Repurposing Artemisinin and its Derivatives as Anticancer Drugs: A Chance or Challenge?

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Cancer has become a global health problem, accounting for one out of six deaths. Despite recent advances in cancer therapy, there is still an ever-growing need for readily accessible new therapies. The process of drug discovery and development is arduous and takes many years, and while it is ongoing, the time for the current lead compounds to reach clinical trial phase is very long. Drug repurposing has recently gained significant attention as it expedites the process of discovering new entities for anticancer therapy. One such potential candidate is the antimalarial drug, artemisinin that has shown anticancer activities in vitro and in vivo. In this review, major molecular and cellular mechanisms underlying the anticancer effect of artemisinin and its derivatives are summarised. Furthermore, major mechanisms of action and some key signaling pathways of this group of compounds have been reviewed to explore potential targets that contribute to the proliferation and metastasis of tumor cells. Despite its established profile in malaria treatment, pharmacokinetic properties, anticancer potency, and current formulations that hinder the clinical translation of artemisinin as an anticancer agent, have been discussed. Finally, potential solutions or new strategies are identified to overcome the bottlenecks in repurposing artemisinin-type compounds as anticancer drugs.

Keywords: artemisinin, artemisinin derivatives, drug repurposing, anticancer therapy, pharmacokinetics, signalling pathways

INTRODUCTION

Cancer has been a growing challenge in the healthcare system and is one of the largest global health problems. It is the second leading cause of death worldwide following ischemic heart disease. In 2018, the disease led to approximately 9.6 million deaths (Organisation 2018). An increase in cancer cases associated with aging population can increase the strain on the healthcare system and is certainly a cause for concern (Board, 2015).

Despite significant breakthrough in cancer therapy in the past decade, chemotherapy is still the mainstay of treatment (National Cancer Institute, 2015). Novel therapies such as targeted therapy and immunotherapy are not readily accessible owing to their high cost. In addition, targeted therapy...
often shows efficacy in specific cancers exhibiting selected biomarkers in a small group of patients and the majority of cancer patients do not respond to immunotherapy (Ventola, 2017). Therefore, there is still an unmet demand to develop more effective and cheaper anticancer drugs and identify the lead compounds for the development of those drugs.

The cost to develop a novel cancer drug is extremely high and the process from target identification to phase III clinical trials is time-consuming. Therefore, drug repurposing is becoming an increasingly explored alternative approach to the traditional drug discovery and development pipeline (Lim et al., 2021; Ren et al., 2021). Since data on existing drugs are largely available, additional studies on its pharmacology, pharmacokinetics and safety are not required (Sleire et al., 2017). Thus, drug repurposing can greatly reduce the duration of the drug development process and time to reach the market as an oncology therapeutic (Parvathaneni et al., 2019), greatly reducing the cost and increasing the patients’ access to the treatment (Parvathaneni et al., 2019).

One group of compounds that is currently being explored for drug repurposing is artemisinin (ARS) and its derivatives (henceforth referred to as artemisinins). Artemisinins are sesquiterpene trioxanes (Figure 1A) that have been clinically used to treat malaria (Augustin et al., 2020; Wang et al., 2020; Wang et al., 2021). The maximum recommended dose is 200 mg daily for 3 days for oral therapy of uncomplicated malaria (Organization, 2015). This dosing regimen has been shown to be safe and effective for the treatment of malaria. However, cancer is a chronic condition that may require long-term treatment with artemisinins in contrast to an acute infection like malaria. In addition, cancer treatment may require a higher dose of the drug to be effective, leading to higher levels of toxicity than that observed in malaria treatment. For the treatment of severe malaria 2.4 mg/kg IV artesunate (ART) administered at 0, 12, and 24 h for up to 7 days is recommended, which is a considerably higher than that required to treat uncomplicated malaria, and adverse reactions of delayed hemolysis at this dose have been reported (Prevention, 2020, May 28). It is unclear whether such side effects will be more prominent at doses used for cancer treatment because no dosing regimen has yet been established for cancer treatment. Therefore, the safety of artemisinins in long-term cancer therapy requires further investigation.

Artemisinins have shown potent anticancer activity in multiple cancers (Wong et al., 2017) (Figure 1B). Artemisinins, ART, and dihydroartemisinin (DHA) exhibited therapeutic effects against multiple tumor types such as breast cancer (Zhang et al., 2015; Yao et al., 2018; Wen et al., 2018), prostate cancer (Xu et al., 2016; Zhou et al., 2017), ovarian cancer (Wu et al., 2012; Zhou et al., 2020), pancreatic cancer (Zhou et al., 2013), and lung cancer (Zhou et al., 2012; Zuo et al., 2014). Artemisinins acts against cancer cells via various pathways such as inducing apoptosis (Zhu et al., 2014; Zuo et al., 2014) and ferroptosis via the generation of reactive oxygen species (ROS) (Zhu et al., 2021) and causing cell cycle arrest.
Therefore, artemisinins can work on multiple targets and affect multiple signaling pathways (Wong et al., 2017). Moreover, ARS has been known to be well tolerated and safe at low doses, lowering the risk of intolerable toxicity (Efferth, 2017). Thus, artemisinins show great potential of repurposing as anticancer drugs.

While most studies showed in vitro and in vivo anticancer efficacy of artemisinins, limited clinical trials in human subjects have been conducted to date. Therefore, the practicality of clinical translation of artemisinins as anticancer agents is uncertain. This review outlines the potential anticancer activity of artemisinins. Additionally, the pharmacokinetic properties of artemisinins, one of the most important aspects in anticancer drug development are discussed in details. This review article will improve our understanding of the limitations in the development of artemisinins as anticancer drugs in human subjects and suggest potential solutions and new strategies to overcome those challenges.

SEARCH STRATEGY

We performed a literature search on PubMed, Scopus, and embase. The first search aimed to identify studies on anticancer effect of artemisinins; thus the search terms (“artemisinins” [Mesh] AND “Neoplasms” [Mesh]) OR (artemisinin [Title/Abstract]) AND (cancer [Title/Abstract]) were used. The search strategy is illustrated in Figure 2.

Another search was performed to understand the pharmacokinetic properties of artemisinins and the following search terms were used ("artemisinins" [Mesh]) OR ((artemisinin [Title/Abstract]) AND ("Pharmacokinetics” [Mesh]) OR (pharmacokinetic [Title/Abstract])). Duplicates were removed using Endnote and titles and abstracts were screened according to the exclusion criteria as illustrated in Figure 2.

PHARMACOKINETICS OF ARTEMISININS

It is important to understand a drug’s pharmacokinetic properties to determine its potential for clinical use. Many studies have been conducted to determine the pharmacokinetic parameters of artemisinins. The main pharmacokinetic characteristics of artemisinins namely absorption, distribution, metabolism, and excretion are elaborated in Absorption of Artemisinins–Elimination of Artemisinins.

Absorption of Artemisinins

An AUC₀₋∞ value (area under the curve from time 0 extrapolated to infinite time) of 657 μg h L⁻¹ was observed in a study on
healthy volunteers administered orally 4 mg/kg of ART (Na-Bangchang et al., 2004). To calculate absolute bioavailability, this value was compared to that of another study on healthy volunteers administered 4 mg/kg IV dose of ART (AUCₘ₀–∞ values of 3.038 μg h L⁻¹) (Li et al., 2009). Therefore, absolute bioavailability was estimated to be 21.6%. In contrast, the AUC₀–∞ value of a group of patients with uncomplicated malaria who received 200 mg oral ART was considerably high (4,868 μg h L⁻¹), indicating that disease condition may affect absorption (Newton et al., 2002) because patients with malaria experience greater exposure than that of healthy volunteers, as indicated by the AUC₀–∞ values.

To better understand the translational potential of artemisinins as anticancer agents, maximum concentration (Cₘₐₓ) values were also evaluated. Cₘₐₓ values of DHA ranged between 0.558–1.270 μM in healthy volunteers (Teja-isavadharm et al., 2001; Na-Bangchang et al., 2004). In healthy volunteers who received oral ART, Cₘₐₓ values ranged between 0.174–1.830 μM (Teja-isavadharm et al., 2001; Batty et al., 2002; Na-Bangchang et al., 2004; Diem Thuy et al., 2008; Li et al., 2009). Moreover, Cₘₐₓ values were compared with IC₅₀ values of promising cancer cell lines obtained in vitro to understand the limitations in clinical translation. Compared to healthy volunteers, patients with uncomplicated malaria showed high Cₘₐₓ values of 3.9–4.6 μM for the use of ART (Binh et al., 2001; Newton et al., 2002) and 3.7–4.03 μM for DHA (Binh et al., 2001; Newton et al., 2002). Thus, the disease state affects the absorption of artemisinins, and further studies are required to better understand the pharmacokinetics of artemisinins in cancer patients.

**Distribution of Artemisinins**

Artesunate has been reported to have small volume of distribution (Vd/F) of 0.0106–0.0920 L/kg because ART has good solubility and is not lipophilic [28]. Therefore, ART would not distribute well to the tissues and might be more effective in treating cancers such as leukemia, hepatocellular carcinoma (HCC), or renal cell carcinoma because the liver and kidney are highly perfused organs. Artesunate might also be useful for the treatment of metastatic cancers. A low Vd/F also implies a short elimination half-life (t₁/₂). In contrast, ARS was recorded to have a much higher Vd/F ranging from 33.7 ± 16.1 to 38.4 ± 18.9 L/kg (Ashton et al., 1998) because ARS is more lipophilic and less water soluble than ART. However, ARS is converted to the active metabolite DHA in the body, which has good solubility with Vd/F of 1.46 L/kg reported in metastatic breast cancer patients (Ericsson et al., 2014).

**Elimination of Artemisinins**

Pharmacokinetic studies showed a relatively short t₁/₂ of artemisinins. For ART, t₁/₂ was 0.41 h (Teja-isavadharm et al., 2001) after an oral dose of 100 mg in healthy volunteers. At a dose of 4 mg/kg, t₁/₂ of 0.74 h was reported (Na-Bangchang et al., 2004). Generally, t₁/₂ has been reported to be less than 1 h and dose-dependent; however, the variations in t₁/₂ with dose are not drastic. A low t₁/₂ value aligns with a low Vd/F value, which implies that a more frequent dosage regimen is required for anticaner treatment with ART because it is cleared from the body relatively quickly. The oral clearance of ART was reported to be 20.6 ± 10.6 L/h/kg (Teja-isavadharm et al., 2001) for 100 mg oral dose, which is considerably high. Because of its high solubility, ART is eliminated by the kidneys. It is important to understand the metabolism and clearance of a drug to determine the recommended dose. However, to successfully determine a dosage regimen, the desired Cₘₐₓ value should be identified.

The challenges in repurposing artemisinins as anticancer drugs can be overcome by using different formulations and combination therapies based on pharmacokinetic properties of these drugs.

**MECHANISMS OF ACTION UNDERLYING ANTICANCER ACTIVITY OF ARTEMISININS**

Artemisinins possess anti-cancer activity, although the underlying mechanisms remain unclear. Generally, artemisinins act via similar pathways because they have a special structure called peroxide bridge, which is strongly associated with the cytotoxicity required for their antimalarial and anticancer activities (Liao et al., 2014; Tran et al., 2014; Xu et al., 2015; Tong et al., 2016). A cell death model revealed a distinguished anticancer mechanism of artemisinins through induction of ferroptotic cell death (Zhu et al., 2021). Other common mechanisms of action include induction of autophagy, cell cycle arrest, and apoptosis. Inhibition of cell proliferation and metastasis was observed in both in vitro and in vivo studies (Hou et al., 2008; Michaelis et al., 2010; Wang et al., 2012; Tran et al., 2014; Xu et al., 2015; Tong et al., 2016) (Figure 3). Hence, multiple signalling pathways are involved in anticancer activities of artemisinins in various cancer types. This section focuses on common mechanisms, which are further detailed in Table 1.

**Induction of Ferroptosis**

Ferroptosis, an oxidative, iron-dependent form of regulated cell death, is characterized by the accumulation of ROS and lipid peroxidation products to lethal levels (Stockwell et al., 2017). Emerging evidence suggests that triggering ferroptosis is a promising therapeutic strategy to kill cancer cells, particularly for eradicating aggressive malignancies that are resistant to the traditional therapies (Liang et al., 2019). Compared to normal cells, ferritin, a major iron storage protein essential for iron homeostasis, is overexpressed in many cancer cells (Buranrat and Connor, 2015). Usually, high ferritin level in blood is a poor prognostic marker in cancer patients, leading to aggressive disease. Other endogenous molecules such as glutathione, nicotinamide adenine dinucleotide phosphate, and glutathione peroxidase 4 (GPX4) have been also closely linked to the regulation of ferroptosis (Stockwell et al., 2017).

Dihydroartemisinin renders cancer cells more sensitive to ferroptosis by increasing the cellular accumulation of free ions due to its ability to induce lysosomal degradation of ferritin in an autophagy-independent manner (Chen X. et al., 2020). Dihydroartemisinin augmented GPX4 inhibition-induced ferroptosis in some cancer cells in both in vitro and in vivo.
models by the inducible knockout of GPX4 (Chen X. et al., 2020). Du et al. revealed that DHA, the main active metabolite of ART, could be a promising therapeutic agent to preferentially target acute myeloid leukemia cells by inducing ferroptosis (Du et al., 2019). Jiang et al. demonstrated that ART could regulate the labile iron pool (LIP) by promoting the lysosomal degradation of ferritin through lysosomal acidification, thereby inducing ROS-dependent cell death in HCC cells. The accumulation of labile iron in the endoplasmic reticulum promoted excessive ROS production and severe endoplasmic reticulum disruption, leading to cell death. These findings suggest ART is a safe anti-HCC agent that disturbs iron homeostasis (Jiang et al., 2021). Besides, artesunate greatly enhanced the anticancer effects of low dose of sorafenib against HCC by inducing oxidative stress and lysosome-mediated ferroptinophagy, two essential aspects of ferroptosis (Li ZJ. et al., 2021). Furthermore, Hamacher-Brady et al. demonstrated that ART could trigger programmed cell death (PCD) in cancer cells in a manner dependent on the level of free iron and the generation of ROS (Hamacher-Brady et al., 2011). Moreover, artesunate could inhibit autophagosome turnover and cause perinuclear clustering of autophagosomes, early and late endosomes, and lysosomes. Lysosomal iron was the lethal source of ROS upstream of mitochondrial outer membrane permeabilization because lysosomal iron chelation blocked all measured parameters of ART-induced PCD, whereas lysosomal iron loading enhanced death. Two lysosomal inhibitors, chloroquine and bafilomycin A1, reduced ART-induced PCD, proving that lysosomal function is required in the process of PCD signaling (Hamacher-Brady et al., 2011). The anticancer effect of ART can be attributed, at least partially, to ferroptosis.

**Induction of Autophagy**

Emerging evidence suggests that autophagy induction is one of the molecular mechanisms underlying anticancer activity of artemisinins (Wang et al., 2012; Chen K. et al., 2014). Mitochondria are important molecular organelles that regulate both apoptosis and autophagy (type II PCD), and ROS generation is one of the triggering factors for mitochondrial dysfunction. DHA-induced autophagy in leukemia K562 cells, evidenced by LC3-II protein expression, was observed to be ROS-dependent (Wang et al., 2012). Inhibitory effect of DHA on the proliferation of leukemia K562 cells was also dependent upon the iron level, indicating an association between autophagy and ferroptosis (Wang et al., 2012).

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**FIGURE 3** | Mechanisms of action underlying anticancer activity of artemisinins. A schematic view of the molecular crosstalk pathways involved anticancer mechanisms of artemisinins, including (A) induction of ferroptosis, (B) induction of autophagy, (C) cell cycle arrest, (D) augment of apoptosis, (E) inhibition of angiogenesis, and (F) invasion and metastasis.
| Cancer type                  | Cell line | IC₅₀ value (µM) | Mechanism of action                                                                 | Ref                      |
|-----------------------------|-----------|-----------------|-------------------------------------------------------------------------------------|--------------------------|
|                             |           | 24H  | 48H  | 72H  |                               |                          |
|                             |           |      |      |      | Artemisin                                                                 |
| Gall bladder Cancer         | GBC-SD    | —    | 49.1 | ±    | 1.69 Upregulate p16, downregulate CDK4 and cyclin D1 to induce G1-phase cell cycle arrest | Jia et al. (2016a)       |
|                             | NOZ       | —    | 58.6 | ±    | 1.77 Activate caspase-3 to induce apoptosis Induce ∆ψₙ collapse of via cytochrome c release Induce the generation of ROS inhibition of cell motility and migration |                          |
| HCC                         | HepG2     | —    | 10.4 | —    | 250 Dose- and time-dependent Dose- and time-dependent | Weifeng et al. (2011)    |
|                             | SMMC-7721 | —    | —    | —    | 290 HCC in cell invasion by altering MMP2 and TIMP2 balance Suppress p-p38, ERK1/2 activation in HCC cells Induce cell invasion by altering MMP2 and TIMP2 balance | Hou et al. (2008)        |
|                             | HepG2     | —    | 14.0 | —    | 290 HCC in cell invasion by altering MMP2 and TIMP2 balance Induce invasion and metastasis of HCC cells Increase production of Cip1/p21 and Kip1/p27 | (Yao et al., 2018b; Chen et al., 2020a) |
|                             | BEL7407   | 9.90 | —    | —    | HCC in cell invasion by altering MMP2 and TIMP2 balance Decrease NF-κB/p65 expression and downregulate VEGF, IL-8, COX-2, and MMP-9 Increase production of Cip1/p21 and Kip1/p27 | (Chen et al., 2010; Wang et al., 2011) |
|                             | Huh-7     | 8.90 | —    | —    | HCC in cell invasion by altering MMP2 and TIMP2 balance Decrease NF-κB/p65 expression and downregulate VEGF, IL-8, COX-2, and MMP-9 Increase production of Cip1/p21 and Kip1/p27 | (Chen et al., 2010; Wang et al., 2011) |
| Lung Cancer                 | A549      | —    | —    | —    | — Regulate metastasis, migration, and invasion by suppressing EMT and CSCs Decrease Wnt/β-catenin signaling pathway | Tong et al. (2018)       |
|                             | H1299     | —    | —    | —    | — HCC in cell invasion by altering MMP2 and TIMP2 balance Suppress p-p38, ERK1/2 activation in HCC cells Induce cell invasion by altering MMP2 and TIMP2 balance | Hou et al. (2008)        |
| Breast Cancer               | NCI-H292  | —    | —    | —    | — Induce depletion of cysteine and inhibit GPX4 Induce depletion of cysteine and inhibit GPX4 Induce depletion of cysteine and inhibit GPX4 | (Yao et al., 2018b; Chen et al., 2020a) |
|                             | MDA-453   | —    | —    | —    | — Induce depletion of cysteine and inhibit GPX4 Induce depletion of cysteine and inhibit GPX4 Induce depletion of cysteine and inhibit GPX4 | (Yao et al., 2018b; Chen et al., 2020a) |
|                             | MCF7      | —    | —    | —    | — Induce depletion of cysteine and inhibit GPX4 Induce depletion of cysteine and inhibit GPX4 Induce depletion of cysteine and inhibit GPX4 | (Yao et al., 2018b; Chen et al., 2020a) |
| Colon Cancer                | HCT116    | —    | >80.0| —    | — Induce production of ROS by reacting with iron Induce production of ROS by reacting with iron Induce production of ROS by reacting with iron | (Yao et al., 2018b; Chen et al., 2020a) |
|                             | SW480     | —    | >80.0| —    | — Induce production of ROS by reacting with iron Induce production of ROS by reacting with iron Induce production of ROS by reacting with iron | (Yao et al., 2018b; Chen et al., 2020a) |
|                             | HT29      | —    | >80.0| —    | — Induce production of ROS by reacting with iron Induce production of ROS by reacting with iron Induce production of ROS by reacting with iron | (Yao et al., 2018b; Chen et al., 2020a) |
| Endometrial Cancer          | Ishikawa  | —    | —    | —    | — Inhibit CDK-4 and induce G1-phase cell cycle arrest Decrease NF-κB/p65 expression and downregulate VEGF, IL-8, COX-2, and MMP-9 Decrease NF-κB/p65 expression and downregulate VEGF, IL-8, COX-2, and MMP-9 Decrease NF-κB/p65 expression and downregulate VEGF, IL-8, COX-2, and MMP-9 | Tran et al. (2014)       |
| Rhabdomyosarcoma            | TE671     | —    | —    | —    | — Generation of ROS Generation of ROS Generation of ROS | Beccafico et al. (2015)  |
| Myeloid Leukaemia           | K562      | —    | 11.3 | —    | — Induce autophagy Autophagy Induce autophagy | Wang et al. (2012)       |
| Pancreatic Cancer           | BxPC-3    | —    | —    | —    | — 40.8 ± 6.8 Induce G0/G1 cell cycle arrest in a dose-dependent manner Decrease NF-κB/p65 expression Decrease NF-κB/p65 expression Decrease NF-κB/p65 expression | (Chen et al., 2010; Wang et al., 2011) |
|                             | AsPC-1    | —    | —    | —    | — 40.8 ± 6.8 Induce G0/G1 cell cycle arrest in a dose-dependent manner Decrease NF-κB/p65 expression Decrease NF-κB/p65 expression Decrease NF-κB/p65 expression | (Chen et al., 2010; Wang et al., 2011) |
|                             | PANC-1    | —    | —    | —    | — 48.9 ± 6.1 Induce G0/G1 cell cycle arrest in a dose-dependent manner Decrease NF-κB/p65 expression Decrease NF-κB/p65 expression Decrease NF-κB/p65 expression | (Chen et al., 2010; Wang et al., 2011) |

(Continued on following page)
| Cancer type          | Cell line | IC₅₀ value (µM) | Mechanism of action                                                                 |
|---------------------|-----------|----------------|-------------------------------------------------------------------------------------|
|                     |           | 24H | 48H | 72H | Ref                               |
| **Hepatocellular Carcinoma** |            |     |     |     |                                   |
| HepG2               | —         | 13.4| —   | —   | Hou et al. (2008)                 |
| Hep3B               | —         | 10.3| —   | —   | Increase production of Cip1/p21 and Kip1/p27 |
| Huh-7               | —         | 9.6 | —   | —   | Downregulate CDKs and cyclins     |
| BEL-7404            | —         | 9.3 | —   | —   | Induce apoptosis by inducing change in the expression of apoptosis related proteins |
| **Lung Cancer**     | A549      | —   | —   | —   | (Liao et al., 2014; Tong et al., 2016) |
|                     | H1229     | —   | —   | —   | Block cell cycle progression from G1 to S phase by suppressing cyclin D1 expression |
|                     |           |     |     |     | Regulate metastasis, migration, and invasion by suppressing EMT and CSCs           |
|                     |           |     |     |     | Decrease expression of Bcl-2 and Bcl-xL which are ant apoptotic proteins         |
|                     | OVCA-420  | 5.64| 0.33| —   | Inhibit cell growth in a dose- and time-dependent manner                          |
|                     | OVCA-439  | 3.83| 0.14| —   | Induce apoptosis by targeting the Bcl-2 family                                   |
|                     | OVCA-433  | 4.48| 0.21| —   | Decrease expression of Bcl-2 and Bcl-xL which are ant apoptotic proteins         |
|                     | OVCAR-10  | 5.72| 0.07| —   | Increase Bax and Bad promoter proteins                                            |
|                     | HEY       | 5.51| 0.27| —   | Induce G2-phase cell cycle arrest                                                |
|                     | OVCA-432  | 14.0| 0.50| —   | Selectively block phosphorylation of Jak2                                         |
|                     | OVCA-3    | 14.9| 0.28| —   | Inhibit constitutive phosphorylation and activation of STAT3                     |
|                     | OCC-1     | 13.8| 0.53| —   | Selectively block phosphorylation of Jak2                                         |
|                     | SK-OV-3   | 14.8| 0.42| —   | Generation of ROS                                                                |
|                     | ALST      | 15.2| 0.37| —   | Induce apoptosis                                                                 |
| **Ovarian Cancer**  | OVCA-440  | —   | —   | —   | Hwang et al. (2010)                                                             |
|                     | OVCA-439  | —   | —   | —   | Inhibit cell growth in a dose- and time-dependent manner                          |
|                     | OVCA-433  | —   | —   | —   | Induce apoptosis by targeting the Bcl-2 family                                   |
|                     | OVCAR-10  | —   | —   | —   | Decrease expression of Bcl-2 and Bcl-xL which are ant apoptotic proteins         |
|                     | HEY       | —   | —   | —   | Induce apoptosis by inducing change in the expression of apoptosis related proteins |
|                     | OVCA-432  | —   | —   | —   | Block PKC/Erk/MAPKs and NF-κB signaling pathways                                  |
|                     | OVCA-3    | —   | —   | —   | Supress PMA-stimulated NF-κB and AP-1                                             |
|                     | OCC-1     | —   | —   | —   | Generate ROS                                                                      |
|                     | SK-OV-3   | —   | —   | —   | Induce apoptosis                                                                   |
|                     | ALST      | —   | —   | —   | (Continued on following page)                                                   |

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| Cancer type       | Cell line                  | IC₅₀ value (µM) | Mechanism of action                                                                 | Ref                                                                 |
|------------------|----------------------------|----------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------|
|                   |                            | 24H       | 48H      | 72H     |                                                 |                                                                      |
| Lung cancer      | NCI-H292                   | —         | —        | —      | Increase degradation of ferritin by lysosomes  | [Yao et al., 2018b; Chen et al., 2020a]                                  |
| Colon Cancer     | HCT116                     | —         | 1.20     | —      | Regulate iron homeostasis via signaling between |                                                                      |
|                  | HT29                       | —         | 1.25     | —      | iron regulatory protein (IRP) and iron-responsive |                                                                      |
|                  | SW480                      | —         | 1.25     | —      | element (IRE)                                   |                                                                      |
|                  | LOVO                       | —         | 1.20     | —      | Inhibit GPX4 and cause cysteine deprivation    |                                                                      |
|                  | RKO                        | —         | 1.80     | —      | Increase sensitivity of cells to RSL3-induced cell |                                                                      |
|                  |                            |           |          |       | death                                         |                                                                      |
| Breast Cancer    | MDA-MB-453                 | —         | —        | —      | Induce cytotoxicity                            | [Luo et al. (2014)]                                                   |
|                  |                            | 5.47      | 25.7     | —      | Induce radiosensitivity of HeLa, but not SiHa |                                                                      |
|                  |                            |           |          |       | Induce apoptosis and necrosis in HeLa          |                                                                      |
|                  | MDA-MB-231                 | —         | —        | —      | Upregulate expression of Beclin1               | [Hamacher-Brady et al., 2011; Chen et al., 2014b; Chen et al., 2020a] |
|                  | T47D                       | —         | —        | —      | Induce autophagy                               |                                                                      |
|                  | MDA-MB-453                 | —         | —        | —      | Induce G2/M-phase cell cycle arrest Cause      |                                                                      |
|                  |                            | 2.69 ±    | 0.10     | —      | lysosomal mitochondrial fragmentation          |                                                                      |
|                  |                            | 0.10      |          |       | Activate cell death of MCF-7                   |                                                                      |
| Neuroblastoma    | UKF-NB-3                   | 2.69 ±    | —        | —      | Activate caspase-3 to induce apoptosis         | [Michaels et al. (2010)]                                              |
|                  |                            | 0.10      |          |       | Induce oxidative stress                        |                                                                      |
|                  | UKF-NB-6                   | 3.54 ±    | —        | —      | Induce apoptosis                               |                                                                      |
|                  |                            | 0.42      |          |       | Suppress angiogenesis                          |                                                                      |
| Kaposi's Sarcoma | KS-IMM                     | —         | —        | —      | Induce apoptosis                               | [Dell’Eva et al. (2004)]                                              |
| Ovarian Cancer   | HEY1                       | —         | —        | 5.80 ± | Induce ROS                                     | [Greenshields et al. (2017)]                                          |
|                  |                            |           |          | 1.62   | Inhibit cell division and induce cell cycle    |                                                                      |
|                  |                            |           |          | 0.56   | arrest                                         |                                                                      |
|                  | HEY2                       | —         | —        | 7.34 ± | Modulate cell cycle regulatory protein expression |                                                                      |
|                  |                            |           |          | 0.64   | and mTOR signalling                            |                                                                      |
|                  | IGROV-1                    | —         | —        | 8.82 ± | ROS and iron-dependent cytotoxicity            |                                                                      |
|                  |                            |           |          | 1.18   | Cause ROS-dependent G2/M-phase                |                                                                      |
|                  | OVCA-R8                    | —         | —        | 5.51 ± | cell cycle arrest                              |                                                                      |
|                  |                            |           |          | 1.06   | 6.38                                           |                                                                      |
|                  | SKOV-3                     | —         | —        | 23.6 ± | Cause ROS-independent G1-phase cell cycle      |                                                                      |
|                  |                            |           |          | 3.86   | arrest                                         |                                                                      |
|                  | TOV-21G                    | —         | —        | 6.11 ± | Interfere with mTORC1 signalling by inhibiting |                                                                      |
|                  |                            |           |          | 0.64   | phosphorylation of downstream p70 S6K1 and     |                                                                      |
|                  | OV-90                      | —         | —        | 31.9 ± | S6 ribosomal protein                          |                                                                      |
|                  |                            |           |          | 4.15   |                                              |                                                                      |
|                  | TOV-112D                   | —         | —        | 0.51 ± | Work through caspase-dependent and caspase-     |                                                                      |
|                  |                            |           |          | 0.03   | independent pathways                           |                                                                      |
|                  | H08910                      | —         | —        | —      | Induce ROS and DNA double-strand               |                                                                      |
|                  | A2780                      | —         | —        | —      | Downregulate RAD51 to increase sensitivity to   |                                                                      |
|                  | HEY                        | —         | —        | —      | cisplatin                                      |                                                                      |
|                  |                            |           |          |       | Sensitise cells to cisplatin by acting         |                                                                      |
|                  |                            |           |          |       | synergistically with cisplatin to induce       |                                                                      |
|                  |                            |           |          |       | double-stranded breaks                        |                                                                      |
|                  |                            |           |          |       | Inhibit formation of RAD51 foci induced by     |                                                                      |
|                  |                            |           |          |       | cisplatin                                      |                                                                      |
(Continued on following page)
| Cancer type               | Cell line       | IC<sub>50</sub> value (µM) | Mechanism of action                                                                 | Ref                                    |
|--------------------------|-----------------|-----------------------------|------------------------------------------------------------------------------------|---------------------------------------|
|                          |                 |                             | 24H | 48H | 72H |                                                   |                                       |
| Pancreatic Cancer        | MiaPaCa-2       | —                           | —   | —   | —   | Induce caspase-independent and non-apoptotic cell death | (Youns et al., 2009; Du et al., 2010) |
|                          | BxPC-3          | 279.3                       | —   | —   | —   | Induce change in mitochondrial membrane potential and ROS-mediated cell death |                                       |
|                          | Panc-1           | 26.8                        | —   | —   | —   | Inhibit growth and proliferation                   |                                       |
|                          | CFPAC-1         | 142.8                       | —   | —   | —   | Induce apoptosis                                     |                                       |
|                          |                 |                             |     |     |     | Induce activation of caspase 3 and caspase 7       |                                       |
|                          |                 |                             |     |     |     | Potentiate effect of gemcitabine in growth inhibition|                                       |
| Renal Cell Carcinoma     | CaKi-1          | —                           | —   | —   | 6.70 | Induce G2/M-phase cell cycle arrest                 | Jeong et al. (2015)                  |
|                          | 786-O           | —                           | —   | —   | 11.0 | Induce cell death by generation of ROS and depletion of intracellular ATP |                                       |
|                          | SN12C-GFP       | —                           | —   | —   | 23.0 | Induce apoptosis                                     |                                       |
| Rhabdomyosarcoma         | TE671           | 10.0                        | —   | —   | —   | Induce apoptosis by causing ROS production         | Beccafico et al. (2015)              |
|                          | RD18            | 10.0                        | —   | —   | —   | Induce expression of myo-miRs, miR-133a and miR-206 that is reliant on ROS and independent of p38 |                                       |
| Osteosarcoma             | HOS             | 52.8                        | —   | —   | —   | Inhibit proliferation                               | Xu et al. (2011)                    |
|                          |                 |                             |     |     |     | Induce G2/M phase cell cycle arrest                |                                       |
| Leukaemia                | J-Jhan          | —                           | —   | —   | —   | Induce G2/M-phase cell cycle arrest                | Steinbrück et al. (2010)            |
|                          | J16             | —                           | —   | —   | 4.39 ± 0.44 | Induce apoptosis via generation of ROS | [Efferth et al., 2007; Steinbrück et al., 2010] |
|                          | SKM-1           | 61.2                        | 38.4| 28.6| —   | Inhibit proliferation                               | Xu et al. (2015)                    |
|                          |                 |                             |     |     |     | Induce apoptosis                                     |                                       |
|                          |                 |                             |     |     |     | Enhance cell adhesion and inhibit metastasis via the Wnt/β-catenin pathway by blocking translocation of subcellular β-catenin and E-cadherin to adherent junctions of the membrane |                                       |
|                          |                 |                             |     |     |     | Enhance chemosensitivity to other agents            | Efferth et al. (2007)               |
|                          |                 |                             |     |     |     | Generate ROS and induce apoptosis via the intrinsic pathway |                                       |
|                          |                 |                             |     |     |     | Synergise with doxorubicin to enhance apoptosis     |                                       |
| Lung Cancer              | H69             | —                           | —   | —   | 2.54 ± 0.23 | Induce G2/M-phase cell cycle arrest                | Steinbrück et al. (2010)            |
|                          | H1299           | —                           | —   | —   | —   | Inhibit migration, invasion, and metastasis by suppressing EMT and CSCs | Tong et al. (2016)                  |
|                          | A549            | —                           | —   | 100 | —   | Suppress Wnt/β-catenin pathway                     |                                       |
|                          |                 |                             |     |     |     | Inhibit cyclin D1 to induce G1-phase cell cycle arrest |                                       |
|                          |                 |                             |     |     |     | Suppress cell viability                             |                                       |
|                          | H1395           | —                           | 150 | —   | —   | Inhibit proliferation                               | Rasheed et al. (2010)               |
|                          | LXF289          | —                           | 60.0| —   | —   | Inhibit u-PA activity, protein and mRNA expression |                                       |
|                          | H460            | —                           | 7.50| —   | —   | Inhibit transactivating capacity of NF-κB           |                                       |
|                          | Calu3           | —                           | 10.0| —   | —   | Inhibit AP-1 transcription factors                 |                                       |
|                          | H1299           | —                           | 12.5| —   | —   | Regulate transcription of MMP-2, MMP-7 and u-PA.    |                                       |
|                          |                 |                             |     |     |     | Regulate invasion and metastasis                    |                                       |
|                          | NCI-H292        | —                           | —   | —   | —   | Increase sensitisation to ferroptosis              | Chen et al. (2020a)                 |
|                          |                 |                             |     |     |     | (Continued on following page)                      |                                       |
In a study on breast cancer cells, ART could inhibit the proliferation of cancer cells by inducing autophagy [53]. Moreover, ART sensitized breast cancer cells to epirubicin chemotherapy. As a result, ART was regarded as a therapeutic candidate in breast cancer therapy [53]. A recent study evaluated the antineoplastic effects of ART in diffuse large B cell lymphoma cells (Chen et al., 2021). The results revealed that ART exhibited anticancer activity through multiple mechanisms of action including autophagy as evidenced by over-expression of LC3B-I/II, whereas p62 expression was downregulated in a dose dependent manner following 24 h of ART treatment. Next, Chen, et al. investigate the antitumor activity of DHA in esophagus cancer cells (Chen X. et al., 2020). The results showed that DHA could inhibit the migration capacity of Eca109 and TE-1 cells by inducing autophagy. Ma et al. also demonstrated similar results that DHA significantly reduced the viability of Eca109 cells in a dose- and time-dependent manner (Ma et al., 2020). Together, these studies indicate that autophagy is one of the key mechanisms underlying death of cancer cells treated with artemisinin and its derivatives.

### Induction of Cell Cycle Arrest

Artemisinins administration resulted in cell cycle arrest in a dose-dependent manner (Willoughby Sr et al., 2009; Chen et al., 2010; Wang et al., 2011). G1-phase cell cycle arrest was observed in GBC-SD and NOZ gallbladder cancer cell lines (Jia J. et al., 2016), LNCaP, PC3, and DU145 prostate cancer cells (Steinbrück et al., 2010), A549 and H1299 lung cancer cells (Liao et al., 2014; Tong et al., 2016), BxPC-3 and AsPC-1 pancreatic cancer cells (Chen et al., 2010), human hepatoma cells (Hou et al., 2008), ovarian cancer cells (Greenshields et al., 2017) and human Ishikawa endometrial cancer cells (Tran et al., 2014).

The induction of G1-phase cell cycle arrest by artemisinins is mediated by several pathways, including downregulation of cyclin-dependent kinase 4 (CDK4) and cyclin D1 expression (Hou et al., 2008; Zeng and Zhang, 2011; Pang et al., 2016). Hep3B: induce ROS-independent apoptosis BEL7404 — 15.0 — Reduce cell viability Huh-7 — 9.22 — Alkylate haem-harbouring nitric oxide synthase in a dose-dependent manner to mitigate proliferation

| Cancer type          | Cell line | IC50 value (µM) | Mechanism of action                                                                 | Ref                                      |
|----------------------|-----------|-----------------|-------------------------------------------------------------------------------------|-----------------------------------------|
| Colon Cancer         | HCT116    | 2.20 — 29.9 ± 2.49 | Inhibit cell viability Inhibit biosynthetic of fatty acid Induce apoptosis via mitochondrial pathway activation and lipid ROS production Inhibit NF-κB pathway Inhibit G2/M-phase cell cycle arrest Inhibit u-PA activity, protein and inhibit proliferation most strongly in CLY, followed by Lovo, then HT-29 | [Steinbrück et al., 2010; Chen et al., 2017b; Chen et al., 2020a] |
|                      | CLY       | — — 20.3 ± 2.20  | Promote apoptosis                                                                   | [Li et al., 2008; Chen et al., 2020a]    |
|                      | Lovo      | — — 30.6 ± 0.73  | Induce G2/M-phase cell cycle arrest prominently in HT-29                             |                                         |
|                      | HT-29     | — — 82.3 ± 3.74  | Induce S-phase cell cycle arrest prominently in CLY. Inhibit hyperactive Wnt pathway |                                         |
|                      | SW480     | — — —            | Increase sensitisation to ferroptosis                                               |                                         |
| Hepatocellular Carcinoma | HepG2    | — 20.5 —         | Huh-7 and Hep3B: induce ROS-dependent apoptosis                                        | [Hou et al., 2008; Zeng and Zhang, 2011; Pang et al., 2016] |
|                      | Hep3B     | — 39.4 —         | HepG2: induce ROS-independent apoptosis Reduce cell viability                         |                                         |
|                      | BEL7404   | — 15.0 —         | Reduce cell viability                                                                |                                         |
|                      | Huh-7     | — 9.22 —         | Alkylate haem-harbouring nitric oxide synthase in a dose-dependent manner to mitigate proliferation |
| Colon Cancer         | U251      | — — 73.3 ± 1.32  | Induce apoptosis and necrosis Induce oxidative DNA damage                            | [Steinbrück et al., 2010; Berdelle et al., 2011] |
|                      | LN-229    | — — —            | Induce G2/M-phase cell cycle arrest                                                  |                                         |
| Melanoma             | SK-Mel-28 | — — 94.4 ± 2.93  | Induce apoptosis                                                                     | Steinbrück et al. (2010)                |
| Prostate Cancer      | DU145     | — — 70.5 ± 5.81 µM| Induce apoptosis                                                                     | Steinbrück et al. (2010)                |
breast cancer (Chen K. et al., 2014), and renal carcinoma (Jeong et al., 2015) cells following the administration of ART. In renal carcinoma cells and ovarian cancer cells, ART-mediated G2/M-phase cell cycle arrest was dependent on ROS generation (Jeong et al., 2015; Greenshields et al., 2017). In breast cancer cells, ART caused G2/M-phase cell cycle arrest by regulating autophagy (Chen K. et al., 2014). Cell cycle arrest is one of the key molecular mechanisms of anticancer activity of artemisinins.

**Augmentation of Apoptosis**

Artemisinins have been reported to induce apoptosis in J16, DU145, SK-Mel-28 (Steinbrück et al., 2010), leukaemia (Efferth et al., 2007; Zhou et al., 2007), HepG2, Hep3B hepatoma (Hou et al., 2008), ovarian cancer (Jiao et al., 2007), Kaposi’s sarcoma-IMM (Dell’Eva et al., 2004), cervical cancer (Luo et al., 2014), SKM-1 (Xu et al., 2015), glioblastoma (Berdelle et al., 2011), neuroblastoma (Michaelis et al., 2010), embryonal rhabdomyosarcoma (Beccafico et al., 2015), pancreatic cancer (Youns et al., 2009), and colorectal cancer (Li et al., 2008; Chen X. et al., 2017) cells. Similar to the cell cycle arrest, apoptosis induction was caused by a myriad of signaling pathways.

One common pathway by which artemisinins induced apoptosis is the generation of ROS which in turn damages organelles, DNA, and proteins, eventually leading to the death of cancer cells (Efferth et al., 2007; Beccafico et al., 2015; Pang et al., 2016; Chen X. et al., 2017). ROS-dependent apoptosis caused by Bax-mediated intrinsic pathway has been observed in Huh-7 and Hep3B cells following treatment with ART (Pang et al., 2016), in which caused mitochondrial activation, and release of cytochrome c and subsequent activation of caspase-9, leading to activation of caspase-3, an executioner caspase that destroys cellular structures such as poly (ADP-ribose) polymerase, an enzyme involved in DNA repair, causing cell death (Hou et al., 2008; Jia J. et al., 2016; Chen X. et al., 2017). In another study, exposure to artemisinins led to a dose dependent increase in caspase-3 cleavage in HepG2 cells (Hou et al., 2008). This process was also evident in K562 leukemia (Zhou et al., 2007) and pancreatic cancer cells (Youns et al., 2009). However, activation of caspase-3 is not always ROS-dependent. Both in vitro and in vivo studies have also shown that ART could induce ROS-independent apoptosis in HepG2 cells (Pang et al., 2016).

**Inhibition of Angiogenesis**

Angiogenesis is a key factor in tumor growth, invasion and metastasis. It is partly mediated by the transcription factor NF-κB and pro-angiogenic factors (including VEGF, IL-8, COX-2 and MMP-9) (Ferrara and Kerbel, 2005; Liu et al., 2021). Dihydroartemisinin showed anti-angiogenic effect in both in vitro angiogenesis models and in vivo pancreatic cancer-derived tumor models (Wang et al., 2011). These effects were likely to be mediated by inhibiting the NF-κB pathway and its downstream pro-angiogenic growth factors. In this study, the results showed that treatment of human umbilical vein endothelial cells with DHA resulted in a dose-dependent inhibition of cell proliferation and capillary tube formation.

The pleiotropic transcription factor NF-κB regulates the expression of multiple genes, including VEGF and IL-8 (Huang et al., 2000). The constitutive NF-κB activity drives the constitutive overexpression of VEGF and IL-8, which contributes to the angiogenic phenotype of human pancreatic cancer. After DHA treatment, decreased expression of VEGF and IL-8 in vitro and in vivo is associated with decreased proliferation and neovascularization.

Artesunate can inhibit the expression of VEGF, which is closely related to the level of VEGF secreted in the conditioned medium. Artesunate has potential anti-leukemia effects for the treatment for chronic myeloid leukemia or as an adjunct to standard chemotherapy regimens (Zhou et al., 2007). Using KS-IMM cells derived from Kaposi’s sarcoma lesions of kidney transplant patients, Dell’Eva et al. proved that ART could inhibit the growth of cancer cells and normal human umbilical cord endothelial cells (Del’Eva et al., 2004) ART also reduces angiogenesis in vivo in terms of vascularization of Matrigel plugs injected subcutaneously into syngeneic mice. In summary, ART is a promising low-cost drug candidate for the treatment of hyper vascularized Kaposi’s sarcoma, and for preventing tumor angiogenesis.

**Inhibition of the Key Signaling Pathways**

NF-κB is a transcription factor that regulates apoptosis, and promotes tumorigenesis, cell proliferation, metastasis, and angiogenesis upon activation (Chen H. et al., 2009). Hence, inhibition of the NF-κB pathway may block these processes and result in cell apoptosis. In BxPC-3 and PANC-1 pancreatic cancer cells, DHA inhibited NF-κB and decreased the production of vascular endothelial growth factor (VEGF), IL-8, COX-2, and MMP-9 (Wang et al., 2011), promoting angiogenesis. NF-κB activates cyclin D1 and Bcl-2 transcription. DHA inhibited both Bcl-2 and cyclin D1 (Chen H. et al., 2009), which are the downstream gene products of NF-κB. The disruption of the NF-κB pathway at different points was also observed in HCT116 (Chen X. et al., 2017) and lung cancer cells (Rasheed et al., 2010), after ART administration, HT-1080 cells (Hwang et al., 2010) after DHA administration, and human Ishikawa endometrial cancer cells (Tran et al., 2014) after ARS administration.

Tong et al. demonstrated that ARS, DHA and ART induced cell cycle arrest in the G1 phase, thereby inhibiting the proliferation of A549 and H1299 cells. Moreover, artemisinins inhibited other malignant tumor markers by migration, invasion, cancer stem cells and epithelial-mesenchymal transition (EMT) and decreased tumor growth in xenograft mouse model. Using IWP-2, Wnt/β-catenin pathway inhibitor and Wnt5a siRNA, Tong et al. showed that anticancer effect of artemisinins partly depends on the inactivation of the Wnt/β-catenin signaling. Artemisinins significantly reduced the protein levels of Wnt5a and β-catenin, and increased the levels of NKD2 and Axin2, and ultimately inhibited the Wnt/β-catenin pathway (Tong et al., 2016). Xu et al. demonstrated that ART induced SMK-1 cell apoptosis in a dose- and time-dependent manner by inhibiting the hyperactive β-catenin signaling pathway (Xu et al., 2015).

Artemisinins inhibit cell proliferation and metastasis (Hou et al., 2008; Xu et al., 2015; Tong et al., 2016). Inhibition of the
### TABLE 2 | Dose and Mechanisms of Action of artemisinins in vivo.

| Animal | Dosing regimen | Disease model | Mechanisms, safety, and efficacy | Reference |
|--------|----------------|---------------|----------------------------------|-----------|
| **Drugs: artemisinin** | | | | |
| Male BALB/c nude mice | 100 mg/kg per day orally over 30 days | GBC-SD and NOZ-derived gallbladder cancer xenograft mouse models | Inhibitory effect on GBC cell-derived tumours<br>Reduce tumour volume and weight<br>Inhibit cell proliferation | Jia et al. (2016b) |
| Male BALB/c athymic nude mice | 100 mg/kg per day orally | LNCaP prostate cancer xenograft model | Inhibit proliferation of LNCaP cells in vivo<br>Inhibited growth of LNCaP xenografts<br>Reduce tumour size and volume<br>Tumours showed no gross vascularity and looked pale yellow, like avascular tissue<br>No adverse side effects observed | Willoughby Sr et al. (2009) |
| Nude BALB/c mice | C0: 0 mg/kg/day C1: 50 mg/kg/day C2: 100 mg/kg/day with stepwise increase in dose | HepG2 hepatocellular carcinoma orthotopic xenograft | Inhibit metastasis<br>Reduce number of tumours found in lungs as compared to the control group<br>Tumour inhibition rate:<br>C1: 51.8%<br>C1: 51.8% | Weifeng et al. (2011) |
| Female BALB/c-nude mice | 60 mg/kg/day | A549 NSCLC xenograft model | Inhibition of tumour growth<br>Reduce tumour weight and volume<br>Did not cause significant weight loss | Tong et al. (2016) |
| Female athymic nude mice | 50 mg/kg/day OR 100 mg/kg/day OR combination with gemcitabine | HepG2 hepatocellular carcinoma xenograft model | Inhibit tumour growth (30.0 and 39.4% for 50 mg/kg/d and 100 mg/kg/d) increase anticancer effect of gemcitabine<br>No observable toxic effects | Hou et al. (2008) |
| Female athymic nude mice | 50 mg/kg/day OR 100 mg/kg/day OR combination with gemcitabine | Hep3B hepatocellular carcinoma xenograft model | Inhibit tumour growth slightly<br>Combination with gemcitabine does not increase inhibition of tumour growth<br>Induce G1-phase arrest and apoptosis | Hou et al. (2008) |
| **Drugs: Dihydroartemisinin** | | | | |
| Female Balb/c-nude mice | 60 mg/kg/day | A549 NSCLC xenograft model | Decrease tumour volume and weight significantly<br>No significant body weight loss | Tong et al. (2016) |
| Male nude BALB/c mice | 2 mg/kg/day 10 mg/kg/day 50 mg/kg/day i.p. injection for 21 days | BxPC-3 pancreatic cancer xenograft | Slow tumour growth<br>Decrease tumour volume<br>2 mg/kg/day: 569 ± 69 mm³<br>5 mg/kg/day: 389 ± 44 mm³<br>10 mg/kg/day: 244 ± 36 mm³<br>Control: 730 ± 90 mm³<br>Decrease microvessel density<br>significantly<br>Inhibit angiogenesis | Wang et al. (2011) |
| Female athymic nude mice | 50 mg/kg/day OR 100 mg/kg/day OR combination with gemcitabine | HepG2 hepatocellular carcinoma xenograft model | Inhibit tumour growth (36.1 and 60.6% for 50 mg/kg/d and 100 mg/kg/d) increase anticancer effect of gemcitabine<br>No observable toxic effects | Hou et al. (2008) |
| Female athymic nude mice | 50 mg/kg/day OR 100 mg/kg/day OR combination with gemcitabine | Hep3B hepatocellular carcinoma xenograft model | Inhibit tumour growth<br>Increase antitumour effect when combined with gemcitabine<br>Induce G1-phase cell cycle arrest<br>Induce apoptosis | Hou et al. (2008) |

(Continued on following page)
TABLE 2 | (Continued) Dose and Mechanisms of Action of artemisinins in vivo.

| Animal | Dosing regimen | Disease model | Mechanisms, safety, and efficacy | Reference |
|--------|----------------|---------------|----------------------------------|-----------|
| Male nude BALB/c mice | 10 mg/kg/day i.p. injection OR combination with gemcitabine 100 mg/kg BD | BxPC-3 pancreatic cancer xenograft model | Reduce tumour volume and suppress tumour growth Combination treatment reduced tumour volume more significantly Decrease Ki-67 Suppress NF-κB DNA binding activity and downregulate related gene products Enhance antitumour effect of gemcitabine | Wang et al. (2010a) |
| BALB/c male mice | 50 mg/kg/day, 5 times per week, for 4 weeks | Cal-27 head and neck squamous cell carcinoma xenograft | Decrease tumour size, volume, and weight significantly No significant body weight loss | Jia et al. (2016b) |
| Female athymic nude Foxn1nu/Foxn1+ mice | 5 mg/kg/day OR in combination with DOX diet intraperitoneal injection | GPX4 iKO H292 lung cancer xenograft model | Suppress tumour growth Decrease expression of Ki-67 Enhance effect of GPX4 targeted therapy | Chen et al. (2020a) |
| Female Balb/c-nude mice | 60 mg/kg/day | A549 NSCLC xenograft model | Inhibit tumour growth to decrease tumour volume and weight significantly Did not cause significant loss in body weight | Tong et al. (2016) |
| Female BALB/c-nude mice | 50 mg/kg/day 100 mg/kg/day 200 mg/kg/day i.p. injection 18 days | HOS human osteosarcoma xenograft model | Inhibit tumour growth dose-dependently and reduce tumour volume Caused some decrease in body weight | Xu et al. (2011) |
| Female BALB/c athymic nude mice | 25 mg/kg/day 50 mg/kg/day 100 mg/kg/day i.p. injection | Panc-1 pancreatic cancer xenograft model | Suppress tumour growth 25 mg/kg/day: 33% 50 mg/kg/day: 44% 100 mg/kg/day: 65% Well tolerated and no observable toxicity | Du et al. (2010) |
| Female CS77BL/6 mice | 100 mg/kg i.p. injection | ID8 murine ovarian cancer model | Inhibit tumour growth and reduce tumour size No overt toxicity or significant loss in body weight | Greenshields et al. (2017) |
| CS7BL/6 &Male (CD-1) BR nude mice | 167 mg/kg/day | KS-IMM xenograft model | Suppress tumour growth and reduce tumour weight significantly | Dell’Eva et al. (2004) |
| Male outbred BALB/c mice | 100 mg/day OR in combination with radiation therapy | HeLa and SiHa cervical cancer xenograft | Inhibit growth of HeLa xenografts in combination with irradiation Enhance radiosensitivity of HeLa xenograft Did not significantly change radiosensitivity of SiHa xenograft | Luo et al. (2014) |
| Athymic BALB/c male nude mice | 50 mg/kg/day oral | HN9 head and neck cancer xenograft model | Inhibit tumour growth Synergise with trigonelline to suppress tumour growth Decrease GSH and increase γH2AX | Roh et al. (2017) |
| Female BALB/c nude mice | 100 mg/kg/day i.p. injection | 786-O renal cell carcinoma xenograft model | Exert antitumour effect and inhibit tumour growth Prevent angiogenesis and metastasis decrease Ki-67 to curb proliferation | Jeong et al. (2015) |
| Female athymic nude mice | 50 mg/kg alone OR in combination with cisplatin 2 mg/kg for 16 days | A2780 and HO8910 ovarian cancer xenografts | Synergise with cisplatin to inhibit tumour growth ARS alone did not exhibit significant antitumour effect | Wang et al. (2015) |

Drugs: Artesunate
The Wnt/β-catenin pathway in lung cancer by DHA and SKM-1 cells by ART led to increased E-cadherin expression (Xu et al., 2015; Tong et al., 2016), which mediates cell-cell adhesion. The increased cell-cell adhesion suppressed tumor metastasis (Xu et al., 2015). In a human fibrosarcoma HT-1080 cell model, anti-invasive effect of DHA was caused by inhibiting the phosphorylation of PKCα/Raf/ERK and JNK and reducing the activation of NF-κB and AP-1, thereby leading to the down-regulation of MMP-9 expression. Therefore, DHA is an effective anti-metastatic agent that works by down-regulating MMP-9 expression (Hwang et al., 2010). In another study on HepG2 cells, ARS activated Cdc42, promoting E-cadherin action which is necessary for cell adhesion (Weifeng et al., 2011). Additionally, artemisinins administration down-regulated proliferating cell nuclear antigen gene expression, MMP2, p-p38, p-ERK1/2, CSC markers, and EMT-related proteins, which promote tumor growth, proliferation, and metastasis in lung cancer and HCC cells and their downregulation would inhibit tumor growth (Rasheed et al., 2010; Weifeng et al., 2011; Liao et al., 2014; Tong et al., 2016). Artemisinins inhibited proliferation in prostate cancer, human osteosarcoma, HepG2, and pancreatic cancer cells (Willoughby Sr et al., 2009; Youns et al., 2009; Xu et al., 2011; Zeng and Zhang, 2011).

Overall, artemisinins act via multiple pathways by regulating the key targets of suppression of cell cycle, induction of apoptosis, inhibition of NF-κB signalling pathway, and suppression of mitogen-activated protein kinase (MAPK) signaling.

### ANTICANCER EFFICACY OF ARTEMISININS IN VITRO AND IN VIVO MODELS

Artemisinins have been recognized as antimalarials, but they have demonstrated great anticancer potential in in vitro and in vivo studies (Table 2).

### In vitro Anticancer Efficacy

Several studies have been conducted to assess the effect-of artemisinins against different types of cancer. For DHA, IC_{50} values ranged between 1.20–15.2 μM (Jiao et al., 2007; Hou et al., 2008; Chen T. et al., 2009; Michaelis et al., 2010; Wang et al., 2012), with the exception of BxPC-3 pancreatic cancer cells (Chen et al., 2010; Wang et al., 2011), TE671 rhabdomyosarcoma cells (Beccafo et al., 2015) and Fadu, Hep-2, and Cal-27 head and neck squamous cancer cells (Jia...
### TABLE 3 | Human clinical trials of artemisinins.

| Study design and population | Dosing regimen | Efficacy data | Safety data | Ref |
|-----------------------------|----------------|---------------|-------------|-----|
| Phase 1 open label study 23 patients with metastatic breast cancer | Oral ART 100 mg OD OR 150 mg OD OR 200 mg OD Add on to guideline-based oncological therapy for 4 weeks | No complete or partial remission | Oral ART 200 mg/d (2.2–3.9 mg/kg/d) was well tolerated and safe | ARTIC M35/2 (von Hagens et al., 2017) |
| Prospective monocentric, and open uncontrolled phase I dose-finding study 13 patients with metastatic breast cancer for long-term compassionate use | Oral ART 100 mg OD OR 150 mg OD OR 200 mg OD Add-on therapy to guideline-based oncological therapy | 6 patients 150 or 200 mg OD (1.8–3.3 mg/kg BW/d), were found to have stable disease until last follow-up | No major safety concerns | von Hagens et al. (2019) |
| Randomised, Double Blind, Placebo-Controlled Pilot Study 23 patients with colorectal cancer 12 received treatment, 11 received placebo | Oral ART 200 mg/d for 14 days | Decreased expression of Ki-67 (probability = 97%) Increased expression of CD31 (probability = 79%) Increased recurrence-free survival probability compared to placebo after 3 years (0.89 vs 0.5) No patients that received ART had increased carcinoembryonic antigen (CEA) levels as compared to the placebo group where 3 patients had increased CEA levels | 6 patients had adverse events, 2 were possibly related to ART. | Krishna et al. (2015) |
| Phase I 19 adult patients with refractory solid tumours | IV ART 8, 12, 25, 34 and 45 mg/kg given on days 1 and 8 of a 21-days cycle administered as a 5-min IV push | No patients had complete or partial response | 18 mg/kg on a Day 1/Day 6, 3-weeks administration cycle was shown to be the maximum tolerated dose Cmax at the maximum tolerated dose was 415 ng/ml Dose limiting toxicities included myelosuppression, liver dysfunction, uncontrolled nausea and vomiting, hypersensitivity Side effects of anaemia, fatigue, N&V, anorexia, dizziness reported | Deeken et al. (2018) |
| Dose-escalation phase I study 28 women with cervical intraepithelial neoplasia 2/3 (CIN2/3) | Intravaginal ART Group 1: one treatment cycle of 50 mg inserts. Next 3 groups: 1, 2, or 3 treatment cycles of 200 mg insert(s), at weeks 0, 2, and 4 of the study Each treatment cycle included a single vaginal insert dose for 5 nights in a row | Histologic regression to CIN1 or less observed in 68% of subjects >60% histologic regression across all 4 dosing groups Mean time to regression shorter in subjects that received multiple treatment cycles compared to only one | No intolerable side effects that led to withdrawal No grade 3 or 4 adverse events reported 3 participants reported no noticeable side effects Treatment generally safe and well-tolerated | Trimble et al. (2020) |

(Continued on following page)
L. et al., 2016) which were highly resistant. This IC₅₀ range was considerably higher than that of Cₘₐₓ in healthy volunteers (0.558–1.27 μM). Only HCT116, HT29, SW480, and LOVO colon cancer cell lines showed IC₅₀ values within the Cₘₐₓ range (Yao Z. et al., 2018; Chen GQ. et al., 2020). For ART, IC₅₀ values range between 2.0–39.4 μM (Effert et al., 2007; Hou et al., 2008; Li et al., 2008; Youns et al., 2009; Du et al., 2010; Michaelis et al., 2010; Rasheed et al., 2010; Steinbrück et al., 2010; Zeng and Zhang, 2011; Luo et al., 2014; Beccafico et al., 2015; Jeong et al., 2015; Xu et al., 2015; Pang et al., 2016; Greenshields et al., 2017; Chen GQ. et al., 2020). Inconsistent with the range of Cₘₐₓ values (0.174–1.83 μM) except in CEM, J-Jhan and Molt-4 leukemia cells (Effert et al., 2007; Steinbrück et al., 2010), and TOV-112D ovarian cancer cells (Greenshields et al., 2017) which are within range. High IC₅₀ value is a significant barrier in the clinical application of use of artemisinins in humans because high doses in vivo may lead to toxicity problems. Combination therapy can also be considered as a therapeutic option because artemisinins can synergize with other drugs to increase efficacy.

**In Vivo Anticancer Efficacy**

Several studies demonstrated the efficacy of artemisinins in tumor-bearing animal models. The cancer types identified in *in vitro* have been effectively treated by artemisinins *in vivo*. The *in vivo* studies used more aggressive dosage regimens of artemisinins with effective doses ranging from 50 to 100 mg/kg/d and showed little toxicity in animals (Hou et al., 2008; Willoughby Sr et al., 2009; Du et al., 2010; Wang et al., 2011; Weifeng et al., 2011; Xu et al., 2011; Jeong et al., 2015; Jia L. et al., 2016; Jia et al., 2016b; Tong et al., 2016). In HepG2 HCC xenografts, tumor inhibition rates of up to 79.6% was observed after administration of 100 mg/kg/d of ARS (Weifeng et al., 2011). Another study reported 60.6% inhibition of tumor growth after administration of 100 mg/kg/d of DHA (Hou et al., 2008). Since HCC cell lines were not highlighted in previous *in vitro* studies, the underlying mechanism of the efficacy of DHA observed in HCC xenografts *in vivo* should be further explored.

At this dosage range, artemisinins showed a significant and conclusive effect on the inhibition of tumor growth. However, 100 mg/kg/dose would translate to 3 g/d for a 60 kg adult, which is significantly greater than the safe and effective dose established for the treatment of malaria (200 mg/d) (Organization, 2015). Another promising result was observed in LOVO colorectal cancer xenografts where the tumor growth inhibition rate was 52.2% (Li et al., 2008) at a dose of 300 mg/kg twice a week. This discrepancy in dosage regimens between malaria cases and *in vivo* studies in xenograft mouse models can make clinical translation challenging.

Notably, among many derivatives of artemisinin, ART has the most extensive data, thus, it has the greatest potential to be developed for future use in cancer treatment in humans.

**CLINICAL APPLICATION OF ARTEMISININS IN CANCER THERAPY**

A few clinical trials conducted were using ART to understand the efficacy of artemisinins in breast cancer, colorectal cancer, and other solid tumors (Table 3) (Krishna et al., 2015; von Hagens et al., 2017; Deeken et al., 2018). The effective dose of ART ranged up to 200 mg/d, which was safe and well tolerated (von Hagens et al., 2017; von Hagens et al., 2019).

A clinical trial conducted in patients with solid tumors revealed the maximum tolerated dose of IV ART as 18 mg/kg in a Day 1/Day 8 regimen with a 3-week administration cycle with dose-limiting toxicities such as myelosuppression, liver dysfunction, and uncontrolled nausea and vomiting (Deeken et al., 2018). Other side effects included anemia, fatigue, dizziness, and anorexia (Deeken et al., 2018) at a much lower dose than the effective dose used in *in vivo* studies. This result indicates that *in vivo* studies do not accurately represent toxicity data in humans. While effective therapeutic range *in vivo* can be as high as 200 mg/kg/d, the same dose cannot be used in humans. Caution should be exercised in proceeding with higher doses of ART that are likely to be more efficacious but less safe.

### TABLE 3 | (Continued) Human clinical trials of artemisinins.

| Study design and population | Dosing regimen | Efficacy data | Safety data | Ref |
|----------------------------|----------------|---------------|-------------|-----|
| Phase I 120 patients with advanced NSCLC | Control: vincristine + cisplatin (NP) Treatment: NP + artesunate 120 mg/day | No significant difference in short-term survival rate, mean survival time disease controlled rate significantly higher in treatment group Time to progression significantly longer in treatment group | Toxicity between treatment and control group not significantly different | Zhang et al. (2008) |
| 2 patients with metastatic uveal melanoma in addition to standard chemotherapy | Artesunate on compassionate use basis | One patient experienced temporary response upon adding ART to fotemustine The other patient experienced stabilization and regression of spleen and lung metastases Promising adjuvant in treatment of melanoma | Well tolerated with no experience of additional side effects | Berger et al. (2005) |
## TABLE 4 | Promising combination therapies of artemisinins.

| Agent combined with DHA/ART | Cell line/disease model | Effect | Ref |
|-----------------------------|-------------------------|--------|-----|
| **Drugs: Dihydroartemisinin** |                         |        |     |
| Onconase                    | MSTO-211H human mesothelioma, NCI-H661, SK-MES-1, SPC-A-1, and A549 NSCLC cells | Significant synergistic antitumour effects with onconase. Drastic decrease in IC50 values from onconase or DHA monotherapy to combination therapy. In SK-MES-1 cells, IC50 value of both dropped from ~1,200 to ~10 µM. In Spc-A-1 cells, IC50 value of onconase was as low as 0.001 µM when administered together with DHA. | Shen et al. (2016) |
| Doxorubicin                 | Hep3b hepatocellular carcinoma cells, MCF-7 breast cancer cells, HeLa cervical cancer, OVCAR-3 ovarian, MCF-7 breast, PC-3 prostate, and A549 lung cancer cells | Increase apoptosis-inducing effects of doxorubicin, inhibit P-gp expression which causes resistance to doxorubicin. Combination therapy activated caspase cascades more than monotherapy. DHA sensitised apoptosis triggered by doxorubicin. Decrease cell viability. Synergistic effect to induce apoptosis. | Yang et al. (2019b), Wu et al. (2013), Tai et al. (2016) |
| Gemcitabine                 | A2780 ovarian cancer cells | Induce ROS generation and increase expression of HO-1, a marker of oxidative stress, hence suppression of CDA expression. Downregulation of CDA causes inhibition of metabolic inactivation of gemcitabine and an overall synergistic effect. CI ranges from 0.6–0.9 depending on the concentration ratio which drugs were administered, with an outlier at 1.3 when the ratio of gemcitabine to DHA was 1:1. | Yang et al. (2019a) |
|                            | Panc-1 and BxPC-3 pancreatic cancer cells | DHA significantly blocks NF-κB activation by gemcitabine, augmenting antitumour effect of gemcitabine | Wang et al. (2010b) |
| Cisplatin                   | A549 and A549/DDP NSCLC cells | Increase apoptosis in combination therapy. Synergistic effect on inhibition of cell proliferation. Combination therapy has lower IC50 value compared to monotherapy. CI = 0.67/06 in A549 and 0.5674 in A549/DDP. | Zhang et al. (2013b) |
| Cytarabine                  | HEL92.1.7, MV4-11, U937, ML-2, M07e, MOLM-13, CMK, CMS, mFLT3, MOLM-13-RES, and M07e acute myeloid leukaemia cells | Potentiate cytarabine activity. Synergistic effect in MV4-11 and ML-2 cells. Better synergistic effect observed when DHA was administered as a pre-treatment, followed by cytarabine. | Drenberg et al. (2016) |
| 5-fluorouracil              | HCT116, HCT116 TP53−/−, SW480, and HT29 colorectal cancer cells | DHA potentiates antitumour activity of 5-FU, combination therapy causes stronger cytotoxic effects and decreases IC50 values, even for HCT116 TP53−/− which is resistant to 5-FU. Combination therapy reduces number of reproducing HCT116 TP53−/− cells. Increase generation of ROS intracellularly, inducing apoptosis. | Yao et al. (2016b) |
| Carboplatin                 | A2780 and OVCAR-3 ovarian carcinoma cells | Decrease viability when used in combination–by 69% in A2780 cells, and by 72% in OVCAR-3 cells. Synergistic increase in apoptosis of OVCAR-3 cells. Additive effect on A2780 cells. | Chen et al. (2009b) |
| Dictamine                   | A549 lung cancer cells | DHA enhances cytotoxicity induced by dictamine. DHA enhances apoptosis induced by dictamine by the caspase-3 dependent pathway. | An et al. (2013) |
| Apo2L/TRAIL                 | PANC-1 and BxPC-3 pancreatic cancer cells | Synergistic inhibition of growth. DHA enhances apoptosis induced by Apo2L/TRAIL by ROS pathway. Combination index <1 indicating synergistic effect. | Kong et al. (2012) |

(Continued on following page)
| Agent combined with DHA/ART | Cell line/disease model          | Effect                                                                 | Ref                      |
|-----------------------------|--------------------------------|----------------------------------------------------------------------|--------------------------|
| Gefitinib                   | NCI-H1975 NSCLC cells          | Potentiates apoptotic effect of gefitinib                             | Jin et al. (2017)        |
|                             |                                | Potentiates effect of gefitinib on downregulation of expression of Cdk1 and cyclin B1 |                          |
|                             |                                | Enhanced effect of gefitinib on inhibition of cell migration and invasion |                          |
|                             |                                | Enhanced effect of gefitinib on downregulation of p-Akt, p-mTOR and p-STAT3 |                          |
|                             |                                | Enhanced effect of gefitinib on upregulation of Bax and downregulation of Bcl-2 |                          |

| Arsenic Trioxide            | A549 lung cancer cells         | Synergistic effect on cell viability                                  | Chen et al. (2017a)      |
|                             |                                | Synergistic effect on DNA damage                                     |                          |
|                             |                                | Synergistic effect on ROS production intracellularly                |                          |
|                             |                                | Synergistic effect in inducing apoptosis and cell cycle arrest       |                          |

| Onconase                    | A549 NSCLC xenograft           | Mice that were treated with combination (onconase 3 mg/kg followed by DHA 10 mg/ml the next day) experienced enhanced suppression of tumour growth and angiogenesis | Shen et al. (2016)      |
|                             |                                | Mean body weight only slightly changed and no obvious adverse effects observed |                          |

| Gemcitabine                 | A2780 ovarian cancer xenograft | Mice that were treated with combination (DHA 95 mg/kg and gemcitabine10 mg/kg injected on days 0, 3, 6, and 9 experienced an enhanced effect on inhibition of tumour growth leading to complete elimination of tumour | Yang et al. (2019a)     |
|                             |                                | No change in body weight                                             |                          |

| Carboplatin                 | A2780 and OVCAR-3 ovarian cancer xenograft | Mice that were treated with the combination (DHA 10 or 25 mg/kg/5 days/week for 3 weeks with carboplatin at a single dose of 120 mg/kg, once on day 0) experienced enhanced inhibition of tumour growth (70%) in both A2780 and OVCAR-3 models, as compared to monotherapy with DHA (41% in the A2780 xenograft and 37% in the OVCAR-3 xenograft) with minimal change in body weight | Chen et al. (2009b)     |
|                             |                                | Decrease in Bcl-2/Bax ratio and pro-caspase 8                         |                          |

| Cisplatin                   | A549 and A549/DDP NSCLC xenografts | Mice that were treated with combination of cisplatin (2 mg/kg/3days) and DHA (50, 100, or 200 mg/kg/day) were demonstrated to have greater suppression of VEGF expression and significant decrease in the number of blood vessels compared to monotherapy | Zhang et al. (2013b)    |
|                             |                                | DHA enhanced chemotherapeutic effect of cisplatin resulting in significant regression compared to monotherapy |                          |
|                             |                                | Increasing doses of DHA also increased the concentration of cisplatin in tumour cells |                          |

| Doxorubicin                 | HeLa cervical cancer heterologous tumour model | Mice that received combination therapy (15 mg/kg DHA and 15 mg/kg doxorubicin) experienced synergistic inhibition of tumour size and more significant reduction in size | Tai et al. (2016)       |
|                             |                                | No toxicity observed in heart, spleen, liver, and kidneys, and no change in weight |                          |

| Apo2L/TRAIL                 | BxPC-3 pancreatic cancer xenograft | Mice that received combination therapy (DHA 10 mg/kg/day and Apo2L/TRAIL 50 µg/day) experienced a significantly larger reduction in tumour volume compared to those that received DHA or Apo2L/TRAIL monotherapy | Kong et al. (2012)      |
|                             |                                | DHA potentiates antitumour effect of Apo2L/TRAIL. Combination therapy had higher apoptosis and lower expression of PCNA, a cell proliferation marker, than monotherapy |                          |

(Continued on following page)
TABLE 4 | (Continued) Promising combination therapies of artemisinins.

| Agent combined with DHA/ART | Cell line/disease model | Effect | Ref |
|-----------------------------|-------------------------|--------|-----|
| **Drugs:artesunate**        |                         |        |     |
| Cisplatin                   | A549 lung cancer cells  | Synergistic effect on antiproliferation induced by cisplatin Cl values < 1, Cl values decrease as concentration of drugs increase ART sensitised A549 cancer cells to apoptosis and G2/M cell cycle arrest induced by cisplatin Upregulation of expression of P21, PS3, and Bax, and downregulation of expression of Bcl-2 in combination treatment Increase caspase activity in combination therapy | Li et al. (2021b) |
| Bortezomib                  | MV4-11 acute myeloid leukaemia cells | Synergistic effect on antiproliferation, apoptosis, and autophagy Upregulation of pro-apoptotic protein Bim and autophagy related protein LC3B in combination therapy Increase activation of caspsases Downregulate expression of Bcl-2 | Hu et al. (2019) |
| Bromocriptine               | GH3 and MMQ rat pituitary adenoma cells | Synergistic effect on cell growth inhibition and inducing cell death Synergistic effect on reduction of cell viability Inhibit cell proliferation and G1-phase cell cycle arrest Combination therapy induced apoptosis in a caspase-dependently | Wang et al. (2017) |
| Triptolide                  | PANC-1, CFPAC-1 pancreatic cancer cells | Enhanced inhibitory effects and synergetic effect on cell viability Synergistic effect on activation of caspsases and hence apoptosis Synergistic effect on downregulation of heat shock proteins Hsp20 and Hsp27 | Liu and Cui, (2013) |
| Doxorubicin                 | J16, CEM, MolM-4, Hut78, J-Neo, J-Bcl-2, J-caspase-8−/−, Jurkat A3 FADD−/−, parental Jurkat A3, and CEM-DoxR leukaemia cells | Synergise to enhance apoptosis | Efferth et al. (2007) |
| Sorafenib                   | Caki-1, 786-O, and SN12C-GFP metastatic renal cell carcinoma cells | Synergistic effect on cytotoxicity Sorafenib sensitisises RCC cells to oxidative stress mediated by ART. Synergistic effect on apoptosis due to dual inhibitory effects on RAF/MAPK and PI3K/AKT/mTOR pathways Combination index <1 | Jeong et al. (2015) |
|                            | SK-hep1 and SM-7721 hepatocellular carcinoma cells | | Yao et al. (2020) |
| Temozolomide                | LN229, A172, and U87MG glioblastoma cells | ART enhances cell death induced by temozolomide | Berte et al. (2016) |
| Aliicin                     | MG-63, U20S, 143-B, SaOS-2 and HOS osteosarcoma cells | Synergistic effect on inhibition of cell viability Synergistic effect on induction of apoptosis Upregulation of caspase activation in combination therapy | Jiang et al. (2013) |
| Oxaliplatin                 | MCF7 breast cancer, HCT116 colon cancer and A549 lung cancer cells | ART exerts additive effect to reduce cell number and cell viability Lenalidomide enhanced effect of ART on A549 and MCF7 cells | Liu et al. (2011) |
| Lenalidomide Gemcitabine    | Malignant B cells | Rituximab increases susceptibility of ART-induced apoptosis | Sieber et al. (2009) |
| Cytarabine                 | HEL92.1.7, MV4-11, U937, ML-2, M07e, MOLM-13, CMK, CMS, mFLT3, MOLM-13-RES, and M07e acute myeloid leukaemia cells | Synergistic effect when administered both simultaneously and sequentially Combination therapy enhanced antileukemic activity | Drenberg et al. (2016) |
| Cisplatin                  | A549 lung cancer xenograft | ART sensitises A549 cells to cisplatin and combination treatment of cisplatin at 3 mg/kg/dose every 3 days and ART at 200 mg/kg/dose daily orally for 3 weeks. led to a more significant inhibition of tumour growth than monotherapy No difference in body weight in combination therapy | Li et al. (2021b) |
| Aliicin                     | MG-63 human osteosarcoma xenograft | Mice that received the combination therapy of ART 50 mg/kg OD and allicin 5 mg/kg OD had significantly suppressed tumour growth compared to monotherapy | Jiang et al. (2013) |

(Continued on following page)
TABLE 4 (Continued) Promising combination therapies of artemisinins.

| Agent combined with DHA/ART | Cell line/disease model | Effect | Ref |
|-----------------------------|-------------------------|--------|-----|
| Cytarabine                  | MV4-11-luc, ML-2, and MOLM-13 acute myeloid leukaemia xenografts | Mice that received the combination therapy of ART 120 mg/kg/day for 5 days and cytarabine 6.25 mg/kg/day for 5 days experienced a decrease leukemic infiltration though there was no prolonging of overall survival rate | Drenberg et al. (2016) |
| Sorafenib                   | SK-7721 HCC xenograft   | Combined treatment of sorafenib 2.5 mg/kg and ART 100 mg/kg reduced tumour growth to a larger extend than monotherapy ART potentiates antitumour effects of sorafenib | Jing et al. (2019) |
| Temozolomide                | U87MG glioblastoma xenograft | Repeated concomitant treatment extended mean survival period Combination treatment of temozolomide 5 mg/kg 5 times a week for 6 weeks and ART 100 mg/kg for 9 weeks inhibited tumour growth more effectively than monotherapy | Berte et al. (2016) |
| Triptolide                  | PANC-1 and CFPAC-1 pancreatic cancer xenograft | Mice that received combination therapy (tripтолide 50 μg/kg and ART 50 mg/kg, OR triptolide 100 μg/kg and ART 50 mg/kg, OR triptolide 100 μg/kg and ART 100 mg/kg) experienced synergistic effect on inhibition of tumour growth which caused greater decrease in tumour size than monotherapy No significant change in body weight in combination treatment | Liu and Cui, (2013) |

Another study showed anticancer activity of ART in colorectal cancer patients, which is consistent with the previous in vitro and in vivo studies (Li et al., 2008; Chen GQ. et al., 2020). Treatment with 200 mg oral ART increased recurrence-free survival rate compared to placebo after 3 years (Krishna et al., 2015), but two patients at the lower weight limit developed leukopenia.

The ARCTIC M33/2 study conducted in patients with metastatic breast cancer used ART as an adjuvant to the patients’ guideline-based cancer therapy for 4 weeks; 10 out of 23 patients had stable disease, whereas five patients experienced disease progression (von Hagens et al., 2017). Therefore, while 200 mg oral ART has been established as a relatively safe dose, efficacy at this dose remains inconclusive. The ARCTIC M33/2 study was extended for long-term compassionate use in 13 patients who did not experience any clinically relevant adverse events in the original phase I study. Results from the follow-up study suggested the dose dependent effects of ART; a greater number of patients administered lower dose (100 mg/kg/d) experienced disease progression than patients administered higher doses (von Hagens et al., 2019). In some patients, up to 37 months of use of ART has been reported, demonstrating the safety of the long-term use of oral ART at this dosage range.

Few clinical trials that have been conducted to date are limited to phase I trials which involved relatively small study populations. Hence, phase II trials are required to investigate the effect of artemisinins on a larger number of patients and gain better insight into the safety and efficacy of the use of artemisinins, in particular ART, as potential anticancer agents in large populations.

FUTURE PERSPECTIVES

Artemisinins, in particular ART, have been proven to promising drugs to repurpose for cancer treatment. Additional phase II and III trials should be conducted in future to gain a better understanding of the long-term safety and efficacy profile of artemisinins in large populations. Further strategies should be explored to expedite the development of artemisinins as anticancer agents.

Combination Therapy

Combination therapy makes use of multiple agents to treat a single condition, a strategy that is commonly employed in cancer treatment. The use of combination therapy has advantages of synergistic and additive effects because different drugs can work on different molecular pathways to exert a greater anticancer effect, thereby leading to greater efficacy. Since IC50 values of artemisinins cancer treatment are relatively high, combination therapy can be used to take advantage of the synergistic effect and lower IC50 and minimise any dose-related toxicities because combination therapy allows the use of lower doses of multiple agents.

Several drugs have demonstrated synergistic effects in vitro when administered in combination with either DHA or ART or both (Table 4). Many studies also reported that the use of artemisinins sensitized cancer cells to conventional chemotherapy and exerted a synergistic effect on apoptosis, inhibition of cell growth, and a reduction of cell viability, leading to a lower IC50 value (Chen T. et al., 2009; Zhang YJ. et al., 2013; Liu and Cui, 2013; Shen et al., 2016; Tai et al.,
2016; Chen H. et al., 2017; Wang et al., 2017; Yang et al., 2019a; Yang et al., 2019b; Hu et al., 2019). Combination index, which measures the degree of drug interactions (Zhang JL. et al., 2013) was used to understand the potential of combination therapy. The combination of DHA with cisplatin (Zhang YJ. et al., 2013), DHA with onconase (Shen et al., 2016), DHA with gemcitabine (Yang et al., 2019a), DHA with Apo2L/TRAIl (Kong et al., 2012), and ART with sorafenib (Yao et al., 2020), which were used to treat lung, lung, ovarian, pancreatic, and liver cancer, had combination index values < 1, which indicates synergism.

Animal xenograft models showed that the combination of artesimisinins with onconase (Shen et al., 2016), gemcitabine (Yang et al., 2019a), carboplatin (Chen T. et al., 2009), cisplatin (Zhang YJ. et al., 2013; Li W. et al., 2021), doxorubicin (Tai et al., 2016), Apo2L/TRAIl (Kong et al., 2012), allicin (Jiang et al., 2013), cytarabine (Drenberg et al., 2016), sorafenib (Jeong et al., 2015; Jing et al., 2019), triptolide [17], and temozolomide (Berte et al., 2016) can exert a synergistic effect on leukemia (Drenberg et al., 2016), renal cell carcinoma (Jeong et al., 2015), glioblastoma (Berte et al., 2016), lung (Zhang YJ. et al., 2013; Shen et al., 2016; Li W. et al., 2021), ovarian (Chen T. et al., 2009; Yang et al., 2019a), cervical (Tai et al., 2016), pancreatic (Kong et al., 2012; Liu and Cui, 2013), and liver (Jing et al., 2019) cancer. Many studies reported the synergistic effect of ART with conventional chemotherapy on the inhibition of tumor growth without a significant decrease in body weight (Kong et al., 2012; Liu and Cui, 2013; Shen et al., 2016; Tai et al., 2016; Yang et al., 2019a; Li W. et al., 2021), suggesting improved efficacy without an overt increase in toxicity. The complete elimination of an ovarian cancer tumor was observed in a study that used DHA and gemcitabine combination therapy.

In summary, combination therapy is a promising strategy to advance the repurposing of artesimisinins as anticancer therapeutics. Since more combination therapy studies have been conducted for DHA than for ART, the use of DHA in human clinical trials should also be explored in future research. Clinical trials exploring ART or DHA as an adjuvant to the conventional chemotherapy should also be conducted.

**Nanoformulation**

To overcome the limitations that result from poor pharmacokinetic properties of artesimisinins, novel delivery methods that could improve the absorption and elimination profile of artesimisinins should be explored. Several in vitro and in vivo studies have been conducted to investigate the use of nanoparticles, nanocarriers, and liposomes as carriers for ARS, ART, and DHA to improve their delivery to the cancer cells. These new formulations improved solubility, exposure, and stability, increased cellular uptake, and enhanced permeability and retention in breast, colorectal, liver, lung, and cervical cancer cells (Chen J. et al., 2014; Chen et al., 2015; Tran et al., 2015; Leto et al., 2016; Liu et al., 2016; Tran et al., 2016; Tran et al., 2017; Wang et al., 2018; Wang et al., 2019; Phung et al., 2020). Both in vitro and in vivo studies revealed promising results with low IC50 values (Zhang et al., 2015; Leto et al., 2016) and high rates of tumor inhibition (Jin et al., 2013; Chen et al., 2015; Wang et al., 2016b; Liu et al., 2016; Dong et al., 2019; Wang et al., 2019; Li et al., 2020).

In a study conducted on BT474 (HER2+) breast tumor cells made using liposomal nanoparticles for drug delivery, IC50 values ranged between 0.07–0.39 µM (Zhang YJ. et al., 2013), indicating high potency. In another study, IC50 values decreased from 127 ± 8.5 µM when free ARS was administered to 69 ± 23 µM when liposomes were administered (Leto et al., 2016), demonstrating the ability of liposomes to increase the efficacy of ARS. Many formulations used pH-dependent drug release in the slightly acidic environment of tumor cells (Wang et al., 2016a; Wang et al., 2016b; Dong et al., 2019; Wan et al., 2019; Wang et al., 2019) for targeted drug delivery and increased accumulation of the drug in the tumor cells while simultaneously reducing unintended off-target interactions. This might have contributed to the greater cytotoxicity observed with the use of novel nanoformulations than with the use of free drug (Chen J. et al., 2014; Chen et al., 2015; Tran et al., 2015; Wang et al., 2016b; Tran et al., 2017; Dong et al., 2019).

After nanoformulation administration, the same efficacy was demonstrated in in vivo studies, whereas an increase in antitumor effect was observed in tumor-bearing mice models (Jin et al., 2013; Chen et al., 2015; Zhang et al., 2015; Wang et al., 2016a; Wang et al., 2016b; Liu et al., 2016; Wang et al., 2018; Dong et al., 2019; Wang et al., 2019; Li et al., 2020; Phung et al., 2020). Antitumor effect was measured by using the tumor volume and tumor growth inhibition rate. In a study that used nanoconjugates, breast tumor volume was 989 ± 164 mm³ after treatment with nanoconjugate formulation compared to 1,417 ± 148 mm³ after treatment with the free drug (Li et al., 2020). Another study conducted on Lewis lung carcinoma tumor bearing mice model reported a tumor growth inhibition rate of 84.6% after treatment with polyethylene DHA nanoparticles compared to 29.9% after treatment with free DHA. Survival rate was also markedly higher (83.3%) than that of free DHA (16.7%) (Liu et al., 2016).

In the future research, combination therapy and nanotechnology should be further explored. The combinations of DHA with oxaliplatin (Duan et al., 2019), DHA with sorafenib (Wang et al., 2019), DHA with docetaxel (Li et al., 2020), and DHA with paclitaxel (Phung et al., 2020) along with the use of nanoparticles have been studied, and in vitro and in vivo data are promising, implying their viability for human trials.

**CONCLUDING REMARKS**

Despite challenges, repurposing artesimisinins for cancer treatment is possible. Artemisinin and its derivatives have anticancer effects against multiple cancer types. Because they act through various pathways, although their potency varies across cancer types. Their efficacy has also been demonstrated in in vivo studies with evidence of inhibition of tumor growth in tumor bearing mice models. A few human trials have also shown promising results that artemisinins, in particular ART, are safe for use, although their efficacy is still relatively limited. The limitations due to their pharmacokinetic properties such as
low tissue distribution, short half-life, and unpredictable toxicity at high doses hinder their clinical translation. However, there are viable options such as the use of combination therapy and nanoformulations that can overcome the pharmacokinetic barriers of artemisinins. At high doses of artemisinins are used in cancer treatment, toxicity prediction models should be used to ensure that severe toxicity is controlled (Li S. et al., 2021). Although artemisinins have great potential as anticancer agents, additional extensive human trials are required before the drug can be established as an anticancer agent.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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AUTHOR CONTRIBUTIONS

JM and CW equally contributed to drafting the article; C-GL, J-TC, MY, GS, and AW contributed to acquisition of data, figure preparation, analysis and interpretation of data, and manuscript revision. PH, DZ, PO, LW, B-CG contributed to the structure design and the conception and design of the study.

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