Expert Opinion

Oncologic, Endocrine and Metabolic

Targeted treatment of cancer with artemisinin and artemisinin-tagged iron-carrying compounds

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Artemisinin is a chemical compound that reacts with iron to form free radicals which can kill cells. Cancer cells require and uptake a large amount of iron to proliferate. They are more susceptible to the cytotoxic effect of artemisinin than normal cells. Cancer cells express a large concentration of cell surface transferrin receptors that facilitate uptake of the plasma iron-carrying protein transferrin via endocytosis. By covalently tagging artemisinin to transferrin, artemisinin could be selectively picked up and concentrated by cancer cells. Futhermore, both artemisinin and iron would be transported into the cell in one package. Once an artemisinin-tagged transferrin molecule is endocytosed, iron is released and reacts with artemisinin moieties tagged to transferrin. Formation of free radicals kills the cancer cell. The authors have found that artemisinin-tagged transferrin is highly selective and potent in killing cancer cells. Thus, artemisinin and artemisinin-tagged iron-carrying compounds could be developed into powerful anticancer drugs.

Keywords: artemisinin, cancer, iron-carrying molecule

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1. Introduction

In 1972, artemisinin was discovered to be the active ingredient responsible for the antimalarial action of the Chinese medicinal herb qing-hao (*Artemisia annua L*), which has been used in China for centuries for afflictions such as fevers, haemorrhoids, and malaria. Artemisinin has a strong effect on chloroquine-resistant cases of malaria and shows no evidence of serious toxicity [1,2]. To date, artemisinin and its analogues have been used for the treatment of > 2 million cases of malaria infection.

Artemisinin is a sesquiterpene lactone peroxide containing an endoperoxide moiety (-C-O-O-C-) which forms free radicals when it reacts with iron [3]. This carbon-based radical, when formed in cells, can lead to cellular damage and cell death by reacting with cellular macromolecules such as proteins and membrane lipids. Because malaria parasites contain large amount of haem-iron, a product from the digestion of haemoglobin within host red blood cells, a hypothesis is that interaction of artemisinin with haem leads to death of the parasite [4-6]. However, a recent experiment [7] indicates that activation of artemisinin inside the parasite is by ferrous iron independent of haem. The source of the iron is not known.

The pharmacology and pharmacokinetics of artemisinin and some of its analogues have been well-studied and documented [8-10]. Various analogues of artemisinin and endoperoxide-containing compounds have been synthesised and some are being used in malaria treatment [11-19]. **Figure 1** shows the chemical structures of artemisinin and two analogues, dihydroartemisinin (DHA) and artelinic acid.

The rationale of the targeted therapy discussed in this paper is based on a concept that takes advantage of the iron-catalysed free radical-forming property of artemisinin and targets it toward cancer cells.

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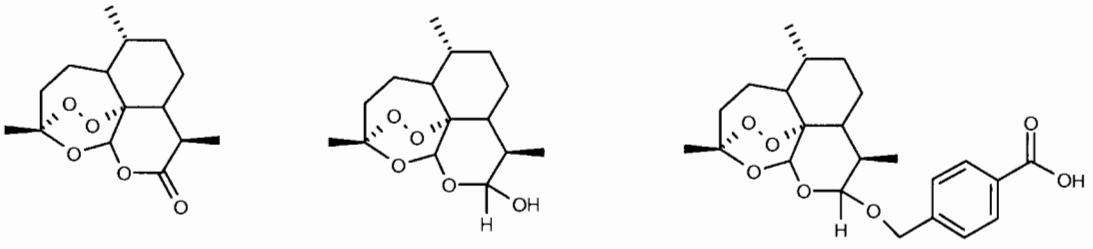

Figure 1. Structures of artemisinin and two analogues.

2. Artemisinin, iron and cancer cells

Because iron plays a vital role in cell physiology and growth (e.g., in energy metabolism and DNA synthesis, as iron is a cofactor of the enzyme ribonuclease reductase that converts ribose to deoxyribose), special molecular mechanisms have evolved for the transportation of iron into cells. In vertebrates, a major iron transport system involves a specific interaction between the iron-carrying protein transferrin, in the plasma, and cell surface transferrin receptor, that results in a facilitated transport of iron into the cell via endocytosis [20].

Iron, in its free form, is toxic because it catalyses the formation of free radicals; notably, formation of a hydroxyl free radical from hydrogen peroxide (the Fenton reaction) that is generated as a metabolic by-product in the mitochondria. Most iron in cells is rendered inactive by chelating to other molecules or stored in ferritin as iron oxide. Iron is transported in the blood by binding to transferrin. Cells in need of iron express transferrin receptors on their surface. Binding of holotransferrin (i.e., iron-loaded transferrin) to the receptor, triggers endocytosis and transports holotransferin inward in intracellular endosomes. Once the pH inside an endosome drops, iron is released in the ferrous form and actively transported from the endosome into the cytoplasm. Apotransferrin (iron-free transferrin) remains attached to the receptor at low pH and is recycled to the cell surface and released. Released iron is immediately used in cellular processes, for example, incorporation into enzymes and cytochromes. Excess iron is converted into an oxide form and stored in ferritin. Feedback mechanisms, involving transcription and post-transcription processes, are involved in iron metabolism and maintain intracellular iron in a tightly controlled state.

Due to their rapid rate of division, most cancer cells have high rates of iron intake [21] and express a high cell surface concentration of transferrin receptors [22] than normal cells. This occurs during the G1 and S phases of the cell cycle to prepare the cell for DNA replication and subsequent mitosis. In general, the aggressiveness of a tumour is positively correlated with transferrin receptor concentration and the proliferation index of its cells. As cancer cells have a higher iron influx via the transferrin receptor mechanism, cancer cells would be more susceptible to the cytotoxic effect of artemisinin. This concept suggests that artemisinin could be effective for the treatment of many types of cancer.

Many examples of increased requirement and dependency on iron by cancer cells to proliferate can be found in the research literature. The following is a description of several common types of cancer. For example, breast cancer cells have 5 – 15 times more transferrin receptors on their cell surface than normal breast cells [23]. Transferrin receptors are overexpressed only on the cell surface of breast carcinoma cells, but not on benign breast tumour cells [24]. More importantly, it has been shown that breast cancer cells do take up more iron than normal breast cells [25]. In addition, the dependence of breast cancer cells on iron intake is suggested by the finding that antibody to transferrin receptors can retard the growth of breast tumour [26]. Artemisinin has been shown to be selectively toxic to human breast cancer cells [27]. Iron also plays an important role in brain tumour cell proliferation, particularly involving the transferrin iron uptake mechanism. For instance, transferrin receptors are present in high concentrations on the cell surface of glioblastomata and meningliomas [28-30]. Transferrin has been shown to be endocytosed into neuroblastoma cells [31]. Cancer cells that metastasised to the brain also express high concentration of transferrin receptors [32]. There is a positive correlation between transferrin receptor expression and Ki-61 growth fraction in glioma [29]. Artemisinin and its analogues have been show to be toxic to brain cancer cells [33]. Artemisinin anlogues have been shown to inhibit proliferation of undifferentiated neuroblastoma (Nb2a) and glioma (C6) cells [34] and glioblastoma multiforme cell line [35]. In chronic myelogenous leukaemia (CML), cells also express more transferrin receptors on their cell surface than normal cells [36-40]. In addition, the dependence of CML cells on iron intake via the transferrin mechanism is suggested by the finding that antibody to transferrin receptors retards the growth or kills CML cells [41,42] and conjugates of transferrin with cytotoxins are effective at killing CML cells [43,44]. Artemisinin is selectively toxic to human leukaemia cells [45]. Leukaemia cells have been shown to be the most sensitive to artesunate, an artemisinin analogue, among various other cancer cells [33]

Thus, because of this increased requirement of iron by cancer cells, artemisinin would be selectively toxic to cancer cells and constitutes a targeted-therapy for cancer.

3. Artemisinin and cancer: experimental results

The authors have shown that Molt-4 cells, a human leukaemia cell line, are more susceptible to the cytotoxic effect of artemisinin than their normal counterpart, (i.e., normal human lymphocytes) [45]. The LC (lethal concentration)$_{50}$ of artemisinin for Molt-4 cells is ~ 100 times lower than that of lymphocytes. An important observation in this experiment is that addition of holotransferrin to the cell culture significantly enhanced the toxicity of artemisinin on cancer cells, suggesting the involvement of intracellular iron in the effect. Further research in the authors' laboratory has shown that artemisinin induces mainly apoptosis in Molt-4 cells [46].

In another experiment, the authors tested artemisinin on two human breast cell lines [27]: HTB 125, a normal human breast cell line with epithelial cell morphology, and HTB 27, a radiation-resistant human breast cancer cell line, also with epithelial cell morphology. The authors found that only 2% of breast cancer cells were still alive after a 16-hour treatment with both DHA and holotransferrin. On the other hand, treatment with DHA alone or 'DHA + holotransferin' had little effect on normal human breast cells. These data indicate that artemisinin is selectively toxic to the radiation-resistant human breast cancer cell line HTB 27, but not to normal breast cells. Furthermore, it was found that holotransferrin significantly enhanced the cytotoxicity of artemisinin on breast cancer cells. Thus, iron plays an important role in the effect as the authors hypothesised. More recently, Efferth *et al.* showed that ferrous iron enhanced the cytotoxic effect of artemisinin toward leukaemia and astrocytoma cells [47].

Other research indicating the role of iron is an *in vivo* study carried out by the authors [48] in which rats implanted with fibrosarcoma were treated with DHA. The authors found that administering iron (ferrous sulfate) orally to the rats enhanced the effect of DHA in retarding tumour growth (oral iron supplement could increase the concentration of holotransferrin in the plasma, because normally only 30% of the transferrin in the blood carries iron). However, in a similar experiment on implanted breast tumour in the rat, the authors found iron administration did not significantly affect the effect of artemisinin (unpublished data).

In addition to the authors' findings, other researchers have also reported the potential selective anticancer properties of artemisinin and its analogues both *in vitro* and *in vivo* on various forms of cancer [33,48-66]. There are also two case reports of patients with laryngeal squamous cell carcinoma [67] and metastatic uveal melanoma [68] treated with artesunate.

Artemisinin has also been shown to impede angiogenesis [51,52,69-74]. This may be another mechanism of the anticancer effect of artemisinin *in vivo*. The antiangiogenesis effect has been shown in chicken chorioallantoic membrane, human ovarian tumour implanted in nude mice, mouse embryonic stem cell-derived embryoid bodies and tube formation of human umbilical vein endothelial cells. The effect apparently involves downregulation of vascular endothelial growth factor-related processes and free radicals.

Artemisinin is a relatively safe drug with no obvious adverse reactions or noticeable side effects [1,2,75,76]. This is supported by the above *in vitro* experiments in which no obvious toxic reaction to normal cells was observed. Nonetheless, it must be pointed out that neurotoxicity has been reported with some analogues of artemisinin. For example, intramuscular administration of arteether at 20 mg/day for 8 days, has been reported to induce neurological deficits in the dog, and artemether at 12.5 – 50 mg/kg/day for 28 days, also induced neurological syndromes in the rat [77]. However, oral and subcutaneous administrations of the synthetic analogue Ro42-1611 (arteflene) to the rat at 400 mg/kg/day for 4 weeks were well-tolerated and did not induce any mutagenic effect [78]. Existing data seem to indicate that oral artemisinin is relatively safe and causes no side effects [79].

The safety of artemisinin *in vivo* is born out in a recent experiment that the authors performed to study the use of artemisinin for cancer prevention [80]. In the experiment, the effect of daily oral artemisinin intake on preventing the development of breast cancer in the rat was studied. Rats were treated with the carcinogen 9,10-dimethyl-1,2-benzanthracene (DMBA) that is known to induce multiple breast tumours. After DMBA treatment, some rats were provided with a powdered rat-chow mixed with 0.02% artemisinin, whereas other animals (controls) were provided with the powdered food without artemisinin. The daily oral intake of artemisinin of the experimental animals was estimated to be 8 – 10 mg/kg. Development of breast cancer in the rats was monitored for 40 weeks. Data show that oral artemisinin significantly delayed (i.e., artemisinin-fed rats that developed breast cancer took significantly longer time for the first tumour to appear) and prevented (i.e., significantly more of the artemisinin-fed rats did not develop cancer within 40 weeks after carcinogen treatment) the development of breast cancer in the rat. In addition, artemisinin-fed rats developed significantly fewer numbers of tumours, and the total volume of tumours in an animal was significantly smaller. These data indicate that daily oral intake of artemisinin is effective in the prevention of breast cancer. Even after 40 weeks of daily oral intake of artemisinin, no apparent health effect was observed in animals that did not developed cancer.

4. Artemisinin-tagged transferrin

As mentioned above, in mammalian cells, iron is transported into cells via a receptor-mediated endocytosis process [20]. Binding of the plasma iron-carrying protein holotransferrin to cell surface transferrin receptors triggers endocytosis that

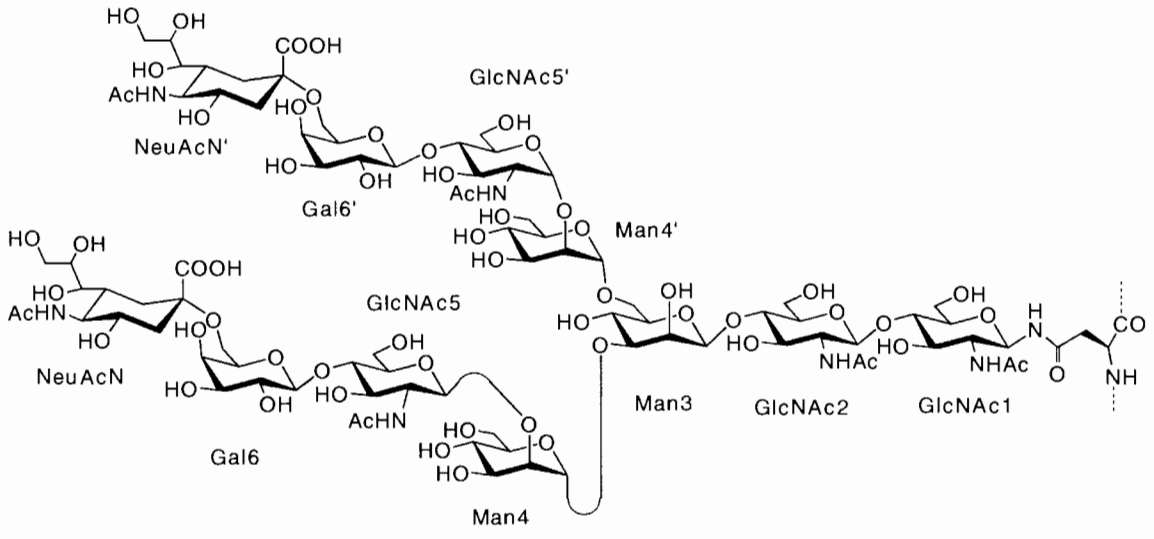

Figure 2. Structure of diantennary saccharide of transferrin.

transports transferrin and the iron it carries into cells. Because cancer cells require a large amount of iron, they express a high number of transferrin receptors on their surface and uptake more iron during G1 and S phases of the cell cycle.

The authors speculate that if artemisinin is covalently attached to holotransferrin, it would be transported as a package into cells and react with iron within the endosome where iron is released from holotransferrin. This may enhance the cytotoxic potency and selectivity of artemisinin on cancer cells.

Transferrin is a glycoprotein. Its protein moiety is mainly involved in its binding to cell surface transferrin receptors, whereas the carbohydrate chains are not involved in receptor binding [81,82]. Transferrin has two N-glycosides attached to the asparagine residues in the C-terminal domain [83]. Periodate oxidation of these carbohydrate chains generates reactive aldehyde groups that can be modified with a variety of hydrazine or aminoxy derivatives of artemisinin. Thus, the artemisinin analogue artelinic acid has been tagged to the glycosylate-moiety of holotransferrin using a relatively simple process. The authors estimate that as many as 10 artemisinin derivatives could be tagged to a molecule of transferrin. The following is a brief description of the procedures that one may use to synthesise artemisinin-tagged transferrin.

4.1 Method of tagging artemisinin to transferrin

The two N-glycosides attached to the asparagine residues in the C-terminal domain of a transferrin molecule are not involved in binding of transferrin to its receptor. Periodate oxidation of these carbohydrate chains generates reactive aldehyde groups that can react with hydrazine or aminoxy derivatives of artemisinin. The carbohydrate chain of transferrin is primarily a diantenary saccharide (**Figure 2**). Assuming that all 1,2-diol moieties are oxidised to the corresponding aldehyde group, the authors estimate that > 10 artemisinin units could be tagged to a molecule of holotransferrin. Artemisinin has also been tagged to amino acid side chains, such as lysine, on the protein surface. Such lysine-based tagging strategy could, however, interfere with receptor binding. The authors have shown that carbohydrate-based tagging chemistry produced a more potent tagged protein compared to lysine-based tagging chemistry [84].

Artelinic acid was used for the tagging. Artelinic acid has a rigid spacer to keep the bulky artemisinin moiety away from the carboxylic acid group. Artelinic acid methyl ester was first synthesised [85], and then reacted with hydrazine to produce the desired artelintic acid hydrazine (ART-Ph-NH_2) (**Figure 3**).

Holotransferrin was first oxidised by sodium periodate in acetate buffer, pH 5.5, for 30 min. Excess reagents and by-products were removed by gel filtration chromatography on Sephadex G-25. The protein fractions were combined, and immediately reacted with artelinic acid hydrazine to produce the tagged transferrin (**Figure 4**). The product was purified by the second gel filtration column (Sephadex G-25) that had been equilibrated with saline buffer, pH 7.5. The protein fractions were collected and stored at 4°C. Unlike oxidised transferrin, tagged protein was found to be very stable, and can be stored at 4°C for at least 12 months without any significant change in its biological activities.

Ultraviolet/visual and circular dichroism spectra of artemisinin-tagged transferrin showed that the protein structure remained largely intact during the protein modification. However, the iron content decreased from ~ 100 to 40% during the periodate oxidation step. The iron content remained unchanged during the tagging step. Periodate, therefore, damages the iron binding site, probably by oxidising some coordinating amino acid residues such as Tyr and His. Procedures to prevent this damage are now being developed in the authors' laboratory.

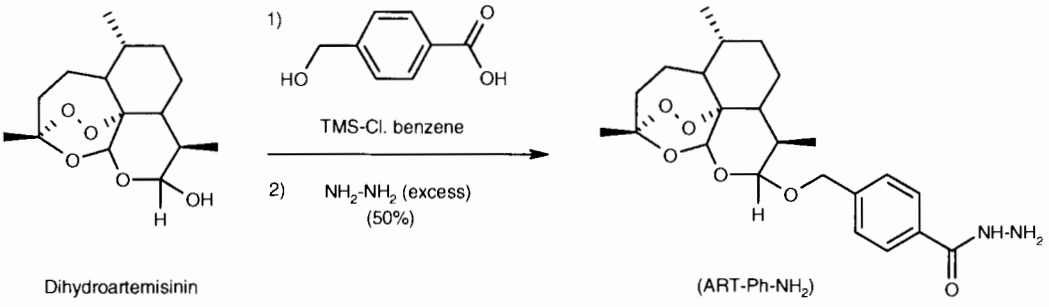

Figure 3. Synthesis of artelinic acid hydrazine.

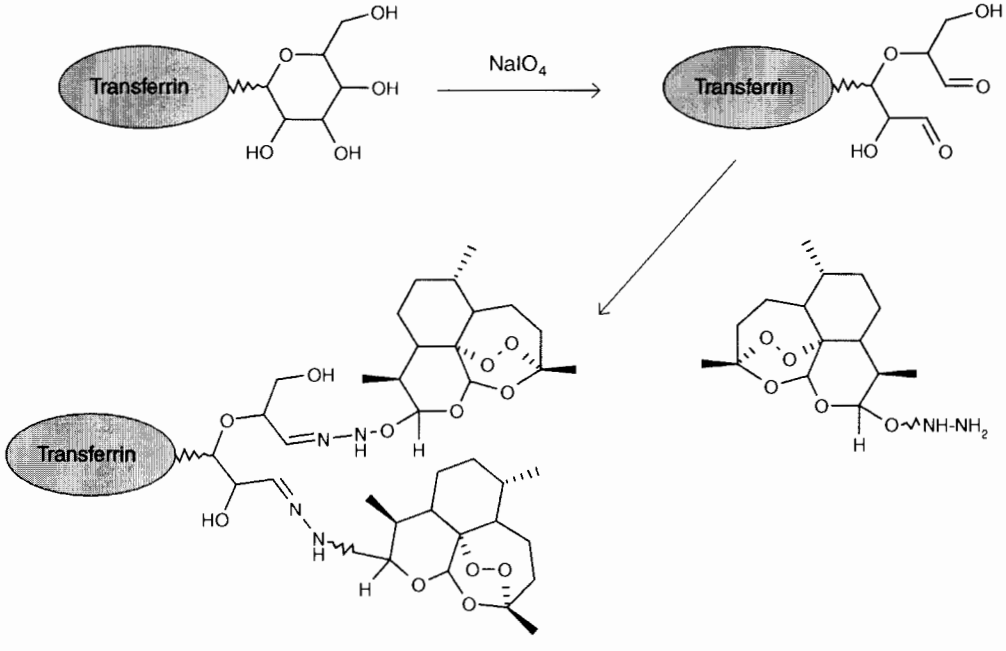

Figure 4. Chemical modification of human transferrin with artemisinin.

Hydrophobic interaction high performance liquid chromatography (HPLC) was used to determined the purity of the tagged protein. Because artemisinin is a hydrophobic compound, artemisinin-tagged protein moves slower on the HPLC condition. The peak of tagged transferrin was much broader than native transferrin, suggesting that the authors' sample was a mixture of tagged proteins with different numbers of artelinic acid moiety on the protein surface. Matrix-assisted laser desorption/ionisation mass spectrometry confirmed the HPLC data. Tagged transferrin and native transferrin gave their molecular ion peaks at 77,619 Da and 75,828 Da, respectively. The mass difference corresponds to the tagging of 4.2 artemisinin moieties per protein on average. The peak shape of tagged protein was, again, much broader than that of native transferrin.

The authors were able to show that artemisinin moieties in tagged protein contain the intact endoperoxide bond. Both DHA and artemisinin produce chemiluminescence [86] when mixed with hematin and luminol due to their endoperoxide bond. When tagged protein was reacted with the chemiluminescence reagent (hematin and luminol), the solution produced a time-dependent chemiluminescence similar to that of dihydroartemisinin.

4.2 Effect of artemisinin-tagged transferrin on cancer cells

The authors have tested this artemisinin-tagged transferrin ('tagged-compound') on the human leukaemia cell line Molt-4 and normal human lymphocytes [84]. The potency of

the 'tagged-compound' was compared with that of dihydroartemisinin.

The following are the LC_{50} values of the 'tagged-compound' and DHA on Molt-4 cells and lymphocytes, as determined by the Probit analysis: Molt-4-'tagged-compound' 0.98 μM; Molt-4-DHA 1.64 μM; lymphocyte-'tagged compound' 33 mM; lymphocyte-DHA 58.4 μM. Thus, compared with DHA, the 'tagged-compound' is more potent in killing Molt-4 cells and less potent in killing normal lymphocytes.

DHA is 36 times more potent in killing Molt-4 cells than its normal counterpart, whereas for the 'tagged-compound', it is 34,000 times. Therefore, tagging artemisinin to holotransferrin significantly increases the specificity of the cancer cell killing efficacy of artemisinin. A preliminary study has also shown that the 'tagged-compound' is ~ 140 times more selective in killing human breast cancer cells than normal breast cells (unpublished data).

The half-life of plasma transferrin in normal human subjects has been estimated to be ~ 11 days [87]. A concern is whether or not the 'tagged-compound' may be neurotoxic, similar to that of long-acting artemisinin analogues [79]. However, with its large therapeutic window, it is likely that a dosage could be found which is safe.

5. Conclusion

A major consideration in effective drug therapy is selectivity in action. Thus, less drug would be needed and less side effects would be produced. Artemisinin fulfills this criterion by taking advantage of a major physiological difference between cancer cells and normal cells, that is, the former uptakes more iron. Because this is true for most cancer cells, artemisinin is conceivably effective against many types of cancer. A comparison of artemsinin-like compounds and other cancer chemotherapy compounds is shown in **Table 1**. In general, the *in vitro* effective doses of artemisinin-like compounds are comparable to those of traditional chemotherapeutic agents. However, the artemisinin-like compounds have much higher selectivity.

Further advantages of using artemisinin for cancer treatment are that it is effective orally and is inexpensive. In addition to extraction from the Artemisia plant, a technology is being developed to genetically engineer bacteria to synthesise artemisinin precursor, which would then be chemically converted to artemisinin [90].

Artemisinin-tagged transferrin provides a further improvement in selectivity. In this case, artemisinin is selectively brought into cancer cells, and release of iron from the transferrin molecule immediately triggers the cytotoxic action. Tagging of artemisinin to transferrin also increases the half-life of artemisinin in the body [87]. The short half-life of artemisinin and its analogues [8] has been a problem in its therapeutic effectiveness. On the other hand, because transferrin is a natural compound and iron is an important component in cellular functions, it is less likely that tolerance would be developed to artemisinin-tagged transferrin. However, the anticancer effectiveness of artemisinin-tagged transferrin *in vivo* remains to be studied.

6. Expert opinion

Artemisinin is an effective anticancer compound that produces few side effects. Because it is inexpensive and effective orally, it could be a useful anticancer agent for patients in developing countries where traditional treatments are inaccessible or unaffordable. Various analogues of artemisinin with different pharmacokinetics are available. Some may be more effective against certain types of cancer than others. For example, the methylated analogue artemether is more hydrophobic and readily crosses the blood–brain barrier. It may be more suitable for the treatment of brain cancer. The highest concentration of artesunate was found to be in the intestine after intravenous administration in the rat [91]. If this is also true in humans, artesunate may be a better analogue for the treatment of colon cancer. Artemisinin-tagged transferrin is more selective than artemisinin, but it is more expensive to produce. However, future technological developments, such as recombinant technique, may possibly lower the cost of this compound. Furthermore, it can also be easily administered to patients as an aerosol inhalant or nasal spray [92]. Thus, both artemisinin and artemisinin-tagged transferrin are promising candidates of targeted therapy for cancer.

The transferrin-receptor iron-transport system has been explored for targeted delivery of drugs to cancer cells [93]. For example, anticancer drugs [94] and toxins [95-97] have been tagged to transferrin for selective delivery to cancer cells. Drugs have been bound to antibodies against transferrin receptors and shown to become more selective against cancer cells [98]. However, a unique feature of artemisinin-tagged transferrin, that is different from these other compounds, is that it brings the prodrug (artemisinin) and its activating factor (iron) simultaneously and selectively into cancer cells, and the prodrug is activated immediately once it gets into a cell.

More research is needed to understand the mechanism and cellular sites of action of artemisinin and artemisinin-tagged transferrin. Possible mechanisms of the anticancer effect of artemisinin have recently been reviewed by Efferth [99]. Oxidative stress apparently plays a role [54,100,101] as would be predicted by the oxidant property of activated artemesinin. In the malaria parasite, artemisinin has been shown to form covalent adducts with various macromolecules [102,103], and it particularly inhibits the parasite orthologue of sarco-endoplasmic reticulum Ca^{2+}-ATPase [7]. Whether or not similar molecular interactions occur in cancer cells is not known. The mode of action of artemisinin is probably different from that of the 'tagged compound'. The 'tagged compound' exerts its effect mainly within the endosome as transferrin remains bound to the receptor and is generally recycled back to the plasma membrane. Thus, the 'tagged compound' probably causes cell death by damaging the plasma membrane, whereas for

Table 1. Cancer cell *in vitro* growth inhibition activity and selectivity of anticancer compounds and endoperoxides.

Compound	LC_{50} (μM)	Selectivity*	Reference
Doxorubicin	0.1‡ NA	2.5 4 – 6§	[88-89]
Cisplatin	1.4‡	5.4	[88]
Paclitaxel	0.01‡ NA	5.0 1 – 2§	[88-89]
Thioguanine	2.0‡	10.0	[88]
Vincristine	0.15‡	6.7	[88]
Mitomycin C	3.5‡	2.9	[88]
Etoposide	10.0‡ NA	1.0 2 – 15§	[88-89]
Staurosporine	0.035‡	0.7	[88]
Dihydroartemisinin + holotransferrin‡‡‡	2.59¶ 1.64¶	88 36	[45,84]
Artemisinin + holotransferrin	0.0026** 0.0054‡‡ 0.71§§ 0.7¶¶	NA NA NA NA	[47,63]
Artemisinin	0.0023** 0.0243‡‡ 6.4 – 11.5§§ 3.3 – 3.8¶¶	NA NA NA NA	[47,63]
Artemisinin + Fe(II)glycine sulfate	0.38§§ 0.42¶¶	NA NA	[47]
Artesunate	0.73 – 1.1§§ 3.5 – 3.8¶¶	NA NA	[47]
Artesunate + Fe(II)glycine sulfate	0.38§§ 0.42¶¶	NA NA	[47]
Artesunate + holotransferrin	0.49§§ 0.37¶¶	NA NA	[47]
Artemisinin-tagged Transferrin‡‡‡	0.98¶	34,000	[84]
Trioxane dimer	0.009 – 0.0023***	NA	[61]

* Ratio of drug concentrations exerting similar effects on normal and cancer cells. ‡ At 48 h, MDA-MB-468 breast cancer cells. § At 48 h, DU145 prostate cancer cells, MDA-MB-435 breast carcinoma cells, RKO colon cancer cells, and SW480 colon cancer cells. ¶ At 48 h, Molt-4 leukaemia cells. ** At 96 h, H69 drug-sensitive small-cell lung cancer cells. ‡‡ At 96 h, H69VP drug-resistant small-cell lung cancer cells. §§ At 7 days (168 h), CCRF-CEM leukaemia cells. ¶¶ At 7 days (168 h), U373 astrocytoma cells. *** At 72 h, TRAMP-C1A, -C2D, -C2G and -C2H prostate cancer cells. ‡‡‡ In studies 45 and 84, LC_{50} values were calculated as a 50% decrease in cell concentration from the original concentration of the cell sample, whereas in the other studies on artemisinin, LC_{50} is the concentration of artemisinin/artesunate that caused a decrease of 50% of cell concentration compared to that of untreated samples. This may explain why higher LC_{50} values are reported in studies [45] and [84]. LC: Lethal concentration; NA: Not available.

artemisinin, it diffuses into cells and its sites of action are intracellular locations where free iron is abundant (e.g., in mitochondria and lysosomes). Leakage of lysosomal enzymes could possibly cause cell death. A puzzling fact is that artemisinin does not seem to be toxic to fast dividing normal cells. This is shown in the authors' cancer prevention study [80], in which rats were provided with food mixed with artemisinin for 40 weeks. No apparent adverse effect, and particularly hair loss was observed. Also, in the authors' study on breast cancer cells [27], the effect of artemisinin was studied in both breast cancer cells and normal breast cells at log phase when they were dividing at comparable rates. It was found that artemisinin was toxic to the cancer cells and not the normal cells. Why are dividing normal cells less sensitive to artemisinin? One possibility is that iron metabolism is less well-controlled in cancer cells. Thus, mote free iron is available to react with artemisinin. It has been reported that some cancer cells express placental-like isoferritin [25,104], which is less effective than ferritin in storing iron. On the other hand, it is also possible that cancer cells intake iron at a faster rate than dividing normal cells, thus, increasing the chance of spill over. As this selectivity is also true with artemisinin-tagged transferrin, it is

Table 2. Receptors for iron-carrying proteins in some human pathogens.

Pathogen	Disease	Receptor
Moraxella catarrhalis	Otitis media	Transferrin, lactoferrin [120]
Moraxella lacunata	Kerato-conjunctivitis	Transferrin, lactoferrin [120]
Neisseria meningitidis	Meningitis	Transferrin, lactoferrin [120]
Neisseria gonorrheae	Gonorrhea	Transferrin, lactoferrin [120]
Actinobacillus actinomycetecomitans	Juvenile periodontitis	Transferrin [120]
Haemophilus influenzae	Meningitis, otitis media	Transferrin [120]
Staphylococcus aureus	Bacteremia, pneumonia, endocarditis, septic arthritis, osteomyelitis, deep abscesses,food poisoning	Transferrin [121]
Staphylococcus epidermidis	Endocarditis, endopthalmitis, septicaemia, cystitis	Transferrin [121]
Streptococcus pneumoniae	Pneumonia, meningitis, bacteremia, otitis media	Lactoferrin [122]
Leishmania chagasi	Leishmaniasis	Transferrin, lactoferrin [123]
Treponema pallidum	Syphilis	Lactoferrin [124]
Mycoplasma pneumonia	Pneumonia	Lactoferrin [125]
Bordetella pertussis	Whooping cough	Lactoferrin [126]
Trichonomas vaginalis	Vaginosis	Lactoferrin [127]
Helicobacter pylori	Gastritis, gastric and duodenal ulcers, gastric adenocarcinoma, lymphoma	Lactoferrin [128]

possible that endosomes in cancer cells are less effective in transporting released iron out to the cytoplasm.

One possible drawback of artemisinin-tagged transferrin is that it has to compete with endogenous transferrin for binding to receptor sites [105]. A possible method to overcome this, is to increase the tagging efficiency such that more artemisinin moieties would be attached to a molecule of transferrin. This has to be done such that the tagged load does not affect the affinity of the transferrin to its receptor.

By manipulating the transferrin-receptor iron-transport system and the oxidative state of a cell, the effects of artemisinin and artemisinin-tagged transferrin can be enhanced. As transferrin receptors on the cell surface are coupled to other membrane components, means can be applied to modify the rate of iron delivery into cells. For example, the K562 cell line has been widely used as an *in vitro* model for CML. Various compounds have been shown to enhance iron content in K562 cells. IFN-γ has been shown to upregulate surface transferrin receptors, thus enhancing iron uptake in K562 cells [106]. Erythopoietin also increases transferrin receptor expression and intracellular iron content in K562 cells [107]. Thapsigargin has been shown to speed up calcium-dependent iron uptake and increase the overall capacity of K562 cells in taking iron by accelerating the transferrin receptor endocytosis process [108]. Conceivably, these compounds could enhance the potency and specificity of artemisinin for the treatment of CML. Another interesting membrane component is the receptor for insulin-like growth factor I (IGF-I), which is endocytosed together with transferrin receptor. IGF has been shown to enhance iron influx in cells, paradoxically, by reducing the rate of endocytosis, and allows more time for cell surface transferrin receptors to pick up transferrin. Furthermore, IGF-I receptors are expressed in higher amount in cancer cells than in normal cells. Thus, co-administration of IGF-I and artemisinin-tagged transferrin could further enhance the potency and selectivity of the 'tagged-compound' in killing cancer cells. Because artemisinin works as a free radical, its effectiveness could also be enhanced by increasing oxygen tension, decreasing intake of antioxidants, and blockade of peroxidase and catalase by drugs such as micronazole [109-111]. However, these treatments have to be titrated such that they would not also increase the toxicity of artemisinin towards normal cells.

Due to the emergence of multiple drug-resistant bacteria, there is an urgent need to develop new antibiotics [112,113]. Some of these drug-resistant bacteria, for example, *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa,* and *Mycobacterium tuberculosis*, are major problems of human health, particularly in developing countries.

Because iron is an essential nutrient for growth, development and cellular functions, living organisms have evolved sophisticated mechanisms to obtain iron from the environment [114-119]. A strategy used by some bacteria is to hijack iron from iron-containing compounds endogenous to their hosts. For example, certain bacteria (see **Table 2**) have receptors on the surface to bind host transferrin and lactoferrin (an iron-carrying protein that is abundant in body fluids such as mucus). After binding, iron is released and actively transported into a bacterium [120]. These bacterial iron assimilation mechanisms can provide a basis for effective drug targeting and delivery.

Studies have shown that, in general, a high concentration of artemisinin is required to kill bacteria [129,130]. This is probably because iron is very tightly regulated in bacteria. Artemisinin is ineffective without iron. The question is whether or not artemisinin-tagged transferrin and lactoferrin are effective in killing bacteria that utilise these iron-transport proteins. The authors' preliminary data have shown that artemisinin-tagged transferrin is quite effective in inhibiting the growth of *M. tuberculosis* compared with isoniazid. *M. tuberculosis* has been shown to use human transferrin and lactoferrin as sources of iron [131].

Artemisinin could also be tagged to other physiological iron-carrying molecules. For example, bacteria have two basic mechanisms to pick up iron from the environment. The first is to hijack iron from iron-containing compounds endogenous to their hosts, such as transferrin and lactoferrin, as discussed above. Another mechanism is secretion and retrieval of highly specific iron-binding chemicals known as siderophores. Two major forms of bacterial siderophores are catecholate and hydroxamate that have very high iron affinity. They are synthesised and secreted by certain bacteria to scavenge iron from the environment. Bacteria have developed specific receptors to bind and pick up siderophores and transport them inside through molecular channels. Once inside, iron is released from the siderophores. Examples of bacteria utilising the siderophore mechanism are *Escherichia coli, Salmomella typhimurium, Klebsiella pneumoniae* and *P. aeruginosa*. There are also bacteria that use both host iron-carrying proteins and siderophores. Examples are *E. coli* and *M. tuberculosis*. There are bacteria (e.g., *Campylobacter jejuni*) that do not produce siderophores, but pick up siderophores released by other bacteria. By the same token as the artemisinin-tagged transferrin, artemisinin can be tagged to siderophores and these compounds may be useful for treatment of bacterial infection. Tagging artemisinin to siderophores is relatively easy. Because the molecules are small, they can be administered orally. Also, as mammalian cells do not bind or react with siderophores, they should be nontoxic to humans. Miller and co-workers have exploited the receptor-mediated transport of siderophores to deliver antibacterial agents, by tagging β-lactams, 5-fluorouridine and other antibiotic compounds to siderophores [132-134]. These compounds kill bacteria at mid-to-high micromolar concentrations. Thus, the use of artemisinin-tagged siderophores for treatment of bacterial infection is feasible. Again, they provide the advantage and efficiency of bringing both artemisinin and its triggering agent iron together simultaneously into a bacterium. It should also be pointed out that certain fungi also use siderophores to obtain iron [135]. Artemisinin-tagged siderophores may, therefore, also be useful for the treatment of fungal infection.

In general, compounds that contain iron-sensitive endoperoxide and physiological iron-carrying compounds tagged with endoperoxide, fit into the scheme of targeted therapy discussed in this paper.

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