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REVIEW ARTICLE

Artemisinin-type drugs for the treatment of hematological malignancies

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Abstract

Qinghaosu, known as artemisinin (ARS), has been for over two millennia, one of the most common herbs prescribed in traditional Chinese medicine (TCM). ARS was developed as an antimalarial drug and currently belongs to the established standard treatments of malaria as a combination therapy worldwide. In addition to the antimalarial bioactivity of ARS, anticancer activities have been shown both in vitro and in vivo. Like other natural products, ARS acts in a multi-specific manner also against hematological malignancies. The chemical structure of ARS is a sesquiterpene lactone, which contains an endoperoxide bridge essential for activity. The main mechanism of action of ARS and its derivatives (artesunate, dihydroartemisinin, artemether) toward leukemia, multiple myeloma, and lymphoma cells comprises oxidative stress response, inhibition of proliferation, induction of various types of cell death as apoptosis, autophagy, ferroptosis, inhibition of angiogenesis, and signal transducers, as NF-κB, MYC, amongst others. Therefore, new pharmaceutically active compounds, dimers, trimers, and hybrid molecules, could enhance the existing therapeutic alternatives in combating hematologic malignancies. Owing to the high potency and good tolerance without side effects of ARS-type drugs, combination therapies with standard chemotherapies could be applied in the future after further clinical trials in hematological malignancies.

Keywords *Artemisia annua* L. · Artemisinin · Artesunate · Combination therapies · Hematological malignancies · Leukemia

Abbreviations

AML	Acute myeloid leukemia
AMoL	Acute monocytic leukemia
AMPK	AMP-activated protein kinase
AP-1	Activator protein-1
Ara-C	Cytarabine
ARM	Artemether
ARS	Artemisinin
ART	Artesunate
ATO	Arsenic trioxide
B-ALL	B-acute lymphoblastic leukemia
BM	Bone marrow
CML	Chronic myeloid leukemia
CuZnSOD	Copper, zinc-superoxide dismutase
DA	Decitabine
DHA	Dihydroartemisinin
DLBCL	Diffuse large B-cell lymphoma
DM	Dexamethasone
DX	Deferoxamine
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
GEM	Genetically-engineered mouse
GpA	Glycophorin A receptor
GPX1/2	Glutathione peroxidases 1 and 2
HBO_2	Hyperbaric oxygen
HET	Dihydroethidine
H_2O_2	Hydrogen peroxide
IFN-α	Interferon-α
JNK	C-Jun-N-terminal kinase
MAPK	Mitogen-activated protein kinases
MM	Multiple myeloma
MMP	Mitochondrial membrane potential
MnSOD	Manganese-superoxide dismutase
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
O_2^-	Superoxide

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PBMC	Peripheral blood mononuclear cells
PBN	*N*-Tert-butyl-alpha-phenylnitrone
P-gp	*P*-Glycoprotein
PPP	Pentose phosphate pathway
ROS	Reactive oxygen species
SS	Sodium salicylate
STAT3	Signal transducer and activator of transcription-3
TCM	Traditional Chinese medicine
TfR	Transferrin receptor
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Introduction

For over two millennia Qinghaosu, known as artemisinin (ARS) in Western cultures, has been one of the most common herbs prescribed to treat febrile symptoms in traditional Chinese medicine (TCM). The earliest mention occurs in the *Hou Bei Ji Fang* (A Handbook of Prescriptions for Emergency Treatment) by Ge Hong (317–420 A.D.) as a remedy with anti-pyretic activity. Subsequently, qinghaosu soup, pills, and powders have been described for relieving malaria symptoms. Later, the herbalist Li Shi Zhen published in *Compendium of Materia Medica* (1596) that "fever and colds" could be treated with qinghaosu preparations [1].

Artemisia annua L. came under the spotlight during the Vietnam War when the Vietnamese government asked China for help to manage the effects of malaria that infected nearly half of the militaries. A program for the discovery of new anti-malarial drugs, known as National Project 523 was launched in 1967 by the Chinese government that established a screening program of traditional Chinese plants. A modified extraction procedure was later able to isolate ARS, the active principle of *Artemisia annua* L. (sweet wormwood) [2]. The structure of *Artemisia annua* L. was elucidated in 1974, showing a sesquiterpene lactone, with an endoperoxide bridge essential for activity. Together with the corresponding derivatives, ARS attracted worldwide attention, and ARS-based combination therapies nowadays belong to the globally established standard treatments for malaria [1, 3]. Following the elucidation of this structure, many derivatives were synthesized with substitutions at the lactone carbonyl group to improve solubility in both water and oil. Water-soluble derivative such as artesunate (ART) and dihydroartemisinin (DHA), the latter considered to be the main active metabolite of ARS, and oil-soluble derivatives such as artemether (ARM) and arteether (Fig. 1) have been synthesized [1, 4].

In the 1980s, the World Health Organization (WHO) officially recommended ARS-type derivatives for malaria treatment, particularly as a part of combination therapies with

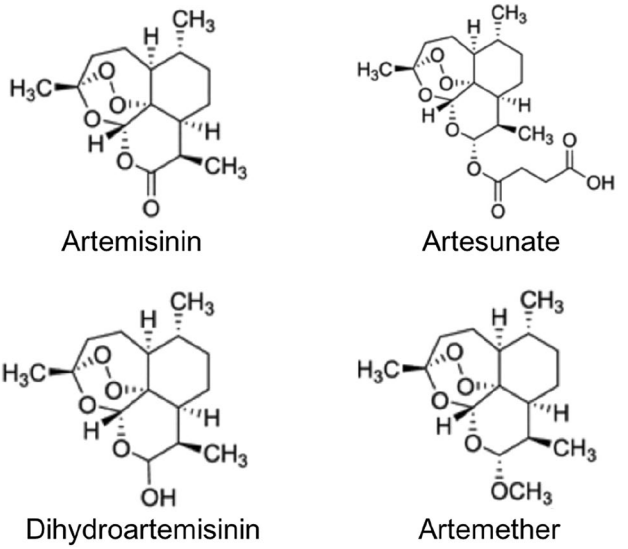

Fig. 1 Chemical structure of compounds derived from traditional Chinese medicine (TCM)

other antimalarial drugs for their impressive activity against multidrug-resistant forms of *Plasmodium falciparum* both in vitro and in vivo [1]. Tu Youyou was honored with the 2015 Nobel Prize for Physiology or Medicine [3] for having isolated and studied ARS which saved the lives of millions of malaria patients.

Numerous natural products originated from Chinese medicine exhibit anti-cancer activities which include anti-proliferative, pro-apoptotic, anti-angiogenic effects, as well as regulating autophagy, reversing multidrug resistance, balancing immunity, and enhancing chemotherapy. ARS-like compounds also exert profound activity against tumor cells in vitro and in vivo [5–8] suggesting that ARS-type drugs could be applied in clinical oncology, probably as part of combination therapy [9–12].

Our group has been working over the past 2 decades with ARS-type compounds and has demonstrated the antiulcerogenic effects of two isolates from *A. annua* [13], activity against parasite [14] and the toxicity effects in pregnant mice [15]. Recently, the development of a transdermal bioadhesive treatment for children's treatment has been demonstrated [16]. Driven by the demand for alternative drugs to improve the survival and decrease the relapse rate of acute myeloid leukemia (AML), our group has also demonstrated the antileukemic effects of natural products such as catechins and quercetin over the last decade [17, 18].

In addition to acute leukemias, other hematological malignancies can also benefit from natural products. Hematologic malignancies are cancers that begin in the cells of blood-forming tissue such as the bone marrow, or in the cells of the immune system and may be considered liquid tumors. A single germline or somatic mutation in

the lymphohematopoietic stem cell may be prone to clonal expansion depending on new mutations acquired [19].

Multiple myeloma (MM), for example, is a malignancy of terminally differentiated plasma cells that primarily reside in the bone marrow (BM); however, in a later stage of the disease, they can also be detected in peripheral blood and extramedullary sites [20]. Lymphomas arise from lymphocytes that are at different stages of development and the subtype characteristics, B-cell and T-cell neoplasms reflect the cell from which they originated [21]. Leukemias are, in general, a heterogeneous group of disorders characterized by clonal expansion, abnormal proliferation of undifferentiated myeloid or lymphoid progenitors, and variable response to therapy [22].

Currently, chemotherapy, immunotherapy, and hematopoietic stem cell transplantation are the main therapeutic approaches against these diseases. Despite the progress in treatment, the outcome of AML in adult patients remains dismal [22]. Approximately 35%–40% of patients under the age of 60 years old are cured. The prognosis for the elderly is improving but remains grim. Furthermore, older patients have unfavorable cytogenetic features probably reflecting antecedent myeloid disorders which underwent clonal evolution. Relapse and/or disease refractoriness and drug resistance to standard chemotherapies are the major causes of treatment failure [23].

One current challenge in the treatment of cancer is to overcome cancer drug resistance. Combination therapies were applied to prevent resistance by combining drugs with different targets, modes of action and distribution of the side effects in the body to reduce toxicities. Natural products act in a multi-specific manner, exhibiting several modes of action simultaneously [11, 12]. For example, ARS-type drugs induce oxidation, and can lead to DNA damage and breaking of double-strands. Thus, many studies have shown the additive or synergistic effects of ARS-type drugs with standard chemotherapies. Doxorubicin, frequently used for chemotherapy of hematological cancers, exhibited profound synergism with ART in various MM cell lines [24]. Moreover, a synergistic interaction was observed with cytarabine plus ART or DHA in AML [25], as well as in other tumor cell lines. Interestingly, the spectrum of drugs that can be combined with ARS and its derivatives is remarkably broad and comprises natural products, radiotherapy, and photodynamic therapy, and also antibodies or recombinant proteins [11, 12].

A PubMed search using the keywords leukemia, lymphoma, multiple myeloma, artemisinin, artesunate, artemether, and dihydroartemisinin yielded a result of 82 papers published over the period of 25 years.

This review supplies an update on the mechanism of action, synergistic effects, and new hybrid compounds of ARS-type drugs, as well as a possible role in the treatment of hematological malignancies.

Cytotoxicity activity of ARS and derivatives

In the 1990s, many researchers described the cytotoxicity of ARS and its derivatives in leukemia cell lines (Table 1). Nine compounds isolated from *Artemisia annua* L., including terpenoids and flavonoids, were tested in vitro on a P388 murine lymphocytic leukemia cell line and ARS showed the highest cytotoxicity [26]. Furthermore, a panel of 55 tumor cell lines showed that ART, a semi-synthetic derivative, was most active against leukemia and colon cancer cell lines [5].

Efflux pumps of ATP-binding cassette (ABC) transporter family extrudes drug molecules that passively diffuse into cancer cells in an active ATP-consuming manner out of the cells. These pumps reduce the intracellular accumulation of many anticancer drugs to sub-therapeutic levels leading to the survival of cancer cells and consequently failure of chemotherapy. ARS-type drugs exhibit multi-specific interaction with P-gp. The derivatives can act on both sides, as a substrate or an inhibitor of P-gp. The currently ARS derivatives, ART, DHA, and ARM, are not transported by P-gp. In additional, they can act as an inhibitor of P-gp, which exhibits the potential to reverse MDR [27]. Efferth and collaborators tested 22 TCM derived compounds in multidrug-resistant acute lymphoblastic leukemia cell lines (CCRF-CEM), such as doxorubicin-selected P-glycoprotein (P-gp)/MDR1-expressing CEM/ADR5000, vinblastine-selected P-gp/MDR1-expressing CEM/VLB$_{100}$, and epirubicin-selected multidrug resistance-related protein 1 (MRP1)-expressing CEM/E1000 sublines. ART, homoharringtonine, and bufalin were the most active and potent compounds and had the lowest IC_{50} in wild-type CCRF-CEM cells. ART modulated multidrug resistance and increased daunorubicin uptake in CEM/E1000 cells [28].

Ethanolic leaf extracts of *A. annua* from Brazilian (hybrid CPQBA 2/39 × PL5) and Chinese origins were tested on leukocytes and Molt-4 (human acute lymphoblastic leukemia) cell line for comparison with DHA. An increased biological activity was expected since *A. annua* leaves have flavonoids that may synergize with ARS. Both extracts had high antioxidant capacity and toxicity toward leukemia cells; however, whereas ethanolic extracts were more potent in killing Molt-4 cells at 24 h, DHA was significantly more potent than ethanolic extracts in killing Molt-4 cells at 48 and 72 h. Furthermore, DHA presented less toxicity in leukocytes than the Brazilian and Chinese extracts with the Brazilian extract revealing a better safety index (LD_{50} value 28.23 µg/ml) compared to Chinese extract at 24 h [29].

The combination of DHA and sodium salicylate (SS) significantly reduced cancer cell proliferation [30]; however,

Table 1 Cytotoxicity activity of ARS-type drugs

Cell line	Derivative	Treatment	Effect	References
In vitro				
6 Leukemia cell lines	ART	Concentration range from 10^{-8} to 10^{-4} M	Cytotoxicity↑, IC_{50} value 1.11 ± 0.56 μM	[5]
HL-60 and PBMC	DHA, PBrDHA, PFDHA, dPFDHA	0–100 μM treated up to 72 h	Cytotoxicity↑ (leukemia cells over PBMC)	[35]
HL-60, THP-1, and KG1	DHA	0, 5, 10, and 15 μM treated for 12 h	Cytotoxicity↑; IC_{50} value 6.82 μM, 9.05 μM, and 9.98 μM, respectively	[36]
MV4-11, MOLM-13, OCI-AML3, NB4, and KG1a	ART, ARM, DHA	0–5 μM treated for 72 h	Proliferation↓ (ART); IC_{50} value 0.82 μM for MOLM-13 and IC_{50} value 1.1 μM for MV4-11	[32]
Molt-4	DHA, ethanolic extracts	0–36.92 μM treated for 24, 48, and 72 h	DHA more potent in killing at 48 and 72 h than ethanolic extracts	[29]
Molt-4	DHA + SS	5.87 μM DHA + 4.89 μM SS treated for 24, 48, and 72 h	Proliferation↓ (additive interaction)	[30]
Molt-4	DHA + pro-oxidant and antioxidant molecules	12.4 μM DHA + 256 μM ascorbate or 75 nM vitamin D_3 or 10 μM DM or 8.82 μM H_2O_2 treated for 24 and 48 h	Cytotoxicity↑	[31]
Jurkat	ARS	2 μM treated for 48 and 72 h	Proliferation↓, BMI1↓, NOTCH1↓, cleaved NOTCH1↓, HES1↓, MYC↓	[33]
K562	DHA	0, 2, 5, 10, 20, and 40 μM treated for 48 and 72 h	Proliferation↓	[37]
CCRF-CEM, CEM/E1000, CEM/ADR5000, CEM/VBL_{100}	ART	Treated for 96 h	Viability↓, ART increased daunorubicin uptake	[28]
P388 (murine leukemia)	ARS	NS	Cytotoxicity↑	[26]
P388 (murine leukemia)	ARS containing cyano and aryl groups	Treated for 48 and 96 h	Proliferation↓	[38]
P-388 (murine leukemia)	DHA containing cyano and aryl groups	NS	Proliferation↓	[39]
U266	DHA	0, 1, 3, 10, 30, and 100 μM treated for 12, 24, and 48 h	Proliferation↓	[40]
RPMI-8226	ART	0–80 μM treated for 24 and 48 h	Proliferation↓	[41]
RPMI-8226	DHA	0–80 μM treated for 24 and 48 h under hypoxia	Proliferation↓	[42]
INA-6, ANBL-6, OH-2, IH-1, VOLIN, CAG, U266, JJN-3, and RPMI-8226	ART	0–100 μM treated for 72 h	Proliferation↓	[34]
SP2/0 (murine myeloma)	ART	0–104 μM treated for 24, 48, and 72 h	Proliferation↓	[43]
OCI-Ly3, OCI-Ly10, SUDHL-4, SUDHL-6, and Karpas-422	ART	0–100 μM treated for 72 h	Proliferation↓	[34]
SUDHL-4 and DB	ARM	0–0.1 mM treated for 24, 48, and 72 h	Proliferation↓	[44]
18 B-cell lymphoma cell lines	ART	0–10 μM treated for 72 h	Proliferation↓	[45]
Jurkat	DHA + siNotch1	20 μM DHA + siNotch1 treated for 24 h	Proliferation↓	[46]
Jurkat and HuT-78	DHA	15 μM and 30 μM, respectively, treated for 48 h	Proliferation↓	[47]

Table 1 (continued)

Cell line	Derivative	Treatment	Effect	References
Ramos	ART + mAb rituximab	0-208 nM rituximab (pretreated for 24 h) + 0–100 μM ART treated for 24 h	Proliferation↓ (compared with ART or mAb alone)	[48]
In vivo				
HL-60 xenograft model (BALB/c Nude mice) (sc.)	DHA	50 mg/kg, ip., treated for 28 days	Tumor growth↓	[36]
MLL-AF9 knock in xenograft model (C57/B6 mice) (retro orbital injection)	ART	25 mg/kg, ip., on days 10, 12, 14, 16, 18 after transplant; irradiated (7.0 Gy)	Survival↑(median survival 26 *vs.* 30 days), leukemic cell engraftment↓, splenomegaly↓	[32]
MV4-11 xenograft model (DKO mice) (intrahepatically injection)	ART	25 mg/kg, ip., on days 12, 14, 16, 18 after transplant; irradiated (1.5 Gy)	Survival↑ (median survival 20 *vs.* 23 days)	
Primary AML xenograft model (NSG mice) (retro orbital injection)	ART	50 mg/kg, ip., on days 7, 9, 12, 14 after transplant; irradiated (3.0 Gy)	Survival↑ (median survival 35 *vs.* 39 days)	
MOLM14 xenograft model (NSG mice) (sc.)	ART, ART-838	200 mg/kg ART and 50 mg/kg ART-838, gavage, two 5-day cycles	Tumor growth↓, (83% of reduction of ART-838-treated tumors and 44% of reduction of ART-treated tumors compare to vehicle-treated tumors)	[6]
B-ALL primagraft model (NSG mice) (iv.)	ART, ART-838	150 mg/kg ART and 40 mg/kg ART-838, gavage, four 5-day cycles	Survival↑ (55% prolongation for mice treated with ART-838 and 51% prolongation for mice treated with ART), delays leukemia onset	
B-ALL primagraft model (NSG mice) (iv.)	ART, ART-838	200 mg/kg ART and 50 mg/kg ART-838, gavage, two 5-day cycles	Similar extent to previous treatment, delays leukemia onset	
MV4-11 xenograft model (NSG mice) (iv.)	Regimen 1	ART 120 mg/kg, oral gavage, once daily (5 days); or Ara-C 6.25 mg/kg, ip., once daily (5 days); or combination; treated for 4 weeks	Lower radiance (luciferase) at day 33 in mice treated with combination treatment; ART alone or in combination with Ara-C failed to provide survival benefit	[25]
ML-2 xenograft model (NSG mice) (iv.)	Regimen 2	ART 120 mg/kg, oral gavage, twice daily (5 days) followed by 100 mg/kg, twice daily (5 days); treated for 2 weeks	Lower leukemic infiltration compared to regimen 3; Survival benefit compared with vehicle	
	Regimen 3	ART 100 mg/kg, oral gavage, twice daily; treated for 9–10 days		
MOLM-13 xenograft model (NSG mice) (iv.)	Regimen 1 and 3	Comparing both regimen (above)	Lower leukemic infiltration in mice treated with regimen 3; no survival benefit	
HUT-102 xenograft model (CB-17/SCID mice) (sc.)	ART	150 mg/kg, ip., 5 times per week between days 1 and 28	Tumor growth↓ (49% of reduction)	[49]
SP2/0 xenograft model (BALB/c mice) (sc.)	ART	50, 100, and 200 mg/kg, ip., for 15 days	Tumor growth↓ (17.83, 36.54, and 47.95% of reduction, respectively)	[43]
DB xenograft model (NOD/SCID mice) (sc.)	ARM	200 mg/kg/day, ip., for 10 days	Tumor growth↓ (69% of reduction)	[44]

Table 1 (continued)

Cell line	Derivative	Treatment	Effect	References
BL-41 xenograft model (NSG mice) (sc.)	ART	200 mg/kg/day, ip., for 12 to 16 days of treatment after inoculation	Tumor growth↓ (70% reduction)	[45]

Arrows ↑ indicate increase and arrows ↓ indicate decrease compared to the control group

no interaction between DHA and SS was indicated. Nutritional supplements that affect the oxidative status of cells such as vitamin C, with antioxidant proprieties, and vitamin D_3, and hydrogen peroxide (H_2O_2) with pro-oxidant properties, cause significant Molt-4 cell death when combined with DHA [31]. Furthermore, the interaction between H_2O_2 and DHA was found to be additive, which may be due to mechanisms similar to apoptosis mediation by reactive oxygen species (ROS).

Moreover, ART showed high cytotoxicity toward MV4-11 and MOLM-13 cell lines with the downregulation of SRC, a key protein in cell proliferation and survival. The antileukemic activity of ART was confirmed in various xenograft models resulting in significant survival prolongation [32]. Xenograft mice models transplanted with MOLM14 cells treated with ART-838, a semi-synthetic ARS-derived trioxane diphenylphosphate dimer, presented repression of tumor growth (83% less growth than in controls). Indeed, a B-acute lymphoblastic primagraft leukemia model treated with ART-838 had an extended mean survival of 20 days (55% prolongation) compared to controls [6]. Drenberg and coauthors also showed lower leukemic infiltration in a xenograft mice leukemia (ML-2) model treated with ART twice daily, in another xenograft model (MOLM-13), ART treatment added no survival benefit [25].

Interestingly, ARS suppressed proliferation via the downregulation of NOTCH1 signaling which may be up-regulated in several cancers [33]. In addition, ARS and derivatives also inhibited cell growth in MM and lymphoma cells. Holien and coauthors observed that ART decreased cell growth in nine MM and five lymphoma cell lines [34].

Role of iron

Since the onset of studies with ARS derivatives and cancer, many studies have shown a crucial role of iron in the anticancer activity of ARS-type drugs. Lai and Singh showed, for the first time in leukemia cell lines, that combined incubation of holotransferrin and DHA can selectively destroy cancer cells, whereas the effect was significantly less on normal lymphocytes [50]. The same group also showed that the addition of sodium butyrate (1 mM) to the culture medium together with DHA (20 µM) and holotransferrin (12 µM) acted synergistically in Molt-4 cell lines [51]. Moreover, Efferth and collaborators showed that iron (II)-glycine sulfate (Ferrosanol®) and transferrin enhanced the cytotoxicity of ART, maltosyl-β-cyclodextrin-encapsulated-ART (ART-MCD) and ARS against leukemia cell lines compared to each derivative without iron. Furthermore, expression levels of mitochondrial aconitase (*ACO2*) and ceruloplasmin (*CP*) correlated with the IC_{50} values of several artemisinin derivatives when administered with ferrous iron [52]. ART

treatment also regulated the expression of drug efflux pumps involved in iron homeostasis as observed after ART incubation with the CCRF-CEM cell line which increased the expression of the ATP-binding cassette (ABC) transporter *ABCB6* and reduced *ABCB7* expression [53].

In fact, tumor cells express significantly more transferrin receptor on their cell surface than normal cells and transferrin endocytosis is higher in tumor cells compared to normal cells. Tagging an ARS analog to transferrin, both iron and ARS were transported into cancer cells and the "tagged-compound" was very potent and selective in killing cancer cells [54]. To improve the cytotoxicity of ARS, the same group of researchers covalently tagged ARS to a peptide that binds to a cavity on the surface of the transferrin receptor (ARS-TfR). After endocytosis, iron released from TfR reacted with the ARS moiety and formed free radicals, leading to cell death. They also demonstrated that ARS-TfR was more potent and an extremely selective anti-tumor agent compared to ARS itself [55]. Two synthetic ARS compounds, ARS dimer-alcohol (dimer-OH) and ARS-tagged holotransferrin (ART-TF), were more potent and showed no significant cross-resistance toward a DHA-resistant Molt-4 (RTN) cell line [56]. Indeed, combined ARS, pretreated with holotransferrin, and hyperbaric oxygen, resulted in an additional 22% decrease in growth of Molt-4 cell line compared with ARS treated alone [57].

Moreover, DHA, 10β-(*p*-bromophenoxy) DHA (PBrDHA), and 10β-(*p*-fluorophenoxy) DHA (PFDHA) demonstrated selective cytotoxicity activity of the endoperoxide group toward leukemia cells over normal peripheral blood mononuclear cells (PBMC). In HL-60 cells, the compounds with endoperoxide induced caspase-dependent apoptotic cell death characterized by mitochondrial membrane depolarization, caspases-3 and -7 activation, and sub-G_0/G_1 DNA formation, concentration- and time-dependent. Deoxy-10β-(*p*-fluorophenoxy) DHA (dPFDHA), which lacks the endoperoxide bridge, was less active, confirming the importance of this functional group [35]. In addition, ARS-type drugs exerted effects in lymphoma cells. Wang and coauthors demonstrated enhanced cytotoxicity effects of co-treatment of DHA plus holotransferrin in T-cell lymphoma cells with inhibition of *TfR* mRNA expression [58] (Table 2).

Oxidative stress response

Efferth showed that ART induced apoptosis of leukemia cells mainly through the mitochondrial pathway via generation of reactive oxygen species (ROS) [32, 63]. Furthermore, the induction of ROS by ART was accompanied by increased phosphorylation of histone H2AX (γ-H2AX), a marker for double-strand DNA damage, and activation of c-Jun N-terminal kinase (JNK), a mitogen-activated protein kinases (MAPK) family member [32], suggesting a strong pro-oxidant effect of ART on leukemic cells.

Moreover, Zhang and collaborators showed that the cytotoxicity induced by DHA correlated with superoxide (O_2^-) levels, measured by dihydroethidine (HET) fluorescence, in a concentration-dependent way. Furthermore, the co-incubation with the superoxide scavenger TEMPOL dramatically reduced the HET fluorescence. Indeed, an increase in protein levels of four antioxidant enzymes, catalase, copper/zinc-superoxide dismutase (CuZnSOD), manganese-superoxide dismutase (MnSOD), and glutathione peroxidases 1 and 2 (GPX 1/2) were observed in Molt-4 cell lines [64]. Another evidence of the antileukemic mechanism of ARS derivatives through ROS generation was obtained using N-tert-butyl-alpha-phenylnitrone (PBN), a compound that effectively sequesters free radicals, or deferoxamine (DX), an iron chelating agent, which attenuated the cytotoxicity of DHA. Altogether, these results suggest that DHA induced the formation of toxic-free radicals via an iron-mediated process [65] (Table 3).

Cell cycle arrest

Two novel derivatives, containing cyano and aryl groups at C-10 carbon of the ARS structure, showed a potent antiproliferative in vitro effect, leading to cell cycle arrest (Table 4) in G_0/G_1 phase [38]. The active metabolite of ARS, DHA, also arrested the cell cycle at G_0/G_1 phase with downregulation of cyclin D, CDK2, and CDK4 [36]. Furthermore, ARS-type drugs induced cell cycle arrest in MM and lymphoma cells. DHA induced cell cycle arrest at sub-G_0/G_1 phase in U266 MM cells [40]. Moreover, ARM induced cell cycle arrest at G_0/G_1 phase in diffuse large B-cell lymphoma (DLBCL) cells with a decrease of cyclin D1, CDK2, and CDK4 [44]. In addition, the hydrophilic ARS-derivative, ART, caused cell cycle arrest in both G_0/G_1 and G_2/M phase in adult T-cell leukemia/lymphoma cells with decreased levels of activator protein-1 (AP-1) and NF-κB signaling [49].

Programmed cell death

Apoptosis

Numerous studies have demonstrated the role of ARS and derivatives in the induction of apoptosis (Table 5). Singh and Lai demonstrated that DHA induces apoptosis, but not necrosis in Molt-4 cell line [61]. Increased phosphorylation of p38 mitogen-activated protein kinases (MAPK), but not JNK, or extracellular signal-regulated kinase (ERK) is required for DHA-induced apoptosis through both intrinsic

Table 2 Role of iron of ARS-type drugs.

Cell line	Derivative	Treatment	Effect	References
In vitro				
HL-60	DHA	0.5 μM treated up to 24 h	Induction of apoptosis iron dependent	[59]
HL-60	DHA	0–1 μM treated for 48 h	TfR expression↓	[60]
HL-60	DHA, X-11	0.8 μM DHA and 0.2 μM X-11 treated for 24 h	Iron and endoperoxide were required for apoptosis induction	[7]
Molt-4	DHA	12 μM holotransferrin + 0–200 μM DHA treated up to 8 h	Holotransferrin increased DHA cytotoxicity	[50]
Molt-4	DHA	12 μM holotransferrin (pretreated for 1 h) + 200 μM DHA or 200 μM DHA alone treated up to 8 h	Induction of apoptosis, but no necrosis	[61]
Molt-4	DHA	12 μM holotransferrin (pretreated for 1 h) + 20 μM DHA + 1 mM sodium butyrate treated for 24 and 48 h	Cytotoxicity in leukemia cells and no effect in lymphocytes	[51]
Molt-4	DHA and "tagged-compound"	0–12.4 μM treated for 24, 48, and 72 h	Increased potency and selective killer of cancer cell comparing to DHA treatment	[54]
Molt-4	ART-peptide, ART-peptide$_2$	6.2 μM treated for 72 h	Improve potency and selectivity; IC_{50} value 1.06 ± 0.08 μM and 0.61 ± 0.05 μM, respectively	[55]
Molt-4	ARS + hyperbaric oxygen	12 μM holotransferrin (pretreated for 1 h) + 10 μM ARS + 90 min in different oxygen condition; treated up to 48 h	Decreased of 22% in growth comparing with ARS treated alone	[57]
Molt-4 (RTN)	DHA Dimer-OH ART-TF	0–100 μM treated for 24 and 48 h	RTN resistant against DHA RTN no resistant against DHA RTN no resistant against DHA	[56]
CCRF-CEM	ART, ARS, ART-MCD	0.001–1000 μM + transferrin or iron (II)-glycine sulfate	Cytotoxicity↑, *ACO2* and *CP* expression levels correlated with ARS IC_{50}	[52]
CCRF-CEM	ART	0.512–124.295 μM treated up to 24 and 96 h	Correlation between ART and TfR receptor; *ABCB6*↑ and *ABCB7*↓	[53]
K562	DHA	20 nM holotransferrin (pretreated for 1 h) + 10 μM DHA treated for 48 h	TfR↓ (mRNA and protein levels), intracellular iron↓	[62]
JJN3 and RPMI-8226/R5	ART	125 μM treated up to 24 h	Efficacy depends on intracellular bivalent iron	[24]
Jurkat	DHA + holotransferrin	0, 10, 20, 40, and 80 μM DHA + 20 μM holotransferrin treated for 48 h	*TfR*↓	[58]

Arrows ↑ indicate increase and arrows ↓ indicate decrease compared to the control group; RTN, DHA-resistant Molt-4 cell

Table 3 Oxidative stress response induced by ARS-type drugs

Cell line	Derivative	Treatment	Effect	References
In vitro				
HL-60	DHA	0.5 μM treated up to 12 h	Induction of apoptosis independent of ROS but slightly increased O_2^-	[59]
HL-60	DHA, X-11	0.8 μM DHA and 0.2 μM X-11 treated for 15 h	O_2^-↑	[7]
HL-60 and THP-1	DHA	0, 5, 10, and 15 μM treated for 12 h	ROS↑	[36]
MOLM14	ART, ART-838	5 μM ART and 0.1 μM ART-838 treated for 24 h	ROS↑	[6]
MV4-11, KG1a, and AML primary blast	ART	1–2 μM treated for 24 h	ROS↑, H2AX↑, p-JNK↑	[32]
ML-2, MV4-11, and MOLM-13	ART, DHA	1–10 μM treated for 24 h	ROS↑ (MV4-11) and minimal increase in other cell lines	[25]
Jurkat and CCRF-CEM	ART	10.4 μM and 1.3 μM, respectively, treated for 30 min	ROS-mediated apoptosis	[63]
Molt-4	DHA	0–20 μM treated for 16 h	O_2^-↑, antioxidant enzymes↑	[64]
Molt-4	DHA + PBN	12.2 μM DHA + 0.25, 0.5, and 1.0 mM PBN treated for 24 h	Cytotoxicity of DHA↓	[65]
	DHA + DX	12.2 μMDHA + 10, 20, and 30 μM DX treated for 24 h	Cytotoxicity of DHA↓	
K562	DHA	20 nM holotransferrin (pretreated for 1 h) + 10 μM DHA or DHA alone treated for 48 h	ROS↑ both groups, but higher levels in group pretreated with holotransferrin	[62]
MT-2 and HUT-102	ART	0, 0.4, 2, and 10 μM and 0, 2, 10, and 50 μM, respectively, treated for 24 and 48 h	ROS↑, γH2AX↑	[49]
JJN3 and RPMI-8226/R5	ART	125 μM treated up to 24 h	ROS↑ (early) and O_2^-↑ (late)	[24]
DAUDI and CA-46	ART	0, 5, 10, and 20 μM treated up to 24 h	ROS↑ (ferroptosis related)	[66]
Ramos	ART + mAb rituximab	140 μM rituximab (pre-incubated for 3 h) + 50 μM or 100 μM ART treated for 6 h	ROS↑, MnSOD↓, catalase↓	[48]
Jurkat	DHA + holotransferrin	0, 10, 20, 40, and 80 μM DHA + 20 μM holotransferrin treated for 48 h	ROS↑	[58]

Arrows ↑ indicate increase and arrows ↓ indicate decrease compared to the control group

and extrinsic pathways in HL-60 cells [59] while ROS is dispensable. Furthermore, DHA induced apoptosis of U937, Jurkat, and HL-60 cell lines, primary human AML and acute lymphoma leukemia (ALL) cells in vitro accompanied by inactivation of MEK/ERK, MCL-1 down-regulation, caspase activation and finally apoptosis. Moreover, DHA-mediated inhibition of xenograft tumor growth associated with apoptosis induction, MCL-1 down-regulation, and ERK inactivation [67].

The active metabolite, DHA and its derivative X-11, acted through a NOXA-mediated pathway and downregulated MCL-1 through a new cascade of O_2^-/FOXO3a/NOXA [7]. The endoperoxide moiety of DHA or X-11 interacted with iron to form carbon-center radicals. Thereby, increased levels of O_2^- through the endoperoxide moiety contributed to NOXA induction and finally apoptosis. NOXA protein bound to MCL-1 leads to Bak activation, and finally apoptosis [7].

The *Bcr/Abl* fusion gene is the pathogenic factor for chronic myeloid leukemia development. Lee and colleagues showed that DHA blocked BCR/ABL tyrosine phosphorylation and suppressed, in a concentration-dependent manner, downstream signaling pathways such as AKT and ERK, and also suppressed NF-κB protein expression, leading to the release of cytochrome *c* from mitochondria and activation of caspase cascade [68]. Furthermore, DHA suppresses *Bcr/Abl* mRNA amplification in imatinib-resistant cell lines [69].

DHA further downregulated MCL-1 expression, though not mRNA expression, suggesting a MCL-1 turnover by the proteasome. *Ddit3*, which encodes CHOP protein, a key

Table 4 Cell cycle arrest induced by ARS-type drugs

Cell line	Derivative	Treatment	Effect	References
In vitro				
HL-60, THP-1, and KG1	DHA	0, 5, 10, and 15 μM treated for 12 h	G_0/G_1 phase arrest, cyclin D↓, CDK2↓, CDK4↓	[36]
MOLM14	ART and ART-838	5 μM ART and 0.1 μM ART-838 treated for 24 h	Sub-G_0/G_1 phase arrest and G_0/G_1 phase arrest	[6]
K562	DHA	20 nM holotransferrin (pretreated for 1 h) + 10 μM DHA treated for 48 h	G_2/M phase arrest and partially arrested in sub-G_0/G_1 phase	[62]
MT-2 and HUT-102	ART	0, 0.4, 2, and 10 μM treated for 24 h	G_0/G_1 and G_2/M phase arrest, AP-1↓, NF-κB↓, CDK1↓, CDK2↓, CDK4↓, CDK6↓, cyclin B1↓, cyclin D2↓, cyclin E↓, p21↑	[49]
P388 (murine leukemia)	ARS derivatives containing cyano and aryl groups	12 nM and 11 nM treated for 21 h	G_0/G_1 phase arrest	[38]
P388 (murine leukemia)	DHA containing cyano and aryl groups	NS	G_0/G_1 phase arrest	[39]
U266	DHA	0, 1, 3, 10, 30, and 100 μM treated for 48 h	Sub-G_0/G_1 phase arrest	[40]
SP2/0 (murine myeloma)	ART	0–104 μM treated for 24 h	G_0/G_1 phase arrest	[43]
OCI-Ly3	ART	Treated for 24 h	G_0/G_1 phase arrest	[34]
SUDHL-4 and DB	ARM	0.1 mM treated for 48 h	G_0/G_1 phase arrest, cyclin D1↓, CDK2↓, CDK4↓	[44]
Jurkat	DHA + holotransferrin	0, 10, 20, 40, and 80 μM DHA + 20 μM holotransferrin treated for 48 h	G_0/G_1 phase arrest	[58]

Arrows ↑ indicate increase and arrows ↓ indicate decrease compared to the control group

regulator of the endoplasmic reticulum (ER) stress pathway contributed to repression of MCL-1 protein by DHA in B-acute lymphoblastic leukemia (B-ALL) mice cells containing the BCR-ABL protein (BCR-ABL$^+$). Interestingly, DHA synergized with ABT-263, a BH3-mimetic, inducing apoptosis, supporting the hypothesis that DHA can repress MCL-1 protein expression. Combined treatment of DHA and ABT-263 extended survival and showed repression in circulating leukemia cells of mice transplanted with BCR-ABL$^+$B-ALL cells. Furthermore, a decrease of MCL-1 protein was observed in *ex vivo* analysis of splenic blast cell of BCR-ABL$^+$B-ALL mouse after in vivo DHA treatment [70]. Indeed, in HL-60 cells, DHA treatment decreased TfR expression at the mRNA and protein level, upregulated the proapoptotic protein Bax and downregulated the antiapoptotic protein BCL-2, resulting in the activation of caspase-3 and apoptosis [60].

Combined treatment of ARS derivatives with other compounds has also shown effects on the apoptosis of leukemic cells. For example, DHA and an inhibitor of 6-phosphogluconate dehydrogenase (6PGD) induced synergistic apoptosis of the chronic myeloid leukemia K562 cell line through AMPK (AMP-activated protein kinase) signaling pathway. Moreover, combined treatment significantly decreased tumor growth in a xenograft model beyond the increased levels of phospho-AMPK [71]. ART and arsenic trioxide (ATO) also increased K562 cell line apoptosis and necrosis [72].

ART induced apoptosis of leukemic T cells through the intrinsic pathway with cytochrome *c* release and caspase-9 activation [63]. In another study, ART treatment of KBM-5 (chronic myeloid leukemia) cell line exerted apoptosis effects through suppression of multiple signaling pathways including suppression of p38/ERK/STAT5/CREB phosphorylation [8]. Moreover, ART showed high cytotoxicity toward MV4-11 and MOLM-13 cells, *Bcl-2* reduction, loss of mitochondrial membrane potential (MMP) and induction of the intrinsic mitochondrial pathway [32]. In vitro studies also showed that, in the acute monocytic leukemia cell line THP-1, ART decreased STAT3 protein levels and activated caspase-3 and -8. These results were reproduced in a xenograft model [73]. Furthermore, Cao and collaborators described DHA-induced apoptosis of THP-1 cell line through AKT and ERK downregulation at mRNA and protein levels and activation of caspase-3 [74].

Drenberg and collaborators showed potent activity of ART and DHA in AML cell lines (ML-2, CMS, MV4-11, U937, M07e, MOLM-13) representing subtypes and genetic lesions associated with high risk and poor prognosis.

Table 5 Programmed cell deaths induced by ARS-type drugs

Cell line	Derivative	Treatment	Effect	References
In vitro				
Apoptosis				
HL-60	DHA, PBrDHA, PFDHA	0–10 μM treated up to 24 h	Induction of apoptosis, MMP↓, caspase-3 and -7↑	[35]
HL-60	DHA	0–1 μM treated for 48 h	Induction of apoptosis, TfR expression↓, Bax↑, BCL-2↓, caspase-3↑	[60]
HL-60	DHA	0.2 μM treated up to 24 h, 0.5 μM treated up to 12 h for MMP	Induction of apoptosis, caspase-3, -8, and -9↑, MMP↓, p38 MAPK↑	[59]
THP-1	ART	0, 10, 25, 50, and 100 μM treated for 48 h	Induction of apoptosis, STAT3↓, caspase-3 and -8↑	[73]
THP-1	DHA	0, 25, 50, 100, 150, and 200 μM treated for 24 h	Induction of apoptosis, p-AKT↓, p-ERK↓, caspase-3↑, *Bcl-2*↓, *Bax*↑	[74]
MOLM14	ART, ART-838	5 μM ART and 0.1 μM ART-838 treated for 24 h	Induction of apoptosis, caspase-3 and -7↑	[6]
HL-60, U937, and NB4	DHA, X-11	0.8 μM DHA and 0.2 μM X-11 treated for 24 h	Induction of apoptosis, FOXO3a↑, NOXA↑, caspase-3, -8, -9↑, Bim (weakly)↑	[7]
HL-60 and THP-1	DHA	0, 5, 10, and 15 μM treated for 12 h	Induction of apoptosis, MMP↓, BCL-2↓, Bax↑, caspase-3↑	[36]
U937, HL-60, Jurkat and AML and ALL primary blast	DHA	0–60 μM treated up to 24 h	Induction of apoptosis, MEK/ERK↓, MCL-1↓, caspases-3, -8, and -9↑, cytochrome *c* release	[67]
MV4-11 and MOLM-13	ART	0–5 μM treated for 48 and 72 h	Induction of apoptosis, MMP↓, *Bcl-2*↓, caspase-3↑	[32]
AML primary blast	ART	5 μM	Induction of apoptosis	
ML-2 and MV4-11	ART, DHA	0.15–5 μM for ML-2 and 0.03–10 μM for MV4-11 treated for 6, 24, and 48 h	Induction of apoptosis, caspase-3 and -7↑, lysosomal induction	[25]
CMS, MV4-11, and U937	ART or DHA + Ara-C	Ara-C (pretreated for 24 h) + ART or DHA (pretreated for 24 h) + Ara-C for 48 h	Synergistic effects (CI range 0.49–0.9 and CI range 0.37–0.72, respectively) (CI value < 1.0, synergistic)	
AML primary blast (MLL-positive and FLT3-ITD-positive)	ART	Treated for 48 h	IC_{50} range 3.2–46.7 μM (*MLL-positive*) and IC_{50} range 0.7–31.8 μM (*FLT3-ITD-positive*)	
AML primary blast (MLL-positive and FLT3-ITD-positive)	ART + Ara-C	0–100 μM treated for 96 h	Synergistic effects (CI value < 1.0, synergistic)	
K562 and primary human leukemia cells	DHA + Physcion	0–2 μM DHA + 5 μM Physcion treated for 24 and 48 h	Induction of apoptosis, p-AMPK↑	[71]
Molt-4	DHA	12 μM holotransferrin (pretreated for 1 h) + 200 μM DHA or 200 μM DHA alone treated up to 8 h	Induction of apoptosis, but not necrosis	[61]
Jurkat and CCRF-CEM	ART	0–15.6 μM treated up to 48 h	Intrinsic pathway of apoptosis, cytochrome *c* release, caspase-9↑	[63]
K562	DHA	0, 2, 5 and 10 μM treated for 48 h	Induction of apoptosis, morphological changes	[37]
K562	ART + ATO	32.5 μM ART + 1.0 μM ATO treated for 6 h	Induction of apoptosis and necrosis	[72]

Table 5 (continued)

Cell line	Derivative	Treatment	Effect	References
K562	DHA	0–10 μM treated for 48 h	BCR/ABL (mRNA, protein, and tyrosine kinase activity)↓, p-AKT↓, p-ERK↓, NF-κB↓, cytochrome *c* release, caspase-3 and -9↑	[68]
K562, K562/RI, and CML-T315I	DHA	0–10 μM treated for 48 h	Induction of apoptosis, BCR/ABL↓ (mRNA, protein, and tyrosine kinase activity), p-AKT↓, p-ERK↓, cytochrome *c* release, Bax↑, BCL-2↓, caspase-3 and -9↑	[69]
KBM-5	ART	0, 50, and 100 μM treated for 4 or 24 h	Induction of apoptosis, p-p38↓, p-ERK↓, p-CREB↓, p-STAT5↓, BCL-2↓, BCL-xL↓, Survivin↓, IAP-1 and -2↓, Bax↑, p21↑	[8]
BCR-ABL$^+$ B-ALL	DHA DHA + ABT-263	0–10,000 nM treated up to 24 h 0–1250 nM DHA + 0–156 nM ABT-263 treated for 24 h	Induction of apoptosis, MCL-1↓, CHOP↑, NOXA↑ Synergistic effect, induction of apoptosis	[70]
MT-2 and HUT-102	ART	0, 0.4, 2, and 10 μM and 0, 2, 10, and 50 μM, respectively, treated for 24 and 48 h	Induction of apoptosis, caspase-3, -8, and -9↑, BCL-xL↓, BCL-2↓, MCL-1↓, Survivin↓, XIAP↓, Bak↑, NF-κB↓, p-IκBα↓, AP-1↓, JunB↓, JunD↓,	[49]
U266	DHA	0, 1, 3, 10, 30, and 100 μM treated for 48 h	Induction of apoptosis, caspase-3↑, c-Jun↑	[40]
SP2/0 (murine myeloma)	ART	26 μM treated up to 96 h	Induction of apoptosis, NF-κB↓ (protein and mRNA), IκBα↑	[43]
INA-6	ART	0, 5, and 10 μM treated for 12 and 72 h	Induction of apoptosis, MYC↓, BCL-2↓, MCL-1↓, BCL-xL↓, caspase-3↑	[34]
JJN3 and RPMI-8226/R5	ART	125 μM treated up to 24 h	Induction of apoptosis (non-caspase pathway), AIF and EndoG (cytoplasmic and nuclear translocation), AIF$^{\Delta\ 1-102/118}$ (cytoplasmic fraction)	[24]
OCI-Ly3	ART	0, 5, and 10 μM treated for 12 and 72 h	Induction of apoptosis, MYC↓, BCL-2↓, MCL-1↓, BCL-xL↓, caspase-3↑	[34]
SUDHL-4 and DB	ARM	0.3 mM treated for 48 h	Induction of apoptosis, c-MYC↓, caspase-3↑	[44]
Various B-cell lymphoma cell lines	ART	5 μM treated for treated up to 18 h	Induction of apoptosis, *ATF-4*↑, *ATF-6*↑, *CHOP*↑, ATF-4↑, ATF-6↑, CHOP↑, cell metabolism (respiration and glycolysis)↓	[45]
Jurkat	DHA	0, 10, and 20 μM treated for 12 or 24 h	Induction of apoptosis (intrinsic pathway)	[75]
Jurkat	DHA + siNotch1	20 μM DHA + siNotch1 treated for 24 h	Induction of apoptosis, c-Myc↓, Notch1↓, caspase-3↑	[46]
Jurkat and HuT-78	DHA	15 μM and 30 μM, respectively, treated for 48 h	Induction of apoptosis, c-Myc↓ (mRNA and protein), Bax/BCL-2 ratio↑, p-AKT↓, p-GSK3β↓	[47]
Ramos	ART + mAb rituximab	140 μM rituximab (pretreated for 24 h) + 50 μM or 100 μM of ART treated for 24 h	Induction of apoptosis, transcriptional factors YY1 and Sp1↓, Fas/CD95↑, MMP↓, caspase-3↑, catalase↓, MnSOD↓	[48]
Autophagy				

Table 5 (continued)

Cell line	Derivative	Treatment	Effect	References
K562	DHA	20 nM holotransferrin (pretreated for 1 h) + 10 μM DHA treated for 48 h	Induction of autophagy, LC3-II↑	[62]
RPMI8226 and NB4	DHA	0, 10, 20, and 40 μM treated for 12 and 24 h	Induction of autophagy, LC3-II↑, p-IκBα↓, p62↓, translocation of Rel/p65 to nucleus↓	[78]
Ferroptosis				
HL-60, THP-1, and KG1	DHA	0, 5, 10, and 15 μM treated for 12 h	Induction of autophagy, p-AMPK↑, p-mTOR↓, p-p70S6K↓, S6K↓, LC3↑, Beclin↑, ATG5↑, ATG7↑, FTH↓	[36]
DAUDI and CA-46	ART	0–20 μM treated up to 24 h	Induction of ferroptosis, ROS↑, lipid peroxidation↑, ATF4↑, CHOP↑, and CHAC1↑ (increased mRNA and protein levels), GSH↓	[66]
In vivo				
U937 xenograft model (NOD/SCID mice) (sc.)	DHA	50 mg/kg, ip., five times per week	Tumor growth↓ (70% of reduction after 20 days treatment), induction of apoptosis, p-ERK↓, MCL-1↓, caspase-3↑	[67]
THP-1 xenograft model (Nude mice) (sc.)	ART	100 and 200 mg/kg, ip., thrice/week	Tumor growth↓, STAT3↓	[73]
KBM-5 xenograft model (Nude mice) (sc.)	ART	50, 100, and 200 mg/kg, ip., thrice/week	Tumor growth↓ (55–70% of reduction dose-dependent), p-p38↓, p-ERK↓, p-CREB↓, p-STAT5↓, BCL-2↓, BCL-xL↓, Survivin↓, IAP-1 and -2↓, Bax↑, p21↑	[8]
K562 xenograft model (Nude mice) (sc.)	DHA + Physcion	2.5 mg/kg/day DHA + 5 mg/kg/day physcion, ip., for 15 days	Tumor growth↓ (75% of reduction), pAMPK↑	[71]
BCR-ABL^{+} B-ALL xenograft model (NSG mice)	DHA + ABT-263	200 mg/kg DHA + 100 mg/kg ABT-263, oral gavage, daily, treated for 15 days	Survival↑, radiance (luciferase)↓, leukemic circulating cells↓, MCL-1 *ex vivo*↓	[70]
CA-46 xenograft model (NOD/SCID mice) (sc.)	ART	200 mg/kg/day, ip., for 15 days	Tumor growth↓ (65.8% of reduction), CHAC1↑	[66]

Arrows ↑ indicate increase and arrows ↓ indicate decrease compared to the control group

MV4-11 cells were the most sensitive with IC_{50} value of 0.092 µM and 0.24 µM for ART and DHA, respectively. Both derivatives induced apoptosis, caspase-3 and -7 activation, in addiction, ROS and lysosomal induction. ARS derivatives also showed synergistic interaction with cytarabine (Ara-C) when drugs were administered sequentially, pre-treatment with DHA followed by Ara-C (CI range 0.37–0.72) and pre-treatment with Ara-C followed by ART (CI range 0.49–0.9). ART produced modest inhibition in patient samples with the same mutations of the cell lines and the combination therapy with Ara-C demonstrated synergistic effects [25]. The semi-synthetic ARS-derived trioxane diphenylphosphate dimer 838 (ART-838) was more potent than ART in 23 leukemia cell lines and caspase-dependent apoptosis was observed [6].

ART also induced apoptosis in MM and lymphoma cell lines, with downregulation of MYC and anti-apoptotic proteins of BCL-2 family beyond caspase-3 activation [34]. Furthermore, ART overcame drug resistance in MM lineage and induced apoptosis predominantly through the non-caspase-mediated pathway by increasing ROS levels and leading to a loss of mitochondrial membrane permeabilization early in time. Thereafter, ART translocated AIF and EndoG, factors of this type of apoptosis, from the mitochondria to the cytoplasm and subsequently to the nucleus inducing apoptosis together with increased levels of superoxide [24]. Wang and the authors observed an apoptosis induction through JNK signaling pathway activation in MM cell line by DHA [40]. Li and collaborators showed apoptosis induction and proliferation inhibition of SP2/0 (murine myeloma) cells together with decreased NF-κB protein and transcriptional activity in addition to increased IκBα, which inactivated NF-κB when they were combined, by ART [43].

The c-Myc transcriptional factor regulated cell proliferation, differentiation, and apoptosis. The combined use of DHA and siNotch1 therapy induced the reduction of Notch1 and c-Myc levels, the last downstream target of Notch1, at mRNA and protein levels, and also increased caspase-3 mRNA and protein levels, subsequently inducing apoptosis and suppressed cell proliferation in T-cell lymphoma cells [46]. Furthermore, DHA reduced c-Myc protein expression at the transcriptional level and could exert antitumor effect by inhibiting the AKT/GSK3β pathway in T-cell lymphoma cells. DHA also induced apoptosis with increased Bax/BCL-2 ratio [47] and through Bak-dependent intrinsic pathway [75].

ART further demonstrated a potent anti-tumor effect against B-cell lymphoma cells. ART induced ER stress and unfolded protein response (UPR) through increased levels of ATF-4, ATF-6, and CHOP at mRNA and protein levels in BL-41 (Burkitt lymphoma) and SU-DHL-6 (large cell lymphocyte) cells, leading to apoptosis. In addition, ART suppressed the overall metabolism, affecting both respiration and glycolysis [45]. Moreover, Sieber and collaborators showed a potent cytotoxic effect of combination therapy using mAb rituximab and ART in B-cell lymphoma cells in which upstream transcriptional factors of apoptosis process, YY1 and Sp1, regulated Fas/CD95 expression leading to the intrinsic apoptosis pathway. Combination therapy also induced downregulation of antioxidant proteins [48]. Indeed, ART induced apoptosis through caspase-dependent and -independent pathway together with a suppression of NF-κB and activator protein-1 (AP-1) signaling in adult T-cell leukemia/lymphoma [49] cells. Furthermore, the lipophilic ARS-derivative, ARM, induced apoptosis in DLBCL cell lines with caspase-3 induction [44].

Autophagy

Regulation of cancer cell autophagy by ARS-type drugs involves decreased phosphorylation of proteins of the PI3K/AKT/mTOR pathway and increased levels of Beclin1 mediated by the JNK pathway. ARS and derivatives also inhibit NF-κB activity through blocking Rel/p65 translocation to the nucleus and activate ER stress through the stress-regulated protein p8 [76]. In leukemia, Wang and collaborators reported that DHA induced autophagy of K562 cells, pretreated with holotransferrin, through ROS generation followed by LC3-II expression and caspase-3 activation [62].

A new ARS-derivative, SM1044, induced autophagy mediated by the CaMKK2/AMPK/ULK1 pathway through promoting de novo synthesis of ceramide in lymphoma cells. Furthermore, the new derivative also induced autophagy-dependent apoptosis through acetylation of Survivin protein and enhanced interaction of Survivin and LC3-II [77]. Moreover, DHA treatment suppressed NF-κB activity by preventing the translocation of the Rel/p65 subunit to the nucleus consequently contributing to autophagy in MM and leukemia cell lines [78].

Ferroptosis

Ferroptosis is an iron-dependent programmed cell death pathway dependent on the lipid peroxidation process. In contrast with other programmed cell deaths, reduction of glutathione peroxidase 4 (GPX4) repairs enzymes, accumulation of lipid peroxidation products and ROS derived from iron metabolism characterizes ferroptosis. ARS-type drugs are capable of reversing ferroptosis resistance of head and neck cancer cells, through inhibition of the Nrf2-ARE antioxidant signaling [79]. Moreover, in pancreatic cells, the induction of ferroptosis by ART is enhanced by GRP78 knockdown, chaperone of ER [80]. Recently, Du and collaborators described that DHA treatment of the HL-60 leukemic cell line induced ferroptosis through degradation of the heavy ferritin chain by autophagy [36]. Indeed, ART

activated ATF4/CHOP/CHAC1 pathway, an ER stress response, and enhanced ferroptosis [66] in Burkitt's lymphoma cell lines.

Anti-angiogenic effects

Angiogenesis plays a fundamental role in the neoplastic process. Effects of ARS-type drugs on cancer cells rely on perturbations of the MAPK pathway, which comprise ERK1/2 reduction or JNK and p38 MAPK activation, NF-κB inhibition and also AKT and mTOR inhibition promoting induction of proliferation and apoptosis of endothelial cell and reduction on the vascular endothelial growth factor (VEGF) production [81]. VEGF is the most potent angiogenic factor and plays an important role in the development and progression of leukemias (Table 6) and MM. Interestingly, ART and DHA reduced the expression and secretion of VEGF in RPMI8226 myeloma and K562 leukemia cell lines, respectively [37, 41] and inhibited the formation of new microvessels in an in vivo model of chick chorioallantoic membrane (CAM) loaded with RPMI8226 conditioned medium (CM) under hypoxic conditions [42]. Furthermore, ART, the other water soluble ARS-derivative compound, inhibited the production of new microvessel on aortic sprouting, reduced CML angiogenesis in an in vivo model of CAM [82], inhibited vascular endothelial cell (HUVEC) migration and reduced expression of VEGF and angiopoietin-1 (Ang-1) proteins [41].

Differentiation induced by ARS and derivatives

ARS potentiated 1α,25-dihydoxyvitamin D_3 and all-*trans* retinoic acid induced differentiation in HL-60 promyelocytic leukemia cells. ARS in combination with low concentration of 1,25-$(OH)_2D_3$ increased HL-60 differentiation into monocytes through ERK and PKC pathways with an increased PKCβ1 isoform. Indeed, ARS plus all-*trans* retinoic acid induced cell differentiation into granulocytes through ERK pathway [83]. ARS has a synergistically effect with interferon-α (IFN-α) in enhancing HL-60 cell differentiation through PKCα/ERK signaling pathway [84]. On the other hand, DHA inhibited erythroid cell differentiation with a decreased expression of glycophorin A (GpA) surface receptor and γ-globin synthesis [85] (Table 7).

Table 6 Angiogenesis proprieties of ARS-type drugs

Cell line	Derivative	Treatment	Effect	References
In vitro				
K562	DHA	0, 2, 5, and 10 μM treated for 48 h	VEGF↓ (expression and secretion)	[37]
RPMI8226	DHA	0–12 μM treated for 48 h under hypoxia	VEGF↓ (mRNA and protein levels, and secretion)	[42]
RPMI8226	ART	0–12 μM treated for 48 h	Microvessel↓, VEGF and Ang-1secretion↓, migration↓	[41]
Jurkat	DHA + holotransferrin	0, 10, 20, 40, and 80 μM DHA + 20 μM holotransferrin treated for 48 h	*VEGF*↓	[58]
In vivo				
KBM-5 xenograft model (Nude mice) (sc.)	ART	50, 100, and 200 mg/kg, ip., thrice/week	Tumor growth↓ (55–70% of reduction dose-dependent), VEGF↓	[8]
K562 CAM model	ART	1.2 μM/100 μl per egg treated for 48 h	Microvessel↓	[82]
K562 CAM model	ART	CM pretreated with 0, 3, 6, and 12 μM (25 ng protein/5 μl per embryo) treated for 48 h	Microvessel↓ (28.2, 44.3, 72.3% of reduction, respectively), VEGF secretion (CM)↓	[82]
RPMI8226 CAM model	DHA	CM pretreated with 0, 3, 6, and 12 μM under hypoxic conditions and loaded onto CAM on day 8	Microvessel↓ (28.6, 41.3, 61.4% of reduction, respectively)	[42]
RPMI8226 CAM model	ART	CM pretreated with 0, 3, 6, and 12 μM under hypoxic conditions and loaded onto CAM on day 8	Microvessel↓ (21.9, 38.24, and 76.9% of reduction, respectively)	[41]

Arrows ↑ indicate increase and arrows ↓ indicate decrease compared to the control group

Table 7 Differentiation induced by ARS-type drugs

Cell line	Derivative	Treatment	Effect	References
HL-6O	ARS + 1,25-$(OH)_2D_3$ ARS + all-trans RA	0, 1.25, 2.5, 5.0, and 10.0 μM ARS + 5 nM 1,25-$(OH)_2D_3$ or 50 nM all-trans RA treated up to 72 h	CD11b↑, PCK and ERK activation, PKCβ1↑ CD11b↑, CD14↑, ERK activation	[83]
HL-60	ARS + IFN-α	20 μM ARS + 10^4U IFN-α	PKCα/ERK activation	[84]
K562	DHA	0–10 μM treated 24, 48, and 72 h for cytotoxic effects; for further tests DHA 0.5 and 2.0 μM treated 24, 48, and 72 h	Inhibition of erythroid differentiation, GpA receptor↓, γ-globin↓	[85]

Arrows ↑ indicate increase and arrows ↓ indicate decrease compared to the control group

New hybrid compounds

Hybridization is a new approach used to improve the activity of chemical compounds [86] (Table 8). Homodimers of two artesunic acid molecules and heterohybrids of artesunic acid and betulin, a natural product that exhibited cytotoxicity activity toward human lung cancer, were tested in human sensitive and multidrug-resistant cells. CEM/ADR5000, multidrug-resistant leukemia cells, were not cross-resistant to the novel compounds and sensitivity was also observed for artesunic acid homodimer in CCRF-CEM cell line. Furthermore, artesunic acid and artesunic acid homodimer increased ROS formation and induced apoptosis and G_0/G_1 cell cycle arrest [87].

As structure modification may improve ARS anti-cancer activity, a series of DHA chalcone hybrids and derivatives were synthetized [88] and demonstrated a high antiproliferative and cytotoxicity effect in HL-60 cell lines [89]. Additionally, new ARS–spermidine conjugates were designed to upregulate polyamine transporter. Amine-linked conjugates were approximately 1.5–2 times more active than amide-linked conjugates in HL-60 leukemia cells and all of them were higher than DHA [90]. Furthermore, new dimers phosphate ester, screening against human leukemia and normal cell lines, exhibited very high potency against cancer cells with no toxicity to normal cells [91]. Dimers and trimers were more active against CCRF-CEM cells [92]. Interestingly, molecular docking showed that most derivatives revealed similar binding sites at the transmembrane region of the multidrug P-glycoprotein [93] transporter.

Three hybrid molecules having aliphatic, aromatic, or alcoholic linkers were analyzed for their activity against human multidrug-resistant leukemia cells. The multidrug-resistant cells were not cross-resistant to any of the dimers [94]. Therefore, new ARS-derived hybrids incorporating cholic acid moieties were efficient against the CCRF-CEM and multidrug-resistant CEM/ADR5000 cells. The majority of the compounds proved to be more active than ARS and ART alone [95]. Furthermore, a series of novel 1,2,4-trioxane-based hybrids incorporating egonol and/or ferrocene fragments were synthesized and showed remarkable cytotoxicity toward CCRF-CEM cells or against multidrug-resistant leukemia cells [96, 97].

ARS-based hydroxamic acids were synthesized with a possible dual mechanism of both endoperoxide bridge and hydroxamic acid moiety. All the compounds exhibited moderate inhibition against HL-60 cells and, interestingly, docking studies of two very active compounds showed that they were capable of binding to HDAC2 with high affinity, even higher than the HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA) [98].

Another modification of ARS derivative that has been demonstrated to improve their activity was a sugar attachment which enhanced specificity of drug delivery, polarity, and solubility, to attenuate toxicity. Thus, N-glycosylated DHA-piperazine, glucose, maltose, and ribose were most active and specific against leukemic cells than DHA and artemisone [99].

A novel synthesis of quinazoline–ARS [100] and thymoquinone–ARS [101] hybrids were investigated and showed promising results for multidrug-resistant cell line [100, 101]. Furthermore, a hybrid containing egonol, isolated from *Styrax officinalis L.* and homoegonol, with anti-inflammatory, antioxidant and anticancer activity, combined with thymoquinone and ARS were synthetized and demonstrated high activity [102].

The antimalarial and anticancer mechanism of ARS-type drugs

This class of compounds acts in a multi-specific manner, exhibiting several modes of action simultaneously. Interestingly, mechanisms of induction of malaria parasite death can be transposable to cancer cells. During the erythrocyte infection, plasmodia consume hemoglobin as a source of amino acids, leading to the generation of ROS by heme–iron which cleaves the endoperoxide moiety of the ARS-type drugs by a Fe^{+2} Fenton-type reaction, with more free radical intermediate formation [103]. This can lead to macromolecular damage and, consequently, death to the parasite.

Table 8 New hybrid compounds

Cell line	Derivative	Treatment	Effect	References
HL-60	DHA chalcones linked by ether or ester	0–0.8 μM treated up to 72 h	Compounds linked by ether are more potent than compounds linked by ester	[88]
HL-60	DHA containing substituted chalcones	0.5, 1, 10, and 30 μM treated for 48 h	More cytotoxic than DHA	[89]
HL-60	DHA and ARS-spermidine conjugates	0.01–100 μM treated for 72 h	Amine-linked conjugates were 1.5–2 times more active than amide-linked conjugates	[90]
HL-60	ARS-based hydroxamic acids compounds	NS	Cytotoxicity↑; high binding affinities compared to SAHA	[98]
Jurkat and PBMC	Dimers phosphate ester	NS	High anticancer potencies without toxicity to normal cells	[91]
CCRF-CEM CEM/ADR5000	ARS, artesunic acid, artesunic acid homodimer	Treated for 72 h	No cross-resistant to any of the dimers; IC_{50} value 1.2 ± 0.1 μM for CCRF-CEM and 0.2 ± 0.03 μM for CEM/ADR5000 treated with artesunic acid homodimer	[87]
CCRF-CEM, CEM/ADR5000	1,2,4-trioxane-egonol and/or ferrocene hybrids	NS	Cytotoxicity↑; IC_{50} value 0.07, 0.25, 0.18 μM for CCRF-CEM and 147.27, 0.57, 1.12 μM for CEM/ADR5000	[96]
CCRF-CEM, CEM/ADR5000	Second generation 1,2,4-trioxane-ferrocene hybrids	0.001–100 μM	Cytotoxicity↑; IC_{50} value 0.13 and 0.01 μM for CCRF-CEM and 0.53 and 1.96 μM for CEM/ADR5000	[97]
CCRF-CEM, CEM/ADR5000	Dimers and trimers	0.001–100 μM treated for 72 h	Cytotoxicity↑; IC_{50} value 2.75, 0.09, 0.002, 0.002 μM for CCRF-CEM and 2.79, 0.2, 0.2, 0.49 μM for CEM/ADR5000	[92]
CCRF-CEM, CEM/ADR5000	ARS-derivatives which incorporate cholic acid moieties	0.001–100 μM	More active against both cell lines than ART and ARS	[95]
CCRF-CEM, CEM/ADR5000	Egonol and homoegonol hybrids	0.001–100 μM treated for 72 h	High anticancer potency of ARS, egonol, and homoegonol hybrids	[102]
CCRF-CEM, CEM/ADR5000	Quinazoline-ARS hybrids	0.001–100 μM treated for 72 h	Antileukemia effect (low micromolar range); 45 times more active toward CEM/ADR5000 comparing to doxorubicin	[100]
CCRF-CEM, CEM/ADR5000	Thymoquinone-ARD hybrids	0.001–100 μM treated for 72 h	Antileukemia effect; low toxicity	[101]
CEM/ADR 5000	Artesunic acid homodimers with aliphatic, aromatic, or alcoholic linkers	0.001 – 100 μM	No cross-resistance	[94]
K562	N-glycosylated DHA-piperazine (glucose, maltose, and ribose)	NS	Cytotoxicity↑; IC_{50} value 0.81, 0.78, and 0.87 μM, respectively; less toxicity to human fibroblast than artemisone and DHA	[99]

Arrows ↑ indicate increase and arrows ↓ indicate decrease compared to the control group

Another mechanism of action is based on its structural similarity with thapsigargin, which is a highly specific inhibitor of sarcoendoplasmic reticulum Ca^{+2}-ATPase (SERCA). Thus, ARS has been shown to inhibit the SERCA orthologue (PfATP6) of the *P. falciparum* [104] and that DHA, the active metabolite of ARS-type drugs, damages proteins and inhibits the proteasome, causing ER stress response [105].

Cancer cells have more intracellular iron than normal cells, thus ARS-like drugs can react with intracellular free iron to form cytotoxic free radicals that promote the death of cancer cells. A plethora of articles has revealed the importance of endoperoxide moiety for anticancer activity. Thus, the cleavage of the endoperoxide bridge leads to reactive oxygen species (ROS) formation and consequently, oxidative stress that induces cancer cell death. Moreover, the endoperoxide-reduced form of ARS, deoxyartemisinin, which lacks the peroxide, is inactive [7] against leukemic cells. Furthermore, similar to the mechanism proposed for antimalarial activity, the anticancer activity of ARS-type drug can be targeting the unfolded protein response (UPR) and endoplasmic reticulum (ER) response of the cancer cell leading to apoptosis or ferroptosis [66].

Most of the changes in the ARS structure that improve the pharmacological properties and pharmacokinetics of ARS compounds occur at C-10 carbon of DHA, enabling derivation to drugs with increased biological efficacy, reduced undesired side effects, selected profile (e.g., lower toxicity), and better bioavailability.

Conclusions

In conclusion, ARS and its derivatives are active against hematological malignancy cells in vitro and in vivo [6, 7, 25]. Hybrids, dimers, and trimers improved cytotoxicity against leukemia cells, proposing high potency even in MDR cells [87, 92]. It is speculated that the main mechanism of action is the bioactivation of the endoperoxide pharmacophore group by iron that leads ROS formation and free radical intermediates formation [50, 54, 63], inducing cell death as occurs with the malaria parasite. ARS-type drugs also enhance chemotherapeutic anticancer activity contributing mechanistically to additive or synergistic effects [25]. Collectively, this review summarizes the main studies that indicated the role of ARS-type drugs in the treatment of leukemia, lymphoma, and multiple myeloma through apoptotic and non-apoptotic cell death (downstream mechanism), mainly by inducing cell cycle arrest at G_0/G_1 phase, inhibition of proliferation, inhibition of angiogenesis with down-regulation of VEGF, and signal transduction modulation (Fig. 2). Importantly, combination therapies with standard chemotherapy drugs enhanced the potential of ARS-type drugs in adjuvant therapy in clinical oncology. No clinical trial for hematological malignancies has been conducted yet. Thus, clinical trials should be encouraged to provide more compelling evidence regarding the use of ARS-derivatives in hematological cancer treatment.

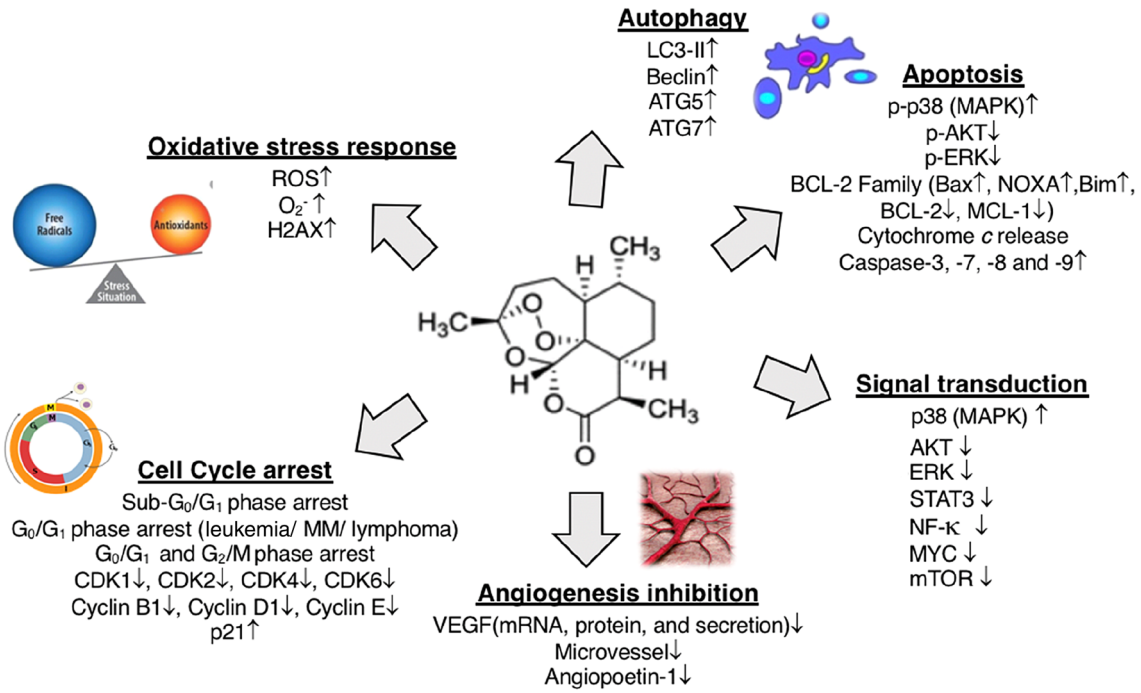

Fig. 2 Summary of the mechanisms of action of ARS-type drugs against hematological malignancies

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Klayman DL (1985) Qinghaosu (artemisinin): an antimalarial drug from China. Science 228:1049–1055
2. Miller LH, Su X (2011) Artemisinin: discovery from the Chinese herbal garden. Cell 146:855–858
3. Tu Y (2016) Artemisinin—a gift from traditional chinese medicine to the world (nobel lecture). Angew Chem. https://doi.org/10.1002/anie.201601967
4. Hien TT, White NJ (1993) Qinghaosu. Lancet 341:603–608
5. Efferth T, Dunstan F, Sauerbrey A, Miyachi H, Chitambar CR (2001) The anti-malarial artesunate is also active against cancer. Int J Oncol 18:767–773
6. Fox JM, Moynihan JR, Mott BT, Mazzone JR, Anders NM, Brown PA, Rudek MA, Liu JO, Arav-Borger R, Posner GH, Civin CI, Chen X (2016) Artemisinin-derived dimer ART-838 potently inhibited human acute leukemias, persisted in vivo, and synergized with antileukemic drugs. Oncotarget 7:7268–7279
7. Zhao X, Zhong H, Wang R, Liu D, Waxman S, Zhao L, Jing Y (2015) Dihydroartemisinin and its derivative induce apoptosis in acute myeloid leukemia through Noxa-mediated pathway requiring iron and endoperoxide moiety. Oncotarget. https://doi.org/10.18632/oncotarget.3336
8. Kim C, Lee JH, Kim S-H, Sethi G, Ahn KS (2015) Artesunate suppresses tumor growth and induces apoptosis through the modulation of multiple oncogenic cascades in a chronic myeloid leukemia xenograft mouse model. Oncotarget. https://doi.org/10.18632/oncotarget.3004.
9. Lam NS, Long X, Wong JW, Griffin RC, Doery JCG (2019) Artemisinin and its derivatives: a potential treatment for leukemia. Anticancer Drugs 30:1–18
10. Efferth T (2017a) From ancient herb to modern drug: artemisia annua and artemisinin for cancer therapy. Semin Cancer Biol. https://doi.org/10.1016/j.semcancer.2017.02.009
11. Efferth T (2017b) Cancer combination therapies with artemisinin-type drugs. Biochem Pharmacol. https://doi.org/10.1016/j.bcp.2017.03.019
12. Kumar MS, Yadav TT, Khair RR, Peters GJ, Yergeri MC (2019) Combination therapies of artemisinin and its derivatives as a viable approach for future cancer treatment. Curr Pharm Des 25:3323–3338
13. Foglio MA, Dias PC, Antônio MA, Possenti A, Ferreira Rodrigues RA, Ferreira da Silva E, Rehder VL, de Carvalho JE (2002) Antiulcerogenic activity of some sesquiterpene lactones isolated from artemisia annua. Planta Med 68:515–518
14. Ives CS, Pedro MM, Ilza MOS, Mary AF, Eduardo L, Sérgio NE, Chagas ACS (2017) Anthelmintic activity of Artemisia annua in sheep-model. J Med Plants Res 11:137–143
15. Boareto AC, Muller JC, Bufalo AC, Botelho GGK, de Araujo SL, Foglio MA, de Morais RN, Dalsenter PR (2008) Toxicity of artemisinin [*Artemisia annua* L.] in two different periods of pregnancy in Wistar rats. Reprod Toxicol 25:239–246
16. Volpe Zanutto F, McAlister E, Marucci Pereira Tangerina M, Fonseca-Santos B, Costa Salles TH, Oliveira Souza IM, Brisibe A, Vilegas W, Chorilli M, d'Ávila MA, Donnelly RF, Foglio MA (2019) Semisynthetic derivative of *Artemisia annua*-Loaded transdermal bioadhesive for the treatment of uncomplicated malaria caused by *Plasmodium falciparum* in children. J Pharm Sci 108:1177–1188
17. Torello CO, Shiraishi RN, Della Via FI, de Castro TCL, Longhini AL, Santos I, Bombeiro AL, Araujo Silva CL, Queiroz ML, Rego EM, Saad STO (2018) Reactive oxygen species production triggers green tea-induced anti-leukaemic effects on acute promyelocytic leukaemia model. Cancer Lett 414:116–126
18. Alvarez MC, Maso V, Torello CO, Ferro KP, Saad STO (2018) The polyphenol quercetin induces cell death in leukemia by targeting epigenetic regulators of pro-apoptotic genes. Clin Epigenetics. https://doi.org/10.1186/s13148-018-0563-3
19. Bowman RL, Busque L, Levine RL (2018) Clonal Hematopoiesis and Evolution to Hematopoietic Malignancies. Cell Stem Cell 22:157–170
20. Kumar SK, Rajkumar V, Kyle RA, Van Duin M, Sonneveld P, Mateos MV, Gay F, Anderson KC (2017) Multiple myeloma. Nat Rev Dis Prim. https://doi.org/10.1038/nrdp.2017.46
21. Armitage JO, Gascoyne RD, Lunning MA, Cavalli F (2017) Non-Hodgkin lymphoma. Lancet 390:298–310
22. Gurnari C, Voso MT, Maciejewski JP, Visconte V (2020) From bench to bedside and beyond: therapeutic scenario in acute myeloid leukemia. Cancers (Basel) 12:1–20
23. De Kouchkovsky I, Abdul-Hay M (2016) Acute myeloid leukemia: a comprehensive review and 2016 update. Blood Cancer J. https://doi.org/10.1038/bcj.2016.50
24. Papanikolaou X, Johnson S, Garg T, Tian E, Tytarenko R, Zhang Q, Stein J, Barlogie B, Epstein J, Heuck C (2014) Artesunate overcomes drug resistance in multiple myeloma by inducing mitochondrial stress and non-caspase apoptosis. Oncotarget 5:4118–4128
25. Drenberg CD, Buaboonnam J, Orwick SJ, Hu S, Li L, Fan Y, Shelat AA, Guy RK, Rubnitz J, Baker SD (2016) Evaluation of artemisinins for the treatment of acute myeloid leukemia. Cancer Chemother Pharmacol 77:1231–1243
26. Zheng G-Q (1993) Cytotoxic terpenoids and flavonoids from *Artemisia annua*. Planta Med. https://doi.org/10.1055/s-2006-959408
27. Wang Y, Li Y, Shang D, Efferth T (2019) Interactions between artemisinin derivatives and P-glycoprotein. Phytomedicine. https://doi.org/10.1016/j.phymed.2019.152998
28. Efferth T, Davey M, Olbrich A, Rücker G, Gebhart E, Davey R (2002) Activity of drugs from traditional Chinese medicine toward sensitive and MDR1- or MRP1-overexpressing multidrug-resistant human CCRF-CEM leukemia cells. Blood Cells, Mol Dis 28:160–168
29. Singh NP, Ferreira JFS, Park JS, Lai HC (2011) Cytotoxicity of ethanolic extracts of *Artemisia annua* to molt-4 human leukemia cells. Planta Med 77:1788–1793
30. Wickerath M, Singh NP (2014) Additive cytotoxic effects of dihydroartemisinin and sodium salicylate on cancer cells. Anticancer Res 34:3399–3401

31. Gerhardt T, Jones R, Park J, Lu R, Chan HW, Fang Q, Singh N, Lai H (2015) Effects of antioxidants and pro-oxidants on cytotoxicity of dihydroartemisinin to Molt-4 human leukemia cells. Anticancer Res 35:1867–1872
32. Kumar B, Kalvala A, Chu S, Rosen S, Forman SJ, Marcucci G, Chen CC, Pullarkat V (2017) Antileukemic activity and cellular effects of the antimalarial agent artesunate in acute myeloid leukemia. Leuk Res 59:124–135
33. Ohtaka M, Itoh M, Tohda S (2017) BMI1 inhibitors downregulate NOTCH signaling and suppress proliferation of acute leukemia cells. Anticancer Res 37:6047–6053
34. Holien T, Olsen OE, Misund K, Hella H, Waage A, Rø TB, Sudan A (2013) Lymphoma and myeloma cells are highly sensitive to growth arrest and apoptosis induced by artesunate. Eur J Haematol 91:339–346
35. Mercer AE, Maggs JL, Sun XM, Cohen GM, Chadwick J, O'Neill PM, Park BK (2007) Evidence for the involvement of carbon-centered radicals in the induction of apoptotic cell death by artemisinin compounds. J Biol Chem 282:9372–9382
36. Du J, Wang T, Li Y, Zhou Y, Wang X, Yu X, Ren X, An Y, Wu Y, Sun W, Fan W, Zhu Q, Wang Y, Tong X (2019) DHA inhibits proliferation and induces ferroptosis of leukemia cells through autophagy dependent degradation of ferritin. Free Radic Biol Med 131:356–369
37. Lee J, Zhou HJ, Wu XH (2006) Dihydroartemisinin downregulates vascular endothelial growth factor expression and induces apoptosis in chronic myeloid leukemia K562 cells. Cancer Chemother Pharmacol 57:213–230
38. Li Y, Shan F, Wu JM, Wu GS, Ding J, Xiao D, Yang WY, Atassi G, Léonce S, Caignard DH, Renard P (2001) Novel antitumor artemisinin derivatives targeting G1 phase of the cell cycle. Bioorg Med Chen Lett 11:5–8
39. Li Y, Wu JM, Shan F, Wu GS, Ding J, Xiao D, Han JX, Atassi G, Leonce S, Caignard DH, Renard P (2003) Synthesis and cytotoxicity of dihydroartemisinin ethers containing cyanoarylmethyl group. Bioorg Med Chem 11:977–984
40. Wang Y, Xu X, Wu X, Chen W, Huang F, Gui X (2018) Dihydroartemisinin treatment of multiple myeloma cells causes activation of c-jun leading to cell apoptosis. Oncol Lett 15:2562–2566
41. Chen H, Shi L, Yang X, Li S, Guo X, Pan L (2010) Artesunate inhibiting angiogenesis induced by human myeloma RPMI8226 cells. Int J Hematol 92:587–597
42. Wu XH, Zhou HJ, Lee J (2006) Dihydroartemisinin inhibits angiogenesis induced by multiple myeloma RPMI8226 cells under hypoxic conditions via downregulation of vascular endothelial growth factor expression and suppression of vascular endothelial growth factor secretion. Anticancer Drugs 17:839–848
43. Li S, Xue F, Cheng Z, Yang X, Wang S, Geng F, Pan L (2009) Effect of artesunate on inhibiting proliferation and inducing apoptosis of SP2/0 myeloma cells through affecting NFκB p65. Int J Hematol 90:513–521
44. Zhao X, Guo X, Yue W, Wang J, Yang J, Chen J (2017) Artemether suppresses cell proliferation and induces apoptosis in diffuse large B cell lymphoma cells. Exp Ther Med 14:4083–4090
45. Våtsveen TK, Myhre MR, Steen CB, Wälchli S, Lingjærde OC, Bai B, Dillard P, Theodossiou TD, Holien T, Sudan A, Inderberg EM, Smeland EB, Myklebust JH, Oksvold MP (2018) Artesunate shows potent anti-tumor activity in B-cell lymphoma. J Hematol Oncol. https://doi.org/10.1186/s13045-018-0561-0
46. Huo L, Wei W, Wu S, Zhao X, Zhao C, Zhao H, Sun L (2018) Effect of dihydrarteminin combined with siRNA targeting-notch1 on Notch1/c-Myc signaling in T-cell lymphoma cells. Exp Ther Med 15:3059–3065
47. Wei W, Zhao X, Wu S, Zhao C, Zhao H, Sun L, Cui Y (2018) Dihydroartemisinin triggers c-Myc proteolysis and inhibits protein kinase B/glycogen synthase kinase 3β pathway in T-cell lymphoma cells. Oncol Lett 16:6838–6846
48. Sieber S, Gdynia G, Roth W, Bona Vida B, Efferth T (2009) Combination treatment of malignant B cells using the anti-CD20 antibody rituximab and the anti-malarial artesuante. Int J Oncol 35:149–158
49. Ishikawa C, Senba M, Mori N (2020) Evaluation of artesunate for the treatment of adult T-cell leukemia/lymphoma. Eur J Pharmacol. https://doi.org/10.1016/j.ejphar.2020.172953
50. Lai H, Singh NP (1995) Selective cancer cell cytotoxicity from exposure to dihydroartemisinin and holotransferrin. Cancer Lett 91:41–46
51. Singh NP, Lai HC (2005) Synergistic cytotoxicity of artemisinin and sodium butyrate on human cancer cells. Anticancer Res 25:4325–4331
52. Efferth T, Benakis A, Romero MR, Tomicic M, Rauh R, Steinbach D, Hafer R, Stamminger T, Oesch F, Kaina B, Marschall M (2004) Enhancement of cytotoxicity of artemisinins toward cancer cells by ferrous iron. Free Radic Biol Med 37:998–1009
53. Kelter G, Steinbach D, Konkimalla VB, Tahara T, Taketani S, Fiebig HH, Efferth T (2007) Role of transferrin receptor and the ABC transporters ABCB6 and ABCB7 for resistance and differentiation of tumor cells towards artesunate. PLoS ONE. https://doi.org/10.1371/journal.pone.0000798
54. Lai H, Sasaki T, Singh NP, Messay A (2005) Effects of artemisinin-tagged holotransferrin on cancer cells. Life Sci 76:1267–1279
55. Oh S, Kim BJ, Singh NP, Lai H, Sasaki T (2009) Synthesis and anti-cancer activity of covalent conjugates of artemisinin and a transferrin-receptor targeting peptide. Cancer Lett 274:33–39
56. Park J, Lai HC, Singh M, Sasaki T, Singh NP (2014) Development of a dihydroartemisinin-resistant molt-4 leukemia cell line. Anticancer Res 34:2807–2810
57. Ohgami Y, Elstad CA, Chung E, Shirachi DY, Quock RM, Lai HC (2010) Effect of hyperbaric oxygen on the anticancer effect of artemisinin on molt-4 human leukemia cells. Anticancer Res 30:4467–4470
58. Wang Q, Wu S, Zhao X, Zhao C, Zhao H, Huo L (2015) Mechanisms of dihydroartemisinin and dihydroartemisinin/holotransferrin cytotoxicity in T-cell lymphoma cells. PLoS ONE 10:1–12
59. Lu JJ, Meng LH, Cai YJ, Chen Q, Tong LJ, Lin LP, Ding J (2008) Dihydroartemisinin induces apoptosis in HL-60 leukemia cells dependent of iron and p38 mitogen-activated protein kinase activation but independent of reactive oxygen species. Cancer Biol Ther 7:1017–1023
60. Zhou H-J, Wang Z, Li A (2007) Dihydroartemisinin induces apoptosis in human leukemia cells HL60 via downregulation of transferrin receptor expression. Anti Cancer Drugs. https://doi.org/10.1097/CAD.0b013e3282f3f152.
61. Singh NP, Lai HC (2004) Artemisinin induces apoptosis in human cancer cells. Anticancer Res 24:2277–2280
62. Wang Z, Hu W, Zhang JL, Wu XH, Zhou HJ (2012) Dihydroartemisinin induces autophagy and inhibits the growth of iron-loaded human myeloid leukemia K562 cells via ROS toxicity. FEBS Open Biol 2:103–112
63. Efferth T, Glaisi M, Merling A, Krammer PH, Li-Weber M (2007) Artesunate induces ROS-mediated apoptosis in Doxorubicin-resistant T leukemia cells. PLoS ONE. https://doi.org/10.1371/journal.pone.0000693
64. Zhang S, Chen H, Gerhard GS (2010) Heme synthesis increases artemisinin-induced radical formation and cytotoxicity that can be suppressed by superoxide scavengers. Chem Biol Interact 186:30–35

65. Chan HW, Singh NP, Lai HC (2013) Cytotoxicity of dihydroartemisinin toward Molt-4 cells attenuated by *N*-tert-butyl-alpha-phenylnitrone and deferoxamine. Anticancer Res 33:4389–4394
66. Wang N, Zeng GZ, Yin JL, Bian ZX (2019) Artesunate activates the ATF4-CHOP-CHAC1 pathway and affects ferroptosis in Burkitt's lymphoma. Biochem Biophys Res Commun 519:533–539
67. Gao N, Budhraja A, Cheng S, Liu EH, Huang C, Chen J, Yang Z, Chen D, Zhang Z, Shi X (2011) Interruption of the MEK/ERK signaling cascade promotes dihydroartemisinin-induced apoptosis in vitro and in vivo. Apoptosis 16:511–523
68. Lee J, Zhang G, Wu X, Xu F, Zhou J, Zhang X (2012) Growth inhibitory effect of dihydroartemisinin on Bcr/Abl+chronic myeloid leukemia K562 cells involve AKT, ERK and NF-κB modulation. J Cancer Res Clin Oncol 138:2095–2102
69. Lee J, Shen P, Zhang G, Wu X, Zhang X (2013) Dihydroartemisinin inhibits the Bcr/Abl oncogene at the mRNA level in chronic myeloid leukemia sensitive or resistant to imatinib. Biomed Pharmacother 67:157–163
70. Budhraja A, Turnis ME, Churchman ML, Kothari A, Yang X, Xu H, Kaminska E, Panetta JC, Finkelstein D, Mullighan CG, Opferman JT (2017) Modulation of navitoclax sensitivity by dihydroartemisinin-mediated MCL-1 repression in BCR-ABL+ B-lineage acute lymphoblastic leukemia. Clin Cancer Res 23:7558–7568
71. Elf S, Lin R, Xia S, Pan Y, Shan C, Wu S, Lonial S, Gaddh M, Arellano ML, Khoury HJ, Khuri FR, Lee BH, Boggon TJ, Fan J, Chen J (2017) Targeting 6-phosphogluconate dehydrogenase in the oxidative PPP sensitizes leukemia cells to antimalarial agent dihydroartemisinin. Oncogene 36:254–262
72. Li Y, Feng L, Jiang W, Shan N, Wang X (2014) Artesunate possesses anti-leukemia properties that can be enhanced by arsenic trioxide. Leuk Lymphoma 55:1366–1372
73. Tan M, Rong Y, Su Q, Chen Y (2017) Artesunate induces apoptosis via inhibition of STAT3 in THP-1 cells. Leuk Res 62:98–103
74. Cao JT, Mo HM, Wang Y, Zhao K, Zhang TT, Wang CQ, Xu KL, Han ZH (2018) Dihydroartemisinin-induced apoptosis in human acute monocytic leukemia cells. Oncol Lett 15:3178–3184
75. Handrick R, Ontikatze T, Bauer KD, Freier F, Rübel A, Dürig J, Belka C, Jendrossek V (2010) Dihydroartemisinin induces apoptosis by a bak-dependent intrinsic pathway. Mol Cancer Ther 9:2497–2510
76. Sun X, Yan P, Zou C, Wong YK, Shu Y, Lee YM, Zhang C, Yang ND, Wang J, Zhang J (2019) Targeting autophagy enhances the anticancer effect of artemisinin and its derivatives. Med Res Rev 39:2172–2193
77. Cheng C, Wang T, Song Z, Peng L, Gao M, Hermine O, Rousseaux S, Khochbin S, Mi JQ, Wang J (2018) Induction of autophagy and autophagy-dependent apoptosis in diffuse large B-cell lymphoma by a new antimalarial artemisinin derivative, SM1044. Cancer Med 7:380–396
78. Hu W, Chen SS, Zhang JL, Lou XE, Zhou HJ (2014) Dihydroartemisinin induces autophagy by suppressing NF-κB activation. Cancer Lett 343:239–248
79. Roh JL, Kim EH, Jang H, Shin D (2017) Nrf2 inhibition reverses the resistance of cisplatin-resistant head and neck cancer cells to artesunate-induced ferroptosis. Redox Biol 11:254–262
80. Wang K, Zhang Z, Wang M, Cao X, Qi J, Wang D, Gong A, Zhu H (2019) Role of GRP78 inhibiting artesunate-induced ferroptosis in KRAS mutant pancreatic cancer cells. Drug Des Dev Ther 13:2135–2144
81. Wei T, Liu J (2017) Anti-angiogenic properties of artemisinin derivatives. Int J Mol Med 40:972–978
82. Zhou HJ, Wang WQ, Wu GD, Lee J, Li A (2007) Artesunate inhibits angiogenesis and downregulates vascular endothelial growth factor expression in chronic myeloid leukemia K562 cells. Vascul Pharmacol 47:131–138
83. Kim SH, Kim HJ, Kim TS (2003) Differential involvement of protein kinase C in human promyelocytic leukemia cell differentiation enhanced by artemisinin. Eur J Pharmacol 482:67–76
84. Kim SH, Chun SY, Kim TS (2008) Interferon-α enhances artemisinin-induced differentiation of HL-60 leukemia cells via a PKCα/ERK pathway. Eur J Pharmacol 587:65–72
85. Finaurini S, Basilico N, Corbett Y, D'Alessandro S, Parapini S, Olliaro P, Haynes RK, Taramelli D (2012) Dihydroartemisinin inhibits the human erythroid cell differentiation by altering the cell cycle. Toxicology 300:57–66
86. Lu X, Efferth T (2020) Repurposing of artemisinin-type drugs for the treatment of acute leukemia. Semin Cancer Biol. https://doi.org/10.1016/j.semcancer.2020.05.016
87. Horwedel C, Tsogoeva SB, Wei S, Efferth T (2010) Cytotoxicity of artesunic acid homo- and heterodimer molecules toward sensitive and multidrug-resistant CCRF-CEM leukemia cells. J Med Chem 53:4842–4848
88. Yang X, Wang W, Tan J, Song D, Li M, Liu D, Jing Y, Zhao L (2009) Synthesis of a series of novel dihydroartemisinin derivatives containing a substituted chalcone with greater cytotoxic effects in leukemia cells. Bioorg Med Chem Lett 19:4385–4388
89. Gaur R, Pathania AS, Malik FA, Bhakuni RS, Verma RK (2016) Synthesis of a series of novel dihydroartemisinin monomers and dimers containing chalcone as a linker and their anticancer activity. Eur J Med Chem 122:232–246
90. Chadwick J, Jones M, Mercer AE, Stocks PA, Ward SA, Park BK, O'Neill PM (2010) Design, synthesis and antimalarial/anticancer evaluation of spermidine linked artemisinin conjugates designed to exploit polyamine transporters in Plasmodium falciparum and HL-60 cancer cell lines. Bioorg Med Chem 18:2586–2597
91. Mott BT, He R, Chen X, Fox JM, Civin CI, Arav-Boger R, Posner GH (2013) Artemisinin-derived dimer phosphate esters as potent anti-cytomegalovirus (anti-CMV) and anti-cancer agents: A structure-activity study. Bioorg Med Chem 21:3702–3707
92. Reiter C, Fröhlich T, Gruber L, Hutterer C, Marschall M, Voigtländer C, Friedrich O, Kappes O, Efferth T, Tsogoeva, (2015) Highly potent artemisinin-derived dimers and trimers: Synthesis and evaluation of their antimalarial, antileukemia and antiviral activities. Bioorg Med Chem 23:5452–5458
93. Gruber L, Abdelfatah S, Frohlich T, Reiter C, Klein V, Tsogoeva SB, Efferth T (2018) Treatment of multidrug-resistant leukemia cells by novel artemisinin-, egonol-, and thymoquinone-derived hybrid compounds. Molecules. https://doi.org/10.3390/molecules23040841
94. Reiter C, Herrmann A, Çapci A, Efferth T, Tsogoeva SB (2012) New artesunic acid homodimers: Potent reversal agents of multidrug resistance in leukemia cells. Bioorg Med Chem 20:5637–5641
95. Letis AS, Seo EJ, Nikolaropoulos SS, Efferth T, Giannis A, Fousteris MA (2017) Synthesis and cytotoxic activity of new artemisinin hybrid molecules against human leukemia cells. Bioorg Med Chem 25:3357–3367
96. Reiter C, Çapci Karagöz A, Fröhlich T, Klein V, Zeino M, Viertel K, Held J, Mordmuller OSE, Anil H, Efferth T, Tsoegoeva SB (2014) Synthesis and study of cytotoxic activity of 1,2,4-trioxane- and egonol-derived hybrid molecules against *Plasmodium falciparum* and multidrug-resistant human leukemia cells. Eur J Med Chem 75:403–412
97. Reiter C, Fröhlich T, Zeino M, Marschall M, Bahsi H, Leidenberger M, Friedrich O, Kappes B, Hampel F, Efferth T, Tsogoeva SB (2015) New efficient artemisinin derived agents against human leukemia cells, human cytomegalovirus and *Plasmodium falciparum*: 2nd generation 1,2,4-trioxane-ferrocene hybrids. Eur J Med Chem 97:164–172
98. Ha VT, Kien VT, Binh LH, Tien VD, My NTT, Nam NH, Baltas M, Hahn H, Han BW, Thao DT, Vu TK (2016) Design, synthesis

and biological evaluation of novel hydroxamic acids bearing artemisinin skeleton. Bioorg Chem 1(66):63–71. https://doi.org/10.1016/j.bioorg.2016.03.008
99. Wu Y, Parapini S, Williams ID, Misiano P, Wong HN, Taramelli D, Basilico N, Haynes RK (2018) Facile preparation of *N*-glycosylated 10-piperazinyl artemisinin derivatives and evaluation of their antimalarial and cytotoxic Activities. Molecules. https://doi.org/10.3390/molecules23040841
100. Fröhlich T, Reiter C, Ibrahim MM, Beutel J, Hutterer C, Zeitträger I, Bahsi H, Leidenberger M, Friedrich O, Kappes B, Efferth T, Marschall M, Tsogoeva SB (2017) Synthesis of novel hybrids of quinazoline and artemisinin with high activities against plasmodium falciparum, human cytomegalovirus, and leukemia cells. ACS Omega 2:2422–2431
101. Fröhlich T, Reiter C, Saeed MEM, Hutterer C, Hahn F, Leidenberger M, Friedrich O, Kappes B, Marschall M, Efferth T, Tsogoeva SB (2018) Synthesis of thymoquinone-artemisinin hybrids: new potent antileukemia, antiviral, and antimalarial agents. ACS Med Chem Lett 9:534–539
102. Çapcı Karagöz A, Reiter C, Seo EJ, Gruber L, Hahn F, Leidenberger M, Klein V, Hampel F, Friedrich O, Marschall M, Kappes B, Efferth T, Tsogoeva SB (2018) Access to new highly potent antileukemia, antiviral and antimalarial agents via hybridization of natural products (homo)egonol, thymoquinone and artemisinin. Bioorg Med Chem 26:3610–3618
103. Posner GH, Cumming JN, Ploypradith P, Oh CH (1995) Evidence for Fe(IV)=O in the molecular mechanism of action of the trioxane antimalarial artemisinin. J Am Chem Soc 117:5885–5886
104. Eckstein-Ludwig U, Webb RJ, van Goethem DA, East JM, Lee AG, Kimura M, O'Neill PM, Bray PG, Ward SA, Krishna S (2003) Artemisinin target the SERCA of *Plasmodium falciparum*. Nature 424:957–961
105. Bridgford JL, Xie SC, Cobbold SA, Pasaje CFA, Herrmann S, Yang T, Gillett DL, Dick LR, Ralph SA, Dogovski C, Spillman NJ, Tilley L (2018) Artemisinin kills malaria parasites by damaging proteins and inhibiting the proteasome. Nat Commun. https://doi.org/10.1038/s41467-018-06221-1