above image)(162)

Similar translocation of Beta-Catenin from cell nucleus to outer membrane was seen in a colo-rectal cancer model reported by Dr. Lin-Na Li in her 2007 study,"Artesunate attenuates the growth of human colorectal carcinoma and inhibits hyperactive Wnt/β-catenin pathway." (157)

Inflammatory Signals From Micro-Environment in Mantle Cell Lymphoma

In Blood 2012, Dr Liang Zhang studied the role of the microenvironment in mantle cell lymphoma. Dr Zhang found that the inflammatory cytokine IL-6 activated the Jak2/STAT3 and PI3K/Akt pathways in MCL, mantle cell lymphoma. IL-6 is a key cytokine for MCL growth and survival. (176) Dr Lai reports in 2003 J Pathology that STAT3 (signal transducer and activator of transcription 3) is the signal transducer of IL-10 another cytokine which is upregulated in the tumor micro-environment, and this induces proliferation in Mantle cell Lymphoma.(177)

Artesunate down-regulated the expression of STAT3 in leukemia

Dr Mei Tan in 2017 Leukemia Research wrote: "Artesunate induces apoptosis via inhibition of STAT3 in THP-1 (leukemia) cells." (179) Dr Tan found that 30 leukemia patients had significantly increased STAT3 protein levels compared to controls. In addition, Artesunate significantly inhibited the proliferation of leukemia cells, and increases apoptosis, by down-regulating STAT3.(179) Similarly in a hepatocellular cancer mouse model, Artesunate obliterated the cancer by suppressing IL-6-JAK-STAT signalling (180) In 2007, Dr Xu reported that artesunate inhibits TNF-α-induced production of pro-inflammatory cytokines via inhibition of NF-κB and PI3 kinase/Akt signal pathway in human rheumatoid arthritis fibroblast-like synoviocytes." (181)

Conclusion:

In my opinion, with the state of our knowledge of non-toxic targeted agents, malignant cancer is now a curable disease, and suffering or death from cancer should become a thing of the past. We have discussed Artemisinin, the Chinese anti-malaria drug, which is also a targeted non-toxic anti-cancer agent. The Artemisinin endoperoxide bridge reacts with iron in the cancer cell to produce oxidative free radicals, which induce apoptotic pathways. Down regulating the glutathione system with **sulfasalazine**, **allicin** (from garlic), **Feverfew** (parthenolide) or **sulforaphane** (from Brocolli) renders the cancer cell more susceptible to oxidative damage from Artemisinin and augments the cancer cell killing effects. Combinations of these agents may prove useful, and should be funded for future NIH research. We have discussed Ivermectin, an astonishingly safe drug taken by 200 million people,

with potent anti-cancer effects, at micromolar concentrations, for both tumor bulk as well as cancer stem cells. Another useful effect of Artesunate is down-regulation inflammatory pathways needed for cancer cell proliferation.

The cancer patient of today may not have the luxury of time, and may be inclined to proceed before prospective randomized studies are completed. Indeed, prospective randomized trials may never be forthcoming due to the nature of the drug discovery and approval system in the US.

Even though Artemisinin and co-agents are considered relatively safe, there may be toxicity at higher dosage, and the user must remain vigilant, and reduce dosage should toxicity occur. It is recommended that you work closely with a knowledgeable clinician.

Update Sept 2017: Synergy of ALA (Aminolevulinic Acid) with Artemsinin, which increases activity ten times. See : Wang, Jigang, et al. "Mechanistic investigation of the specific anticancer property of artemisinin and its combination with aminolevulinic acid for enhanced anticolorectal cancer activity." ACS central science 3.7 (2017): 743-750.(172) ALA is precursor molecule for heme synthesis, allowing the cancer cells to make more heme which is then reactive with the Artesunate/Artemisinin. Note: ALA was FDA approved June 2017 as a photo-imaging tool during neurosurgery for malignant glioma. The patient takes an oral dose of Gleolan 3 hours before surgery, allowing fluorescent visualization of tumor at surgery.(172-175)

Credit and Thanks goes to **Robert Jay Rowen, MD** who brought Artemisin to my attention in a 2002 article in Townsend letter: Artemisinin: From Malaria to Cancer Treatment .

Thanks to Stephen Levine, PhD, founder of Allergy Research Group for introducing high quality Artemisinin products for the general public.

This article is part one, for part two **click here.** For part three click here.

Financial Disclosure: I have none. I receive no monetary inducements from, nor have any financial interests in any manufacturer of artemisinin products. I do however receive stipends from Amazon for links to products on this page.

Buy Allergy Research ARTEMISININ on Amazon

Buy Nutricology Artemisinin on Amazon.

Sources for Artemisinin and Derivatives

Artemisinin Products: Hepalin.com

Artemix (Artesunate 50mg, Artemisinin 50mg, Artemether 40mg combo) contains 30 capsules in a bottle:$60

Link to :Useful Artemisinin information page

Articles with related interest:

Clarithromycin Anti-Cancer Antibiotic

Itraconazole Anti-Fungal Anti-Cancer Drug

Celecoxib Anti-Cancer Drug

Metformin Repuroposed as AntiCancer Drug

Doxycycline Vitamin C AntiCancer Synergy

IV Vitamin C as Chemotherapy

Alpha Lipoic Acid AntiCancer Agent

Ivermectin AntiCancer Wonder Drug From Japan

Cancer as a Parasitic Disease

Cancer as a Metabolic Disease

How do Cannabinoids Kill Cancer Cells

Artemisinin Support Group on Facebook

Jeffrey Dach MD
7450 Griffin Road Suite 190
Davie, Florida 33314
954-792-4663

Links and References

video by Dr Henry Lai – May 2014

artemisinin- discovered by chinese in 1970's used as anti-malaria compound. Used for 30 years with safe profile. Leukemia cell will pick up 1000 times more iron than a normal cell. Artemisinin is approximately 100 times more potent in killing leukemia cells than normal lymphocytes. Similar results for breast cancer cells.

Artemesisnin is very selective against cancer cells, Effective at low dose. Few side effects. Oral capsule. Inexpensive. 1-2 mg/kg per day effective in treating cancer, (in animals)

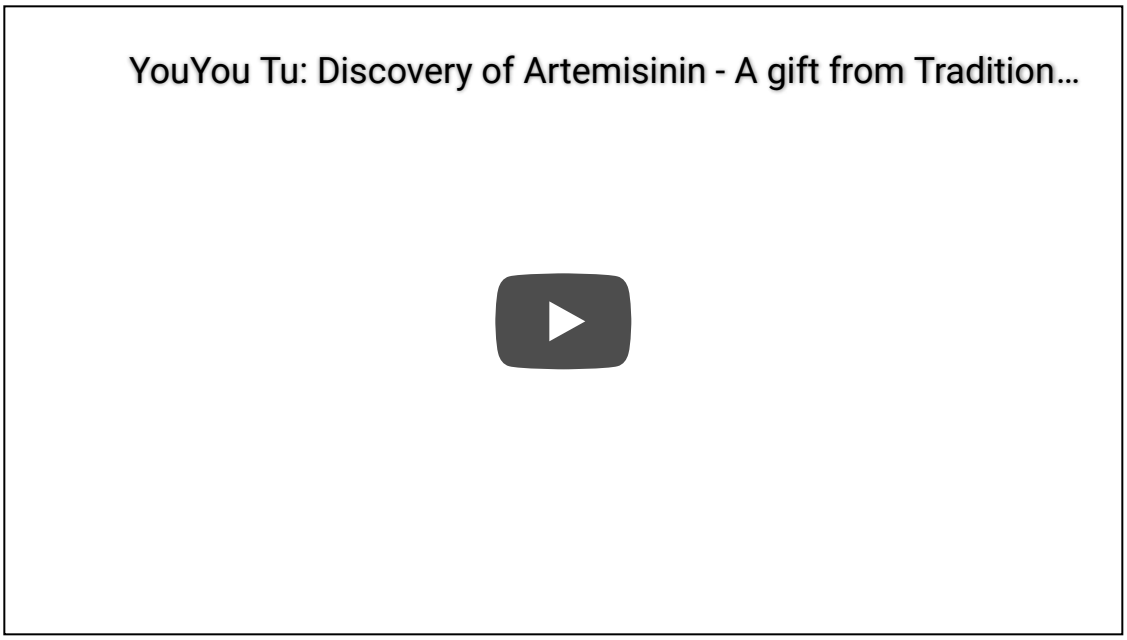
Discovery of Artemisinin video

The 2011 Lasker~DeBakey Clinical Medical Research Award honors a scientist who discovered artemisinin and its utility for treating malaria. Tu Youyou (China Academy of Chinese Medical Sciences, Beijing) developed a therapy that has saved millions of lives across the globe, especially in the developing world. An artemisinin-based drug combination is now the standard regimen for malaria, and the World Health Organization (WHO) lists artemisinin and related agents in its catalog of “Essential Medicines.” Each year, several hundred million people contract malaria. Without treatment, many more of them would die than do now. Tu led a team that transformed an ancient Chinese healing method into the most powerful antimalarial medicine currently available.

Nobel Prize in Medicine for Discovery of Artemisinin Video Published on Dec 8, 2015

Youyou Tu delivered her Nobel Lecture on 7 December 2015 at Aula Medica, Karolinska Institutet in Stockholm. The lecture was presented in Mandarin.

Youyou Tu: Discovery of Artemisinin – A gift from Traditional Chinese Medicine to the World



Artemisinin 2015

1) Das A K. Anticancer effect of antimalarial artemisinin compounds. Ann Med Health Sci Res [serial online] 2015 [cited 2016 Feb 13];5:93-102.

Anticancer activity of artemisinin has been demonstrated primarily in vitro and in animal models. In a study, testing **55 cell lines** showed that artesunate showed inhibitory effects against **leukemia, colon, melanoma, breast, ovarian, prostate, central nervous system, and renal cancer cells**. [39] The semisynthetic derivative DHA showed remarkable antineoplastic activity against pancreatic, leukemic, osteosarcoma, and lung cancer cells. [40] Moreover, **artemisone was superior to artemisinin** and showed better synergism with other anti-cancer agents. [41]

Furthermore, artesunate proapoptotic effect is not affected in a doxorubicin-resistant leukemia cell line; rather it **potentiates doxorubicin's apoptotic effects.** [8]

how the antitumor activity is exerted following artemisinin activation is still not well-understood but is associated with **multiple mechanisms, including reactive oxygen species (ROS), oxidative DNA damage, sustained DNA double-strand breaks, and apoptosis.** A better understanding of common mechanisms under similar conditions in different cell systems will greatly help developing targeted artemisinin derivatives.

Experimental evidences, mostly animal studies indicate that artemisinins and its derivatives may be a therapeutic alternative in the future, **particularly in highly metastatic and aggressive cancers without developing drug resistance.**

2011

2) Mercer, Amy E., et al. "The role of heme and the mitochondrion in the chemical and molecular mechanisms of mammalian cell death induced by the artemisinin antimalarials." Journal of Biological Chemistry 286.2 (2011): 987-996.

2012

3) J Biomed Biotechnol. 2012; 2012: 247597. Antitumor Activity of Artemisinin and Its Derivatives: From a Well-Known Antimalarial Agent to a Potential Anticancer Drug. Maria P. Crespo-Ortiz 1 ,* and Ming Q. Wei 2

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.

2015

4) Hongbao, Ma, Margaret Ma, and Yang Yan. "Cancer and Artemisinin Research Literatures." Cancer Biology, 2015;5(3), Brookdale Hospital, Brooklyn, New York 11212, USA; 2 Cambridge, MA 02138, USA Hongbao Cancer Artemisinin Research Cancer Biology 2015 . Nice listing of research articles

2015 Iron-dependent cell death – **Ferroptosis(53)**

5) Ooko, E., et al. "Artemisinin derivatives induce iron-dependent cell death (ferroptosis) in tumor cells." Phytomedicine: international journal of phytotherapy and phytopharmacology 22.11 (2015): 1045.

The ferroptosis inhibitor ferrostatin-1 and the iron chelator deferoxamine led to a significantly reduced cytotoxicity of artenimol, indicating ferroptosis as cell death mode. Treatment with **ferroptosis-inducing agents such as artemisinin** derivatives represents an attractive strategy for cancer therapy.

2014 **Lysosomal Degradation of Ferritin**

Cell mechanisms of artemisinin

6) Yang, Nai-Di, et al. "Artesunate induces cell death in human cancer cells via enhancing lysosomal function and lysosomal degradation of ferritin." Journal of Biological Chemistry 289.48 (2014): 33425-33441. (full free)

Artesunate (ART) is an anti-malaria drug that has been shown to exhibit anti-tumor activity, and **functional lysosomes are reported to be required for ART-induced cancer cell death**, whereas the underlying molecular mechanisms remain largely elusive. In this study, we aimed to elucidate the **molecular mechanisms underlying ART-induced cell death.** We first confirmed that **ART induces apoptotic cell death in cancer cells.**

Interestingly, we found that **ART preferably accumulates in the lysosomes** and is able to **activate lysosomal function via promotion of lysosomal V-ATPase assembly.** Furthermore, we found that lysosomes function upstream of mitochondria in reactive oxygen species production. Importantly, we provided evidence showing that **lysosomal iron is required for the lysosomal activation and mitochondrial reactive oxygen species production induced by ART**. Finally, we showed that **ART-induced cell death is mediated by the release of iron in the lysosomes, which results from the lysosomal degradation of ferritin, an iron storage protein.**

Meanwhile, overexpression of ferritin heavy chain significantly protected cells from ART-induced cell death. In addition, knockdown of nuclear receptor coactivator 4, the adaptor protein for ferritin degradation, was able to block ART-mediated ferritin degradation and rescue the ART-induced cell death. In summary, our study demonstrates that ART treatment activates lysosomal function and then promotes ferritin degradation, subsequently **leading to the increase of lysosomal iron that is utilized by ART for its cytotoxic effect on cancer cells.** Thus, our data reveal a new mechanistic action underlying ART-induced cell death in cancer cells.

Repression of NFkB – stimulates autophagy

7) Hu, Wei, et al. "Dihydroartemisinin induces autophagy by suppressing NF-κB activation." Cancer letters 343.2 (2014): 239-248.
Nuclear factor-kappa B (NF-κB) and autophagy are two major regulators involved in both tumor initiation and progression. However, the association between these two signaling pathways still remains obscure. In this work, we demonstrate that **dihydroartemisinin (DHA) stimulates the induction of autophagy in several cancer cell lines through repression of NF-κB activity.** We also show that **inhibiting NF-κB results in an accumulation of reactive oxygen species (ROS)**, which participate in the stimulation of autophagy. These findings present a pathway by which DHA promotes autophagy in cancer cells and provide evidence for the DHA-induced sensitization effect of some chemotherapeutics.

8) Toyokuni, Shinya. "**Iron and thiols** as two major players in carcinogenesis: friends or foes?." The Changing Faces of Glutathione, a Cellular Protagonist (2015): 114. Toyokuni Shinya Iron thiols carcinogenesis Cellular Protagonist 2015

Leukemia 2012

9) Wang, Zeng, et al. "Dihydroartemisinin induces autophagy and inhibits the growth of iron-loaded human myeloid leukemia K562 cells via ROS toxicity." FEBS open bio 2 (2012): 103-112.

In summary, DHA induces autophagy and inhibits the growth of iron-loaded human myeloid leukemia K562 cells via ROS toxicity,

which includes the down-regulation of TfR expression and induction of apoptosis through the mitochondrial pathway.

Artemisinin synergistic with Resveratrol

10) Li, Peichun, et al. "Synergic effects of artemisinin and resveratrol in cancer cells." Journal of cancer research and clinical oncology 140.12 (2014): 2065-2075.

The aim of this study was to investigate whether resveratrol (Res) combined with artemisinin (ART) possess synergistic effect on different cancer cells.
MATERIALS AND METHODS:The viability of HepG2 and HeLa cells treated with ART and Res was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Combination index (CI) analysis and isobologram were used to assess the synergistic effect of ART and Res in different ratios. Wound-healing assay was used to investigate the migration rate. AO staining and fluorescent microscopy measurements were performed to detect the cell apoptosis. Reactive oxygen species (ROS) was measured with 2'7′-dichlorofluorescein diacetate (DCFH-DA).
RESULTS:MTT assay indicated that ART and Res inhibited the growth of HeLa and HepG2 cells in a dose-

dependent manner. The combination of ART and Res exhibited the strongest anticancer effect at the ratio of 1:2 (ART to Res). The combination of the two drugs also markedly reduced the ability of cell migration. Apoptosis analysis showed that combination of ART and Res significantly increased the apoptosis and necrosis rather than use singly. Additionally, ROS levels were elevated by combining ART with Res.
CONCLUSIONS:**Taken together, the present study suggested that ART and Res possessed the synergistic anti-tumor effect. ART in combination with Res could be an effective therapeutic strategy for cancer.**

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11) Berdelle, Nicole, et al. "Artesunate induces oxidative DNA damage, sustained DNA double-strand breaks, and the ATM/ATR damage response in cancer cells." Molecular cancer therapeutics 10.12 (2011): 2224-2233.

12) Chen, Kai, et al. "Artesunate induces G2/M cell cycle arrest through autophagy induction in breast cancer cells." Anti-cancer drugs 25.6 (2014): 652-662.

We found that artesunate (ART) inhibited the growth of MCF-7 and MDA-MB-231 breast cancer cells. **ART arrested the cell cycle in the G2/M phase, which was accompanied by an upregulation of p21. ART upregulated the expression of Beclin1, an initiator of autophagy (type II programmed cell death).** In addition, ART stimulated the aggregation of LC3, which is considered to be a marker of autophagosome formation. We further verified the transformation of LC3 from type I into type II. 3-MA, a classical autophagy inhibitor, attenuated ART-induced autophagosome formation, cell growth repression, G2/M arrest, and p21 upregulation. Autophagy induction and p21 upregulation were also repressed by knockdown of Beclin1. Furthermore, ART sensitized breast cancer cells to the chemotherapeutic agent epirubicin through an autophagy-dependent cascade. Our study showed that ART induced autophagy in breast cancer cells and indicated that the anticancer effects of ART were exerted through an autophagy pathway. Moreover, ART sensitized breast cancer cells to epirubicin chemotherapy. Our results provide a basis for further development of ART as a novel therapeutic agent for the treatment of breast cancer.

Patents Dr Henry Lai

13) Use of artemisinin and its derivatives in cancer therapy US 20100279976 A1
Abstract A method for treating cancer in a mammal includes administering to the mammal in need thereof a therapeutically effective amount of artemisinin (ART) or its derivative, such as dihydroartemisinin (DHA), artemether (ARM), or artesunate (ARS) alone or in combination with a chemotherapeutic agent, such as gemcitabine and carboplatin. **A method for inhibiting tumor cell proliferation includes contacting a tumor cell with ART or its derivative, such as DHA, ARM, and ARS, in an amount effective to inhibit tumor cell proliferation or in combination with a chemotherapeutic agent, such as gemcitabine and carboplatin.**

Dr Henry Lai

full pdf free

14) http://www.ncbi.nlm.nih.gov/pubmed/22935909
Lai, Henry C., Narendra P. Singh, and Tomikazu Sasaki. "Development of artemisinin compounds for cancer treatment." Investigational new drugs 31.1 (2013): 230-246.

Artemisinin contains an endoperoxide moiety that can react with iron to form cytotoxic free radicals. Cancer cells contain significantly more intracellular free iron than normal cells and it has been shown that artemisinin and its

analogs selectively cause apoptosis in many cancer cell lines. In addition, artemisinin compounds have been shown to have anti-angiogenic, anti-inflammatory, anti-metastasis, and growth inhibition effects. These properties make artemisinin compounds attractive cancer chemotherapeutic drug candidates. However, simple artemisinin analogs are less potent than traditional cancer chemotherapeutic agents and have short plasma half-lives, and would require high dosage and frequent administration to be effective for cancer treatment. More potent and target-selective artemisinin-compounds are being developed. These include artemisinin dimers and trimers, artemisinin hybrid compounds, and tagging of artemisinin compounds to molecules that are involved in the intracellular iron-delivery mechanism. These compounds are promising potent anticancer compounds that produce significantly less side effect than traditional chemotherapeutic agents.

artemisone 2011

15) Cancer Chemother Pharmacol. 2011 Mar;67(3):569-77.
In vitro study of the anti-cancer effects of artemisone alone or in combination with other chemotherapeutic agents. Gravett AM1, Liu WM, Krishna S, Chan WC, Haynes RK, Wilson NL, Dalgleish AG. anticancer effects of artemisone 2011 Gravett

Artemisinins are now established drugs for treatment of malaria. These agents have been shown to possess impressive anti-cancer properties. We have investigated the role of artemisone (ATM), a novel derivative of artemisinin (ART) in a cancer setting both alone and in combination with established chemotherapeutic agents.
METHODS:The anti-proliferative effects of ART and ATM were tested on a panel of human cancer cells in vitro using the methylthiazoletetrazolium assay, and the effect on cell cycling established by flow cytometry. Immunoblot analyses were performed to determine effects at the molecular level. Finally, ART and ATM were combined with the common anti-cancer agents oxaliplatin, gemcitabine and thalidomide.
RESULTS:ART and ATM caused dose dependent decreases in cell number. ATM was consistently superior to ART, with IC50 s significantly lower in the former. Neither drug caused significant changes to the cell viability (%viable cells >95%), but arrested cell cycling. Blockade was either exclusively at the level of G1, or at all phases of the cell cycle, and associated with reductions in cyclin D1, CDK4 and pRb. Combination studies showed the anti-proliferative effect of ATM was often enhanced by addition of the other drugs, whilst ART exhibited antagonistic properties.
CONCLUSIONS:ART and ATM are active in cancer cell lines, with ATM displaying the greater anti-proliferative effect when used alone. ATM also enhances the effects of the above drugs, with ART being less likely to improve activities. Taken together, ATM should be thought of as the ART-derived compound next in line for further study.

16) Lai, Henry, et al. “Artemisinin-transferrin conjugate retards growth of breast tumors in the rat.” Anticancer research 29.10 (2009): 3807-3810. Lai Henry Artemisinin transferrin conjugate retards breast tumors 2009

AML – **antimalarial agent mefloquine – Kills stem cells**

17) Sukhai, Mahadeo A., et al. “Lysosomal disruption preferentially targets acute myeloid leukemia cells and progenitors.” The Journal of clinical investigation 123.1 (2013): 315-328.

To identify new therapeutic strategies for AML, we screened a library of on- and off-patent drugs and identified the **antimalarial agent mefloquine** as a compound that selectively kills AML cells and **AML stem cells** in a panel of leukemia cell lines and in mice.

Would mefloquin act synergisticaly with artemisinin ?

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transferrin receptors over-expressed in mantle cell lymphoma

18) Cancer Res. 2007 Feb 1;67(3):1145-54.
Prevention of mantle lymphoma tumor establishment by routing transferrin receptor toward lysosomal compartments. Lepelletier Y1, Camara-Clayette V, Jin H, Hermant A, Coulon S, Dussiot M, Arcos-Fajardo M, Baude C, Canionni D, Delarue R, Brousse N, Benaroch P, Benhamou M, Ribrag V, Monteiro RC, Moura IC, Hermine O.

Herein, we show that **TfR is highly expressed on MCL cells**,

The emergence of tumour-specific, molecularly targeted agents signifies a paradigm shift in cancer therapy. Taken together with immunohistochemistry data from MCL patients, **these results reveal the high level of TfR expression in MCL cells in vivo and in vitro**.

Interestingly, the combined effect of A24 with VP-16 or with ara-C was significantly more pronounced than that of any of these agents alone. This difference was more markedly observed in EBV− cells (Fig. 3B and C). Thus, A24 showed an inhibiting effect on MCL cell proliferation that was both comparable with and synergistic with conventional chemotherapeutic agents.

In spite of recent progress in MCL treatment, the disease prognosis remains poor mainly due to the intrinsic resistance of malignant cells to conventional chemotherapy (37– 39), and virtually, all patients, even those who exhibit complete response, will relapse.

19) Obrador-Hevia, Antònia, et al. "Molecular biology of mantle cell lymphoma: from profiling studies to new therapeutic strategies." Blood reviews 23.5 (2009): 205-216.

Transferrin receptor

Prevention of MCL establishment by routing the transferrin receptor toward intracellular lysosomal compartments has been shown in an in vitro model. 137 In fact this is an interesting approach that merits further clinical studies for rapidly growing tumour cells such as some MCL forms. Transferrin receptors are highly expressed in rapidly growing tumours including MCL but not in their normal counterparts. These tumours require transferrin for growth and survival. Therefore, impairing the function of these iron uptake receptors promotes apoptosis, and the activation of caspase 3 and 9 has been reported to account for this effect. 137 A correlation between expression of transferrin membrane receptor and Ki-67 has also been reported, 137 suggesting that this protein could represent an interesting target. The implementation of this new therapeutic modality is favoured even further by the fact that synergism with active cytotoxic drugs in MCL has been found, such as ara-C (Aracytine) or VP-16 (Etoposide). 137 It has also been reported that the transferrin receptor gene is up-regulated in MCL relapses. 138 Accordingly, multiple therapeutic avenues can be envisioned including the design of radioimmunoisotypes, or coupling of monoclonal antibodies targeting this receptor with cytotoxic agents.

addition of iron renders artemsisin more cytotoxic

20) Free Radic Biol Med. 2004 Oct 1;37(7):998-1009.ENHANCEMENT OF CYTOTOXICITY OF ARTEMISININS BY FERROUS IRON KAINA 2004

Enhancement of cytotoxicity of artemisinins toward cancer cells by ferrous iron.
Efferth T1, Benakis A, Romero MR, Tomicic M, Rauh R, Steinbach D, Häfer R, Stamminger T, Oesch F, Kaina B, Marschall M.
Iron(II) heme-mediated activation of the peroxide bond of artemisinins is thought to generate the radical oxygen species responsible for their antimalarial activity. We analyzed the role of ferrous iron in the cytotoxicity of artemisinins toward tumor cells. **Iron(II)-glycine sulfate (Ferrosanol)** and transferrin increased the cytotoxicity of free artesunate, artesunate microencapsulated in maltosyl-beta-cyclodextrin, and artemisinin toward **CCRF-CEM leukemia** and U373 astrocytoma cells **1.5- to 10.3-fold compared with that of artemisinins applied without iron.** Growth inhibition by artesunate and ferrous iron correlated with induction of apoptosis. Cell cycle perturbations by artesunate and ferrous iron were not observed. Treatment of p53 wild-type TK6 and p53 mutated WTK1 lymphoblastic cells showed that mutational status of the tumor suppressor p53 did not influence sensitivity to artesunate. The effect of ferrous iron and transferrin was reversed by monoclonal antibody RVS10 against the transferrin receptor (TfR), which competes with transferrin for binding to TfR. CCRF-CEM and U373 cells expressed TfR in 95 and 48% of the cell population, respectively, whereas TfR expression in peripheral mononuclear blood cells of four healthy donors was confined to 0.4-1.3%. This indicates that artemisinins plus ferrous iron may affect tumor cells more than normal cells.

iron in cancer cells

21) Kwok, Juliana C., and Des R. Richardson. "The iron metabolism of neoplastic cells: alterations that facilitate proliferation?." Critical Reviews in Oncology/Hematology 42.1 (2002): 65-78.

For many years it has been known that **neoplastic cells express high levels of the transferrin receptor 1 (TfR1) and internalize iron (Fe) from transferrin (Tf) at a tremendous rate.** Considering the high requirement of neoplastic cells for Fe, understanding its metabolism is vital in terms of devising potential new therapies. Apart from TfR1, a number of molecules have been identified that may have roles in Fe metabolism and cellular proliferation. These molecules include transferrin (Tf), the oestrogen-inducible transferrin receptor-like protein, transferrin receptor 2 (TfR2), melanotransferrin (MTf), ceruloplasmin, and ferritin. In the present review these latter molecules are discussed in terms of their potential functions in tumour cell Fe metabolism and proliferation. Further studies are essential to determine the specific roles of these proteins in the pathogenesis of cancer. **Cancer cells generally have higher numbers of the TfR1 than their normal counterparts** [[3], [4], [5]] **and take up Fe at a higher rate** [[6], [7], [8]]. This is reflected by the **ability of tumours to be radiolocalized using 67Ga [9], that binds to the Tf Fe-binding site** and is delivered via its binding to the TfR1 [[4], [10]].

22) Kundu, Chanakya Nath, et al. "Anti-malarials are anti-cancers and vice versa–One arrow two sparrows." Acta tropica 149 (2015): 113-127.Antimalarials are anticancers Kundu Acta tropica 2015

Transferrin Factor Synthesized in Testicles, kidney, spleen

23) J Biol Chem. 1980 Oct 25;255(20):9523-5. Sertoli cells synthesize and secrete transferrin-like protein.Skinner MK, Griswold MD. Sertoli cells of the testes synthesize Tf to provide proliferating spermatocytes with Fe [71]. kidney, spleen also.

Curcumin anti-cancer Effects

24) Hasanali, Zainul, Kamal Sharma, and Elliot Epner. "Flipping the cyclin D1 switch in mantle cell lymphoma." Best Practice & Research Clinical Haematology 25.2 (2012): 143-152.

Curcumin, a plant flavonoid that is available naturally in turmeric and as an herbal supplement, has been shown in vitro to **downregulate cyclins D1** and D3 at both the transcriptional and post-transcriptional levels in MCL and MM cell lines [50] . Curcumin is known to inhibit the COP9 signalosome, a multiprotein complex similar to the proteasome [51], [52], and [53]. Our laboratory has demonstrated that **curcumin and bortezomib synergize in downregulating** protein levels of cyclins D1 and D3 in MM and MCL cells [35] . Clinical trials of this agent in combination in lymphoid malignancies alone or in combination with bortezomib are ongoing.

Curcumin against Four MCL cell lines

25) Shishodia, Shishir, et al. "Curcumin (diferuloylmethane) inhibits constitutive NF-κB activation, induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma." Biochemical pharmacology 70.5 (2005): 700-713. Biochem Pharmacol. 2005 Sep 1;70(5):700-13.
Shishodia S1, Amin HM, Lai R, Aggarwal BB.

Human mantle cell lymphoma (MCL), an aggressive B cell non-Hodgkin's lymphoma, is characterized by the **overexpression of cyclin D1** which plays an essential role in the survival and proliferation of MCL. Because of MCL's resistance to current chemotherapy, novel approaches are needed. Since MCL cells are known to **overexpress NF-kappaB regulated gene products** (including cyclin D1), we used curcumin, a pharmacologically safe agent, to target NF-kappaB in a **variety of MCL cell lines**. All four MCL cell lines examined had overexpression of cyclin D1, constitutive active NF-kappaB and IkappaB kinase and phosphorylated forms of IkappaBalpha and p65. This correlated with expression of TNF, IkappaBalpha, Bcl-2, Bcl-xl, COX-2 and IL-6, all regulated by NF-kappaB. On treatment of cells with curcumin, however, **downregulated constitutive active NF-kappaB** and inhibited the consitutively active IkappaBalpha kinase (IKK), and phosphorylation of IkappaBalpha and p65. Curcumin also inhibited constitutive activation of Akt, needed for IKK activation. **Consequently, the expression of all NF-kappaB-regulated gene products, were downregulated by the polyphenol leading to the suppression of proliferation, cell cycle arrest at the G1/S phase of the cell cycle and induction of apoptosis as indicated by caspase activation, PARP cleavage, and annexin V staining.** That NF-kappaB activation is directly linked to the proliferation of cells, is also indicated by the observation that peptide derived from the IKK/NEMO-binding domain and p65 suppressed the constitutive active NF-kappaB complex and inhibited the proliferation of MCL cells. Constitutive **NF-kappaB activation was found to be due to TNF**, as anti-TNF antibodies inhibited both NF-kappaB activation and proliferation of cells. Overall, our results indicate that curcumin inhibits the constitutive NF-kappaB and IKK leading to suppression of expression of NF-kappaB-regulated gene products that results in the suppression of proliferation, cell cycle arrest, and induction of apoptosis in MCL.

26) Choudhuri, Tathagata, et al. "Curcumin selectively induces apoptosis in deregulated cyclin D1-expressed cells at G2 phase of cell cycle in a p53-dependent manner." Journal of Biological Chemistry 280.20 (2005): 20059-20068. **All of these data suggest that curcumin can exert its apoptogenic effect in those cells whose cyclin D1 expression is deregulated due to genetic manipulations.**

27) Singh, Amareshwar TK, et al. "Curcumin nanodisk-induced apoptosis in mantle cell lymphoma." Leukemia & lymphoma 52.8 (2011): 1537-1543.

28) Tadmor, Tamar, and Aaron Polliack. "Mantle cell lymphoma: curcumin nanodisks and possible new concepts on drug delivery for an incurable lymphoma." Leukemia & lymphoma 52.8 (2011): 1418. Mantle cell lymphoma curcumin nanodisks Tadmor Tamar Aaron Polliack Leukemia lymphoma 2011

29) Yallapu, Murali M., Meena Jaggi, and Subhash C. Chauhan. "Curcumin nanomedicine: a road to cancer therapeutics." Current pharmaceutical design 19.11 (2013): 1994.

Cannabinoid Extracts Anti-Cancer Effects

30) Semin Cancer Biol. **2011** Nov;21(5):313-21. The role of cannabinoid receptors and the endocannabinoid system in mantle cell lymphoma and other non-Hodgkin lymphomas. Wasik AM1, Christensson B, Sander B.

The initiating oncogenic event in mantle cell lymphoma (MCL) is the translocation of cyclin D1, t(11;14)(q13;q32). However, other genetic aberrations are necessary for an overt lymphoma to arise. Like other B cell lymphomas, MCL at some points during the oncogenesis is dependent on interactions with other cells and factors in the microenvironment. The G protein coupled receptors cannabinoid receptors 1 and 2 (CB1 and CB2) are expressed at low levels on non-malignant lymphocytes and at higher levels in MCL and other lymphoma subtypes. In this review we give an overview of what is known on the role of the cannabinoid receptors and their ligands in lymphoma as compared to non-malignant T and B lymphocytes. In MCL cannabinoids mainly reduce cell proliferation and induce cell death. Importantly, our recent findings demonstrate that cannabinoids may induce either apoptosis or another type of programmed cell death, cytoplasmic vacuolation/paraptosis in MCL. The signalling to death has been partly characterized. Even though cannabinoid receptors seem to be expressed in many other types of B cell lymphoma, the functional role of cannabinoid receptor targeting is yet largely unknown. In non-malignant B and T lymphocytes, cannabinoid receptors are up-regulated in response to antigen receptor signalling or CD40. For T lymphocytes IL-4 has also a crucial role in transcriptional regulation of CB1. In lymphocytes, cannabinoid act in several ways – by affecting cell migration, cytokine response, at high doses inhibit cell proliferation and inducing cell death. The possible role for the endocannabinoid system in the immune microenvironment of lymphoma is discussed.

31) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4278325/
Wasik AM, Nygren L, Almestrand S, et al. Perturbations of the endocannabinoid system in mantle cell lymphoma: correlations to clinical and pathological features. Oncoscience. **2014**;1(8):550-557.

32) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4171598/
Chakravarti B, Ravi J, Ganju RK. Cannabinoids as therapeutic agents in cancer: current status and future implications. Oncotarget. **2014**;5(15):5852-5872.

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NICE IMAGE OF GREEN STAIN FOR CB1 RECEPTOR

33) http://www.nature.com/leu/journal/v17/n9/full/2403057a.html
Leukemia (**2003**) 17, 1880–1890. doi:10.1038/sj.leu.2403057
High level of cannabinoid receptor 1, absence of regulator of G protein signalling 13 and differential expression of Cyclin D1 in mantle cell lymphoma
T C Islam1, A C Asplund1, J M Lindvall1, L Nygren2, J Liden3, E Kimby4, B Christensson2, C I E Smith1 and B Sander2

34) full pdf Flygare, Jenny, et al. "Cannabinoid receptor ligands mediate growth inhibition and cell death in mantle cell lymphoma." FEBS letters 579.30 (**2005**): 6885-6889.

We have earlier reported **overexpression of the central and peripheral cannabinoid receptors CB1 and CB2 in mantle cell lymphoma (MCL)**, a B cell non-Hodgkin lymphoma. In this study, treatment with cannabinoid receptor ligands caused a decrease in viability of MCL cells, while control cells lacking CB1 were not affected. Interestingly, equipotent doses of the CB1 antagonist SR141716A and the CB1/CB2 agonist anandamide inflicted additive negative effects on viability. Moreover, treatment with the CB1/CB2 agonist Win-55,212-2 caused a decrease in long-term growth of MCL cells in culture. **Induction of apoptosis**, as measured by FACS/Annexin V–FITC, contributed to the growth suppressive effect of Win-55,212-2. **Our data suggest that cannabinoid receptors may be considered as potential therapeutic targets in MCL.**

35) http://molpharm.aspetjournals.org/content/70/5/1612.long
Gustafsson, Kristin, et al. “Cannabinoid receptor-mediated apoptosis induced by R (+)-methanandamide and Win55, 212-2 is associated with **ceramide accumulation** and p38 activation in mantle cell lymphoma.” Molecular pharmacology 70.5 (**2006**): 1612-1620.

36) Int J Cancer. **2008** Sep 1;123(5):1025-33.
Expression of cannabinoid receptors type 1 and type 2 in non-Hodgkin lymphoma: growth inhibition by receptor activation.
Gustafsson K1, Wang X, Severa D, Eriksson M, Kimby E, Merup M, Christensson B, Flygare J, Sander B.
Endogenous and synthetic cannabinoids exert antiproliferative and proapoptotic effects in various types of cancer and in mantle cell lymphoma (MCL). In this study, we evaluated the expression of cannabinoid receptors type 1 and type 2 (CB1 and CB2) in non-Hodgkin lymphomas of B cell type (n = 62). A majority of the lymphomas expressed higher mRNA levels of CB1 and/or CB2 as compared to reactive lymphoid tissue. **With the exception of MCL, which uniformly overexpresses both CB1 and CB2**, the levels of cannabinoid receptors within other lymphoma entities were highly variable, ranging from 0.1 to 224 times the expression in reactive lymph nodes. Low levels of the splice variant CB1a, previously shown to have a different affinity for cannabinoids than CB1, were detected in 44% of the lymphomas, while CB1b expression was not detected. In functional studies using MCL, Burkitt lymphoma (BL), chronic lymphatic leukemia (CLL) and plasma cell leukemia cell lines, the stable anandamide analog R(+)-methanandamide (R(+)-MA) **induced cell death only in MCL and CLL cells, which overexpressed both cannabinoid receptors,** but not in BL. In vivo treatment with R(+)-MA caused a significant reduction of tumor size and mitotic index in mice xenografted with human MCL. Together, our results suggest that therapies **using cannabinoid receptor ligands will have efficiency in reducing tumor burden in malignant lymphoma overexpressing CB1 and CB2.**

37) Gustafsson, Kristin, et al. Potentiation of cannabinoid-induced cytotoxicity in mantle cell lymphoma through modulation of ceramide metabolism.” Molecular Cancer Research 7.7 (**2009**): 1086-1098. Cannabinoid induced cytotoxicity mantle cell lymphoma ceramide metabolism Gustafsson Kristin 2009
Ceramide levels are elevated in mantle cell lymphoma (MCL) cells following treatment with cannabinoids. Here, we investigated the pathways of ceramide accumulation in the MCL cell line Rec-1 using the stable endocannabinoid analogue R(+)-methanandamide (R-MA). We further interfered with the conversion of ceramide into sphingolipids that promote cell growth. Treatment with R-MA led to increased levels of ceramide species C16, C18, C24, and C24:1 and transcriptional induction of ceramide synthases (CerS) 3 and 6. The effects were attenuated using SR141716A, which has high affinity to cannabinoid receptor 1 (CB1). The CB1-mediated induction of CerS3 and CerS6 mRNA was confirmed using Win-55,212-2. Simultaneous silencing of CerS3 and CerS6 using small interfering RNA abrogated the R-MA-induced accumulation of C16 and C24. Inhibition of either of the enzymes serine palmitoyl transferase, CerS, and dihydroceramide desaturase within the de novo ceramide pathway reversed ceramide accumulation and cell death induced by R-MA treatment. To enhance the cytotoxic effect R-MA, sphingosine kinase-1 and glucosylceramide synthase, enzymes that convert ceramide to the pro-proliferative sphingolipids sphingosine-1-phospate and glucosylceramide, respectively, were inhibited.

Suppression of either enzyme using inhibitors or small interfering RNA potentiated the decreased viability, induction of cell death, and ceramide accumulation induced by R-MA treatment. Our findings suggest that **R-MA induces cell death in MCL via CB1-mediated up-regulation of the de novo ceramide synthesis pathway.** Furthermore, this is the first study were the cytotoxic effect of a cannabinoid is enhanced by modulation of ceramide metabolism. (Mol Cancer Res 2009;7(7):1086–98)

38) Wasik, A. M., et al. "WIN55, 212-2 induces cytoplasmic vacuolation in apoptosis-resistant MCL cells." Cell death & disease 2.11 (2011): e225.
Cannabinoid receptors 1 (CB1) and/or 2 (CB2) are overexpressed in many types of human malignancies including mantle cell lymphoma (MCL). Agonists to CB1 and CB2 promote ceramide de novo synthesis, p38–mitogen-activated protein kinase-dependent activation of caspase-3 and apoptotic cell death in most MCLs. However, in this report we describe that in some MCLs the response to treatment with cannabinoids decreased cell viability as assessed by metabolic activity but did not involve the caspase-3 cascade or loss of plasma membrane integrity. Both primary cells from one MCL patient and the MCL cell line Granta519 responded to treatment with cannabinoids by formation of cycloheximide-sensitive cytoplasmic vacuoles, but did not enter apoptosis. The persistent expression of mammalian homolog of Atg8 with microtubule-associated protein-1 light chain-3 II (LC3 II) and p62, as well as the lack of protection from chloroquine, indicates that lysosomal degradation is not involved in this cytoplasmic vacuolation process, distinguishing from classical autophagy. Transmission electron microscopy images and immunofluorescence staining of endoplasmic reticulum (ER) chaperone calreticulin showed that the vacuoles were of ER origin and that chromatin remained normal. These features resemble paraptosis-like cell death—a third type of a programmed cell death not previously described in response to cannabinoids.

Sulfazalazine

39) Ishimoto, Takatsugu, et al. "CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc− and thereby promotes tumor growth." Cancer cell 19.3 (2011): 387-400. CD44 regulates redox status in cancer cells by stabilizing xCT Cancer cell 2011 Ishimoto Takatsugu The xCT Inhibitor **Sulfasalazine** Suppresses CD44-Dependent Tumor Growth and Promotes Activation of p38MAPK in Tumor Cells In Vivo – Stem Cells !!!

40) Lewerenz, Jan, et al. "The cystine/glutamate antiporter system xc− in health and disease: from molecular mechanisms to novel therapeutic opportunities." Antioxidants & redox signaling 18.5 (2013): 522-555. The cystine glutamate antiporter system xc in health and disease Lewerenz Jan 2013

Early evidence suggested that nonsteroidal anti-inflammatory drugs also inhibit system xc – (15). On this basis, the Gout lab identified the FDA-approved drug **sulfasalazine,** commonly used to treat chronic inflammatory diseases such as rheumatoid arthritis, as a potent system xc – inhibitor (79). However, this compound is also a **potent inhibitor of nuclear factor kappa B (NF-jB) activation** (283).

41) Gout, P. W., et al. "Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the x c-cystine transporter: a new action for an old drug." Leukemia (08876924) 15.10 (2001). 1Department of Cancer Endocrinology, BC Cancer Agency, Vancouver, BC, Canada

42) Doxsee, Daniel W., et al. "Sulfasalazine-induced cystine starvation: Potential use for prostate cancer therapy." The Prostate 67.2 (2007): 162-171.

43) Chung, W. Joon, and Harald Sontheimer. "Sulfasalazine inhibits the growth of primary brain tumors independent of nuclear factor-κB." Journal of neurochemistry 110.1 (2009): 182-193.

44) Guan, Jun, et al. "The x c− cystine/glutamate antiporter as a potential therapeutic target for small-cell lung cancer: use of sulfasalazine." Cancer chemotherapy and pharmacology 64.3 (2009): 463-472.

45) Dixon, Scott J., et al. "Pharmacological inhibition of cystine–glutamate exchange induces endoplasmic reticulum stress and ferroptosis." Elife 3 (2014): e02523.Pharmacological inhibition of cystine glutamate exchange induces endoplasmic reticulum stress and ferroptosis.Elife 2014 Dixon SCott

46) Narang, Vishal S., et al. "Sulfasalazine-induced reduction of glutathione levels in breast cancer cells: enhancement of growth-inhibitory activity of doxorubicin." Chemotherapy 53.3 (2007): 210-217. Sulfasalazine induced reduction of glutathione levels in breast cancer cells Narang 2007 Chemotherapy

Background: We previously showed that the anti-inflammatory drug, sulfasalazine (salicylazosulfapyridine, SASP), **can arrest proliferation of MCF-7 and MDA-MB-231 mammary cancer cells** by inhibiting uptake of cystine via the xc– cystine/glutamate antiporter. Here we examined SASP with regard to reduction of cellular glutathione (GSH) levels and drug efficacy-enhancing ability. Methods: GSH levels were measured spectrophotometrically. Cellular drug retention was determined with 3H-labeled methotrexate, and drug efficacy with a colony formation assay. Results: Incubation of the mammary cancer cells with SASP (0.3–0.5 mM) led to reduction of their GSH content in a time- and concentration-dependent manner. Similar to MK-571, a multidrug resistance-associated protein inhibitor, SASP increased intracellular accumulation of methotrexate. Preincubation of cells with SASP (0.3 mM) significantly enhanced the potency of the anticancer agent doxorubicin (2.5 nM). Conclusions: SASP-induced reduction of cellular GSH levels can lead to growth arrest of mammary cancer cells and enhancement of anticancer drug efficacy.

We also showed, for the first time, that sulfasalazine (salicylazosulfapyridine, SASP), an anti-inflammatory drug used against inflammatory bowel disease
and rheumatoid arthritis, is a potent inhibitor of x c – – mediated cystine uptake [16] , and that it can markedly inhibit proliferation of human breast carcinoma cells at relatively low concentrations (0.2–0.5 m M) via cystine starvation [17] .

The human breast carcinoma cell lines, MCF-7 (estrogen receptor positive) and MDA-MB-231 (estrogen receptor negative, highly invasive),

From Rheumatoid artritis.net: **How is sulfasalazine taken?**

Sulfasalazine comes in a 500-mg tablet for oral administration and it typically started at a dose of **500 mg per day** and increased by 500 mg every week, while monitoring for side effects, until a daily target dose (this is determined by your weight, approximately 40 mg/kg) is reached. For most adult patients with RA, the final daily dose ranges from **2000-3000 mg (2-3 grams)**. If gastrointestinal (GI) side effects are problematic, divided doses can be used or a special enteric-coated form (Azulfidine EN-tabs) to protect against GI effects. To help prevent stomach upset, you should take sulfasalazine with food, followed by a full glass of water.1,2

Chloroquin synergistic with Artemisinin ?

47) Ganguli, Arnab, et al. "Inhibition of autophagy by chloroquine potentiates synergistically anti-cancer property of artemisinin by promoting ROS dependent apoptosis." Biochimie 107 (2014): 338-349. Inhibition of autophagy by chloroquine potentiates synergistically anti-cancer property of artemisinin by promoting ROS dependent apoptosis Ganguli Biochimie 2014

The appropriate manipulation of autophagy by using CQ provides a powerful strategy to increase the Potency of selective anticancer property of ART.

However, the low toxicity and low bio-availability make it unsuitable for use as an anticancer agent. Works are going on to try to increase the potency of artemisinin and make it acceptable as an anticancer drug.

One of the well known late phase autophagy inhibitor is chloroquine (CQ). For more than six decades CQ has been using to treat several diseases (like, malaria, rheumatoid arthritis, lupus) because of its high effectiveness and well tolerated by human [23]

Chloroquin, its protonated forms are trapped within the acidic vacuoles (late endosome and lysosome), causing increased in pH, inactive lysosomal hydrolysates, thus increase the autolysosomes accumulation by inhibiting the autophagy.

For **pretreatment regime**, we treated A549 cells (lung cancer) with 25-100 μM CQ for 12 h and subsequently after washing the cells, treatment with 75 μM ART for 72 h was performed.

For co-treatment, we used 75 μM ART and different concentration of CQ (2.5-25 μM) simultaneously to treat A549 cells for 72 h. In both the cases, we measured cell viability by MTT assay and calculated combination index (CI) (Fig 5A-D). We found that CI value of pre-treatment (Fig. 5A and C) were much more lower than CI values of co-treatment (Fig.5 B and D), indicating pre-treatment had synergistic effect where as co-treatment had nearly an additive effect.

Furthermore co-treatment also reduced cell viability of normal cells (Fig. 5E) but pretreatment did not have any cytotoxic effect towards normal cells (Fig. 5F).

In control experiments, when cells were treated with separately CQ (50
μM for 12 h) and ART (75 μM for 72 h), and the apoptotic populations were 3% and 16% respectively, but in case of combination treatment, apoptotic population was increased to 40% (Fig. 6C).

We observed that NAC significantly reduced AVOs in both single and double treated A549 cells (Fig. 8C). Furthermore, scavenging ROS by NAC decreased the cleaved caspase 3 expression (Fig. 8E) and subsequently blocked apoptotic cells death (Fig. 8D)

pretreatment of CQ was found to be more effective to increase the potency of artemisinin than co-treatment (Fig.5A-E).

48) Solomon, V. Raja, and Hoyun Lee. “Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies.” European journal of pharmacology 625.1 (2009): 220-233. Chloroquine and its analogs a new promise of an old drug for effective and safe cancer therapies Solomon Raja Hoyun Lee European journal of pharmacology 2009

49) Kimura, Tomonori, et al. Chloroquine in cancer therapy: a double-edged sword of autophagy.” Cancer research 73.1 (2013): 3-7.

The dosage of chloroquine usually ranges between 100 and 500 mg/day. Side effects are minimal at low doses, while many more toxic effects occur at higher doses, such as visual disturbances, gastrointestinal upset,

electrocardiographic changes, headache, and pruritus.

50) Eur J Pharmacol. 2016 Jan 15;771:139-44. Time to use a dose of Chloroquine as an adjuvant to anti-cancer chemotherapies. Pascolo S1.
Chloroquine, a drug used for **over 80 years** to treat and prevent malaria and, more recently, to treat autoimmune diseases, is very safe but has a plethora of dose-dependent effects. **By increasing pH in acidic compartments it inhibits for example lysosomal enzymes.** In the context of cancer, Chloroquine was found to have direct effects on different types of malignancies that could potentiate chemotherapies. For example, the anti-malaria drug may inhibit both the multidrug-resistance pump and autophagy (mechanisms that tumor cells may use to resist chemotherapies), intercalate in DNA and enhance the penetration of chemotherapeutic drugs in cells or solid cancer tissues. However, these activities were mostly demonstrated at high doses of Chloroquine (higher than 10mg/kg or 10mg/l i.e. ca. 31μM). Nevertheless, it was reported that daily uptake of clinically acceptable doses (less than 10mg/kg) of Chloroquine in addition to chemo-radio-therapy increases the survival of glioblastoma patients (Sotelo et al., 2006; Briceno et al., 2007). However, **the optimal dose and schedule of this multi-active drug with respect to chemotherapy has never been experimentally determined.** The present article reviews the several known direct and indirect effects of different doses of Chloroquine on cancer and how those effects may indicate that a fine tuning of the dose/schedule of Chloroquine administration versus chemotherapy may be critical to obtain an adjuvant effect of Chloroquine in anti-cancer treatments. We anticipate that the appropriate (time and dose) addition of Chloroquine to the standard of care may greatly and safely potentiate current anti-cancer treatments.

51) Sukhai, Mahadeo A., et al. “Lysosomal disruption preferentially targets acute myeloid leukemia cells and progenitors.” Journal of Clinical Investigation 123.1 (2013): 315.

mefloquin and artemisinin combination

However, strikingly, 10 of these, including the artemisinin class of antimalarials (27, 28), proved to be compounds that are known to increase the production of ROS.

Synergistic combinations of mefloquine and artenimol or artesunate also synergistically increased ROS production

mefloquine specifically targets lysosomal function. This finding is consistent with mefloquine’s known ability to preferentially accumulate in lysosomes of the malarial parasite

Mefloquine directly disrupted lysosomes isolated from AML cell lines and primary AML patients’ samples, in a dose-dependent manner, as measured by release of cathepsins B and L

The effects of mefloquine were specific to lysosomes, as mefloquine treatment did not disrupt isolated mitochondria

In a dose-dependent manner, mefloquine disrupted lysosomes in TEX leukemia cells and mefloquine-sensitive cells from AML patients, but not normal hematopoietic cells

Taken together, these data point to mefloquine-mediated lysosomal disruption as the cellular mechanism underlying antileukemic action.

AML cells have increased lysosomal mass compared with normal hematopoietic cells.

TEM revealed that lysosomes are **larger in primary human AML cells**, including the CD34+ AML cells, as well as in AML cell lines, in comparison to the lysosomes found in normal human CD34+ hematopoietic cells

Here we report that mefloquine, a quinoline approved for the treatment and prevention of malaria (23, 24), **has toxicity for human AML cells including AML progenitors, while sparing normal human hematopoietic cells treated with the same doses.**
serum concentrations of mefloquine up to 5 μM have been reported in individuals receiving 250 mg weekly for malaria prophylaxis (59, 60). Thus, antileukemia concentrations of mefloquine may be pharmacologically achievable.

The antimalarial chloroquine is structurally similar to mefloquine and inhibits the degradation of autophagy targets in the autophagolysosome. Through this mechanism, **chloroquine can induce cell death and sensitize cells to chemotherapy** (including imatinib mesylate in chronic myeloid leukemia; refs. 63–66) and radiation. However, as it involves induction of lysosome disruption, the mechanism of action of mefloquine appears distinct from that of chloroquine and other inhibitors of autophagy.

52) Neuro Oncol. 2010 May;12(5):473-81. Chloroquine-induced autophagic vacuole accumulation and cell death in glioma cells is p53 independent.
Geng Y1, Kohli L, Klocke BJ, Roth KA.

Glioblastoma (GBM) is a high-grade central nervous system malignancy and despite aggressive treatment strategies, GBM patients have a median survival time of just 1 year. **Chloroquine** (CQ), an antimalarial lysosomotropic agent, has been identified as a potential adjuvant in the treatment regimen of GBMs. However, the mechanism of CQ-induced tumor cell death is poorly defined. We and others have shown that CQ-mediated cell death may be p53-dependent and at least in part due to the intrinsic apoptotic death pathway. Here, we investigated the effects of CQ on 5 established human GBM lines, differing in their p53 gene status. CQ was found to induce a concentration-dependent death in each of these cell lines. Although CQ treatment increased caspase-3-like enzymatic activity in all 5 cell lines, a broad-spectrum caspase inhibitor did not significantly attenuate death. Moreover, **CQ caused an accumulation of autophagic vacuoles in all cell lines and was found to affect the levels and subcellular distribution of cathepsin D, suggesting that altered lysosomal function may also play a role in CQ-induced cell death.** Thus, **CQ can induce p53-independent death in gliomas that do not require caspase-mediated apoptosis**. To potentially identify more potent chemotherapeutics, various CQ derivatives and lysosomotropic compounds were tested on the GBM cells. **Quinacrine and mefloquine were found to be more potent than CQ in killing GBM cells in vitro and** given their superior blood-brain barrier penetration compared with CQ may prove more efficacious as chemotherapeutic agents for GBM patients.

What is the usual dose of Chloroquine? From rheuminfo.com

Chloroquine is available in 250 mg tablets. The dose is based on your lean body weight with the standard dose ranging from one-half (125 mg) to one tablet (250 mg) per day. The dose should be no more than 3 mg/kg/day of lean body weight.

Why do I need to see an Ophthalmologist (Eye Doctor) when I am taking Chloroquine?

As mentioned above, Chloroquine can cause damage to the back of the eye (retina). Early eye toxicity is not usually a serious problem. However, once serious damage occurs, it is permanent. Therefore, appropriate monitoring of the eye by a medical specialist familiar with the side-effects of Chloroquine is essential. The eye examination includes regular tests such as vision and eye pressure. It also includes tests which are not part of a

regular eye check-up such as testing for colour vision and testing your visual fields. Your doctor will usually ask you to see an ophthalmologist every 6-24 months.

Ferroptostis

53) Xie, Y., et al. "Ferroptosis: process and function." Cell Death & Differentiation (2016).

56) Nandakumar, Dalavaikodihalli Nanjaiah, et al. "Curcumin-artemisinin combination therapy for malaria." Antimicrobial agents and chemotherapy 50.5 (2006): 1859-1860.

Synergy with allicin full pdf

57) .Synergistic Anticancer Effect of Artesunate with Allicin in Osteosarcoma Jiang_2013 Asian Pac J Cancer Prev. 2013;14(8):4615-9
The synergistic anticancer effect of **artesunate combined with allicin** in osteosarcoma cell line in vitro and in vivo. Jiang W1, Huang Y, Wang JP, Yu XY, Zhang LY.

Artesunate, extracted from Artemisia annua, has been proven to have anti-cancer potential. Allicin, diallyl thiosulfinate, the main biologically active compound derived from garlic, is also of interest in cancer treatment research. This object of this report was to document synergistic effects of artesunate combined with allicin on osteosarcoma cell lines in vitro and in vivo.
METHODS:After treatment with artesunate and allicin at various concentrations, the viability of osteosarcoma cells was analyzed by MTT method, with assessment of invasion and motility, colony formation and apoptosis. Western Blotting was performed to determine the expression of caspase-3/9, and activity was also detected after drug treatment. Moreover, in a nude mouse model established with orthotopic xenograft tumors, tumor weight and volume were monitored after drug administration via the intraperitoneal (i.p.) route.
RESULTS:The viability of osteosarcoma cells in the combination group was significantly decreased in a concentration and time dependent manner; moreover, invasion, motility and colony formation ability were significantly suppressed and the apoptotic rate was significantly increased through caspase-3/9 expression and activity enhancement in the combination group. Furthermore, suppression of tumor growth was evident in vivo.
CONCLUSION:Our results indicated that artesunate and allicin in combination exert synergistic effects on osteosarcoma cell proliferation and apoptosis.

synergy with resveratrol

58) J Cancer Res Clin Oncol. 2014 Dec;140(12):2065-75.
Synergic effects of **artemisinin and resveratrol** in cancer cells.
Li P1, Yang S, Dou M, Chen Y, Zhang J, Zhao X.
The aim of this study was to investigate whether resveratrol (Res) combined with artemisinin (ART) possess synergistic effect on different cancer cells.
MATERIALS AND METHODS:The viability of HepG2 and HeLa cells treated with ART and Res was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Combination index (CI) analysis and isobologram were used to assess the synergistic effect of ART and Res in different ratios. Wound-healing assay was used to investigate the migration rate. AO staining and fluorescent microscopy measurements were performed to detect the cell apoptosis. Reactive oxygen species (ROS) was measured with 2'7'-dichlorofluorescein diacetate (DCFH-DA).
RESULTS:MTT assay indicated that ART and Res inhibited the growth of HeLa and HepG2 cells in a dose-dependent manner. The combination of ART and Res exhibited the strongest anticancer effect at the ratio of 1:2

(ART to Res). The combination of the two drugs also markedly reduced the ability of cell migration. Apoptosis analysis showed that combination of ART and Res significantly increased the apoptosis and necrosis rather than use singly. Additionally, ROS levels were elevated by combining ART with Res.
CONCLUSIONS:Taken together, the present study suggested that **ART and Res possessed the synergistic anti-tumor effect.** ART in combination with Res could be an effective therapeutic strategy for cancer.

59) Tolomeo,M. et al. (2005) Pterostilbene and 3-hydroxypterostilbene are effective apoptosis-inducingagents in MDR and BCR-ABL-expressing leukemia cells. Int. J. Biochem. Cell Biol. 37, 1709–1726 Pterostilbene apoptosis in MDR leukemia cells Tolomeo 2005

Gleditsia sinensis

60) Oncol Rep. 2003 Sep-Oct;10(5):1601-7.
Gleditsia sinensis fruit extract is a potential chemotherapeutic agent in chronic and acute myelogenous leukemia. Chow LM1, Chui CH, Tang JC, Teo IT, Lau FY, Cheng GY, Wong RS, Leung TW, Lai KB, Yau MY, Gou D, Chan AS.
The anti-leukemia activity of the saponin rich Gleditsia sinensis Lam. fruit extract (GSE) was investigated on cancer cell lines and bone marrow cells obtained from consented patients with chronic myelogenous leukemia (CML) and acute myelogenous leukemia (AML) during presentation. The growth inhibitory activity of the extract was determined by [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) assay. Colony formation assay was performed to investigate the regeneration potential. Cellular morphology change was studied. Apoptosis was demonstrated by DNA electrophoresis, reverse transcription polymerase chain reaction (RT-PCR) and flow cytometry. The mean concentration to inhibit the cell growth by 50% (MTS50) was 18+/-1.6 micro g/ml for K562 CML cell line and 12+/-1.3 micro g/ml for HL-60 acute promyelocytic leukemia cell line. Patient samples showed a mean MTS50 of 13-28 micro g/ml. Non-malignant hematological disorder bone marrow samples showed a mean MTS50 from 45 to 53 micro g/ml. Loss of regeneration property after treatment with GSE of these two cancer cell lines were confirmed by colony formation assay. GSE was able to induce cell shrinkage in K-562. DNA laddering was observed by incubating the leukemia cells with GSE. RT-PCR demonstrated that the pro-apoptic gene bax was induced while the anti-apoptic gene bcl-2 and cell cycle active gene PCNA were reduced. Flow cytometric analysis showed that the apoptotic effect of GSE on leukemia cell line was time- and dose-dependent. **Thus GSE might be potentially used as a chemotherapeutic drug to treat patients with acute and chronic myelogenous leukemi**a.

61) Int J Mol Med. 2005 Mar;15(3):539-43.
Gleditsia sinensis fruit extract-induced apoptosis involves changes of reactive oxygen species level, mitochondrial membrane depolarization and caspase 3 activation. Chui CH1, Lau FY, Chan AS, Cheng GY, Wong RS, Lai KB, Kok SH, Yeung TT, Teo IT, Yau MY, Cheung F, Cheng CH, Tang JC.
Recently, we have shown that the anomalous fruit extract of Gleditsia sinensis (GSE) processes apoptotic activity on numerous solid tumour and leukaemia cell lines as well as primary cultured leukaemia cells obtained from bone marrow aspirate of patients. GSE treated cancer cells exhibited apoptotic features as readily illustrated by morphological investigation, DNA fragmentation analysis and TUNEL labelling methods. Elevation of intracellular superoxide dismutase activity was observed. However, the detailed mechanism still remains undefined. Here we further demonstrated that cell cycle arrest, increment of hydrogen peroxide production, changes of intracellular acid-base equilibrium and mitochondrial membrane potential depolarization (DeltaPsi(m)) were induced from cancer cells after GSE incubation. Caspase 3 protease activity was significantly enhanced upon GSE treatment. Taken together, a defined signaling pathway for the mechanistic action of GSE on cancer cells was worked out.

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Ivermectin

62) Sharmeen, Sumaiya, et al. "The antiparasitic agent ivermectin induces chloride-dependent membrane hyperpolarization and cell death in leukemia cells." Blood 116.18 (2010): 3593-3603.
To identify known drugs with previously unrecognized anticancer activity, we compiled and screened a library of such compounds to identify agents cytotoxic to leukemia cells. From these screens, we identified ivermectin, a derivative of avermectin B1 that is licensed for the treatment of the parasitic infections, strongyloidiasis and onchocerciasis, but is also effective against other worm infestations. As a potential antileukemic agent, **ivermectin induced cell death at low micromolar concentrations in acute myeloid leukemia cell lines** and primary patient samples preferentially over normal hematopoietic cells. Ivermectin also delayed tumor growth in 3 independent mouse models of leukemia at concentrations that appear pharmacologically achievable. As an antiparasitic, ivermectin binds and activates chloride ion channels in nematodes, so we tested the effects of ivermectin on chloride flux in leukemia cells. Ivermectin increased intracellular chloride ion concentrations and cell size in leukemia cells. Chloride influx was accompanied by plasma membrane hyperpolarization, but did not change mitochondrial membrane potential. Ivermectin also increased reactive oxygen species generation that was functionally important for ivermectin-induced cell death. Finally, **ivermectin synergized with cytarabine and daunorubicin** that also increase reactive oxygen species production. Thus, given its known toxicology and pharmacology, ivermectin could be rapidly advanced into clinical trial for leukemia.

63) Draganov, Dobrin, et al. "Modulation of P2X4/P2X7/pannexin-1 sensitivity to extracellular ATP via ivermectin induces a non-apoptotic and inflammatory form of cancer cell death." Scientific reports 5 (2015).

64) Furusawa, Shinobu, et al. "Potentiation of Doxorubicin-Induced Apoptosis of Resistant Mouse Leukaemia Cells by Ivermectin." Pharmacy and Pharmacology Communications 6.3 (2000): 129-134.

65) Melotti, Alice, et al. "The river blindness drug Ivermectin and related macrocyclic lactones inhibit WNT-TCF pathway responses in human cancer." EMBO molecular medicine (2014): e201404084.

Patent – Ivermectin used with chemotherapy for lymphoma

66) Use of synergistic combinations of an avermectin and an antineoplastic compounds for the treatment of hematological malignancies
EP 2498785 A1 (text from WO2011054103A1)
The disclosure relates to a method of treating a hematological malignancy comprising administering to a subject in need thereof a synergistic combination of a first compound comprising an effective amount of one or more **Avermectins and a second compound comprising a chemotherapeutic, preferably daunorubicin, cyarabine, doxorubicin, idarubicin mitoxantrone, amsacrine**, and mixtures thereof

67) FURUSAWA SHINOBU ET AL: "Potentiation of doxorubicin-induced apoptosis of resistant mouse leukaemia cells by ivermectin", PHARMACY AND PHARMACOLOGY COMMUNICATIONS, vol. 6, no. 3, March 2000 (2000-03), pages 129-134, XP9167795, ISSN: 1460-8081

68) YURI N. KORYSTOV ET AL: "Avermectins inhibit multidrug resistance of tumor cells", EUROPEAN JOURNAL OF PHARMACOLOGY, vol. 493, no. 1-3, 1 June 2004 (2004-06-01), pages 57-64, XP055055776, ISSN: 0014-2999,

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Diclofenac

69) The COX inhibitor, diclofenac induces mantle cell lymphoma apoptosis independent of p53 status. Hesham M. Hassan1, Michelle L. Varney2, Aalia M. Aly3, Shantaram S. Joshi2, Rakesh K. Singh2, and Bhavana J. Dave2
Author Affiliations 1Assiut University, Assiut, Egypt;
2university of Nebraska Medical Center, Omaha, NE;
3South Egypt Cancer institute, Assiut, Egypt.

Proceedings: AACR 106th Annual Meeting 2015; April 18-22, 2015; Philadelphia, PA
Abstract **Mantle cell lymphoma** (MCL) is characterized by a clinically aggressive course with frequent relapses and poor survival. The p53 pathway is frequently dysregulated and p53 status predicts clinical outcome in MCL. Diclofenac has been reported to increase p53 pathway activity, upregulates p73 expression and inhibit cell growth; hence we investigated the ability of diclofenac to induce apoptosis and/or cell cycle arrest in MCL, and its dependence on p53 status. To investigate the response of MCL to diclofenac, wild-type p53 [Granta-519 and JVM-2] and mutant p53 [Jeko-1 and Mino-1] expressing cells, therapy resistant cell lines and primary human cells isolated from MCL patients were used. Cell proliferation, apopotosis, and expression of down-stream signaling p53 targets were analyzed. Nontoxic concentrations of diclofenac induced concentration- and duration-dependent inhibition of MCL cell growth independent of p53 status. Diclofenac treatment also resulted in cell cycle arrest and cell death. Molecularly, diclofenac treatment was associated with increased activity of caspases 3, 7, and 8. Increased p53 pathway activity was demonstrated by duration-dependent induction of p53 transcriptional target genes, including the cell cycle regulatory molecule p21 and pro-apoptotic molecules including, PUMA, NOXA, BIM, and CD95. Most importantly, diclofenac treatment was associated with enhanced expression of the pro-apoptotic isoforms of the p53 homologue TAp73. Our data demonstrates that diclofenac treatment results in decreased cell cycle progression, and increased apoptosis that is associated with increased p53 pathway activity, independent of p53 status, and increased activity of its family member p73.
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Crowd Funding

70) Can Artesunate, an antimalarial drug, provide an effective and affordable treatment for Bowel Cancer ? By Professor Sanjeev Krishna

(71) Krishna, Sanjeev, et al. “A randomised, double blind, placebo-controlled pilot study of oral artesunate therapy for colorectal cancer.” EBioMedicine 2.1 (2015): 82-90.

This was a single centre, randomised, double-blind, placebo-controlled trial. Patients planned for curative resection of biopsy confirmed single primary site Colo-rectal Cancer (CRC) were randomised (n = 23) to receive preoperatively either 14 daily doses of oral artesunate (200 mg; **n = 12**) or placebo (**n = 11**).

During a median follow up of 42 months 1 patient in the artesunate and 6 patients in the placebo group developed recurrent CRC. Till this analysis, there have been **no deaths in artesunate recipients (despite some patients having relatively poor prognosis), and 3 deaths in placebo recipients.**

Animal Study – Veterinary Medicine Whole leaf extract 30:1 artemesia

72) Breuer, Elmar, and Thomas Efferth. “Treatment of iron-loaded veterinary sarcoma by Artemisia annua.” Natural products and bioprospecting 4.2 (2014): 113-118.
Veterinary Clinic for Pets, Müllheim/Baden, Germany Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, Germany
1:30 A.annua extract for the treatment of spontaneous tumors and dogs and cats

This indicates that adjuvant iron-triggered A.annua impacts the overall survival rates of dogs suffering from sarcoma and improves the treatment success over that of surgery alone.
An interesting result was that artemisinin represented only a minor constituent in A.annua and that scopoletin was the most abundant phytochemical. It is well known that A.annua contains a large panel of different secondary metabolites, including scopoletin, which are cytotoxic [5, 57, 76–79]. The activity of the A.annua preparation in pet tumors and the presence of large amounts of scopoletin raise the question of whether scopoletin might contribute to the anticancer effect of this plant rather than artemisinin. The in vitro cytotoxicity of scopoletin towards cancer cells has been previously described [80–82].

Verterinary Artemsinin

73) LupArte – Artemisia Annua Extrakt 30:1
Einzelfuttermittel mit Zusatzstoff für Hunde und Katzen
pro 450 mg- Kapsel: Aromastoff 450 mg konzentrierten Artemisia Annua Pulverextrakt (30:1), prebiotisches Inulin aus Artischockenherzen (50 mg)

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Targeting progenitor cells Parthenolide (Feverfew)

74) Guzman, Monica L., et al. "The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells." Blood 105.11 (2005): 4163-4169. Recent studies have described malignant stem cells as central to the initiation, growth, and potential relapse of acute and chronic myelogenous leukemia (AML and CML). Because of their important role in pathogenesis, rare and biologically distinct leukemia stem cells (LSCs) represent a critical target for therapeutic intervention. However, to date, very few agents have been shown to directly target the LSC population. The present studies demonstrate that **parthenolide** (PTL), a naturally occurring small molecule, induces robust apoptosis in primary human AML cells and blast crisis CML (bcCML) cells while sparing normal hematopoietic cells. Furthermore, analysis of progenitor cells using in vitro colony assays, as well as stem cells using the nonobese diabetic/severe combined immunodeficient (NOD/SCID) xenograft model, show that PTL also preferentially targets AML progenitor and stem cell populations. Notably, in comparison to the standard chemotherapy drug cytosine arabinoside (Ara-C), PTL is much more specific to leukemia cells. **The molecular mechanism of PTL-mediated apoptosis is strongly associated with inhibition of nuclear factor κ B (NF-κB), proapoptotic activation of p53, and increased reactive oxygen species (ROS).** On the basis of these findings, we propose that the activity of PTL triggers LSC-specific apoptosis and as such represents a potentially important new class of drugs for LSC-targeted therapy.

Parthenolide (PTL) is a sesquiterpene lactone found as the major active component in Feverfew (Tanacetum parthenium), an herbal medicine that has been used to treat migraine and rheumatoid arthritis for centuries.12 More recently, PTL has been found to have several other properties, including antitumor activity, inhibition of DNA synthesis, and inhibition of cell proliferation in different cancer cell lines.13-16 In addition, PTL sensitizes cancer cells to other antitumor agents17-20 and acts as a chemopreventive agent in a UVB-induced skin cancer animal model.21 PTL is a potent inhibitor of NF-κB activation and has been shown to directly bind IκB-kinase (IKK)22,23 and to modify the p50 and p65 NF-κB subunits.24,25 PTL can also block signal transducers and activators of transcription 3 (STAT3) phosphorylation on Tyr705,26 sustain c-Jun N-terminal kinase (JNK) activation,17,18 and increase intracellular reactive oxygen species (ROS).13,27

Targeting cancer stem cells
75) Naujokat, Cord, and Roman Steinhart. "Salinomycin as a Drug for Targeting Human Cancer Stem Cells."

Journal of Biomedicine and Biotechnology 2012 (2012).

In particular, the biomolecules **salinomycin and parthenolide** as well as the biguanide **metformin** have been demonstrated to induce apoptosis in various types of human cancer cells [108, 123, 124], suggesting that these compounds may contribute to the eradication of cancer more effectively than compounds targeting either CSCs or regular cancer cells. Moreover, the **ionophore antibiotic salinomycin** seems to have even extended capabilities of eliminating cancer (Table 1), because this compound has been demonstrated to effectively target regular cancer cells [16, 125–127], highly multidrug and apoptosis-resistant cancer cells [16, 85, 125], and CSCs [16, 84, 87, 88, 128–131].

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synergy artemisinin with butyrate

76) Anticancer Res. 2005 Nov-Dec;25(6B):4325-31.
Synergistic cytotoxicity **of artemisinin and sodium butyrate** on human cancer cells.Singh NP1, Lai HC. Butyric acid is a short chain fatty acid produced by large bowel bacterial flora. It serves as an antiinflammatory agent and nutrient for normal colon cells. Butyric acid has also been shown to induce apoptosis in colon and many other cancer cells. Artemisinin is a compound extracted from the wormwood Artemisia annua L. It has been shown to selectively kill cancer cells in vitro and to be effective in treating animal and human cancer. We and others have found that the artemisinin analog, dihydroartemisinin (DHA), kills cancer cells by apoptosis. In the present study, the efficacy of a combined treatment of DHA and butyric acid at low doses in killing cancer cells was investigated.
MATERIALS AND METHODS:Molt-4 cells (a human **lymphoblastoid leukemia cell** line) and freshly isolated human lymphocytes, cultured in complete RPMI-1640 medium, were first incubated with 12 microM of human holotransferrin at 37 degrees C in a humid atmosphere of 5% CO2 for one hour to enhance the iron concentration in the cells. Cells from each cell type were then divided into 20 flasks. These flasks were grouped into four sets of five cultures each. Zero, 5, 10 or 20 microM of DHA was added, respectively, to these sets and the cells were incubated at 37 degrees C for one hour. Zero, 1, 5, 10, or 20 mM of sodium butyrate was then added to the five cultures of each set, respectively. Thus, the treatments involved a combination of 4 doses of DHA and 5 doses of sodium butyrate. The cells were counted immediately before the addition of DHA, and at 24 and 48 hours after the addition of sodium butyrate.
RESULTS:DHA alone at the 24-hour time-point and 20 microM concentration significantly reduced the number of Molt-4 cells in the culture by approximately 40% ($p < 0.001$, compared to non-treated control), whereas it did not significantly affect the number of normal human lymphocytes. Similarly, 1 mM sodium butyrate alone at 24 hours reduced the number of Molt-4 cells by approximately 32% ($p < 0.001$, compared to non-treated control), without significantly affecting normal human lymphocytes. **The combination of 20 microM DHA and 1 mM sodium butyrate killed all Molt-4 cells at the 24-hour time-point and did not significantly affect lymphocytes. CONCLUSION:DHA in combination with butyric acid acts synergistically at low doses. The combination may provide a less toxic, inexpensive and effective cancer chemotherapy.**

The oral intake of artemisinin and its analogs (7, 43) and butyric acid (44) is safe. Oral administration of the artemisinin analogs, artesunate (100 mg in a healthy adult) resulted in micromolar plasma concentration of DHA (45). The basal plasma concentration of butyric acid is normally >1 ÌM (42). Oral or intravenous administration of sodium butyrate (50 mg/kg) has been shown to increase the plasma butyrate concentration to the millimolar range in rats (46).
The artemisinin-butyrate combination may work well, particularly for colon cancer where butyrate concentrations can be raised by simple oral probiotic intake of butyric acid producing Lactobacilli.

Artemisinin Synergy with Rituximab anti-CD-20 Ab

77) Sieber, Sebastian, et al. "Combination treatment of malignant B cells using the anti-CD20 antibody **rituximab** and the anti-malarial **artesunate**." International journal of oncology 35.1 (2009): 149-158. Combination treatment of malignant B cells Rituximab and Artesunate Sieber Sebastian Int j oncology 2009

Chemotherapy of non-Hodgkin's lymphoma is frequently hampered by drug resistance. The monoclonal antibody rituximab specifically targets the CD20 antigen and sensitizes B-cell lymphoma cells to standard anticancer drugs. In the present investigation, we analyzed, whether a combination of rituximab and artesunate may act in a complementary manner and eventually synergize in tumor cell killing. Artesunate is an anti-malarial drug, which also exerts profound activity towards cancer cells. While rituximab alone was minimally cytotoxic, rituximab increased cytotoxicity to artesunate in Ramos cells. Artesunate induced apoptosis, induced Fas/CD95 expression and the formation of reactive oxygen species (ROS) and resulted in a breakdown of mitochondrial membrane potential. This argues for the involvement of both receptor-driven extrinsic and mitochondrial intrinsic routes of apoptosis. Rituximab increased Fas/CD95 expression and ROS formation and decreased mitochondrial membrane potential ultimately leading to increased apoptosis induced by artesunate. The transcription factors YY1 and Sp1 are upstream regulators of apoptosis by controlling the expression of apoptosis-regulating genes. YY1 and Sp1 were down-regulated and Fas/CD95 was up-regulated by rituximab and artesunate indicating that artesunate activated the Fas/CD95 pathway and that rituximab increased the susceptibility of tumor cells to artesunate-induced apoptosis. Furthermore, rituximab affected the expression of antioxidant genes. The antibody decreased artesunate-induced up-regulation of catalase expression and increased artesunate-induced down-regulation of glutathione S-transferase-phi expression. Manganese-dependent superoxide dismutase expression was not changed by artesunate. Antioxidant proteins may help to detoxify artesunate-induced ROS. Rituximab reversed the artesunate-induced expression changes of antioxidant genes and, hence, reduced the detoxification capacity of Ramos cells. The effects of rituximab on antioxidant genes represent a novel mechanism of rituximab for chemosensitization.

Lymphoma and Myeloma Highly Sensitive to Artemisinin

78) Eur J Haematol. 2013 Oct;91(4):339-46. Lymphoma and myeloma cells are highly sensitive to growth arrest and apoptosis induced by artesunate.
Holien T1, Olsen OE, Misund K, Hella H, Waage A, Rø TB, Sundan A.

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 IC50 values of artesunate in cell lines varied between 0.3 and 16.6 μm**. Furthermore, some primary myeloma cells were also sensitive to artesunate at doses around 10 μm. 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.

Use of Vitamins interacting with Artemisinin

Vitamin C, Vit D, H202 potentiates artemisinin leukemia cell death.
NOT vit E. though.

79) Anticancer Res. 2015 Apr;35(4):1867-71. Effects of antioxidants and pro-oxidants on cytotoxicity of dihydroartemisinin to Molt-4 human leukemia cells. Gerhardt T1, Jones R1, Park J1, Lu R1, Chan HW1, Fang Q1, Singh N2, Lai H1. Antioxidants and Pro oxidants cytotoxicity of dihydroartemisinin to leukemia cells Gerhardt Anticancer Res 2015
The objective of the present study was to investigate how oxidative status influences the effectiveness of cytotoxicity of artemisinin towards cancer cells. It is hypothesized that antioxidants would reduce, whereas pro-oxidants would enhance, cytotoxicity.
MATERIALS AND METHODS:Molt-4 human leukemia cells were incubated with **vitamins C, E, D3, dexamethasone, or hydrogen peroxide** alone or in combination with dihydroartemisinin (DHA). Concentrations of these compounds studied were similar to those achievable by oral administration. Viable cell counts were performed before (0 h) and at, 24 and 48 h after treatment.
RESULTS:Vitamin C, vitamin D3, dexamethasone, and H2O2 caused significant Molt-4 cell death. Vitamin E caused an increase in Molt-4 cell growth. **Vitamin C and vitamin D3 significantly interacted with DHA at the 48-h time point and with H2O2 at both 24-h and 48-h time points.**
CONCLUSION:Cellular oxidative status could alter the potency of artemisinin in killing cancer cells.

80) Hamacher-Brady, Anne, et al. “Artesunate Activates Mitochondrial Apoptosis in Breast Cancer Cells via Iron-catalyzed Lysosomal Reactive Oxygen Species Production.” J. Biol. Chem 2011.286 (2010): 6587-6601. Artesunate Activates Mitochondrial Apoptosis in Breast Cancer Cells via Iron-catalyzed Lysosomal Reactive Oxygen Species Production J Biol Chem 2011 Hamacher Brady

We thus investigated the role of lysosomes in ART-induced PCD and determined that in MCF-7 breast cancer cells ART activates lysosome-dependent mitochondrial outer membrane permeabilization. ART impacted endolysosomal and autophagosomal compartments, inhibiting autophagosome turnover and causing perinuclear clustering of autophagosomes, early and late endosomes, and lysosomes. **Lysosomal iron chelation blocked all measured parameters of ART-induced PCD, whereas lysosomal iron loading enhanced death,** thus identifying lysosomal iron as the lethal source of reactive oxygen species upstream of mitochondrial outer membrane permeabilization

Moreover, **lysosomal inhibitors chloroquine and bafilomycin A1 reduced ART-activated PCD**, evidencing a requirement for lysosomal function during PCD signaling. **CQ (chloroquin) resulted in enhanced vesicle size and LTR intensity, consistent with previously reported CQ-induced increase in lysosomal activities** (44, 45).

The malaria parasite digests iron-rich hemoglobin in its acidic food vacuole, and the interaction of ART with hemederived iron results in lethal ROS generation (15). **The parasite food vacuole is analogous to eukaryotic lysosomes**, organelles that constitute a major site of intracellular degradation via hydrolytic enzymes. Therefore, we investigated the role of lysosomal iron in ART-activated ROS production and PCD in MCF-7 cells by employing the **iron chelator deferoxamine mesylate (DFO)**. DFO specifically targets lysosomal iron as it enters the cell via endocytosis and accumulates in the lysosome (37).

In combination with ART, DFO decreased ART-triggered ROS production, with a significant difference for ART at 20 . g/ml. Moreover, at 48 h **DFO significantly decreased ART-induced cell death** to the levels of DFO alone at ART concentrations of both 10 and 20 □g/ml.

Endolysosomal Iron Loading Enhances ART-triggered PCD
We employed diferric holotransferrin (HTF), which is actively internalized by receptor-mediated endocytosis; during transit through endolysosomes, bound iron is released due to low pH environment (18)

HTF (holotransferrin) co-treatment with both 10 and 20 □g/ml ART **significantly increased cell death** at 24 h and at 48 h (Fig. 2C). The ensemble of findings given above indicates that lysosomal free iron serves as the major source of ROS and thereby is a critical prerequisite during ART-mediated cell death in MCF-7 breast cancer cells. In response to both ART 10 g/ml and ART 20 □g/ml, **AVs were clustered to the perinuclear region** (Fig. 3A, arrows). DFO (iron chelator) treatment prevented AV clustering in response to ART.

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81) SEE (6) Yang, Nai-Di, et al. “Artesunate induces cell death in human cancer cells via enhancing lysosomal function and lysosomal degradation of ferritin.” Journal of Biological Chemistry 289.48 (2014): 33425-33441. full free
Here, we utilized a blue fluorescence-tagged ART to investigate its localization, and our results clearly showed that ART accumulates in lysosome.

The addition of antioxidant **N-acetylcysteine (NAC)**, which has been shown to directly react with hydrogen peroxide and superoxide (40), was able to **inhibit** the production of ROS induced by ART

As expected, both **NAC and BAF (Baflinomycin)** significantly protected ART-induced cell death (Fig. 6E).

In summary, these data indicate that lysosomal iron plays a critical role in ART-induced lysosomal activation, mitochondrial ROS production, and cell death. These results thus indicate that **ART-induced ferritin degradation is mainly performed by the lysosomes**.

Altogether, these data suggest that it is not the lysosomes per se required for ART-induced cell death, but **delivery and degradation of ferritin in lysosomes is required for ART-induced cell death**.

First, ART accumulates in the lysosomes (Fig. 2); second, ART increases lysosomal acidification, cathepsin enzyme activity, and protein degradation via promoting lysosomal V-ATPase assembly . At present, the exact mechanism for the lysosomal accumulation by ART is not known. ART may increase lysosomal V-ATPase activity via promoting the assembly status of the two domains.

we believe that this perinuclear clustering of lysosomes is, in fact, an indication of autophagy induction.
the endoperoxide bridge of ART is broken by ferrous iron, and then ART becomes free radicals to target cellular organelles, such as mitochondria, and eventually leads to cell death

In fact, we also found that **chloroquine inhibited the ferritin degradation and cell death induced by ART as efficiently as BAF (data not shown).**

In summary, we demonstrate a novel mechanism underlying ART-induced cancer cell death. As shown in Fig. 9E, **ART accumulates in lysosomes and reacts with lysosomal iron. The activated ART then promotes**

lysosomal function via enhancing V-ATPase assembly. One major source of iron for the cytotoxicity of ART is attained from the degradation of ferritin by lysosomes. In conclusion, we propose a new model of action on the mechanism of ART-induced cell death by focusing on lysosomal activation and ferritin degradation. At present, there is evidence demonstrating **higher iron levels in cancer cells than in non-cancerous cells**, and iron has been targeted for cancer therapy and prevention (69). Therefore, the results from our study support the development of this important anti-malaria drug as a cancer therapeutic agent.

82) Lai, Henry, et al. “Effects of artemisinin-tagged holotransferrin on cancer cells.” Life sciences 76.11 (2005): 1267-1279.

83) Int J Pharm. 2008 Apr 16;354(1-2):28-33. Epub 2007 Sep 6.
Anticancer properties of artemisinin derivatives and their targeted delivery by transferrin conjugation. Nakase I1, Lai H, Singh NP, Sasaki T.
Artemisinin and its derivatives are well known antimalaria drugs and particularly useful for the treatment of infection of Plasmodium falciparum malaria parasites resistant to traditional antimalarials. Artemisinin has an endoperoxide bridge that is activated by intraparasitic heme-iron to form free radicals, which kill malaria parasites by alkylating biomolecules. In recent years, there are many reports of anticancer activities of artemisinins both in vitro and in vivo. Artemisinins have inhibitory effects on cancer cell growth, including many drug- and radiation-resistant cancer cell lines. The cytotoxic effect of artemisinin is specific to cancer cells because most cancer cells express a high concentration of transferrin receptors on cell surface and have higher iron ion influx than normal cells via transferrin mechanism. In addition, some artemisinin analogs have been shown to have antiangiogenesis activity. Artemisinin tagged to transferrin via carbohydrate chain has also been shown to have high potency and specificity against cancer cells. The conjugation enables targeted delivery of artemisinin into cancer cells. In this review, we discuss the anticancer activities and mechanisms of action of artemisinins and the transferrin-conjugate.

mitochondrial outer membrane permeabilization (MOMP).'[

84) Lai, Henry, et al. “Artemisinin-transferrin conjugate retards growth of breast tumors in the rat.” Anticancer research 29.10 (2009): 3807-3810. Artemisinin can retard cancer growth but not completely stop or reduce the growth. This is shown by the data of this experiment on breast cancer and on fibrosarcoma in rats reported previously by us (18). In most studies on the effect of artemisinin analogs on tumor growth in animals, an approximately 20-60% reduction in growth was generally observed in colorectal carcinoma xenografts (19), hepatoma xenograft (20), ovarian cancer (21), and HL-60 human leukemia xenograft (22). Exceptions are a study by Willoughby et al. (23) in which a complete elimination of prostate cancer xenograft in mice was reported after treatment with artemisinin, and that of Wang et al. (24) in which no significant effect on the growth of implanted Lewis lung cancer in mice was found with artemisinin treatment. **Once the tumor is established, artemisinin is not very effective in reversing the progress. However, artemisinin is effective in the prevention of cancer when the target cells are still small in number.**

85) Mariani, A., et al. “Iron-dependent lysosomal dysfunction mediated by a natural product hybrid.” Chemical Communications 52.7 (2016): 1358-1360.

Artesumycin is a fluorescent hybrid of the natural products marmycin A and artemisinin. It was designed to combine the lysosomotropic properties of the angucycline and the iron-reactive capacity of the endoperoxide to target the lysosomal compartment of cancer cells. Herein, we show that artesumycin inhibits cancer cell proliferation in an iron-dependent manner and chemically fragments in vitro in the presence of redox-active iron(II). Visual detection of artesumycin by fluorescence microscopy provided substantial evidence that the **small**

molecule selectively targets lysosomes. This original approach based on a fluorescent and iron-reactive probe represents a powerful strategy for initiating and, concomitantly, visualizing lysosomal dysfunction in human cells.

(86) Yu, Zuoren, et al. "Cancer stem cells." The international journal of biochemistry & cell biology 44.12 (2012): 2144-2151.

87) Yu, Zuoren, et al. "Cancer stem cells." The international journal of biochemistry & cell biology 44.12 (2012): 2144-2151.

88) Pardal, Ricardo, Michael F. Clarke, and Sean J. Morrison. "APPLYING THE PRINCIPLES OF STEM-CELL BIOLOGY TO CANCER." NATURE REVIEWS| CANCER 3 (2003): 895. APPLYING THE PRINCIPLES OF STEM-CELL BIOLOGY TO CANCER. Pardal 2003

(89) Hsieh, Hsiu-Mei, et al. "Berberine-containing pharmaceutical composition for inhibiting cancer stem cell growth or carcinoma metastasis and application thereof." U.S. Patent Application No. 14/790,154.

90) Moselhy, J., et al. "Natural Products That Target Cancer Stem Cells." Anticancer research 35.11 (2015): 5773.
Epigallocatechin-3-gallate (EGCG) – Green Tea
6-Gingerol – Ginger
β-Carotene – Carrot, Leafy Greens
Baicalein – Chinese Skullcap
Curcumin – Turmeric
Cyclopamine – Corn Lilly
Delphinidin – Blueberry, raspberrry
Flavonoids (Genistein) – Soy, red clover, coffee
Gossypol – Cottonseed
Guggulsterone – Commiphora (myrrh tree)
Isothiocyanates – Cruciferous vegetables
Linalool – Mint
Lycopene – Grapefruit, tomato
Parthenolide – Feverfew
Perylill alcohol – Mint, cherry, lavender
Piperine – Black pepper
Placycodon saponin – Playycodon grandifloruim
Psoralidin – Psoralea corylilyfolia
Quercetin – Capers, onion
Resveratrol – Grapes, plums, berries
Salinomycin – Streptomyces albus
Silibinin – Milk Thistle
Ursolic acid – Thyme, basil, **oregano**
Vitamin D3 – Fish, egg yolk, beef, cod liver oil
Withaferin A – Withania somnifera (ashwaganda)

91) Naujokat, Cord, and Roman Steinhart. "Salinomycin as a drug for targeting human cancer stem cells." BioMed Research International 2012 (2012). Salinomycin as a drug for targeting human cancer stem cells 2012 Naujokat Cord Steinhart

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Feverfew Parthenolide

92) Ghantous, Akram, et al. “What made sesquiterpene lactones reach cancer clinical trials?.” What made sesquiterpene lactones reach cancer clinical trials Ghantous Akram 2010. Drug discovery today 15.15 (2010): 668-678.
Medicinal plants, rich in SLs and having anti-inflammatory properties, are a potential source of NFkB inhibitors [48]. **Parthenolide and artemisinin are established NF-kB inhibitors and render cancer cells sensitive to chemotherapy**
[49,55]. Parthenolide was found to directly modify the NF-kB p65 subunit [58] or to suppress the activity of the upstream IkB kinase complex leading to the stabilization of the NF-kB inhibitors, IkBa and IkBb [59]. The nucleophilic attack by parthenolide occurs through a-methylene-g-lactone ring and epoxide and epoxide moieties that target specific nucleophiles but not others [60].

Parthenolide is the only small molecule, to date, that has been reported to selectively target several cancer and cancer stem cells while sparing normal counterparts [28,38,69]. NF-kB signaling is elevated in leukemic stem cells but not in the normal hematopoietic stem cells. Parthenolide caused robust apoptosis of primary AML cells and **effectively eradicated AML stem and progenitor cells in vitro without affecting hematopoietic stem cells** [28,69]. The mechanism of action of parthenolide was found to be through the **inhibition of NF-kB, the activation of proapoptotic p53 and an increase in reactive oxygen species** [69]. Gleevec or **Cytarabine, treatments commonly used for the most aggressive leukemias, are not as effective as parthenolide because they are not as specific.**
Promising antileukemic drugs should mediate cell death and inhibit leukemic stemcell- specific activity, and parthenolide fulfills these criteria

Parthenolide Phase I trial – No blood levels found ??

93) Invest New Drugs. 2004 Aug;22(3):299-305.
Phase I dose escalation trial of feverfew with standardized doses of parthenolide in patients with cancer. Curry EA 3rd1, Murry DJ, Yoder C, Fife K, Armstrong V, Nakshatri H, O’Connell M, Sweeney CJ.

Feverfew is a botanical product that contains parthenolide. Parthenolide has in vitro and in vivo anti-tumor and anti-angiogenic activity. Feverfew has been used extensively without any formal pharmacokinetic analysis. A Phase I trial was conducted to evaluate the pharmacokinetics and toxicity of parthenolide given as a component of “feverfew.”
PATIENTS AND METHODS:Feverfew (Tanacet trade mark) was administered as a daily oral tablet in a 28-day cycle. A starting dose of 1 mg per day was explored with subsequent dose escalations to 2, 3, and 4 mg. Assessment of plasma pharmacokinetics was performed on patients accrued to the trial. Solid phase extraction and mass spectroscopy were used to evaluate parthenolide plasma concentrations. The limit of detection for parthenolide in plasma was 0.5 ng/ml. Patients were evaluated for response after every two cycles.
RESULTS:Feverfew given on this schedule had no significant toxicity, and the maximum tolerated dose was not reached. When parthenolide was administered at doses up to **4 mg as a daily oral capsule in the feverfew preparation**, there was **not detectable concentration in the plasma.** Because of this, parthenolide pharmacokinetics were not able to be completed.
CONCLUSION:Feverfew, with up to 4 mg of parthenolide, given daily as an oral tablet is well tolerated without dose- limiting toxicity, but does not provide detectable plasma concentrations. Purification of parthenolide for administration of higher doses will be needed.

94) full pdf
GUNN, ELLEN J., et al. “The natural products parthenolide and andrographolide exhibit anti-cancer stem cell activity in multiple myeloma.” The natural products parthenolide and andrographolide exhibit anti-cancer stem cell activity in multiple myeloma Ellen Gunn 2011. Leukemia & Lymphoma 52.6 (2011): 1085-1097.
Abstract Multiple myeloma (MM) is an incurable plasma cell malignancy where **nearly all patients succumb to a relapse**. The current preclinical models of MM target the plasma cells, constituting the bulk of the tumor, **leaving the cancer stem cells to trigger a relapse.** Utilizing a three-dimensional tissue culture system where cells were grown in extracellular matrix designed to reconstruct human bone marrow, we tested the anti-multiple myeloma cancer stem cell (MM-CSC) potential of two natural product inhibitors of nuclear factor kB (NFkB). Here we show that **parthenolide** and andrographolide are potent anti-MMCSC
agents. Both natural products demonstrated preferential toxicity toward MM-CSCs over non-tumorigenic MM cells. Addition of the bone marrow stromal compartment abrogated andrographolide activity while having no effect on parthenolide cytoxicity. This is the first report of a natural product with anti-CSC activity in myeloma, suggesting that it has the potential to improve the survival of patients with MM by eliminating the relapse-causing MM-CSCs.

Crooks PA

95) Bioorg Med Chem Lett. 2009 Aug 1;19(15):4346-9. .
Aminoparthenolides as novel anti-leukemic agents: Discovery of the NF-kappaB inhibitor, **DMAPT** (LC-1).
Neelakantan S1, Nasim S, Guzman ML, Jordan CT, **Crooks PA.**
A series of aminoparthenolide analogs (6-37) were synthesized and evaluated for their anti-leukemic activity. Eight compounds exhibited good anti-leukemic activity with LD(50)’s in the **low microM range** (1.5-3.0microM). Compounds 16, 24 and 30 were the most potent compounds in the series, causing greater than **90% cell death at 10microM concentration against primary AML cells in culture**, with LD(50) values of 1.7, 1.8 and 1.6microM.

96) Zhang, Quan, et al. “Guaianolide sesquiterpene lactones, a source to discover agents that selectively inhibit acute myelogenous leukemia stem and progenitor cells.” Journal of medicinal chemistry 55.20 (2012): 8757-8769.

97) Shanmugam, Rajasubramaniam, et al. “A water soluble parthenolide analog suppresses in vivo tumor growth of two tobacco-associated cancers, lung and bladder cancer, by targeting NF-κB and generating reactive oxygen species.”

98) International Journal of Cancer 128.10 (2011): 2481-2494.
Karmakar, A., et al. “Nanodelivery of parthenolide using functionalized nanographene enhances its anticancer activity.” RSC advances 5.4 (2015): 2411-2420.

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full free
99) J Biol Chem. 2013 November 22; 288(47): 33542–33558.
Targeting Aberrant Glutathione Metabolism to Eradicate Human Acute Myelogenous Leukemia Cells* Shanshan Pei,‡

Our data indicate that CD34+ AML cells have **elevated expression of multiple glutathione pathway regulatory proteins**, presumably as a mechanism to compensate for increased oxidative stress in leukemic cells. Consistent with this observation, CD34+ AML cells have lower levels of reduced glutathione and increased levels of oxidized glutathione compared with normal CD34+ cells. These findings led us to hypothesize that AML cells will be

hypersensitive to inhibition of glutathione metabolism. To test this premise, we identified compounds such as **parthenolide (PTL**) or piperlongumine that **induce almost complete glutathione depletion and severe cell death** in CD34+ AML cells.

Importantly, these compounds only induce limited and transient glutathione depletion as well as significantly less toxicity in normal CD34+ cells. We further determined that PTL perturbs glutathione homeostasis by a multifactorial mechanism, which includes inhibiting key glutathione metabolic enzymes (GCLC and GPX1), as well as **direct depletion of glutathione**. These findings demonstrate that primitive leukemia cells are uniquely sensitive to agents that target aberrant glutathione metabolism, an intrinsic property of primary human AML cells. Our findings indicate agents such as **parthenolide** (PTL) and piperlongumine (PLM) have a dramatic inhibitory effect on the leukemic glutathione system, whereas **only a limited and transient perturbation in normal cells.** This preferential effect is strongly linked to their selective toxicity toward leukemia and other cancer cell types. Importantly, we have previously shown that PTL effectively eradicates AML stem and progenitor populations (11), cells that are typically resistant/refractory to conventional chemotherapy (12, 13).

Thus, we propose that therapeutic targeting of glutathione metabolism represents a potentially powerful strategy to induce selective toxicity toward a broad range of primary leukemia cells, including malignant stem and progenitor populations.

We first studied PTL, which contains an **active α,β-unsaturated-γ-lactone group (Fig. 3B, red circular area) that should readily react with the free thiol group of glutathione**. Indeed, **PTL induced a dose-dependent decrease of cellular glutathione within 2 h of treatment in primary AML cells**

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Mantle cells have high glutathione transferase

100) Bennaceur-Griscelli, Annelise, et al. “High level of glutathione-S-transferase π expression in mantle cell lymphomas.” Clinical cancer research 10.9 (2004): 3029-3034.

The GST-π gene in located at 11q13 and is coamplified with the cyclin D1 gene (in the same amplicon) in some human solid tumors (breast carcinoma, squamous cell lung carcinoma, bladder carcinomas, and esophageal carcinoma; Ref. 19). The unusual resistance to chemotherapy in MCL compared with other lymphoma entities GST-π overexpression has been associated with resistance to alkylating agents and anthracyclines. **A high GST-π expression (>50% cells stained) was observed in the 24 cases of MCL**

MCL is one of the most chemotherapy-drug-resistant lymphomas.

The 10 patients who received sequential CHOP and cytarabine-based chemotherapy at diagnosis did not reach complete remission after CHOP alone but entered into complete remission after high-dose cytarabine (38) . The modest role of the glutathione system in cytarabine resistance compared with alkylating and anthracyclin resistance could be an explanation for the high complete remission rate observed after high-dose cytarabine therapy in MCL (7 , 39)

101) Zhou, Jianbiao, and Wee-Joo Chng. “Identification and targeting leukemia stem cells:The path to the cure for acute myeloid leukemia.” World J Stem Cells 6.4 (2014): 473-484. Identification and targeting leukemia stem cellsThe path to the cure for acute myeloid leukemia Zhou 2014

full pdf
102) FOLIA HISTOCHEMICA ET CYTOBIOLOGICA Vol. 46, No. 2, 2008
pp. 129-135. Molecular basis of parthenolide-dependent proapoptotic activity in cancer cells Beata Paj¹k1, Arkadiusz Orzechowski1,2, Barbara Gajkowska1
1Department of Cell Ultrastructure, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
2Department Molecular basis of parthenolide-dependent proapoptotic activity in cancer cells Beata Pajak 2008

Saadane et al. [32] found that the inhibition of IKKβ resulted in stabilization of cytoplasmic IKKα, which in turn leads to inhibition of NF-κB translocation. In contrast, Hehner et al. [28] speculated that PN interferes with IKC component that is necessary for the sequential transmission of signals leading to the activation of both IKKs. Since the IKC is non-functional in the absence of NK- κB essential modulator/IKKγ, this latter protein was the potential candidate for the inhibitory activity of parthenolide. Moreover, in all cases, the PN administration blocked DNA-binding of NF-κB. Consequently, **lack of NF-κB activity render cancer cells prone to undergo apoptosis** or became sensitized to cytokine- and anti-cancer drug-induced cell death [33-36].

PATENT

103) Use of parthenolide derivatives as antileukemic and cytotoxic agents
US 20150259356 A1
Publication number US20150259356 A1
Publication type Application
Application number US 14/727,126
Publication date Sep 17, 2015
Filing date Jun 1, 2015
Priority date Jul 11, 2003
Also published as US7678904, 9 More »
Inventors Peter A. Crooks, Craig T. Jordan, Xiaochen Wei
Original Assignee University Of Kentucky

104) Zhou, Jianbiao, Ying Qing Ching, and Wee-Joo Chng. “Aberrant nuclear factor-kappa B activity in acute myeloid Leukemia: from molecular pathogenesis to therapeutic target.” Oncotarget 6.8 (2015): 5490.

105) Planta Med. 2003 Nov;69(11):1009-12.
Transport of parthenolide across human intestinal cells (Caco-2).
Khan SI1, Abourashed EA, Khan IA, Walker LA.
This study examined the intestinal epithelial membrane transport of the sesquiterpene lactone parthenolide, a bioactive compound present in the migraine prophylactic herb feverfew. The Caco-2 human colonic cell line was used as an in vitro model of the human intestinal mucosal barrier. The bidirectional transport (apical to basolateral and basolateral to apical) of parthenolide was investigated using Caco-2 monolayers grown on Transwell inserts.

Quantitation of parthenolide was performed using high performance liquid chromatography (HPLC). Apical to basolateral and basolateral to apical permeability coefficients and percent transport were calculated and a potential bioavailability of parthenolide was determined. Sodium fluorescein was used as a marker for paracellular leakage. Parthenolide, at a concentration of 250 microM, demonstrated substantial linear transport across the monolayer. The transport parameters were not affected by the presence of MK-571, an inhibitor of multidrug resistance transporter P-glycoprotein (MRP). Upon comparison of the transport parameters of parthenolide with atenolol under identical conditions and the reported values for model compounds like mannitol and propranolol, it

is concluded that parthenolide is effectively absorbed through the intestinal mucosa via a passive diffusion mechanism.

FeverFew Inhibits Platelets

106) Folia Haematol Int Mag Klin Morphol Blutforsch. 1988;115(4):447-9.
Inhibition of platelet behaviour by feverfew: a mechanism of action involving sulphydryl groups. Heptinstall S1, Groenewegen WA, Spangenberg P, Lösche W.
Extracts of feverfew inhibit platelet aggregation and the platelet release reaction. The active components are believed to be sesquiterpene lactones such as parthenolide. Evidence is presented that inhibition of platelet behaviour is via neutralization of sulphydryl groups either inside or outside the cell. The precise nature of the sulphydryl groups that are susceptible to feverfew and are involved in platelet aggregation and the release reaction have not yet been defined.

107)
DNC NEWS: **Feverfew, Stem cells and the treatment of Cancer**
June 29, 2005 Subject: Research on the common herb feverfew suggests it may play an important role in the treatment of Cancer.

PATENT

108) Stabilized feverfew formulations
WO 2005082393 A1
patent 4% feverfew
http://www.google.com/patents/WO2005082393A1?cl=en
A Feverfew extract containing 4% parthenolide is available from Galilee Herbal Remedies and as OptiPure Brand (Chemco

Industries, Inc.) as “PharmaFew”. [009] The present invention more advantageously provides a “stabilized” Feverfew extract having at least about 4% parthenolide by weight of the total weight of the extract. Roy Bouskila, Soft Gel Technologies Inc, Ronald G Udell
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Sulforaphane

109) Sestili, Piero, and Carmela Fimognari. “Cytotoxic and Antitumor Activity of Sulforaphane: The Role of Reactive Oxygen Species.” BioMed Research International 2015 (2015).
SFR is passively absorbed by cells, where it is rapidly conjugated with glutathione (GSH) by glutathione S-transferases (GSTs).
However, after dietary consumption, SFR levels in humans are lower and closer to 3 μM
SFR administered orally protects against animal carcinogenesis and induces antiproliferative effects in human tumor cells in xenograft models.
A second important antitumor mechanism is the ability of SFR to block cell proliferation and induce apoptosis of cancer cells, thus reducing tumor growth.

depletes glutathione

it is important noting that cells treated with high doses of SFR undergo a situation of increased ROS sensitivity since a peculiar capacity of the isothiocyanate consists in depleting the GSH cellular pool [58, 59] (Figure 1), an effect which is particularly severe with high, supranutritional SFR concentrations. Indeed, depletion of GSH deprives cells of a first line, soluble antioxidant defense [53, 60, 61], giving rise to a “vicious oxidative cycle” (ROS production in cells which at the same time are being depleted in GSH) which is indirectly demonstrated by the fact that N-acetylcysteine (NAC) supplementation enhanced cell survival opposing to GSH depletion [57, 58] rather than acting itself as a mere, direct antioxidant.
loss of mitochondrial transmembrane potential, release of cytochrome C, and mitochondrial damage are in effect induced by SFR
A selective toxicity of SFR to cancer cells has been demonstrated in different experimental models.

Leukemia Lymphoma

110) Fimognari C., Turrini E., Sestili P., et al. Antileukemic activity of sulforaphane in primary blasts from patients affected by myelo- and lympho-proliferative disorders and in hypoxic conditions. PLoS ONE. 2014;9(7)
Sulforaphane caused a dose-dependent induction of apoptosis in blasts from patients diagnosed with acute lymphoblastic or myeloid leukemia.
We found that synthesized SR caused a dose-dependent induction of apoptosis in acute leukemia cell lines and primary lymphoblasts from patients diagnosed with B-ALL, T-ALL, and AML.
SR controlled the expansion of leukemic cells
As to the concentrations of SR used in this study, they could be not achievable from food supply. However, after a single oral dose of SR at 150 μmol/kg in rat, plasma concentrations of SR equivalents increase to 15.2 μM [46].

111) Shang, Hung-Sheng, et al. “Sulforaphane-induced apoptosis in human leukemia HL-60 cells through extrinsic and intrinsic signal pathways and altering associated genes expression assayed by cDNA microarray.” Environmental toxicology (2016).

Breast Cancer

112) Li, Yanyan, et al. “Sulforaphane, a dietary component of broccoli/broccoli sprouts, inhibits breast cancer stem cells.” Clinical Cancer Research 16.9 (2010): 2580-2590.
A NOD/SCID xenograft model was employed to determine whether sulforaphane could target breast CSCs in vivo, Sulforaphane eliminated breast CSCs in vivo, thereby abrogating tumor growth after re-implantation of primary tumor cells into the secondary mice
These findings support the use of sulforaphane for chemoprevention of breast cancer stem cells and warrant further clinical evaluation.

Breast Cencer CAncer stem cells in vitro and in vivo xenograft mouse model

113) Kallifatidis G, Rausch V, Baumann B, et al. Sulforaphane targets pancreatic tumour-initiating cells by NF-kappaB-induced antiapoptotic signalling. Gut. 2009;58:949–63. [PubMed]
An interesting observation is that sulforaphane was able to inhibit stem/progenitor cells at the concentrations (0.5~5 μM) that hardly affected the bulk population of cancer cells, implying that sulforaphane is likely to preferentially target stem/progenitor cells compared to the differentiated cancer cells.
These results suggest that sulforaphane was able to eliminate breast CSCs in primary xenografts, thereby abrogating the re-growth of tumors in secondary mice. Taken together with the in vivo Aldefluor assay results, these findings suggest that **sulforaphane targets breast CSCs with high potency.**
sulforaphane decreased the protein level of β-catenin by up to 85% in MCF7 and SUM159 cells; and the

expression of cyclin D1, one of the Wnt/β-catenin target genes, declined by up to 77% as well
As a chemoprevention agent, sulforaphane possesses many advantages, such as high bioavailability and low toxicity
A recent pilot study detected an accumulation of sulforaphane in human breast tissue, with 1.45 ± 1.12 pmol/mg for the right breast and 2.00 ± 1.95 pmol/mg for the left, in eight women who consumed broccoli sprout preparation containing 200 μmol sulforaphane about 1 hr before the surgery (36). These concentrations of sulforaphane are expected to be effective against breast CSCs, based on our in vitro results

114) Li, Y., and T. Zhang. "Targeting cancer stem cells with sulforaphane, a dietary component from broccoli and broccoli sprouts." Future oncology (London, England) 9.8 (2013): 1097-1103.

115) Wu, S., et al. "Sulforaphane produces antidepressant-and anxiolytic-like effects in adult mice." Behavioural brain research 301 (2015): 55.

Sulfasalazine – Mantle Cell Lymphoma

116) Bebb, G., et al. "Sulfasalazine, inhibits growth of mantle cell lymphoma (MCL) cell cultures via cyst (e) ine starvation and delays tumour growth in a newly developed murine MCL model." BLOOD. Vol. 102. No. 11. 1900 M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA: AMER SOC HEMATOLOGY, 2003.

Introduction: Cyst(e)ine deficiency within lymphoid cells leads to a rapid decline in their levels of glutathione (a major free radical scavenger), loss of defense against oxidative stress, and subsequently apoptosis. Lymphoid cells cannot synthesize the amino acid and depend for growth and viability on its uptake from their micro-environment. Since lymphomas have been shown to retain the inability to synthesize cyst(e)ine they are potentially susceptible to cyst(e)ine starvation-based therapy. We have previously demonstrated that sulfasalazine (SASP), a drug used for treatment of severe inflammatory bowel disease and rheumatoid arthritis, is a potent inhibitor of the cystine/glutamate antiporter, xc-, a major plasma membrane cystine transporter. **SASP abrogated growth of T and B lymphoma cell cultures via cystine starvation; SASP, administered intraperitoneally, markedly inhibited growth of rat Nb2-U17 lymphoma transplants in Nb rats without major toxicity to the hosts** (Anti-Cancer Drugs 14:21, 2003). In the present study we investigated the usefulness of SASP in our newly developed **model of MCL, a B-cell non-Hodgkin lymphoma (NHL), characterized by cyclin D1 and BCL2 over-expression.** Results: Growth of human MCL cultures in Fischer's medium, supplemented with 10% fetal bovine serum and antibiotics, was markedly inhibited by SASP at therapeutic concentrations, showing IC50s of 0.13 and 0.30 for Z138C and Granta MCL cultures, respectively. Culture growth arrest could be largely prevented by enhancing cellular cystine uptake using 66 uM 2-mercaptoethanol (reported to promote cystine uptake via the leucine transporter), indicating that the SASP-induced inhibition resulted from cyst(e)ine starvation. A study into the efficacy of SASP in vivo was initiated using SCID/Rag2-M mice injected subcutaneously with Z138C cells (5 million cells/mouse); such a procedure leads to consistent development of tumours within 28 days. When tumours had reached a weight of about 0.1 gr, groups of six mice were treated for 10 consecutive days with saline (controls) or SASP (250 mg/kg body weight i.p., b.i.d.), a dosage well below the maximally tolerated dosage (300 mg/kg every 8 hr). It was found that treatment with SASP inhibited tumour growth, showing a delay in growth of at least 7 days, without major toxicity. Conclusions: **SASP has a marked inhibitory effect on growth of MCL cell lines in vitro, an effect also seen in vivo in our murine SC MCL model.** SASP may represent a novel approach for MCL treatment. The precise molecular consequences of SASP treatment on MCL cells warrant further investigation. Additional studies on the effect of SASP at higher dosages and in combination with cyclophosphamide and targeted therapies, eg. ASO and monoclonal antibodies against bcl-2, are in progress.

Lymphoma

117) Gout, Peter W., Chris R. Simms, and May C. Robertson. "In vitro studies on the lymphoma growth-inhibitory activity of sulfasalazine." Anti-cancer drugs 14.1 (2003): 21-29.
Sulfasalazine (SASP) is a novel, potent inhibitor of cellular cystine uptake mediated by the x(c)- cystine/glutamate antiporter. Lymphoid cells cannot synthesize cyst(e)ine and depend for growth on its uptake from their micro-environment. We previously showed that SASP (0.2 mM) can abrogate lymphoma cell proliferation in vitro by specifically inhibiting x(c)- -mediated cystine uptake. Intraperitoneal administration of SASP to Noble rats markedly suppressed Nb2-U17 rat lymphoma transplant growth, notably without major toxicity to the hosts. Since Nb2-U17 cells are x(c)- -deficient, the growth arrest was apparently not due to SASP-tumor cell interaction, but possibly to interference with x(c)- -mediated cysteine secretion by somatic cells. In this study we found that replication of x(c)- -deficient Nb2-11 lymphoma cells can be sustained in vitro, in the absence of cystine uptake enhancers, by co-culturing with IMR-90 fibroblasts known to secrete cysteine. SASP, at 0.15 and 0.2 mM, arrested replication of fibroblast-driven Nb2-11 cells by 93 and 100%, respectively, without impeding fibroblast proliferation. Addition of 2-mercapto-ethanol (60 microM), a cystine uptake enhancer, almost completely prevented this growth arrest, indicating that SASP specifically inhibited cysteine secretion by the fibroblasts, a process based on x(c)- -mediated cystine uptake. It is proposed that the lymphoma growth-inhibitory activity of SASP in vivo involves inhibition of cysteine secretion by tumor-associated somatic cells (macrophages, dendritic cells), leading to cysteine starvation of the tumor cells and apoptosis. The difference between the lymphoma cells and fibroblasts in sensitivity to SASP treatment is consistent with the marked antitumor effect of SASP lacking significant side effects.
Small cell Lung cancer full pdf

118) Guan, Jun, et al. "The x c− cystine/glutamate antiporter as a potential therapeutic target for small-cell lung cancer: use of sulfasalazine." Cancer chemotherapy and pharmacology 64.3 (2009): 463-472.
Conclusions The xc- cystine/glutamate antiporter is
potentially useful as a target for therapy of SCLC based on
glutathione depletion. Sulfasalazine may be readily used
for this approach, especially in combination chemotherapy.

pancreatic full pdf

119) Lo, M., et al. "Potential use of the anti-inflammatory drug, sulfasalazine, for targeted therapy of pancreatic cancer." Current Oncology 17.3 (2010): 9-16.

Breast

120) Narang, Vishal S., et al. "Suppression of cystine uptake by sulfasalazine inhibits proliferation of human mammary carcinoma cells." Anticancer research 23.6C (2002): 4571-4579.

Prostate

121) Doxsee, Daniel W., et al. "Sulfasalazine-induced cystine starvation: Potential use for prostate cancer therapy." The Prostate 67.2 (2007): 162-171.

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Butyrate

2016

122) Zhang, J., et al. "Sodium Butyrate Induces Endoplasmic Reticulum Stress and Autophagy in Colorectal Cells: Implications for Apoptosis." PloS one 11.1 (2016): e0147218.

2007

123) Pajak, B., A. Orzechowski, and B. Gajkowska. "Molecular basis of sodium butyrate-dependent proapoptotic activity in cancer cells." Advances in Medical Sciences (De Gruyter Open) 52 (2007).
This review outlines the molecular events that accompany the antitumor action of sodium butyrate (NaBt). Butyrate, alow-molecular weight **four-carbon chain volatile fatty acid** (VFA) has been previously shown to withdraw cells from cell cycle or to promote cell differentiation, and finally to induce programmed cell death. Recent advances in molecular biology indicate, that **this product of large bowel microbial fermentation of dietary fiber**, might evoke the above-mentioned effects by indirect action on genes. NaBt was shown to **inhibit histone deacetylase activity, allowing DNA binding of several transcription factors**. Higher genomic activity leads to the higher expression of **proapoptotic genes**, higher level of their protein products and elevated sensitivity to death ligand-induced apoptosis. Cancer cells might be arrested in G1 phase of cell cycle in a p21-dependent manner. Proapoptotic activity of NaBt includes higher expression of membrane death receptors (DR4/5), higher level and activation of Smad3 protein in TGF-beta-dependent apoptotic pathway, lower level of antiapoptotic proteins (cFLIP, XIAP) and activation of proapoptotic tBid protein. Thus, both intrinsic and extrinsic apoptotic pathways are stimulated to ampify the apoptotic signals. These effects are specific for tumor but not for regular cells. Unique properties of NaBt make this agent a promising metabolic inhibitor to retard tumorigenesis to suppress tumor growth.

full pdf

Inhibits Telormerase Activity

124) Choi, Yung Hyun. "Apoptosis of U937 human leukemic cells by sodium butyrate is associated with inhibition of telomerase activity." International journal of oncology 29.5 (2006): 1207-1213.

Sodium butyrate as a histone deacetylase inhibitor. Exposure of U937 cells to sodium butyrate resulted in growth inhibition and induction of apoptosis in a dose-dependent manner.
The increase in apoptosis was associated with the up-regulation in pro-apoptotic Bax expression, and down-regulation of anti-apoptotic Bcl-2 and Bcl-XL.

sodium butyrate markedly inhibited the activity of telomerase and the expression of human telomerase reverse transcriptase (hTERT), a main determinant of the telomerase enzymatic activity, was progressively downregulated by sodium butyrate.

In addition to the deregulation of apoptosis, it is increasingly clear that the process of neoplasia is characterized by the activation of telomerase that adds telomeric repeats to the ends of replicating chromosomes, telomeres

Critically short telomeres are suggested to cause irreversible cell growth arrest and cellular senescence.

telomerase reactivation is a rate-limiting step in cellular immortality and carcinogenesis, and telomerase repression can act as a tumor-suppressive mechanism.

Sodium butyrate is a short chain fatty acid normally produced as a result of bacterial fermentation of fiber in mammalian intestines, representing one of the end products of carbohydrate breakdown

the ratio between the level of proapoptotic Bax and that of the anti-apoptotic factor Bcl-2 determines whether a cell responds to an apoptotic signal.

there was a concentration-dependent down-regulation of Bcl-2 members such as Bcl-2 and Bcl-XL in sodium butyrate-treated U937 cells, but the levels of Bax were up-regulated, resulting in an increase in the ratio of Bax/Bcl-2 and/or Bcl-XL.

In conclusion, our results indicated that **sodium butyrate potently suppresses the proliferation of U937 human leukemic cells by inducting apoptosis through an increase of Bax expression and activation of caspase-3. The growth inhibitory effects of sodium butyrate were also associated with a specific inhibition of hTERT expression and telomerase activity.**

Lauric Acid – coconut oil

2013 full pdf

125) Fauser, J. K., et al. “Induction of apoptosis by the medium-chain length fatty acid **lauric acid** in colon cancer cells due to induction of oxidative stress.” Chemotherapy 59.3 (2013): 214-224.

Lauric acid, as a component of triglycerides, comprises about half of the fatty acid content in **coconut milk, coconut oil,** laurel oil, and palm kernel oil. Compared to butyrate, lauric acid displayed preferential antineoplastic properties, including induction of apoptosis in a CRC cell line.

The SCFA butyrate has demonstrated the capacity to induce apoptosis by: **inhibiting histone deacetylase activity, inducing cell cycle arrest, promoting differentiation, activating NF-κB, downregulating α 2 β 1 , modifying glucose availability, and inducing caspase activation in CRC cell lines** [1, 2, 9–12] .

126) Jurečeková, Jana, et al. “Targeting of Bcl-2 family proteins for treatment of acute leukaemia.” General physiology and biophysics 30 (2011): S3-S12.

127) Fauser, Jane Kathryn. Medium Chain Fatty Acids and Wnt/β-Catenin Inhibitors as Adjunctive Colorectal Cancer Chemotherapeutic Agents. Diss. The University of Adelaide, 2012.

128) Nohara, Kazunari, Yoshiko Yokoyama, and Kazutaka Kano. “The Important Role of Caspase-10 in Sodium Butyrate.” Kobe J. Med. Sci 53.5 (2007): 265-273.

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Ivermectin

2015

129) Draganov, Dobrin, et al. “Modulation of P2X4/P2X7/pannexin-1 sensitivity to extracellular ATP via ivermectin induces a non-apoptotic and inflammatory form of cancer cell death.” Scientific reports 5 (2015).

We found that Ivermectin kills mouse and human triple-negative breast cancer (TNBC) cells through augmented P2X7-dependent purinergic signaling associated with caspase-1 and caspase-3 activation.
FIg 7 Model of P2X4/P2X7/Pannexin-1-induced cancer cell death.
Ivermectin induces P2X4/P2X7-dependent activation of Pannexin-1 channels and release of ATP. The release of ATP might be transiently protective, but only in cell types that are highly sensitive to Ivermectin-induces cell swelling when ATP and Ca2+ signaling are essential for control of cell volume. In cancer cells where no cell size changes can be observed (for example human TNBC MDA-MB-231 cells), high concentrations of ATP (1–3 mM) immediately enhance Ivermectin cytotoxicity. Potentiated P2X7 receptor signaling drives a fast progressing necrotic/pyroptotic mechanism driven by NADPH oxidases-generated ROS, cytosolic Ca2+/CaMKII activation, and MPTP, and characterized by caspase-1 cleavage, due to possible NLRP3 inflammasome activation. Necrotic killing is followed by a slower progressing apoptotic cell death program mediated by caspase-3 activation. The failure of the default apoptotic pathway might be attributed to faster activation of caspase-1, inadequate autophagic control of mitochondrial MPTP, collapse of cellular energy metabolism, resulting in rapid progression of necrotic cell death. Damage to mitochondria and ER stress as well as potential depletion of cellular ATP reserves simultaneously promote autophagy that might render even the slower apoptotic pathway immunogenic.

2014 full free

130) Melotti, Alice, et al. "The river blindness drug Ivermectin and related macrocyclic lactones inhibit WNT-TCF pathway responses in human cancer." EMBO molecular medicine (2014): e201404084.
as a therapeutic WNT-TCF pathway response blocker to treat WNT-TCF-dependent diseases including multiple cancers.

We find that macrocyclic lactones of the Avermectin family have specific anti-WNT-TCF response activity in human cancer cells and that the clinically approved compound Ivermectin (EMEA- and FDA-approved) is a specific WNT-TCF response blocker at low micromolar concentrations.

cancer stem cells
Pre-treatment with Ivermectin and Selamectin inhibits colon cancer stem cell self-renewal in clonogenic spheroid assays
These results suggest an action on both the bulk of the tumor and its cancer stem cells.

Moreover, they might also be useful as routine prophylactic agents, for instance against nascent TCF-dependent intestinal tumors in patients with familial polyposis and against nascent sporadic colon tumors in the general aging population.

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131) O'neill, Paul M., Victoria E. Barton, and Stephen A. Ward. "The molecular mechanism of action of artemisinin—the debate continues." *Molecules* 15.3 (2010): 1705-1721. Molecular Mechanism of Action of Artemisinine Debate ONeill 2010

132) *Robert, Anne, Jérôme Cazelles, and Bernard Meunier. "Characterization of the alkylation product of heme by the antimalarial drug artemisinin." Angewandte* Chemie International Edition 40.10 (2001): 1954-1957.

133) full free pdf
Mathur, Rohit, et al. "Targeting Wnt pathway in mantle cell lymphoma-initiating cells." Journal of hematology & oncology 8.1 (2015): 63. Targeting Wnt pathway in mantle cell lymphoma initiating cells Mathur 2015

134) Li, Yanyan, et al. "Implications of cancer stem cell theory for cancer chemoprevention by natural dietary compounds." The Journal of nutritional biochemistry 22.9 (2011): 799-806.

135) Rodova, Mariana, et al. "Sonic hedgehog signaling inhibition provides opportunities for targeted therapy by sulforaphane in regulating pancreatic cancer stem cell self-renewal." PloS one 7.9 (2012): e46083.

Given the requirement for Hedgehog in pancreatic cancer, we investigated whether hedgehog blockade by SFN could target the stem cell population in pancreatic cancer. In an in vitro model, human pancreatic CSCs derived spheres were significantly inhibited on treatment with SFN, suggesting the clonogenic depletion of the CSCs. Interestingly, SFN inhibited the components of Shh pathway and Gli transcriptional activity. Interference of Shh-Gli signaling significantly blocked SFN-induced inhibitory effects demonstrating the requirement of an active pathway for the growth of pancreatic CSCs. SFN also inhibited downstream targets of Gli transcription by suppressing the expression of pluripotency maintaining factors (Nanog and Oct-4) as well as PDGFRα and Cyclin D1. Furthermore, SFN induced apoptosis by inhibition of BCL-2 and activation of caspases. Our data reveal the essential role of Shh-Gli signaling in controlling the characteristics of pancreatic CSCs. We propose that pancreatic cancer preventative effects of SFN may result from inhibition of the Shh pathway. Thus Sulforaphane potentially represents an inexpensive, safe and effective alternative for the management of pancreatic cancer.

HDAC Inhibitors in Mantel Cell

136) Heider, Ulrike, et al. "Histone deacetylase inhibitors reduce VEGF production and induce growth suppression and apoptosis in human mantle cell lymphoma." European journal of haematology 76.1 (2006): 42-50.

Three MCL cell lines (JeKo-1, Hbl-2 and Granta-519) were exposed to different concentrations of the HDAC inhibitors **sodium butyrate** (NaB) and suberoylanilide hydroxamic acid vorinostat (SAHA) for 8-72 h.

Mantle cell lymphoma (MCL) is an incurable disease with an aggressive course and novel treatment strategies are urgently needed. The purpose of this study was to evaluate the effects of histone deacetylase (HDAC) inhibitors, a new group of antiproliferative agents, on human MCL cells.
METHODS: Three MCL cell lines (JeKo-1, Hbl-2 and Granta-519) were exposed to different concentrations of the HDAC inhibitors sodium butyrate (NaB) and suberoylanilide hydroxamic acid (SAHA) for 8-72 h. Their effects on cell viability, apoptosis induction and cell cycle proliferation were studied. Moreover, the influence of SAHA on the expression of cyclin D1, the cell cycle regulators p21 and p27 and the production of vascular endothelial growth factor (VEGF) were analyzed.
RESULTS: The HDAC inhibitors induced accumulation of acetylated histones in MCL cells. **MTT assays and Annexin-V staining showed that they potently inhibited viability in a dose-dependent manner and induced apoptosis in all cell lines tested. Cell cycle analysis indicated that their exposure to SAHA or NaB decreased the proportion of cells in S phase and increased the proportion of cells in the G0/G1 and/or G2/M phases. Incubation with the two HDAC inhibitors resulted in downregulation of cyclin D1.** SAHA lead to an upregulation of p21 in all cell lines and an upregulation of p27 in JeKo-1 and Granta-519 cells, while expression of p27 in Hbl-2 was not altered. In addition, SAHA inhibited the production of the angiogenic cytokine VEGF. Treatment with NaB increased the expression of p21 in JeKo-1 and Hbl-2 cells, while in Granta 519 cells no effect was noted. The expression of p27 remained constant in all three cell lines after exposure to NaB.
CONCLUSION: Based on these findings, we provide evidence that HDAC inhibitors have antiproliferative effects in MCL and may represent a promising therapeutic approach.

137) Ivermectin pharmacology and therapeutic applications Sunita Chhaiya 2012 Chhaiya, Sunita B., et al. "IJBCP International Journal of Basic & Clinical Pharmacology." International Journal 2.6 (2013): 799. Ivermectin pharmacology and therapeutic applications Sunita Chhaiya 2012

Pediculosis head lice dosage- Two 200 mcg/kg tablets a week apart. (12 mg tablet for a 60 kg male).

138) NEW ZEALAND DATA SHEET STROMECTOL ivermectin 3 mg tablet 2011 **NEW ZEALAND DATA SHEET STROMECTOL ivermectin 3 mg tablet 2011**

Berberine – Berberine inhibits WNT Pathways

139) Biofactors. 2013 Nov-Dec;39(6):652-62. Berberine acts as a natural inhibitor of Wnt/β-catenin signaling–identification of more active 13-arylalkyl derivatives. Albring KF1, Weidemüller J, Mittag S, Weiske J, Friedrich K, Geroni MC, Lombardi P, Huber O.

Aberrant activation of the canonical Wnt/β-catenin signaling pathway has been reported for numerous tumors of different origins. **In most cases, mutations in components of the Wnt signaling pathway or in β-catenin itself were detected which ultimately induce a genetic program that promotes cell proliferation and attenuates apoptosis.** Thus, targeting of Wnt/β-catenin signaling is of specific therapeutic interest. As a result of berberine treatment, cellular levels of active β-catenin were reduced concomitant with an increase in the expression of E-cadherin.

140) Eur J Pharmacol. 2014 Oct 5;740:584-95. Targets and mechanisms of berberine, a natural drug with potential to treat cancer with special focus on breast cancer.Jabbarzadeh Kaboli P1, Rahmat A2, Ismail P3, Ling KH4.

Berberine was shown to be effective in inhibiting cell proliferation and promoting apoptosis invarious cancerous cells. Some signaling pathways affected by berberine, including the MAP (mitogen-activated protein) kinase and Wnt/β-catenin pathways, are critical for reducing cellular migration and sensitivity to various growth factors.

Epiphany Against cancer

full free pdf
141) Guamán Ortiz, Luis Miguel, et al. “Berberine, an epiphany against cancer.” Molecules 19.8 (2014): 12349-12367.

BBR treatment promotes cell cycle arrest and death in human cancer cell lines, coupled to an increased expression of apoptotic factors

BBR functions as an inhibitor of the telomere elongation by blocking the telomerase activity through formation of a G-quadruplex with telomeric DNA
BBR has the potential to modulate and regulate Wnt/β-catenin pathway [56], which in normal cells is inactivated by ubiquitination and subsequent degradation of the β-catenin protein,
BBR was proved to alter the mitochondrial membrane potential
(MMP), inhibit mitochondrial respiration leading to mitochondrial dysfunction and regulate the expression of Bcl-2 family members, as Mcl-1 [45,47]. Alterations in mitochondrial membrane stimulate the release of cytochrome c promoting the formation of reactive oxygen species (ROS) that trigger apoptosis that requires the activation of caspases and poly(ADP-ribose) polymerase-1 (PARP-1)
cleavage

Silibinin – Inhibits WNT pathway

full free pdf
142) Tiwari, Prabha, and K. P. Mishra. “Silibinin in cancer therapy: A promising prospect.” Cancer Research Frontiers 1.3 (2015): 303-318.

Silibinin also **inhibited Wnt/β-catenin signaling** by suppressing Wnt co-receptor LRP6 expression in human breast cancer cells MDA-MB-231 and T-47D

Exogenous SOD markedly enhanced silibinin-induced apoptosis

clinical trials for the treatment of hepatotoxicity in childhood acute lymphoblastic leukemia (ALL). Silymarin (The target dose of silibinin was 5.1 mg/kg/day) was administered orally for 28 days and it significantly reduced liver toxicity in children with ALL (74). Recently a new silibinin drug formulation Legasil® administration improved hepatic failure due to extensive liver infiltration in a breast cancer patient (75).
A Phase II Study to Assess Efficacy of Combined Treatment with Erlotinib (Tarceva) and Silybin-phytosome (**Siliphos**) in Patients with EGFR mutant lung adenocarcinoma is going on (ClinicalTrials.gov Identifier:

Silibinin has also shown promising results against cancer stem cells, supporting further development of anti-cancer therapeutics that target tumor stem cells.

143) Siegel, Abby B., et al. “A phase I dose-finding study of silybin phosphatidylcholine (milk thistle) in patients with advanced hepatocellular carcinoma.” Integrative cancer therapies (2013): 1534735413490798.

Targeting Cancer Stem Cells – Pterostilbene

144) Wu, Chi-Hao, et al. “Targeting cancer stem cells in breast cancer: potential anticancer properties of 6-shogaol and pterostilbene.” Journal of agricultural and food chemistry 63.9 (2015): 2432-2441.

Breast cancer stem cells (BCSCs) constitute a small fraction of the primary tumor that can self-renew and become a drug-resistant cell population, thus limiting the treatment effects of chemotherapeutic drugs. The present study evaluated the cytotoxic effects of five phytochemicals including 6-gingerol (6-G), 6-shogaol (6-S), 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone (5-HF), nobiletin (NOL), and **pterostilbene (PTE) on MCF-7 breast cancer cells and BCSCs**. The results showed that 6-G, 6-S, and **PTE selectively killed BCSCs and had high sensitivity for BCSCs isolated from MCF-7 cells that expressed the surface antigen CD44(+)/CD24(-).** 6-S and PTE induced cell necrosis phenomena such as membrane injury and bleb formation in BCSCs and inhibited mammosphere formation. In addition, 6-S and PTE increased the sensitivity of isolated BCSCs to chemotherapeutic drugs and significantly increased the anticancer activity of paclitaxel. Analysis of the underlying mechanism showed that 6-S and PTE decreased the expression of the surface antigen CD44 on BCSCs and promoted **β-catenin phosphorylation through the inhibition of hedgehog/Akt/GSK3β signaling, thus decreasing the protein expression of downstream c-Myc and cyclin D1 and reducing BCSC stemness.**

human lung adenocarcinoma

145) Yang, Yang, et al. “Pterostilbene exerts antitumor activity via the Notch1 signaling pathway in human lung adenocarcinoma cells.” PloS one 8.5 (2013): e62652.

antitumor activity of PTE against human lung adenocarcinoma in vitro and in vivo and explored the role of the Notch1 signaling pathway

PTE treatment resulted in a dose- and time-dependent decrease in the viability of A549 cells.

reduced mitochondrial membrane potential (MMP) and a decreased intracellular glutathione content but also by increases in the apoptotic index and the level of reactive oxygen species (ROS).

Activation of the Notch1 Intracellular Domain (NICD) protein and activated Hes1. DAPT (a gamma secretase inhibitor) and Notch1 siRNA prevented the induction of NICD and Hes1 activation by PTE treatment and sensitized the cells to PTE treatment.

Down-regulation of Notch signaling also prevented the activation of pro-survival pathways (most notably the PI3K/Akt pathway) after PTE treatment.

Chloroquin and Pterostilbene – autophagy inhibitor potentiated pterostilbene effect

146) Papandreou, Ioanna, et al. "Plant stilbenes induce endoplasmic reticulum stress and their anti-cancer activity can be enhanced by inhibitors of autophagy." Experimental cell research 339.1 (2015): 147-153.

We performed a screen of 1726 small, drug like molecules to identify those that could activate an ER-stress responsive luciferase gene. After secondary screening, we determined that the **plant stilbenes pterostilbene and piceatannol were the most potent inducers of ER stress from this group.** ER stress can be particularly toxic to cells with high ER load, so we examined their effect on cells expressing the Wnt family of secreted glycoprotein growth factors. Molecular analysis determined that these **ER stress-inducing stilbenes could block Wnt processing and also induce autophagy in acute lymphoblastic leukemia cells expressing Wnt16**. **Combining pterostilbene (to induce ER stress) with chloroquine (to inhibit autophagy) lead to significant cellular toxicity in cells from aggressive acute lymphoblastic leukemia.**
CONCLUSIONS: Plant stilbenes are potent inducers of ER stress. However, their toxicity is more pronounced in cancer cells expressing Wnt growth factors. **The toxicity of stilbenes in these ALL cells can be potentiated by the addition of autophagy inhibitors, suggesting a possible therapeutic application.**

147) **vorinostat HDAC Inhibitors**

Sakajiri, Sakura, et al. "Histone deacetylase inhibitors profoundly decrease proliferation of human lymphoid cancer cell lines." Experimental hematology 33.1 (2005): 53-61.

Methylation of tumor suppressor genes is frequently observed in human cancers. These genes are silenced by histone deacetylase (HDAC) recruited by methylated DNA in their promoter regions. HDAC removes acetyl groups from histones and prevents the basic transcriptional machinary access to the target gene, leading to transcriptional repression. HDAC inhibitors (HDACIs) can restore the expression of the tumor suppressor and/or cell cycle regulatory genes in cancer cells and block the cellular proliferation of these cells. In this study, we investigated the in vitro antiproliferative activities of the HDACIs, suberoylanilide hydroxamic acid (SAHA), and valproic acid against 14 human lymphoid cancer cell lines. All of these cell lines were sensitive to the antiproliferative effects of the HDACI. SAHA induced either G1 or G2-M arrest as well as apoptosis. SAHA downregulated cyclin D1 and D2, and upregulated p53, p21, and p27. Chromatin immunoprecipitation analysis revealed a remarkable increase in the level of acetylated histones associated with the p21 promoter after SAHA treatment. In nude mice, SAHA significantly inhibited growth of a mantle cell lymphoma without major toxic side effects. In summary, HDACIs are promising therapeutic agents for human lymphoid cancers.
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HerbalZym

148) Modulation of β-catenin signaling by natural agents induces apoptotic cell death in many common cancers, including colon cancer, breast cancer and prostate cancer. Part 1

149) PART TWO

Berberine reduces cellular levels of active β-catenin and inhibits β-catenin transcriptional activity. **Silymarin, honokiol, lupeol, genistein, gugulipid, resveratrol, quercetin, EGCG, lycopene, sulforaphane, ellagic acid, Vitamin D, and omega-3-polyunsaturated fatty acids also antagonize or modulate Wnt/β-catenin signaling**. We believe that these natural agents that are non-toxic in nature could be used either alone or in combination with conventional therapeutics for the prevention and/or treatment of a variety of cancers.

Parthenolide

150) Bioavailable Parthenolide Histone Deacetylase (HDAC) Inhibitor, Part 1

151) Bioavailable Parthenolide Histone Deacetylase (HDAC) Inhibitor, Part 2

Ivermectin Effective Ovarian Cancer

152) Hashimoto, Hisashi, et al. "Ivermectin inactivates the kinase PAK1 and blocks the PAK1-dependent growth of human ovarian cancer and NF2 tumor cell lines." Drug discoveries & therapeutics 3.6 (2009). Ivermectin inactivates blocks kinase PAK1 Ovarian Cancer Hashimoto 2009

153) Efferth – Artemisinin artemisinin-second-career-as-anticancer-drug-2016-world-j-tradit-chin-med-2015-efferth-thomas

Efferth, Thomas. "Artemisinin–Second Career as Anticancer Drug?." 2016 World J Tradit Chin Med 2015; 1(4): 2–25
Artemisinin We and others found that this sesquiterpenoid also exerts profound anticancer activity in vitro and in vivo. Artemisinin-type drugs exert multi-factorial cellular and molecular actions in cancer cells. Ferrous iron reacts with artemisinin, which leads to the formation of reactive oxygen species and ultimately to a plethora anticancer effects of artemisinins, e.g. on and non-homologous end-joining, as well as different modes of cell death (intrinsic and extrinsic apoptosis,autophagy, necrosis, necroptosis, oncosis, and ferroptosis). Furthermore, artemisinins inhibit neoangiogenesis in tumors. The signaling of major transcription factors (NF-κB, MYC/MAX, AP-1, CREBP, mTOR etc.) and signaling pathways are affected by artemisinins (e.g. **Wnt/ β-catenin pathway,** AMPK pathway, metastatic pathways, nitric oxide signaling, and others). Several case reports on the compassionate use of artemisinins as well as clinical Phase I/II pilot studies indicate the clinical activity of artemisinins in veterinary and human cancer patients. Larger scale of Phase II and III clinical studies are required now to further develop artemisinin-type compounds as novel anticancer drugs.

Artemisinin as Cancer Stem Cell Agent

154) Oncotarget. 2016 May 24; 7(21): 31413–31428.
Artemisinin and its derivatives can significantly inhibit lung tumorigenesis and tumor metastasis through Wnt/β-catenin signaling

Yunli Tong,#1,2 Yuting Liu,#1,2 Hongming Zheng,2 Liang Zheng,1,2 Wenqin Liu,1,2 Jinjun Wu,2 Rilan Ou,2 Guiyu Zhang,2 Fangyuan Li,2 Ming Hu,2,3 Zhongqiu Liu,1,2 and Linlin Lu2

our findings revealed that ART, DHA, and ARTS could suppress lung-tumor progression by inhibiting **Wnt/β-catenin pathway**, thereby suggesting a novel target for ART, DHA, and ARTS in cancer treatment.

ART, DHA, and ARTS **decreased the expression of these CSCs** markers in lung cancer cells, even in tumor tissues in vivo (Figure (Figure6D),6D), suggesting an inhibition of these compounds on CSCs pluripotency.

155) Cao, Liu, et al. "Dihydroartemisinin exhibits **anti-glioma stem cell activity** through inhibiting p-AKT and activating caspase-3." Die Pharmazie-An International Journal of Pharmaceutical Sciences 69.10 (2014): 752-758. Dihydroartemisinin anti-glioma stem cell activity Cao Liu Die Pharmazie 2014.

We hypothesized that DHA has potential to inhibit glioma CSC and/or other CSC growth. However, there are no studies on the growth inhibition effect of DHA on glioma CSCs and/or other CSCs or on the underlying mechanism of such activity. In this report, we studied the effects of DHA on GL261 GSCs. We found that DHA could significantly influence the morphology of the GSC sphere sin a dose-dependent manner (Fig. 2C), suggesting that **DHA might exert an inhibition effect on the growth of as well as induce the apoptosis of GSCs.**

DHA thus presents a novel therapeutic agent for **targeting GSCs** in the treatment of glioma.

156) Kast, Richard E., Georg Karpel-Massler, and Marc-Eric Halatsch. "CUSP9* treatment protocol for recurrent glioblastoma: aprepitant, **artesunate,** auranofin, captopril, celecoxib, disulfiram, itraconazole, ritonavir, sertraline augmenting continuous low dose temozolomide." Oncotarget 5.18 (2014): 8052.

ART WNT Pathway

157) Li, Lin-Na, et al. "Artesunate attenuates the growth of human colorectal carcinoma and inhibits hyperactive Wnt/β-catenin pathway." International journal of cancer 121.6 (2007): 1360-1365.

Artesunate (ART), a remarkable antimalarial agent, also inhibited the growth of human colorectal carcinoma. As determined by MTT assay, flow cytometry analysis on apoptosis and indirect immunofluorescence analysis on the proliferation-associated marker Ki67, ART suppressed the proliferation and promoted the apoptosis of colorectal cancer cells in a dose-dependent manner. Furthermore, **immunofluorescence analysis on β-catenin and RT-PCR analysis on Wnt/β-catenin target genes demonstrated ART translocated β-catenin from nucleus to adherent junctions of membrane and reduced transcription mediated by β-catenin.** These results suggested the anticancer activity of ART correlated with the **inhibition of hyperactive Wnt/β-catenin** signaling pathway. In vivo, ART significantly slowed the growth of colorectal tumor xenografts. Bioluminescent imaging also revealed that ART decreased the physiological activity of tumor xenografts and delayed spontaneous liver metastasis. *These antitumor effects were related to the membranous translocation of β-catenin and the inhibition of the unrestricted activation of Wnt/β-catenin pathway*, which was confirmed by the immunohistochemical staining of tumor tissues. These results and the known low toxicity are clues that ART might be a promising candidate drug for the treatment of colorectal carcinoma

Artesunate attenuates the growth of human colorectal carcinoma and inhibits hyperactive Wnt/β-catenin pathway

In vitro, ART treatment **strongly inhibited the hyperactive Wnt/β-catenin pathway** and significantly promoted the apoptosis of CLY cells. In vivo, ART not only inhibited the volumetric development of tumor xenografts but also

attenuated their physiological activity. These results and the known low toxicity are clues that ART might be a promising candidate drug for the treatment of colorectal carcinoma.

pdf

158) Xu, H., et al. "Anti-malarial agent artesunate inhibits TNF-α-induced production of proinflammatory cytokines via **inhibition of NF-κB and PI3 kinase/Akt signal pathway** in human rheumatoid arthritis fibroblast-like synoviocytes." (2007): 920-926.

In this study, we explored the effect of artesunate, an artemisinin derivative, **on tumour necrosis factor (TNF)-α-induced production of interleukins, IL-1β, IL-6 and IL-8, in human rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS),** and further investigated the signal mechanism by which this compound modulates those cytokines' production.

Methods. RA FLS obtained from patients with active RA were stimulated with **TNF-α and incubated with artesunate, and IL-1β, IL-6 and IL-8 production was measured by ELISA**. DNA-binding activity and nuclear **translocation of nuclear factor kappa B (NF-κB)** were measured by a sensitive multi-well colourimetric assay and confocal fluorescence microscopy, respectively. Signal transduction proteins expression was measured by western blot.

Results. **Artesunate decreased the secretion of IL-1β, IL-6 and IL-8 from TNF-α-stimulated RA FLS in a dose-dependent manner**. **Artesunate also prevented TNF-α-induced nuclear NF-κB translocation,** DNA-binding activity and gene transcriptional activity, as well as phosphorylation and degradation of IκBα, but phosphorylation of p38 mitogen-activated protein kinase, extracelluar signal-regulated kinase and c-Jun N-terminal kinase were unaffected. The production of IL-1β, IL-6 and IL-8 induced by TNF-α was decreased by pyrrolidine dithiocarbamate (PDTC), a chemical inhibitor of NF-κB. These observations suggest that artesunate inhibits production of IL-1β, IL-6 and IL-8 through inhibition of NF-κB signalling pathway. **We also showed that artesunate prevented Akt phosphorylation.** TNF-α-induced production of IL-1β, IL-6 and IL-8 was hampered by treatment with the phosphatidylinositol 3 (PI3) kinase inhibitor LY294002, suggesting that inhibition of Akt activation might inhibit IL-1β, IL-6 and IL-8 production induced by TNF-α. Conclusions. Our results indicate that artesunate exerts an anti-inflammatory effect in RA FLS and provide the evidence that artesunate may have therapeutic potential for RA.

159) Wang, Shuang-Jia, et al. "Dihydroartemisinin inactivates NF-κB and potentiates the anti-tumor effect of gemcitabine on pancreatic cancer both in vitro and in vivo." Cancer letters 293.1 (2010): 99-108.

Gemcitabine is currently the best known chemotherapeutic option available for pancreatic cancer, but the tumor returns de novo with acquired resistance over time, which becomes a major issue for all gemcitabine-related chemotherapies. In this study, for the first time, we demonstrated that dihydroartemisinin (DHA) enhances gemcitabine-induced growth inhibition and apoptosis in both BxPC-3 and PANC-1 cell lines in vitro. The mechanism is at least partially due to **DHA deactivates gemcitabine-induced NF-κB activation, so as to decrease tremendously the expression of its target gene products, such as c-myc, cyclin D1, Bcl-2, Bcl-xL.** In our in vivo studies, gemcibabine also manifested remarkably enhanced anti-tumor effect when combined with DHA, as manifested by significantly increased apoptosis, as well as **decreased Ki-67 index, NF-κB activity and its related gene products,** and predictably, significantly reduced tumor volume. We concluded that inhibition of gemcitabine-induced NF-κB activation is one of the mechanisms that **DHA dramatically promotes its anti-tumor effect on pancreatic cancer.**

ART plus Lenalidomide

160) Liu, W. M., A. M. Gravett, and A. G. Dalgleish. "The antimalarial agent artesunate possesses anticancer properties that can be enhanced by combination strategies." *International journal of cancer* 128.6 (2011): 1471-1480. Artesunate Anticancer Combination Strategies Lenalidomide Liu W M Int j cancer 2011

We also showed that ART maintained activity in polyploidy cells, and that an **impressive enhancement to its activity was achievable through a combination with the immunomodulatory drug lenalidomide.**

We have noted similarities in the activities between ART and LEN. Both agents are multimodal and efficacious in their disease type. Both are immune modulators, antiangiogenic and antimetastatic, and affect cells at the level of intracellular signalling such as MAPK/ERK, NF-Kappa Beta and p21 waf1/cip1.19

PI3K inhibitors buparlisib Idelalisib

161) Massacesi, Cristian, et al. "PI3K inhibitors as new cancer therapeutics: implications for clinical trial design." OncoTargets and therapy 9 (2016): 203.

nice images of nuclear location of B Cateninin changing to membrane location after treatment with ART

162) Xu, Na, et al. "Artesunate Induces SKM-1 Cells Apoptosis by Inhibiting Hyperactive β-catenin Signaling Pathway." International journal of medical sciences 12.6 (2015): 524.

We also identified the downstream target of Wnt/β-catenin pathway, c-myc and cyclinD1, and we found **c-myc and cyclinD1 protein levels decreased** from 25μg/mL after ART treatment (Fig (Fig4,4, $P<0.05$) Furthermore, this decrease seems to negatively correlate with E-cadherin protein expression level. This may indicate that ART inhibited the Wnt/β-catenin signaling pathway by resuming the loss of E-cadherin expression in SKM-1cells.

Our findings in SKM-1 cells are in agreement with this study, and we also found **ART treatment inhibited Wnt/β-catenin downstream expression of targets such as c-myc and cyclinD1**. C-myc and cyclinD1 are the proto-oncogenes involved in regulating proliferation, differentiation and apoptosis 18.

Strikingly, when we treated SKM-1 cell with **ART, both β-catenin and E-cadherin translocated from the nucleus to the membrane, thereby forming the β-catenin/E-cadherin complex and strengthening cell-cell adhesion.**

In summary, ART treatment demethylated CDH1, which, in turn recovers the E-cadherin activation in SKM-1 cells. Furthermore, by inhibiting the **Wnt/β-catenin pathway, the translocation of subcelluar β-catenin and E-cadherin to adherent junctions of the membrane enhances cell adhesion and inhibits metastasis** and may also enhance chemo-sensitivity to other clinical agents. The expression of Wnt/β-catenin downstream targets; **c-myc and cyclinD1**, **also diminished causing tumor cell growth inhibition and increased apoptosis.** Thereby these anti-tumor effects caused by ART may be a promising therapeutic drug for MDS therapy.

Artesunate inhibits Cancer Stem Cells

163) Subedi, Amit, et al. "High-throughput screening identifies artesunate as selective inhibitor of cancer stemness: Involvement of mitochondrial metabolism." Biochemical and Biophysical Research Communications

4.477 (2016): 737-742.
Cancer stem cells (CSCs) have robust systems to maintain cancer stemness and drug resistance. Thus, targeting such robust systems instead of focusing on individual signaling pathways should be the approach allowing the identification of selective CSC inhibitors. Here, we used the alkaline phosphatase (ALP) assay to identify inhibitors for cancer stemness in induced cancer stem-like (iCSCL) cells. We screened several compounds from natural product chemical library and evaluated hit compounds for their efficacy on cancer stemness in iCSCL tumorspheres. We identified artesunate, an antimalarial drug, as a selective inhibitor of cancer stemness. Artesunate induced mitochondrial dysfunction that selectively inhibited cancer stemness of iCSCL cells, indicating an essential role of mitochondrial metabolism in cancer stemness.

164) Wong, Yin Kwan, et al. "Artemisinin as an anticancer drug: Recent advances in target profiling and mechanisms of action." Medicinal Research Reviews (2017). Artemisinin anticancer Recent advances in mechanisms Wong Medicinal Research Reviews 2017 *Artemisinin targets the p38-MAPK, PI3K/Akt, Ras, NF-KappaB,and Wnt/Beta-catenin pathways*

165) Li, Qigui, Peter Weina, and Mark Hickman. "The use of artemisinin compounds as angiogenesis inhibitors to treat cancer." Research Directions in Tumor Angiogenesis. InTech, 2013.

166) Houh, Youn Kyung, et al. "The Effects of Artemisinin on the Cytolytic Activity of Natural Killer (NK) Cells." International Journal of Molecular Sciences 18.7 (2017): 1600.

Abstract: Artemisinin, a chemical compound used for the treatment of malaria, has been known to show anti-cancer activity. However, the effect of this chemical on natural killer (NK) cells, which are involved in tumor killing, remains unknown. **Here, we demonstrate that artemisinin exerts a potent anti-cancer effect by activating NK cells.** NK-92MI cells pre-treated with artemisinin were subjected to a cytotoxicity assay using K562 cells. The results showed that **artemisinin significantly enhances the cytolytic activity of NK cells in a dose-dependent manner.** Additionally, the artemisinin-enhanced cytotoxic effect of NK-92MI cells on tumor cells was accompanied by the stimulation of granule exocytosis, as evidenced by the detection of CD107a expression in NK cells. Moreover, this enhancement of cytotoxicity by artemisinin was also observed in human primary NK cells from peripheral blood. Our results suggest that artemisinin enhances human NK cell cytotoxicity and degranulation. **This is the first evidence that artemisinin exerts antitumor activity by enhancing NK cytotoxicity.** Therefore, these results provide a deeper understanding of the action of artemisinin and will contribute to the development and application of this class of compounds in cancer treatment strategies.

167) Oncotarget. 2015 Feb; 6(6): 4020–4035. Artesunate suppresses tumor growth and induces apoptosis through the modulation of multiple oncogenic cascades in a chronic myeloid leukemia xenograft mouse model
Chulwon Kim,1 Jong Hyun Lee,1 Sung-Hoon Kim,1 Gautam Sethi,2 and Kwang Seok Ahn1

Artesunate (ART), a semi-synthetic derivative of artemisinin, is one of the most commonly used anti-malarial drugs. Also, ART possesses anticancer potential albeit through incompletely understood molecular mechanism(s). Here, the effect of ART on various protein kinases, associated gene products, cellular response, and apoptosis was investigated. The in vivo effect of ART on the growth of human CML xenograft tumors in athymic nu/nu mice was also examined. In our preliminary experiments, we first observed that phosphorylation of p38, ERK, CREB, Chk-2, STAT5, and RSK proteins were suppressed upon ART exposure. Interestingly, ART induced the expression of SOCS-1 protein and depletion of SOCS-1 using siRNA abrogated the STAT5 inhibitory effect of the drug. Also various dephosphorylations caused by ART led to the suppression of various survival gene products and induced apoptosis through caspase-3 activation. Moreover, ART also substantially potentiated the apoptosis induced by chemotherapeutic agents. Finally, when administered intraperitoneally, ART inhibited p38, ERK, STAT5, and

CREB activation in tumor tissues and the growth of human CML xenograft tumors in mice without exhibiting any significant adverse effects. Overall, our results suggest that ART exerts its anti-proliferative and pro-apoptotic effects through suppression of multiple signaling cascades in CML both in vitro and in vivo.

Artesunate Synergy with Venetoclax BCL-2 inhibitor

168) Kumar, Bijender, et al. "Antileukemic activity and cellular effects of the antimalarial agent artesunate in acute myeloid leukemia." Leukemia Research (2017). Antileukemic activity and cellular effects of the antimalarial agent artesunate in acute myeloid leukemia
Artesunate has potent antileukemic activity against AML in vitro and in murine models of AML.
The cytotoxicity of artesunate against AML is mediated by induction of reactive oxygen species.
Artesunate augments cytotoxicity of chemotherapy as well as the **BCL-2 inhibitor venetoclax** in AML.

The artimisinins are a class of antimalarial compounds whose antiparasitic activity is mediated by induction of reactive oxygen species (ROS). Herein, we report that among the artimisinins, artesunate (ARTS), an orally bioavailable compound has the most potent antileukemic activity in AML models and primary patients' blasts. ARTS was most cytotoxic to the FLT3-ITD+ AML MV4-11 and MOLM-13 cells (IC50 values of 1.1 and 0.82 μM respectively), inhibited colony formation in primary AML and MDS cells and augmented cytotoxicity of chemotherapeutics. **ARTS lowered cellular BCL-2 level via ROS induction and increased the cytotoxicity of the BCL-2 inhibitor venetoclax** (ABT-199). ARTS treatment led to cellular and mitochondrial ROS accumulation, double stranded DNA damage, loss of mitochondrial membrane potential and induction of the intrinsic mitochondrial apoptotic cascade in AML cell lines. The antileukemic activity of ARTS was further confirmed in MV4-11 and FLT3-ITD+ primary AML cell xenografts as well as MLL-AF9 syngeneic murine AML model where **ARTS treatment resulted in significant survival prolongation of treated mice compared to control**. Our results demonstrate the potent preclinical antileukemic activity of ARTS as well as its potential for a rapid transition to a clinical trial either alone or in combination with conventional chemotherapy or BCL-2 inhibitor, for treatment of AML.

Effective Under Hypoxic Conditions

169) Front Oncol. 2014; 4: 116.Published online 2014 May 19.
Dihydroartemisinin is a Hypoxia-Active Anti-Cancer Drug in Colorectal Carcinoma Cells. Teona Ontikatze,1,† Justine Rudner,1,† René Handrick,1,2 Claus Belka,3 and Verena Jendrossek1,*

In contrast to many genotoxic drugs and radiotherapy, which are generally less efficient in hypoxic tumor cells, **DHA exerts pronounced anti-neoplastic effects under severely hypoxic conditions.** While the canonical intrinsic apoptosis pathway seemed to be predominantly activated by DHA in oxygenated cells, DHA induced a caspase-independent apoptosis-like cell death in severe hypoxia. **Since DHA targets normoxic as well as hypoxic cells with equal potency,** the drug might be a promising tool to improve treatment outcome, particularly in hypoxic human tumors resistant to conventional therapies.

170) Immunopharmacol Immunotoxicol. 2017 Feb;39(1):28-36.
Artemisinin inhibits inflammatory response via regulating NF-KappaB and MAPK signaling pathways. Wang KS1, Li J1, Wang Z1, Mi C1, Ma J1, Piao LX1, Xu GH1, Li X1, Jin X1.

Artemisinin, isolated from the Chinese plant Artemisia annua, has been used for many years to treat different forms of malarial parasites. In this study, we explored the anti-inflammatory activity of artemisinin and the underlying mechanism of this action. We demonstrated that the anti-inflammatory effects of artemisinin in TPA-

induced skin inflammation in mice. Then the artemisinin significantly inhibited the expression of NF-KappaB reporter gene induced by TNF-a in a dose-dependent manner. Artemisinin also inhibited TNF-a induced phosphorylation and degradation of I?Ba, p65 nuclear translocation. Artemisinin also has an impact on upstream signaling of IKK through the inhibition of expression of adaptor proteins, TNF receptor-associated factor 2 (TRAF2) and receptor interacting protein 1 (RIP1). Furthermore, pretreatment of cells with **artemisinin prevented the TNF-a-induced expression of NF-KB target genes, such as anti-apoptosis (c-IAP1, Bcl-2, and FLIP), proliferation (COX-2, cyclinD1), invasion (MMP-9), angiogenesis (VEGF), and major inflammatory cytokines (TNF-a, iNOS, and MCP1).** We also proved that artemisinin NFpotentiated TNF-a-induced apoptosis. Moreover, artemisinin significantly impaired the ROS production and phosphorylation of p38 and ERK, but did not affect the phosphorylation of JNK. Taken together, artemisinin may be a potentially useful therapeutic agent for inflammatory-related diseases.

171) Tran, Kalvin Q., Antony S. Tin, and Gary L. Firestone. "Artemisinin triggers a G1 cell cycle arrest of human Ishikawa endometrial cancer cells and inhibits Cyclin Dependent Kinase-4 promoter activity and expression by disrupting NF-kB transcriptional signaling." Anti-cancer drugs 25.3 (2014): 270.

Artemisinin induced a G1 cell cycle arrest in cultured human Ishikawa endometrial cancer cells and downregulated cyclin-dependent kinase-2 (CDK2) and CDK4 transcript and protein levels. Analysis of CDK4 promoter-luciferase reporter constructs showed that the **artemisinin ablation of CDK4 gene expression** was accounted for by the loss of CDK4 promoter activity. Chromatin immunoprecipitation demonstrated that artemisinin inhibited nuclear factor κ-light-chain-enhancer of activated B cells (**NF-κB) subunit p65 and p50** interactions with the endogenous Ishikawa cell CDK4 promoter. Coimmunoprecipitation revealed that **artemisinin disrupts endogenous p65 and p50 nuclear translocation through increased protein-protein interactions with IκB-α, an NF-κB inhibitor**, and disrupts its interaction with the CDK4 promoter, leading to a loss of CDK4 gene expression.

172) Wang, Jigang, et al. "Mechanistic investigation of the specific anticancer property of artemisinin and its combination with aminolevulinic acid for enhanced anticolorectal cancer activity." ACS central science 3.7 (2017): 743-750.

173) HOW DOES GLeOLAN WORK? FDA Approved 2017,

The active substance in Gleolan, 5-aminolevulinic acid, is orally administered, then absorbed by cells in the body, where it is converted into fluorescent chemicals, particularly protoporphyrin IX (PPIX). Since glioma cells take up more of the active substance and convert it more rapidly into PPIX, higher levels of PPIX accumulate in the cancer cells than in normal tissue. When illuminated under blue light of a specific wavelength, the PPIX in the tumor glows an intense red, while the normal brain tissue appears blue. This enables the surgeon to see the tumor more clearly during brain surgery and to remove it more accurately, sparing healthy brain tissue.

174) Aminolevulinic acid hydrochloride, known as ALA HCl (Gleolan, NX Development Corp.) as an optical imaging agent indicated in patients with gliomas

175) PRESCRIBING INFORMATION GLEOLAN [aminolevulinic acid hydrochloride (ALA HCl)]

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IL-6 as a key cytokine for MCL growth and survival

Jak2/STAT3-Micro-Environment Mantle Cell Lymphoma

176) Zhang, Liang, et al. "Role of the microenvironment in mantle cell lymphoma: IL-6 is an important survival factor for the tumor cells." Blood 120.18 (2012): 3783-3792.
IL-6 activated the Jak2/STAT3 and PI3K/Akt pathways in MCL, and the inhibition of these pathways completely or partially abrogated IL-6–mediated protection of MCL cells. Hence, **our study identifies IL-6 as a key cytokine for MCL growth and survival** and suggests that targeting the IL-6 pathway may be a novel way to improve the efficacy of chemotherapy in MCL patients.

STAT3 (signal transducer and activator of transcription 3) is the signal transducer of IL-10, and STAT3 is activated by phosphorylation.

177) J Pathol. 2003 Jan;199(1):84-9. Expression of STAT3 and its phosphorylated forms in mantle cell lymphoma cell lines and tumours.
Lai R1, Rassidakis GZ, Medeiros LJ, Leventaki V, Keating M, McDonnell TJ.
The pathogenesis of mantle cell lymphoma (MCL) is incompletely understood, although cyclin D1 overexpression leading to deregulated cell proliferation is probably important. **Recent data suggest that interleukin (IL)-10 can increase the proliferative activity of MCL cells. STAT3** (signal transducer and activator of transcription 3) is the signal transducer of IL-10, and STAT3 is activated by phosphorylation. The hypothesis of this study is that STAT3 is activated in MCL. The expression of the two phosphorylated (i.e. active) forms of STAT3, pSTAT3-tyr (phosphorylated at the tyrosine(705) residue) and pSTAT3-ser (phosphorylated at the serine(727) residue), was assessed in four MCL cell lines and 12 MCL tumours using western blots and/or immunofluorescence staining techniques. **All MCL cell lines expressed STAT3**, but only one had detectable pSTAT3-tyr and none had pSTAT3-ser. **Addition of IL-10 rapidly resulted in expression of pSTAT3-tyr but not pSTAT3-ser.** All eight cases of frozen MCL tumours examined had detectable pSTAT3-tyr and pSTAT3-ser. Immunofluorescence studies using four formalin-fixed, paraffin wax-embedded MCL tumours demonstrated cytoplasmic localization of STAT3, as opposed to the nuclear localization of the pSTAT3 species. In conclusion, these findings provide evidence that **STAT3 is constitutively activated in MCL,** supporting the concept that STAT3 signalling may be important in the pathogenesis of these tumours.

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Artesunate (ATS) as the best potential inhibitor of STAT-3

178) Ilamathi, M., S. Santhosh, and V. Sivaramakrishnan. "Artesunate as an Anti-Cancer Agent Targets Stat-3 and Favorably Suppresses Hepatocellular Carcinoma." Current topics in medicinal chemistry 16.22 (2016): 2453-2463.

Background: Aberrant signal transducer and activator of transcription 3 (**STAT-3**) molecular signaling elicit hepatocellular carcinoma (HCC) in humans. Therefore, targeting STAT-3 is considered as an attractive option towards suppression of HCC in humans. Objective: Our objective is to identify a potential small molecule inhibitor that can specifically target STAT-3 and suppress HCC. Methods: In this study, we analyze a group of sesquiterpene lactone (STL) candidates that has been recently reported in preclinical trials against cancer by a unified computational and experimental approach. Results: Our virtual analysis of the STL candidates revealed **Artesunate (ATS) as the best potential inhibitor of STAT-3** with comparable potency to specific inhibitor S3I-201. We also observed that **ATS inhibited IL-6 driven STAT-3-DNA binding activity** with comparable potency to S3I-201 in a cell free system. Furthermore ATS was observed to interfere with STAT-3 dimerization and suppression of both constitutive and IL-6 inducible STAT-3 in vitro. Nevertheless, we also observed that **ATS modulated STAT-3 dependent targets (procaspase-3, Bcl-xl and survivin) favoring occurrence of apoptosis in vitro.** Overall, the putative inhibition of STAT-3 by ATS suggested its capacity to interfere with STAT-3

dimerization by binding to the SH2 domain of STAT-3 monomer. It resulted in suppression of STAT-3 and also favored promotion of in vitro cells towards apoptosis. Consequently, ATS also exhibited selective cytotoxicity of cancer cells over normal cells in vitro. Conclusion: All the above observations substantiated by unified computational and in vitro experimental approaches suggested its potential role as a therapeutic anti-cancer agent against HCC.

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ART down-regulated the expression of STAT3 in leukemia

179) Tan, Mei, et al. "Artesunate induces apoptosis via inhibition of STAT3 in THP-1 cells." Leukemia Research (2017).Leuk Res. 2017 Nov;62:98-103.

Our objective was to explore **STAT3 expression** in patients with **acute myeloid leukaemia (AML)**, assess the anti-proliferative effects of **artesunate (ART)** on THP-1 cells in vivo and in vitro, and investigate the underlying mechanisms.
METHODS:In this study, we examined **30 patients** with acute myeloid leukaemia diagnosed in our hospital from January 2015 to January 2016. The 20 control group patients had non-haematological diseases and were hospitalized for the same period. We extracted 2ml bone marrow, separated the mononuclear cells, obtained total proteins, and detected **STAT3 protein levels** with Western blot analyses. The THP-1 cells were treated with different concentrations of **ART(0, 10, 25, 50, 100, 200μM).** Then, THP-1 cell viability was detected with CCK-8 assays, apoptosis was measured with flow cytometry, and the STAT3, caspase-3 and caspase-8 protein levels were assessed using Western blot analyses. THP-1 cells in logarithmic growth phase were subcutaneously **injected into the necks of 5-week-old nude mice**. The control group was subcutaneously injected with 0.1ml PBS. After the nude mouse tumours grew, the mice were divided into the control group and drug intervention groups (ART 100μM group, ART 200μM group). The mice in the intervention groups were **intraperitoneally injected with ART,** and the control group was injected with the same amount of normal saline. Then, changes in the tumours were observed. After the drug intervention, the total protein was extracted, and STAT3 expression was detected by Western blot analysis.
RESULTS:Compared with the control group, the **AML patients had significantly increased STAT3 protein levels** (P<0.01). **ART significantly inhibited the proliferation of THP-1 cells in a dose-dependent and time-dependent manner. ART also increased THP-1cell apoptosis.** After treatment with ART, **STAT3 protein was significantly down-regulated,** and apoptosis of the cells was induced by the activation of caspase-3 and caspse-8.
CONCLUSION:AML patients had higher expression of STAT3 than that of the controls. **ART induced apoptosis in THP-1 cells and inhibited the growth of xenografts in nude mice, and we also observed that ART down-regulated the expression of STAT3** and activated the caspase-3 and caspase-8. We speculated that the effect of ART on THP-1 cells may be related to inhibition of STAT3 and activation of caspase3 and caspase-8.

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180) Ilamathi, M., et al. "Artesunate obliterates experimental hepatocellular carcinoma in rats through suppression of IL-6-JAK-STAT signalling." Biomedecine & pharmacotherapie 82 (2016): 72.
Activation of the **IL-6 mediated JAK-STAT** (Janus associated kinase-signal transducer and activator of transcription) oncogenic signalling plays a major role in hepatocellular carcinoma pathogenesis. The aim of this study is to assess the anti-tumour, anti-proliferative and apoptotic potential of artesunate and its capacity to modulate JAK-STAT pathway in a nitrosodiethylamine mediated experimental hepatocellular carcinoma model. Administration of nitrosodiethylamine (200mg/kg body weight by i.p. Injections) to rats resulted in alterations of

liver pathophysiological parameters such as increased relative liver weight, and increased tumour nodule occurrence. It also increased the levels of serum marker enzymes (AST, ALT, ALP, LDH, and γGT) and tumour biomarker (AFP) levels suggestive of its capacity to cause liver tumourigenesis. Additionally, the immunohistochemistry of liver sections pertaining to nitrosodiethylamine administered animals showed increased detection of AgNOR, PCNA, and GST-Pi positive cells suggestive of its capacity to promote liver proliferation associated tumourigenesis. **On the contrary, artesunate (25mg/kg bodyweight) supplementation** to nitrosodiethylamine administered animals **decreased all the above** mentioned pathophysiological, biochemical, and immunohistochemistry parameters suggesting its **anti-tumour and anti-proliferative potential**. Furthermore, immunoblot analysis showed **significant up-regulation of IL-6, GP130, JAK-2, STAT-3 (pY705), Bcl-xL, Bcl-2 and simultaneous down-regulation of Caspase-3, PARP and SOCS-3** in nitrosodiethylamine administered animals. Nevertheless, the immunoblot analysis revealed **vice-versa on artesunate supplementation** to nitrosodiethylamine administered animals, indicating promotion of the feedback loop inhibition mechanism through SOCS3 up-regulation thereby leading to **suppression of JAK-STAT signalling**. Overall all these findings substantiate that artesunate promotes anti-tumour, anti-proliferation and apoptosis against nitrosodiethylamine mediated hepatocellular carcinoma.

181) Xu, H., et al. "Anti-malarial agent artesunate inhibits TNF-α-induced production of proinflammatory cytokines via inhibition of NF-κB and PI3 kinase/Akt signal pathway in human rheumatoid arthritis fibroblast-like synoviocytes." (2007): 920-926.

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Mariapj
on **August 17, 2016 at 11:25 PM** said:

Thank you for sharing with such great research and information. II would like to comment that my Dr in Colombia told me to treat lyme Disease with anthelmintics (antiparasitics) but to not use antimalarials due to my immune system was very low and the antimalarials work by immune-supression. Was this considered when treating for cancer with Artemisinin? How can the immune system could be taken care while taking Artemisinin? It could be of great interest for the Lyme community too. (Lyme is a multiple infection, ignored by the medical community who decided to treat for only two weeks and if the person is not cured they say it is a chronic disease and the insurances don't have the obligation to treat. This is why we have to go to other Doctors or research by ourselves to try to be able to heal or to have a decent quality of life). Best regards, María Lyme Disease.

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Michael
on **August 8, 2017 at 12:55 AM** said:

Excellent discussion!

Donna luis
on **November 6, 2017 at 4:09 AM** said:

They are only being honest because i actually they were scam not until they help my step mom,if you need their please do and stop having negative thought.
Donna luis

Mahdi
on **December 6, 2017 at 2:58 AM** said:

Can you provide better reference that chlorophyll can replace ALA. The article full text is not available and abstract tell nothing about it

Mahdi
on **December 6, 2017 at 5:50 PM** said:

Edit: I mean probably they use ALA instead of Iron supplement to avoid fueling cancer cells. the source you provided says only chlorophyll increase Iron (nothing about heme precursor)

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