Review Article

Antitumor Activity of Artemisinin and Its Derivatives: From a Well-Known Antimalarial Agent to a Potential Anticancer Drug

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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.

1. Introduction

Cancer remains as a life-threatening disease and a leading cause of death as its control has been difficult. Although, a range of conventional therapies based on chemotherapy, surgery, and radiotherapy are available, these approaches are in many cases of limited efficacy [1]. Moreover, current anticancer regimens are frequently associated with significant levels of toxicity and the emergence of drug resistance. One major challenge to relieve cancer burden is to develop highly effective drugs with specificity on cancers but little or no side effects on normal mammalian cells.

Many research projects have been focused on developing new chemotherapies either by exploring the anticancer ability of novel compounds or by assessing drugs conventionally used in other clinical diseases. Natural products have been found to be a relevant source of novel and potent bioactive compounds with minimal side effects *in vivo*. Plant derivatives have been known to be effective against a range of diseases with broad antimicrobial activity, and some have also exhibited significant antitumor activity. One of the promising compounds is artemisinin, a naturally occurring antimalarial with anticancer properties [2]. Artemisinin and its derivatives, which are commonly used in malaria therapy, have also potent anticancer activity in the nano- to-micromolar range in sensitive and drug- or radiation-resistant cell lines [3–5]. Importantly, artemisinin is one of the very few drugs that have been widely used as antimalarials but has no significant side effects [6] or clinical resistance, although tolerance has been reported [7]. Recently, growing amount of research has focused on the mechanisms underlying the action and response to artemisinin-like drugs.

In this review, we will revisit some of the key issues in the development of artemisinin and its analogs as anticancer agents to better understand the mechanisms of their antitumor effects from the insights of new gained knowledge. By considering the benefits, limitations, and current and future development of artemisinins, we can then identify emerging

questions and address future research needs in this promising field of cancer drug discovery.

2. Artemisinin and Its Derivatives

Artemisinin is a sesquiterpene lactone with a 1,2,4-tiroxane ring system (Figure 1). This endoperoxide compound is extracted from the Chinese herb *qinghaosu* (*Artemisia annua* or annual wormwood) which was used for treating fevers for over two millennia [8]. Despite its efficacy, the prototype drug, artemisinin, has pharmacokinetic limitations. Naturally, artemisinin has low solubility in water or oil, poor bioavailability, and a short half-life *in vivo* (∼2.5 h) [9, 10]. To overcome some of these problems, three generations of artemisinin-like endoperoxides including semisynthetic derivatives and fully synthetic compounds have been developed. So far, two generations of semisynthetic derivatives of artemisinin such as artesunate, arteeter, artemether, and artemisone have been effectively used as antimalarials with good clinical efficacy and tolerability (Figure 1).

Semisynthetic artemisinins are obtained from dihydroartemisinin (DHA), the main active metabolite of artemisinin [11, 12]. The first generation of semisynthetic artemisinins includes arteeter and artemether, the lipophilic artemisinins, whereas artesunate is the water soluble derivative [11, 12]. Artemisone, a second-generation artemisinin, has shown improved pharmacokinetic properties including longer half-life and lower toxicity [13]. So far, artesunate is the derivative that is commonly used in the antimalarial combination therapy.

Fully synthetic artemisinin derivatives have also been designed by preserving the peroxide moiety which confers potent drug activity. These compounds are easily synthesized from simple starting materials, thus being currently under intense development [14–17].

3. Antitumor Mechanism of Action of Artemisinin

In the malaria parasite, the endoperoxide moiety of artemisinin has been shown to be pharmacologically important and responsible of the antimalarial activity [18, 19]. The endoperoxide bond is thought to be activated by reduced heme (FPFeII) or ferrous iron (FeII) [20], leading to cytotoxic carbon-centered radicals which are highly potent alkylating agents [21]. Radicals may target essential parasite macromolecules causing parasite's death. However, the precise mechanism of action and primary target of artemisinin remain under study. In *Plasmodium*, it has been postulated that artemisinin may target organelles such as the mitochondrion, endoplasmic reticulum, and the digestive vacuole (reviewed in [22]). Some postulated molecular targets include heme alkylation, protein alkylation, Ca^{2+} ATPase (SERCA) inhibition, membrane damage, and loss of mitochondrial potential (reviewed in [22]). Despite the continuous debate on artemisinin activation and specific targets, supporting evidence points that heme or ferrous iron is required for potent activity [23]. This observation has been substantiated in other systems. In *Schistosomas*, artemether

has an exquisite action against the tegument; this activity is also enhanced by iron [24].

Interestingly, the potent anticancer action of artemisinin can also be attributed to the endoperoxide bond (red square in Figure 2) and shares the same parasitical chemical base. Lack of the endoperoxide moiety does not completely abrogate anticancer activity [25] but significantly reduces cytotoxicity to only fiftieth compared to those compounds with the trioxane ring [26–28]. Residual anticancer activity may be associated with an alternative peroxide-independent mechanism [26]. In a general consensus, iron and heme or heme-bound proteins have been involved in the bioreductive activation of artemisinin [29–31]. In most of the systems, preloading of cancer cells with iron or iron-saturated holotransferrin (diferric transferrin) triggers artemisinin cytotoxicity [32–35] with an increase in artemisinin activity up to 100-fold in some cell lines [36]. Moreover, artemisinins tagged to iron-carrying compounds exhibit greater activity compared with that of artemisinin alone [37–39]. Recently, it was shown that chemical modulation using succinylacetone, a heme synthesis inhibitor, decreases DHA cytotoxicity in HL-60 (human promyelocytic leukemia cells) [35]. This was consistent with previous studies showing that induction of heme oxidase followed by downregulation of the heme synthesis genes may also inhibit cytotoxicity of novel artemisinin dimers in the same cancer line [40]. Similarly, treatment with desferroxamine (DFO), an iron chelator, renders compounds inactive [41]. Iron and heme metabolism may have a relevant role in the selective antitumor activity of artemisinin. Continued proliferation and growth of malignant cells require higher iron metabolism to achieve processes of cell survival [35]. Therefore, cancer cells exhibit an increase in transferrin receptors (TfR) which are responsible for the iron uptake and regulation of intracellular concentrations. Levels of expression of TfR in cancer cells may vary depending on the cell line. However, they differ substantially from normal cells leading to a high selectivity index of artemisinin and its derivatives. Efferth et al. reported that leukemia (CCRF-CEM) and astrocytoma (U373) cells express TfR in 95% and 43% of the cell population, whereas normal monocytes only account for approximately 1% [42, 43]. Blocking the TfR by pretreatment with specific monoclonal antibodies abrogates artemisinin activity [43].

It has been hypothesized that iron-activated artemisinin induces damage by release of highly alkylating carbon-centered radicals and radical oxygen species (ROS) (Figure 2) [28, 35]. Radicals may play a role in the cell alterations reported in artemisinin-treated cancer cells such as enhanced apoptosis, arrest of growth, inhibition of angiogenesis, and DNA damage (Figure 2). Several studies have also associated artemisinin toxicity with impaired cytokinesis, enhanced levels of oxidative stress, inhibition of tumor invasion, migration, and metastasis (reviewed in [44]). ROS generation may contribute with the selective action of artemisinin on cancer cells. Tumor cells have enhanced vulnerability to ROS damage as they exhibit lower expression of antioxidant enzymes such as superoxide dismutase, catalase, and gluthatione peroxidase compared to that of normal cells [45, 46]. Hence, increasing oxidative stress is a common anticancer
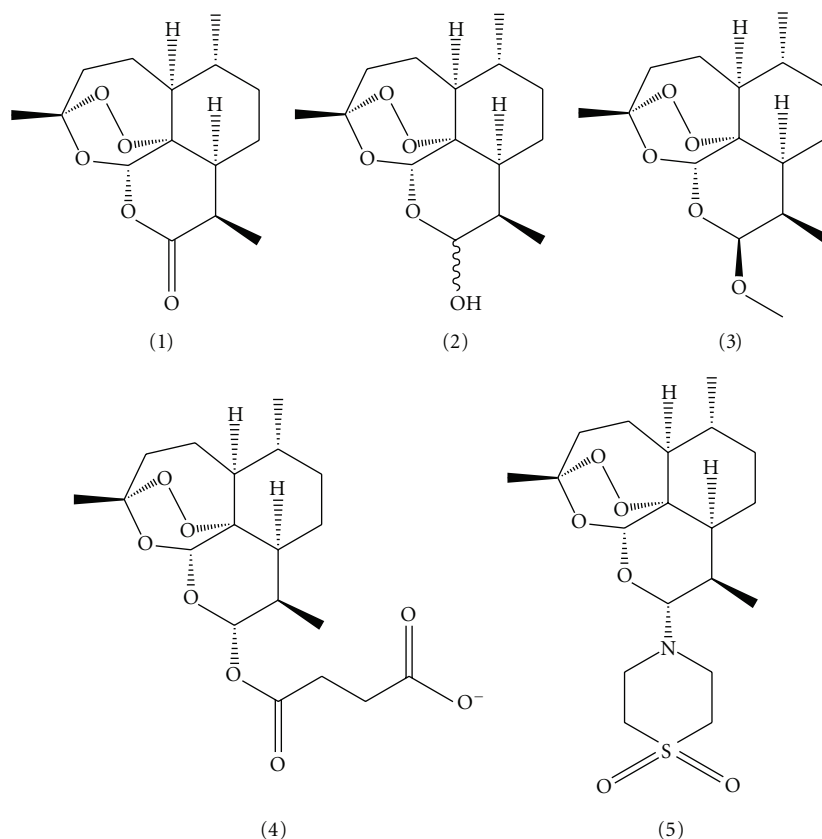
Figure 1: Chemical structure of artemisinin antimalarials (artemisinins) with anticancer activity. Artemisinin (1), dihydroartemisinin (DHA) (2), artemether (3), artesunate (4), and artemisone (5).

mechanism of antitumor agents [47]. In addition, the selectivity of artemisinin may be boosted by preferential targeting of cancer biomarkers or overexpressed cancer genes and proteins which are not detectable in normal differentiated tissues [48].

3.1. Generation of ROS as a Primary Effector of Cytotoxicity. As in *Plasmodium*, the artemisinin molecular targets in cancer cells are debatable. Although artemisinin-induced alterations in some tumor cells are consistent, it is not clear if this toxicity resides in defined molecular targets. Drug concentrations required to have an effect on cancer cells are often higher than those inducing toxicity in malaria parasites. Artemisinin, DHA, artesunate, and artemether exhibit 48 h IC_{50}s (fifty percent inhibitory concentration) up to 15 nM in malaria parasites [49, 50], whereas their anticancer activity is cell-line dependent and IC_{50}s fluctuate between 0.5 and $\geq 200\,\mu$M [5]. The exquisite sensitivity of malaria parasites to artemisinin points to the presence of specific parasitic targets. By contrast, in cancer cells, the artemisinin effect seems to be rather mediated by more general mechanisms through generation of ROS. However, it has been suggested that ROS-mediated damage may be triggered by an initiating event in the vicinity of artemisinin activation [35]. Microscopy analyses in artesunate-treated cells have shown early oncosis-like morphological changes at subcellular structures in which ROS generation may be triggered [51].

Microarray analyses found that the action of artemisinin seems to be modulated by the expression of oxidative stress enzymes including catalase, thioredoxin reductase, superoxide dismutase and the glutathione S-transferase family [5, 52]. Artemisinin-sensitive cells have downregulated oxidation enzymes whereas overexpression of these molecules renders cancer cells less sensitive [5]. Direct evidence in the HL-60 cell line has revealed that early and rapid generation (1 h) of ROS has been associated with apoptosis induction and artemisinin-induced damage. Furthermore, IC_{50} has been directly correlated with ROS levels [52]. Conversely, the action of artemisinin in several experimental systems has been reverted in presence of the antioxidant agents, N-acetyl cysteine, and 1,2-dihydroxybenzene-3,5-disulfonic acid (TIRON, an iron scavenger), which resulted in a delay in cell death [40, 52, 53]. A recent study has demonstrated that generation of ROS in artesunate-treated HeLa cells (16 h) occurring before cytotoxicity is being detected (48 h) [35], suggesting that this may be the starting event in artemisinin-induced damage. The electron transfer chain (ETC) in the mitochondrion has been proposed to play a role in the generation of ROS, however substantial cytotoxicity is still detected in HeLa cells devoid of ETC indicating that other sources of ROS may be available in the cells [35]. Indeed, emerging evidence has postulated that oxidative stress in breast cancer cells is initially generated in the lysosome as consequence of iron-activated artesunate in a process
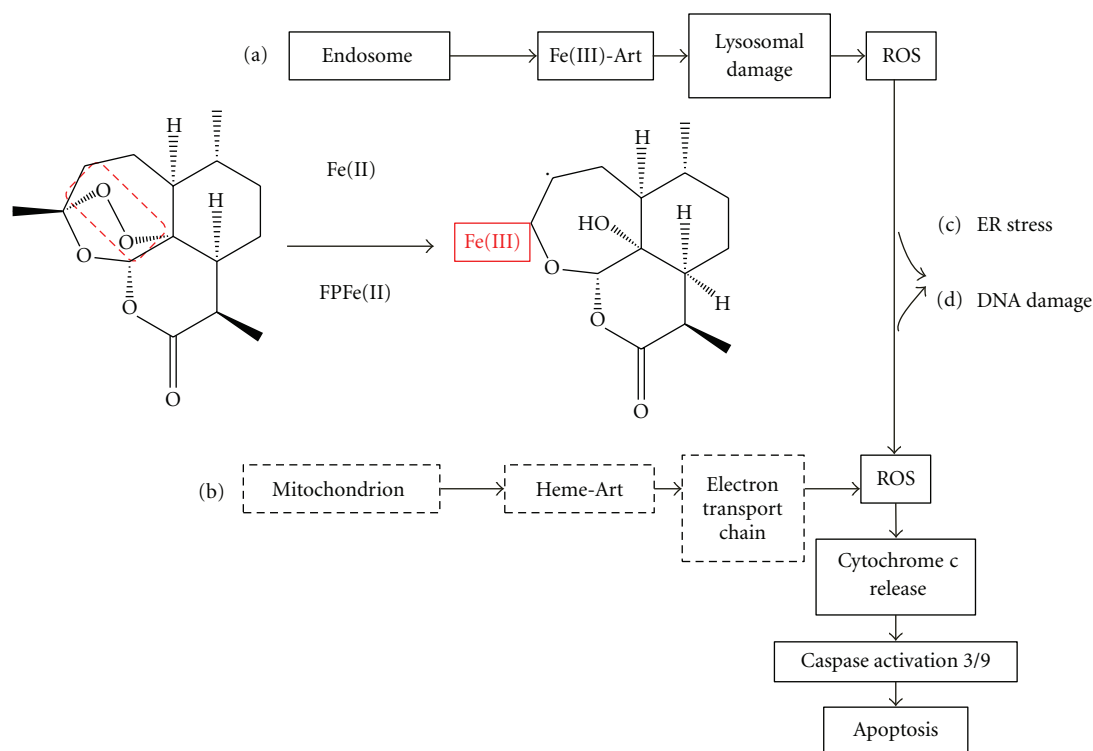
FIGURE 2: Postulated anticancer mechanisms of action of artemisinins. (a) It has been postulated that bioactivation of artemisinin occurs in the endosome after pH-induced release of iron from internalized transferrin. Iron activated-artemisinin generates carbon-centered radicals which may mediate lysosomal disruption and generation of ROS resulting in mitochondrial damage, activation of caspases, and cell death. (b) Alternatively, it has been suggested that only specific activation of artemisinin by heme or heme-bound protein generates cytotoxic-carbon-centered radicals. In the mitochondrion, these adducts interfere with the electron transfer chain (ETC) by interacting with heme or heme-bound proteins leading to generation of ROS and apoptosis. (c) ROS harboring may induce ER stress and (d) genotoxicity.

similar to that suggested in malaria parasites [31]. Thus, activation of the mitochondrial intrinsic apoptotic pathway is a downstream event leading to cell death [31] (Figure 2). In this model, artemisinins may be negatively controlling heme synthesis and further increase cytotoxicity [31].

Despite the growing evidence of ROS-mediated damage in many cell systems [31, 33, 35, 54], cell damage has been also independently associated with oxidative stress [35]. Particularly, novel artemisinin dimers seem to exert antitumor action with little or no ROS generation, however the underlying mechanism of cytotoxicity is still under study [26]. It also remains unclear if artemisinins-induced necrosis may be a ROS-independent mechanism of cell death [35].

The antineoplastic toxicity of artemisinins appears to be also modulated by calcium metabolism [40, 55–57], endoplasmic reticulum (ER) stress [33, 40], and the expression of the translationally controlled tumor protein, TCTP, a binding calcium protein which has been also postulated as a parasite target [5]. Although the expression of the TCTP gen, *tctp,* was initially correlated with cancer cell response to artemisinins, a functional role for TCTP in the artemisinin action has yet to be found [58].

As for malaria parasites, the role of sarcoendoplasmic Ca^{2+} ATPase (SERCA) as artemisinin target in cancer cells has also been explored [40]. Previous evidence has revealed that treatment with $10\,\mu M$ artemisinin increases calcium

concentrations as a result of SERCA inhibition [59]. However, studies on the mechanism of action of two artemisinin dimers have shown that potent ROS-mediated induced ER-stress after treatment was independent of SERCA inhibition [40]. Interestingly, the behavior of a highly active artemisinin dimer and thapsigargin, a well-known SERCA inhibitor, seems to be similar but mediated by different molecular events [40]. In fact, thapsigargin lacks the endoperoxide moiety and only generates discrete ROS levels. Nevertheless the ER appears to be a relevant site for artemisinin action as in HepG2 cells a fluorescent derivative has been shown to preferentially accumulate in this cell compartment [60].

Artemisinins have shown pleiotropic effects through different experimental systems. It is also possible that the underlying mechanisms mediating artemisinins cytotoxicity may vary upon specific hallmarks or shifting characteristics in cancer cell lines (Table 1). This will be only possible to elucidate if the molecular events involved in countering malignant cell proliferation are investigated in different cell lines under similar conditions.

4. Artemisinins as Anticancer Drugs

4.1. Antitumor Effects of Artemisinin and Its Derivatives.
Significant antitumor activity of artemisinin and licensed semisynthetic artemisinin derivatives has been documented

TABLE 1: Factors that may influence artemisinins response in cancer cells.

System	Factor/characteristic
Cancer cell	Proliferating activity
	Expression of transferrin receptors
	Accumulation levels of iron
	Levels of gene expression (i.e., proapoptotic and antiapoptotic genes)
	Shifting hallmarks
	Overexpression of potential molecular targets in some tumors
	Cellular dependence on redox balance
	Expression of antioxidant enzymes
	Expression of estrogen receptors in breast cancer cells
Artemisinin compound	Dose and time of exposure
	Chemical structure: number of trioxane rings, for example, dimeric compounds can be up to >1000 fold-more potent than monomeric artemisinins [61]
	In dimeric endoperoxides: nature and stereochemistry of the linker
	In novel compounds, electrophilic substitutions in the ring or those conferring lipophilicity. Boat/chair conformation

in vitro and in animal models. Considerable research has been focused on the most active compounds, namely, DHA and artesunate. One study that tested 55 cell lines from the Developmental Therapeutics Program of the National Cancer Institute (NCI) showed that artesunate displays inhibitory activity against leukemia, colon, melanoma, breast, ovarian, prostate, central nervous system (CNS), and renal cancer cells [5]. Dihydroartemisinin has also remarkable antineoplastic activity against pancreatic, leukemic, osteosarcoma, and lung cancer cells [62]. Moreover, artemisone has shown better activity than artemisinin and considerable synergistic interactions with other anticancer agents [63].

Artemisinin has been found to act either directly by inducing DNA damage (genotoxicity) or indirectly by interfering with a range of signaling pathways involved in several hallmarks of malignancy. However, direct DNA damage is only described in specific systems, while indirect effects are more commonly refereed in the literature. In pancreatic cells (Panc-1), artesunate caused DNA fragmentation and membrane damage. Interestingly, low doses of artesunate were associated with oncosis-like cell death, whereas higher concentrations induce apoptosis [51]. Extend and type of damage seem to depend on the phenotype and the origin of cell line, and it may also vary in a time- and dose-dependent manner (Table 1). Notably, higher sensitivity to artesunate was observed in rapidly growing cell lines when compared with slow growing cancer cells [5].

Alternatively, DHA, artesunate, and artemether are likely to modulate genes and proteins coordinating growth signals, apoptosis, proliferation capacity, angiogenesis and tissue invasion, and metastasis. A complex network of interactions through different pathways may enhance the anticancer effect of these endoperoxide drugs leading to cancer control and cell death (Table 2).

4.1.1. Artemisinins Counter Cancer Proliferative Capacity. In normal cells, cyclin-dependent kinases (CDK) are the proteins translating signals in order to push cell through the cell cycle. Normal growth relies on the ability to translate signals in order to replicate and divide in an effective manner [64]. Uncontrolled proliferation in cancer cells is the result of mutations inducing amplification of growth signals, deregulation of checkpoints, and loss of sensitivity to growth inhibitors [65]. Abnormal cell growth is also triggered by deregulation of programmed cell death or apoptosis [65]. Artemisinin and its semisynthetic derivatives are able to effectively induce cell growth arrest in cancer lines either by disrupting the cell cycle kinetics or by interfering with proliferation-interacting pathways. Dihydroartemisinin and artesunate are very potent growth inhibitors with multiple studies pointing to DHA as the most potent anticancer artemisinin-like compound (DHA > artesunate > arteeter > artemether) [5, 66]. Recently, artemisone has shown impressive antitumor efficacy in 7 cells lines including melanoma and breast cancer cells [63]. Artemisinin compounds have been shown to exert cytostatic and cytotoxic action on cancer cells [63, 67]. Artemisinin-induced growth arrest has been reported at all cell cycle phases; however, arrest at G_0/G_1 to S transition seems to be more commonly affected [5] (Table 2). Arrest at all cell cycle phases at the same time has been interpreted as a cytostatic effect [63]. Disruption of the cell cycle at G_2/M was observed after DHA treatment in osteosacorma, pancreas, leukemia [68] and ovarian cancer cells [69] (Table 2). Similarly, artesunate interferes with G_2 in osteosarcoma, ovarian, and other different cancer lines (Table 2). The underlying mechanisms of artemisinins-induced growth arrest include alterations in the expression and activity of regulatory enzymes of the cell cycle, such as CDK2-4 and -6 and D type cyclins (G_1-to-S-phase transition) or CDK1, and A-type cyclin (G_2/M) [70–72]. The antiproliferative action of artemisinin induces downregulation of CDK transcription, inhibition of CDK promoters or increase of p21, p27, and CDK inhibitor [72] (Table 2). Inhibition of proliferation may be also attributed to downregulation of interacting proteins targeting multiple pathways [72]. It has

TABLE 2: Antitumor effects of artemisinins.

Cmpd	Cancer/cell line	Effect	Event/mechanism	Refs
DHA/ART	Osteosarcoma	Growth arrest	G_2/M, decreased survivin	[73]
	4 cell lines with different p53 status	Apoptosis	Increased Bax, activation of caspase 3,8,9 Decreased Bc12, Cdc25B, cyclin B1, NF-κB	[44]
DHA	Hepatoma (different cell lines)	Growth arrest	G_1, decreased cyclin D, E, CDK2-4, E2F1 Increased Cip 1/p21, Kip 1/p27	[67]
		Apoptosis	Increased Bax/Bcl2 ratio, activation of caspase 3 Increased poly ADP-ribose polymerase Decreased MDM2	
DHA/ART	Neuroblastoma	Growth arrest	G_1	[52]
		Apoptosis	Activation of caspase 3	
DHA	Pancreas (BxPC3 RFP)	Growth arrest	G_1, decreased cyclin D1, increased p21	[74]
		Apoptosis	Increased Bax, decreased Bcl2	[74, 75]
			Decreased VEGF	[75, 76]
		Angiogenesis	Decreased NF-κB DNA binding	[74, 76]
			IL-8, COX2, MMP9	[76]
DHA	Human promyelocytic Leukemia (HL-60) Colorectal cancer (HT116)	Growth arrest	G_1	[33, 34]
		Apoptosis	ER stress, degradation of c-MYC	[33]
			Increased GRP78	[34]
			DNA damage	[33]
DHA	Lung cancer (SPCA1) (PC-14) (ASTC-a-l)	Apoptosis	Decreased survivin	[56]
			Increased calcium levels, increased p38 MAPK	[57]
			Increased oxidation, activation caspase 3,9,8 Bax translocation	[54]
DHA/ARS	Human ovarian cancer (cell panel, A2780, OVCAR-3)	Growth arrest	G_2	[69]
		Apoptosis	Increased Bax-Bad, decreased Bclx-Bcl2	[69, 77]
			Activation caspase 3/9 pathway	[69, 77]
DHA	Lymphatic endothelial cells	Apoptosis	Increased Bax, decreased Bcl2 Decreased VEGFR-3/FL-4	[78]
DHA	Melanoma (A375, G361, LOX)	Apoptosis	Increased oxidative stress, increased NOXA Activation caspase 3	[79]
DHA	Jurkat T Lymphoma	Apoptosis	DNA damage Increased oxidation, increased NOXA Increased Bak, activation of caspase 9	[80]
DHA	Fibrosarcoma (HT 1080)	Migration/invasion	Decreased NF-κB, AP-1 Decreased activation of MMP2, MMP9 Decreased PKC α/Raf/ERK and JNK	[81]
DHA	Glioma cells (C6)	Apoptosis	Decreased HIF 1α, VEGF	[41]
DHA	Chronic myeloid leukemia (K562 cells)	Growth arrest	G_2, decreased PCNA, cyclin B1, D1, E1	[82]
			CDK2-4, E2F1, DNA-PK, DNA-topo1, JNK VEGF	[68]
ART		Angiogenesis	Decreased VECF	[82]
DHA	Lewis lung carcinoma	Angiogenesis	Decreased VEGF-C, IL-1 β-induced p38 MAPK activation	[83]
			Decreased VEGF receptor KDR/flk-1	[84]
DHA/ART	Cervix carcinoma (HeLa) Human papillomavirus immortalized/transformed cells	Apoptosis	Activation of caspase 9	[85]
ART	Leukemia, melanoma, non-small cell lung cancer, colon, renal, ovarian, prostate, CNS; prostate, breast cancer (NIC cell panel)	Growth arrest	G_0/G_1, decreased CDK2, CDC25A G_2/M, decreased cyclin B1	[5]

TABLE 2: Continued.

Cmpd	Cancer/cell line	Effect	Event/mechanism	Refs
ART	Endometrial carcinoma (HEC-1B)	Growth arrest	G_0/G_1	[86]
		Apoptosis	Activation of caspase 3, decreased COX-2	
		Angiogenesis	Increased E-cadherin	
ART	Pancreatic cancer (BxPC3, MiaPaCa-2)	Apoptosis	Activation of caspases 3, 7 Inhibition of topoisomerase II a	[87]
ART	Non-small cell lung cancer (SPC-A1)	Metastasis	Decreased MMP2, transactivation of AP-1	[56, 88]
			NF-κB	
			uPA promoter	[88]
			MMP7	[56]
ART	Colorectal (CLY, HT29, Lovo)	Metastasis	Increased E cadherin Decreased Wnt-signalling pathway	[89]
ART	Mouse myeloma cell line SP2/0	Growth arrest	G_0/G_1	[90]
		Apoptosis	Decreased NF-κB p65, increased IκBα	
ARS	Hepatocellular cancer cells (HepG2, SMMC-7721)	Metastasis	Increased TIMP2, Cdc42, E cadherin	[91]
			Decreased MMP2	
	Nasopharyngeal cancer lines (CNE-1 and CNE-2)	Growth arrest	G_1	[92]
	Melanoma (A375P, A375M)	Growth arrest	—	[93]
		Migration	Decreased MMP2, $\alpha v\beta 3$ integrin	
ATM	Colorectal (HCT116, SW480)	Growth arrest	G_1, S, G_2, decreased CDK1	[63]
			All phases	
	Breast (MCF-7)		G_1, decreased CDK4, cyclin D1	

Abbreviations: Cmpd: compound; DHA: Dihydroartemisinin; ART: artesunate, ARS: artemisinin, ATM: artemisone.

been shown that DHA treatment in pancreatic cells (BxPC3, AsPC-1) inhibits viability by decreasing levels of proliferating cell nuclear antigen (PCNA) and cyclin D with parallel increase in p21 [74]. Another study in the same system shows that DHA counters NF-κB factor activation leading to inhibition of its targets in the proliferation (c-myc, cyclin D) and apoptotic pathways (Bcl2, Bcl-xl) [94]. Downregulation of survivin, a protein modulating apoptosis and G_2/M cell cycle progression [95], was observed after treatment with DHA in lung cancer cells (SPC-A1) [94]. A similar effect was described by Qiang et al. in artesunate- treated osteosarcoma cells [44]. In prostate cancer, DHA induces cell cycle arrest by disrupting the interaction of Sp1 (specificity protein 1) and the CDK4 promoter [96]. Dissociation of the Sp1-CDK4 complex promotes caspase activation and cell death. In addition one work has identified artesunate as a topoisomerase II, an inhibitor which inhibits growth by interaction with multiple pathways [87]. Overall, artemisinins seem to be interfering with several pathways that are common to different cancer entities.

4.1.2. Proapoptotic Effect of Artemisinins. Apoptosis is a widely studied mechanism in antitumor therapy as its manipulation is an effective strategy for cancer control. This cellular process is mediated by a balance between the Bcl2 family genes, the proapoptotic Bax, and the antiapoptotic Bcl2 and their effects on the mitochondria [97, 98]. An increase in the Bax/Bcl2 ratio induces the release of cytochrome c followed by sequential activation of caspases and culminating with cell death [98].

Apoptosis is a common and rapid artemisinin-induced effect observed in many cancer and cell lines. Treatment with 200μM DHA in leukemia cells induced apoptosis after 1 hour of exposure [32]. Artemisinin sensitivity has been correlated to the level of expression of antiapoptotic (Bcl2) and proapoptotic genes (Bax) in a cancer cell line [61, 97, 99] (Table 1). In general, the apoptotic effects of artemisinin have been attributed to activation of the intrinsic pathway. Hence, mitochondrial membrane damage is thought to have a pivotal role in the cascade of cell death events. Many studies have revealed that artemisinin-like compounds induce apoptosis by modulating the Bax/Bcl2 ratio [33, 44, 54, 63, 75, 77, 78, 86, 99]. Consistent with these observations, DHA and artesunate, in a panel of osteosarcoma cells, caused cytochrome c release, Bax overexpression, increase in Bax/Bcl2 ratio [44, 73], and activation of caspases 3 and 9. DHA also activates caspase 8 and decreases the levels of CDC25B, cyclin B1, and NF-κB [73]. In the same system, artesunate exposure depletes survivin which has also been involved in the apoptotic DHA response in lung cancer cells [56]. Similar events have been described in hepatoma cancer lines treated with DHA, particularly in this system DHA and the prototype drug artemisinin seem to have similar potency [67]. A microarray analysis has correlated the expression levels of c-MYC with enhanced DHA-induced apoptosis. Leukemia (HL-60) and colon cancer cells (HCT116) expressing high levels of c-MYC are significantly more sensitive to the DHA proapoptotic action. Moreover, knockdown of *c-myc* in HCT116 depleted DHA-associated cell death [33]. Downregulation of *c-myc*

may also correlate with induced G_1 arrest in this cell line [33]. Studies in metastatic melanoma (A375, G361 cell line) and Jurkat T lymphoma cells have associated the elevated apoptotic action of DHA with upregulation of NOXA (a proapoptotic protein), caspase 3 activation, and oxidative stress [79, 80]. In lung cells, the apoptotic effect of DHA occurs with increasing calcium concentration and activation of p38 [56, 57].

In some studies alterations on molecules acting on the extrinsic apoptotic pathway have also been described [54]. DHA seems to increase the transcription of the cell death receptor 5 (DR5) promoter and induces DR5 in different prostate cancer lines. In fact, a combination treatment with TRAIL, a DR5 ligand, strongly enhances DHA proapoptotic action by up to 35% on this system [100].

Artemisinins usually promote apoptosis rather than necrosis in most of the systems, however in some cases both apoptosis and necrosis have been reported. Induction of apoptosis is a major benefit of artemisinins' antitumor action as it prevents the collateral effects of inflammation and cell damage caused by necrosis. Artemisinin-induced necrosis has been associated with low levels of ATP and defective apoptotic mechanisms in some cell lines [35].

4.1.3. Artemisinins and Metastasis/Invasion Inhibition. The ability of malignant cells to invade has been associated with high mortality and morbidity in cancer patients. The spread of cancer cells to other organs is a process in which malignant cells readily invade through the extracellular matrix, reach and survive in the bloodstream, and finally seed at distant organs [101]. To achieve invasion, the cancer cell requires the loss of expression or function of E-cadherin, a calcium-binding transmembrane molecule involved in cell-cell adhesion. A range of genes encoding extracellular matrix processing proteases, motility factors, and adhesion proteins are also acting at different steps in the metastatic process [101]. Recently, PAI-1 and TIMP-1 known as endogenous protease inhibitors have also been shown to be involved in cancer metastasis [102]. An invaluable benefit of artemisinin is its relevant antimigratory activity in highly aggressive and invasive cancer entities [56, 59, 88, 91]. Antimetastatic activity of artemisinins has been correlated with modified expression of the matrix metalloproteinases (MMP) gene family and their effects on $\alpha v \beta 3$ integrins [93]. In hepatoma cells (HepG2 and SMMC 7721), treatment with $12.5\,\mu$M artemisinin depleted migration linked to a decrease in MMP2 with concomitant increase in TIMP-2. Inhibition of metastasis is achieved as artemisinin increases cell-cell adhesion by enhancing E-cadherin activity and Cdc42 activation [91]. In addition, it has been found that some cancer cells may have specific proteins cointeracting at different pathways. For example, in non-small cell lung cancer [56] and fibrosarcoma, DHA treatment induced low levels of MMP2, MMP7, or MMP9 driven by AP-1 and NF-κB transactivation or inactivation [81]. Previous studies have shown that MMP2 is regulated by Sp-1 transcription factor activity [103], moreover DHA-induced disruption of Sp-1 molecular interactions has been postulated as a crucial event for DHA regulation effects on different pathways [72]. Other investigations have found

that in mouse lung Lewis cancer, lymphoid node metastasis and lymphangiogenesis were retarded by artemisinin-mediated inhibition of vascular endothelial growth factor C (VGF-C) [83].

4.1.4. Artemisinins and Angiogenesis Inhibition. As malignant tissues grow, metastases and solid tumors require extra blood supply for thriving and survival. Thus, cancer cells induce neovascularisation by regulating proteins and pathways involved in the generation and restructure of new vasculature [101]. Angiogenesis process leads to enhanced proliferation of endothelial cells through induction of vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), its receptors, and cytokines [101]. This event occurs via multiple mechanisms including hypoxia-driven activation of expression of HIF-1α and the aryl hydrocarbon receptor nuclear translocator (ARNT) [104]. Angiogenesis control is mediated by angiostatin, endostatin, thrombospondin, TIMPs, PAI-1, and others [101]. Due to their role in tumor survival, the proangiogenic factors and the molecules involved in their regulatory networks are relevant drug targets. A microarray-based study revealed that artemisinins, artesunate and other derivatives inhibit neovascularisation by modulating gene expression of angiogenic factors [105]. Artemisinins responses seem to be mediated by downregulation of growth factors (VEGF, FGF) [82, 106], HIF-1α [107], new vessel mediator angiogenin (ANG), the cysteine-rich angiogenic inducer (CYR61), some metalloproteinases (MMP9, MMP11, and BMP1), and collagens [105]. In parallel, artemisinins- induced upregulation of angiogenesis inhibitors was observed [105]. These findings have been supported by experimental investigation in different systems, unveiling other molecular interactions. Exposure of human umbilical vein endothelial cells to $50\,\mu$M DHA prevents angiogenesis by depleting the levels of the VEGF flt-1 and KDR/flk-1-receptors. Similar effects were reproduced in lymphatic endothelial cells and Lewis lung carcinoma [78, 84]. In pancreatic cells (BxPc-3) and BalB/c nude mice, DHA induced inhibition of NF-κB DNA binding and downregulation of its angiogenic-related targets such as VEGF, IL-8, COX2, and MMP9 [76]. Reduced levels of NF-κB have been previously associated with proliferation and metastasis inhibition [33, 81, 90, 94] suggesting that NF-κB regulation may be a key role in the multimodal action of DHA in this system. NF-κB is a crucial factor regulating multiple processes and it has a key role in the anticancer drug response. It is activated by DNA damage and it is a mediator of apoptosis resistance in response to drug pressure.

Other anticancer properties have also been attributed to artemisinins. Artesunate has shown its ability to revert cellular transitions allowing re-differentiation of tissues by negative control of Wnt-signaling pathway [89]. Notably, artesunate has been found to be more effective in less differentiated cell lines [89].

4.2. Antitumor Action of Artemisinins in Resistant Cancer Cells. One major obstacle for a successful anticancer therapy is the development of resistance over time. Many aggressive tumors become refractory to anticancer therapy with hardly

any chemotherapeutic alternatives. A leading cause of drug resistance is the drug efflux generated by overexpression of membrane protein pumps, which results in ineffective low drug concentrations [108]. Anticancer activity of artemisinins has shown to be unaffected in otherwise resistant and multiresistant cancer cells. One study using the 55 NCI cell lines and microarray analysis revealed that genes related with resistance to the established anticancer drugs such as *MDR1 (Pgp), MRP1,* and *BCRP* showed no impact on the activity of artemisinins [5]. This was substantiated when no effects on the artesunate growth inhibition profile were observed in multidrug resistance HL-60 cell lines overexpressing *MRP-1* and *BCRP*-overexpressing cells, suggesting that antitumor activity of artemisinin is preserved when resistance to other agents is present [5]. Artemisinins are effective in a broad range of resistant cancer lines including doxorubicin, metrotexate, and hydroxyurea-resistant lines with no cross-resistance [5]. Further investigation has shown that artesunate proapoptotic effect is not affected in a doxorubicin-resistant leukemia cell line; instead artesunate potentiates doxorubicin apoptotic effects [4]. In another study, anticancer potency of artesunate is preserved in chemoresistant and chemosensitive neuroblastoma cell lines and primary neuroblastoma cultures [52]. In this system, sensitivity to artesunate was not affected in vincristin, doxorubicin, cisplastin, topotecan, mephalan, and ectoposide-adapted cells with IC_{50}s ranging from 1.4–2.7 μM similar to that of the parent sensitive cell line [52]. Only one cell line showed low sensitivity to artesunate which was related to low ROS formation and increased expression of gluthatione cysteine ligase (GCL) [52]. Depletion of glutathione mediated by a GCL inhibitor improved artesunate sensitivity in this cell line [52]. P-glycoprotein (Pgp) or p53 attenuation did not affect sensitivity to artesunate [52]. DHA has shown the lowest IC_{50} in some cell lines such as cholangiocarcinoma (CL-6) and hepatocarcinoma (Hep G2) compared to other anticancer agents; moreover, upregulation of *MDR1*, *MRP1-2*, or *MRP3* shows no effect on potency [109]. Lack of cross resistance between anticancer agents and artemisinins might be based on different mechanisms of drug action and/or resistance. Most of the conventional anticancer agents are nucleoside analogs, whereas artemisinins action is thought to be mediated by a ROS-dependent mechanism. Furthermore, in erythromyelogenous leukemia and human small cell lung cancer, artemisinins show no significant inhibition towards Pgp or MRP1 [4], thus in principle overexpression of protein pump may not affect artemisinin's potency. In another system however, artemisinin (the prototype drug) increases doxorubicin resistance by upregulating *mdrp* through a mechanism that will be discussed later.

4.3. Interactions of Artemisinins and Standard Anticancer Chemotherapy: Artemisinin Combination Therapy (ACT) for Cancer?

Existing anticancer therapies predominantly target cancer proliferation either with chemotherapeutic agents, ionizing radiation or direct toxicity on growth factor signaling pathways. In a combination therapy for cancer, the antineoplastic action of artemisinin may contribute to an independent antitumor activity with no additional side effects.

The benefits of combining artemisinins with other anticancer agents have been investigated showing that multifactorial action of artemisinin in different pathways may improve overall activity (synergism).

It has been reported that resistant cancer cell lines become sensitive by adding artemisinin to the conventional treatment (chemosensitization). Interestingly, DHA and artesunate have exhibited the strongest chemosensitizing/synergistic effects [4, 110], whereas the prototype drug artemisinin shows only additive and antagonistic interactions (Table 3). DHA significantly improves the anticancer effect of gemcitabine, an anticancer drug used in pancreatic cancer which develops resistance over time. *In vitro* and *in vivo* analysis in pancreatic cells demonstrated a DHA-induced increase in growth inhibition and apoptosis by 4- and 2-fold, respectively, compared with those obtained with gemcitabine alone [94]. A dual action of DHA in potentiating gemcitabine activity and possibly counteracting resistance has been attributed to DHA inhibition of gemcitabine- induced NF-κB activation and subsequent action on its targets [94]. A similar effect has been shown in hepatoma cancer cell lines irrespective of their p53 status [67]. DHA synergistically enhances tumor growth inhibition by 45% when in combination with gemcitabine, whereas artemisinin, the prototype, only induces additive effects [94].

Consistent with this observation, a greater antitumor activity was observed when DHA was used in a combination with cyclosphosphamide in murine Lewis lung carcinoma cell line or in combination with cisplatin in non-small cell lung cancer A549 in mice [84]. In rat C6 glioma cells, addition of 1 μM DHA increased by 177% the cytotoxic effect of temozolomide, a DNA-alkylating agent used in the treatment of brain cancer. Further investigation found that DHA promotes apoptotic and necrotic activity of temozolomide through ROS generation [107]. Recently, an enhancement of artesunate anticancer activity has been observed in different combination regimens. A striking synergy was achieved in combinations of artesunate and the immunomodulator drug, lenalidomide [111].

However, the benefits of an artemisinin combination therapy need to be carefully dissected. Therapeutic effects are influenced by the mode of action of the drugs and multiple interactions in particular systems and schedules. Recently, Gravetth et al. showed that gemcitabine has only additive effects when combining gemcitabine and artemisone in colon and breast cancer cells [63]. In cancer colon cells (HT-29), it has been suggested that artemisinin may impair doxorubicin activity possibly by countering the doxorubicin effect on NF-κB inhibition [59]. The same authors have reported artemisinin-induced resistance in the same system through a different mechanism. Thus, it has been postulated that artemisinin exposure inhibits SERCA with subsequent accumulation of calcium. As a result, Pgp is upregulated and leads to generation of doxorubicin resistance cells [59]. By contrast, pretreatment with a calcium chelator reverted the cells to a sensitive phenotype [59]. Notably, DHA and artesunate have not been evaluated in this system; it remains to be elucidated whether the most potent chemosensitizers have similar effects on this cell line. So far, artesunate or DHA

TABLE 3: Drug interactions of artemisinins.

Drug combination	Cancer/cell line	Effect	Refs
DHA + Temozolomide	Rat C6 glioma cells	Increased apoptosis, ROS Induced necrosis	[107]
DHA + Cyclophosphamide	Lewis lung carcinoma	Increased apoptosis, decreased VEGF receptor KDR/flk-1 Apoptosis	[84]
DHA + Cisplatin	Human non-small cell lung cancer (A549)	Decreased metastasis	[84]
DHA + Gemcitabine	Pancreas (Panc-1)	Inhibition of proliferation, decreased cyclin D1 Increased apoptosis, increased Bax/Bcl2 ratio, activation of caspase 3	[94]
	Hepatoma (cell panel)	Increased growth inhibition by 45%	[67]
DHA + Butyric acid	Human lymphoblastoid leukemia (Molt-4)	Synergistic. Depletion of cancer cells	[110]
DHA + Radiation	Glioma cells U373MG	Increased cytotoxicity Inhibition of radiation-induced GST	[53]
DHA + Carboplatin	Ovarian cancer cells (A2780, OVCAR-3)	Increased growth inhibition through death receptor and mitochondrial mediated pathways	[77]
DHA + TRAIL	Prostate cancer (DU145, PC-3, LNCaP)	Increased apoptosis extrinsic and intrinsic pathways	[100]
ART/DHA + Doxorubicin + Pirarubicin	Leukemia (K562/adr) Small cell lung cancer (GLC4/adr)	Synergistic	[4]
ART + Lenalidomide	Lung (A549) and breast (MCF-7)	Decreased IC50 by 48%	[111]
ART + Oxiplatin	Colon (HT 1116)	Additive. Sensitising effect	[111]
ART + Gemcitabine	Breast (MCF-7)	Additive	
	Lung (A549)	Additive	
ATM + Oxiplatin ATM + Thalidomide ATM + Gemcitabine	Colon (HCTl16, SW480) Breast (MCF-7)	All additive	[63]
ARS + Hyperbaric oxygen (HBO$_2$)	Molt-4 human leukemia	22% decrease in growth	[112]
ARS + Doxorubicin	Colon cancer(HT29)	Predicted as antagonic, mediated by activation of NF-κB/overexpression of Pgp	[59]
ARS + Oxiplatin	Colon (HCTl16, SW480)	Antagonism	[63]
ARS + Thalidomide		Additive	
ARS + Gemcitabine	Breast (MCF-7)	Antagonism	

Abbreviations: HBO$_2$: hyperbaric oxygen.

in combination with doxorubicin and pirarubicin showed chemosensitizing effect in leukemia and human-small-cell-cancer-resistant cell lines, but no further increase of sensitivity was observed in the sensitive parent cell lines [4]. The chemosensitising effect was independent of Pgp inhibition [4]. Overall, this evidence suggests that DHA and artesunate have remarkable ability to potentiate antitumor agents and to counter tumor resistance.

Artemisinins also improve ionizing-based therapies. In glioma cells U373MG, DHA treatment inhibits the radiation-induced expression of GST with concomitant ROS generation. A combination treatment with DHA has been shown to be more effective than radiation or DHA alone [53]. The adjuvant effect of artemisinin in other cancer treatments including hyperbaric oxygen has also been reported [112].

4.4. Artemisinin Resistance. A salient feature of artemisinin is that artemisinin resistance *in vitro* or in the field has yet to be confirmed after 30 years of use as an antimalarial. Clinically, tolerance has been reported in patients with therapeutic failure. However, *in vitro* tolerant strains are usually unstable and only develop after several years of continuous drug exposure [113]. The multimodal action of artemisinins at different cancer pathways might also predict a delay of induced resistance in malignant cells. Indeed, only few cell lines have shown intrinsic low sensitivity or no response to artemisinin or its derivatives. For example, artemisinin (the prototype drug) seems to be less active in breast cancer cells (MCF-7) and gastric cancer (MKN) [93]. Some studies in breast cancer cells have suggested that artemisinin response may be mediated by estrogen receptors (ERα and ERβ) which

are involved in cell proliferation (reviewed in [72]). Interestingly, it has been documented that in breast cancer cells, disruption of iron metabolism may enhance potency of doxorubicin and cisplatin [114]. The low response to artemisinin has been also associated with overexpression of BMI-1 in highly metastatic nasopharyngeal cancer cell lines (CNE-1, CNE-2) [92]. A recent study found some levels of cross resistance to artesunate and DHA in a unique cisplatin chemo-resistant cell line. This effect was partially reverted by L-buthionine-S,R-sulfoximine, an inhibitor of the antioxidant GLC [52].

However, *in vitro* resistance has already been developed under experimental conditions. Microarray and experimental studies using knockouts and transfected cells indicate that upregulation of the tumor suppressor p16^{INK4A} and the antioxidant protein, catalase, may confer resistance to artesunate independent of the p53 status [115]. Recently, concerns have arisen after Baechmeier et al. showed that a 24 h preincubation with 20 μM artesunate induces resistance in highly metastatic breast cancer cells. Pretreated MDA-MB-231 metastatic cells were completely refractory to further artesunate treatment, whereas a similar treatment in MDA-MB-468, a non-metastatic cell line, renders less sensitive cells. Further investigation on the mechanism of artesunate-acquired resistance indicates that upregulated transcription of NF-κB, AP-1, and NMP-1 overcome artesunate apoptotic and antimetastatic action and allows tumor progression [116]. It is not clear, however, whether artesunate-induced resistance and loss of sensitivity are preserved after long-term cell subculturing. It also remains to elucidate if other semisynthetic endoperoxides may induce a similar effect or whether a combinational therapy may delay or revert the effect on cell lines bearing this phenotype.

4.5. Artemisinins Toxicity. Dose-dependent toxicity is a major drawback that hampers anticancer therapy. This problem may be overcome by enhancing anticancer activity and thus reducing toxic drug concentrations. DHA is the most active and neurotoxic artemisinin derivative [117]. Neurotoxicity has been reported in animal studies in a dose- and time-dependent manner ($\geq$7 days) [118–120]. The toxicity of artemisinin-like compounds has been associated with long-term availability, whereas short-term peak concentrations are not toxic [121]. Thus, rapid elimination of artemisinin in oral formulations is safer than slow-release or oil-based intramuscular formulations [6, 121]. Remarkably, although artemisinins derivatives have been widely used as antimalarials, their toxicity in humans have been shown to be negligible. In cancer therapy, artemisinin may have multiple benefits as it can be used in combination with no additional side effects, but also it enhances potency and reduces doses of more toxic anticancer partners. Clinical doses used in malaria treatment after administration of 2 mg/kg in patients rise plasma concentrations of 2640 $\pm$ 1800 μg/mL (approximately 6.9 $\pm$ 4.7 mM) which can be considered up to 3 orders of magnitude higher than those artemisinin concentrations with antitumor activity [5]. It becomes relevant to closely monitor the safety of long-term artemisinin-based therapies as severe side effects may be highly unusual but significant.

So far, artemisinin treatments for as long as 12 months have been reported with no relevant side effects [30, 122, 123]. However, an extremely rare case of toxic brainstem encephalopathy was described in a patient after a 2-week herbal/ artemisinin combination (400 mg) regimen for breast cancer [124]. Brainstem neurotoxicity has been reported in animal studies and associated with long-term (>28 days) and high-dose treatments [118]. Recently, a fatal case of overdosing in a child who was taking antimalarial treatment was reported [125].

4.6. Artemisinins in Clinical Trials. Antitumor activity of artemisinin has also been documented in human trials [126] and individual clinical cases [30, 122]. Artemether and artesunate have been used in cancer therapy with good tolerability and lack of significant side effects.

Artesunate was successfully used in the treatment of laryngeal squamous cell carcinoma and substantially reduced the size of the tumor (by 70%) after two months of treatment [122]. Furthermore, artesunate increased survival and substantial metastasis reduction when used in combination with standard chemotherapy in patients with malignant skin cancer [30]. Another report describes a beneficial improvement in a patient with pituitary macroadenoma who was treated with artemether for 12 months [123]. Artemether has longer half-life and easily crosses the blood-brain barrier which is crucial for brain tumor treatment.

Similarly, a clinical trial in 120 patients with advanced non-small cell lung cancer has shown that artesunate in combination with a chemotherapy regimen of vinorelbine and cisplatin elevated 1-year survival rate by 13% with a significant improvement in disease control and time to progression [126]. No additional artesunate-related side effects were reported [126]. In Germany, a trial in patients with advanced breast cancer is currently ongoing. Tolerability to a combination therapy of 4-week artesunate will be assessed in this trial. Another trial in UK in colorectal adenocarcinoma to evaluate anticancer action and tolerability of artesunate was completed last year, but the results have not been published.

5. Anticancer Action of Novel Artemisinins Derivatives

5.1. Novel Semisynthetic Derivatives with Antitumor Action. Imperative need of highly effective compounds with enhanced pharmacological properties has led to the design of novel endoperoxide compounds with selective toxicity toward cancer cells. Considerable progress has been made in the design of novel compounds with enhanced potency at the nanomolar range, increased selectivity, and low toxicity *in vitro*. It has been reported that triazolyl substituted artemisinins-induced significant growth inhibitory effect [127]. Independent of stereo- or region-chemistry, strong inhibition was influenced by the functional group attached to the triazole ring. Substituted compounds with a penthyl benzene group showed the highest antiproliferative activity ranging from 0.07 to 0.39 μM 72 h IC$_{50}$ in 6 cancer lines [127]. Recently, Feng et al. synthesized a series of dihydroartemisinin derivatives via an aza-Michael addition reaction with high selectivity index and

IC_{50} in the nanomolar range against HeLa cells ($0.37\,\mu M$) [128]. In a series of deoxoartemisinins and carboxypropyldeoxoartemisinins, antitumor effect was associated with boat/chair conformations and drug-receptor interactions [129].

Different from their antiparasitic activity, it has been found that dimeric and trimeric artemisinin derivatives display much higher antitumor activity than their monomeric counterparts. In the last decade, an increase in the number of outputs in artemisinin dimeric compounds with anticancer activity has been observed. These compounds have shown IC_{50} ranging from 0.014 to $6\,\mu M$ [130, 131]. Potent anticancer toxicity has been correlated with the nature of the linker [132] and with lipophilicity or electrophilic substitutions [66]. Posner et al. developed a series of artemisinin-trioxane derivate dimers from which two phosphate esters displayed nanomolar growth inhibitory values in the NCI 60 human cell line screen. Further investigation *in vitro* showed that in HL-60 cells, these compounds are more potent than doxorubicin, whereas their strongly anti-parasitic monomeric counterparts showed no anticancer activity. As suggested by the authors, two trioxane units in addition to the nature of the linker may be relevant in conferring potent anticancer activity [132]. Homodimers of artesunic acid have also nanomolar inhibitory values when tested in chemo-resistant and sensitive leukemia cells. Notably, the artesunic dimer seems to be 6-fold more potent in the multiresistant Pgp overexpressing cells (CEM/ADR500) than in its sensitive counterparts. Anticancer activity was attributed to apoptosis induction, arrest of cell cycle at G_0/G_1, and ROS generation [131]. In prostate cancer cells (LNCaP, TRAMP CIA, and C2H), two C10 non-acetal trioxane dimers displayed a 3-fold increase in potency compared to doxorubicin (17-18 nM versus 45.3 nM resp.). The dimers induced arrest at G_0/G_1 mediated by decreasing cyclin D1, cyclin E, CDK2, and an increase in p21 and p27. They also show proapoptotic action through upregulation in Bax expression [130].

In many studies, there has been an emphasis on the nature and stereochemistry of the dimer linker which may influence anticancer activity. However, it has also been shown that the linker by its own is inactive. Morrisey et al. have described that an artemisinin dimer exhibits up to 30-fold more activity than artemisinin in prostate cancer lines [61]. This dimer selectively exerted highly antigrowth potential and apoptosis in C4-2 (a cell line derived from LNCaP) and LNCaP cells compared to artemisinin [61]. An enhanced anticancer activity seems to be given by the stereoisomery of the linker [130]. In another study, C12 non-acetal dimers and one trimer of deoxoartemisinin showed similar potency to that of the conventional anticancer drugs against many cell lines. The linker with one amide or one sulfur-centered 2 ethylene groups was essential for potent anticancer activity [133]. Diastomeric-cholic-acid-derived 1,2,4,5-tetraoxanes were also tested and found to have high anticancer activity against human melanoma (Fem X), and cervix cancer (HeLa) [134] cis stereoisomers were twofold more active. The authors further suggested that an amide terminus in the linker confers increased anticancer activity.

Interestingly, Beckman et al. showed that the stereochemistry of the ether linkage of the dimers, of dihydroartemisinin (diDHA), and dihydrodeoxyartemisinin (the respective endoperoxide lacking dimer) was as important for antitumor activity as the endoperoxide moiety. Dimers were tested against 60 cells from 9 different cancers showing that although in general the diDHA was more active than dihydrodeoxyartemisinin toward anticancer growth, asymmetrical dimers of either diDHA or dihydrodeoxyartemisinin were similarly toxic [26]. The mechanism underlying the antiproliferative action of the artemisinin-derived dimers needs further study.

Recently, a series of potent artemisinin-like derivatives of easy synthesis and anticancer activity has been identified. These endoperoxides exhibit high chemical stability and greater cytotoxicity than artesunate. These compounds also exhibit relevant antiangiogenic properties as judged by studies in a zebrafish model [135].

5.2. Fully Synthetic Artemisinins. It is important to recall that some limitations of artemisinins such as short half-life (between 1 and 5 hours [136, 137]), limited affordability, and solubility need to be further addressed. Although semisynthetic compounds have partially overcome these issues, they still rely on the availability of natural precursors. In malaria, some trioxolanes and ozonides with remarkable improved pharmacokinetics are under clinical development [138]. Recently, it has been found that synthetic trioxolanes with enhanced pharmacokinetic properties may exhibit a similar toxicity than artesunate in *Schistosomas* [139]. Given that artemisinins may be potentially used as anticancer drugs and possibly in other parasitic and viral infections, the development of novel compounds with enhanced pharmacokinetic properties and targeted anticancer actions is also paramount. Although novel semisynthetic artemisinins have shown substantial antineoplastic activity, there is still limited information regarding the cytotoxicity of fully synthetic endoperoxides. A series of tetraoxacyclohexanes have been shown to potentially exhibit anticancer properties. A triol substituted compound has displayed prominent antitumor action *in vivo* toward melanoma (LOX IMVI) and ovarian (IGROV1) cancer in nanomolar concentrations (LC_{50} 60 nM) [140]. Other authors have synthesized compounds with dual action (antimalarial/anticancer effect). These deoxycholic-acid-(DCA-) —and cholic acid (CA)—derived mixed tetraoxanes are cytotoxic at very low concentrations and particularly potent against melanoma cancer (LOX IMVI, LC_{50} up to 69 nM) [141].

6. Future Development of Artemisinins as Anticancer Drugs

Artemisinins have been recommended and widely used as antimalarials for several years [142]. This drug class has shown many biological activities, in particular, strong anticancer growth activity. Supporting evidence indicates that artemisinin-like compounds may be a therapeutic alternative in highly metastatic and aggressive cancers [44] with no long-term effective therapy [44, 61] and commonly developing

drug resistance [94]. Furthermore, antimalarial endoperoxides may act synergistically with other anticancer drugs with no additional side effects [143].

6.1. What Do We Need to Know? The ability of artemisinins to kill cancer cells through multiple and heterogeneous molecular events has been well documented. However, some questions about the molecular base of artemisinin-induced cell death need further study. Growing research has been focusing on determination of the mechanism of bioactivation and molecular events underlying the artemisinin effects. However, how the antitumor activity is exerted following artemisinin activation is still not well understood. So far, the precise molecular events involved in how, when, and where ROS production is initially triggered in cancer cells remain to be defined. In addition, the relevance of any ROS-independent mechanism should be also addressed; these might not be obvious but possibly important for artemisinin cytotoxicity in some cancer cells. Some other aspects such as the direct DNA damage induced by artemisinin-like compounds and the role of p53 status in genotoxicity need to be further analysed.

One relevant aim in anticancer therapy is cotargeting multiple pathways minimizing shifting cell hallmarks and side effects. Whereas it remains important to characterize the anticancer effects of existing and novel artemisinins derivatives, research also needs to be focused on unveiling the mechanisms of cytotoxicity by identifying their relation to a particular cancer biomarkers and molecules. Artemisinins seems to regulate key players participating in multiple pathways such as NF-κB, survivin, NOXA, HIF-1α, and BMI-1. These molecules and others are to be revealed, which in turn may be involved in drug response, drug interactions, mechanisms of resistance, and collateral effects in normal cells. A better understanding of common mechanisms under similar conditions in different cell systems will greatly aid the development of targeted artemisinin derivatives. This will improve artemisinins cytotoxicity by lowering IC$_{50}$, emerging of resistance, drug associated toxicity, and potentiating drug interactions.

It is important to connect the molecular interactions and the regulatory effects of artemisinin on the cancer hallmarks and particularly in those tumors with poor prognosis. Some cancer cell biomarkers may be potentially useful to predict success on an artemisinin-based treatment in specific systems. Furthermore, novel endoperoxide compounds and combinational therapies can be addressed to target or cotarget markers of carcinoma progression and prevent invasiveness and metastatic properties in highly recurrent and aggressive tumors or advanced stage cancers.

Although the benefits of artemisinins in the clinical setting have been already assessed, specific interactions with established chemotherapy need to be further dissected in different cancer cells and their phenotypes. This will be crucial to implement clinical trials and treatment of individual cases. In this regard, long-term therapy with artemisinins also requires close monitoring. It is important to note that the prototype drug, artemisinin, seems to modulate responses leading to antagonistic interactions with other anticancer drugs. However, whereas it may be useful to have the prototype drug as a control *in vitro*, its pharmacokinetic properties may differ from the semisynthetic artemisinins. Therefore, artemisinin antagonistic reactions and resistance must be cautiously validated using different semisynthetic derivatives. DHA, artesunate, and artemether are the endoperoxides currently licensed for therapeutic use. So far, artemether has been shown to share similar anticancer properties than DHA and artesunate [144].

Cancer research drives a permanent discovery of new genes and interactions. The study of how artemisinin drives tumor control may become even more complex as immunological hallmarks are also involved in the generation of tumors. Immunological hallmarks in cancer cells include the ability to induce chronic inflammatory response, evasion of tumor recognition, and ability to induce tolerance [145]. Whether artemisinin may participate in the mechanisms involved in these events has yet to be determined.

Overall, the real potential and benefits of the artemisinin drug class remain yet to be uncovered. The imminent possibility of artemisinins being included in the arsenal of anti cancer drugs has opened the door for challenging research in this area, one that seems to fulfill many expectations.

Abbreviations

ADP-ribose polymerase:	Adenosine diphosphate ribose polymerase
AP-1:	Activator protein 1
BAK:	Proapoptotic member of the BCL2 protein family
BAX:	BCL2-associated protein
BCL2:	B-cell lymphoma 2
BCRP:	Breast cancer resistant protein gen
BCLX:	Bcl-2-like protein 1
CDC25B:	Dual specific phosphatase involved in the activation of cyclin-dependent kinases
CDK:	Cyclin-dependent kinase
Cip 1/p21:	Cyclin-dependent kinase inhibitor 1
CDC25A:	Dual specific phosphatase involved in the activation of cyclin-dependent kinases
COX2:	Cyclooxygenase 2
Cdc42:	GTPase of the Rho family
c-MYC:	Transcription factor
DNA-PK:	DNA-dependent protein kinase
DNA topo 1:	DNA topoisomerase 1
E2F1:	Transcription factor
ER:	Endoplasmic reticulum
GST:	Glutathione S-transferase
GRP78:	78 kDa glucose-regulated protein
HIF 1α:	Hypoxia-inducible factor 1 alpha
αvβ3 integrin:	Transmembrane heterodimeric protein expressed on sprouting endothelial cells
IκBα:	Inhibitor of NF-κB
IL-1β:	Interleukin 1 beta

IL8:	Interleukin 8
JNK:	Jun N-terminal kinase
Kip1/p27:	Cyclin-dependent kinase inhibitor 1B
KDR:	Kinase insert domain protein receptor
MMP:	Matrix metalloproteinase
MRP1:	Multidrug resistance-associated protein gene
MDM2:	Murine doble minute oncogene protein
NK-κB:	Nuclear factor of kappa light polypeptide gene enhancer in B cells
NOXA:	Proapoptotic protein, a member of the BH3-only Bcl-2 protein family
p38-MAPK:	Mitogen-activated protein kinase
PAI-1:	Plasminogen activator inhibitor 1
PCNA:	Proliferating cell nuclear antigen
PKCa:	Serine/threonine kinase
ROS:	Radical oxygen species
Raf/ERK:	Signaling pathway
uPA:	Urokinase plasminogen
TIMP2:	Tissue inhibitor of metalloproteinases
TRAIL:	The tumor necrosis factor-related apoptosis-inducing ligand
VEGF:	Vascular endothelial growth factor
VEGFR-3/FL-4:	Vascular endothelial growth factor receptor
Wnt:	Wingless-type signaling pathway.

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