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Artemisinins as a novel anti-cancer therapy: targeting a global cancer pandemic through drug repurposing

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Title Page

Artemisinins as a novel anti-cancer therapy: targeting a global cancer pandemic through drug repurposing

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Abstract

Artemisinins are a unique class of antimalarial drugs with significant potential for drug repurposing for a wide range of diseases including cancer. Cancer is a leading cause of death globally and the majority of cancer related deaths occur in Low and Middle Income Countries (LMICs) where conventional treatment options are often limited by financial cost. Drug repurposing can significantly shorten new therapeutic discovery pathways, ensuring greater accessibility and affordability globally. Artemisinins have an excellent safety and tolerability profile as well as being affordable for deployment in Low and Middle Class Income Countries at around USD1 per daily dose. Robust, well designed clinical trials of artemisinin drug repurposing are indicated for a variety of different cancers and treatment settings.

Key Words

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Introduction

Four decades since the discovery of artemisinins for the treatment of malaria by a collaborative research group led by Nobel Prize winner and eminent Chinese scientist Professor Youyou Tu, this class of drugs continue to fascinate and attract significant scientific interest as novel therapeutic agents that could be repurposed to treat a plethora of medical conditions. Artemisinins are a remarkable class of compounds that have shown promising cytotoxic effects against viruses, fungi and a variety of cancers as well as powerful anti-inflammatory effects in animal models of asthma, sepsis, arthritis, pancreatitis, systemic lupus erythematosus and haemorrhagic shock[1-11]. This diverse therapeutic potential has led to artemisinins being identified as potentially the new 'aspirin'[1].

Artemisinins: from traditional Chinese medicine to anti-malarial blockbuster

Artemisinins are a family of sesquiterpene trioxane anti-malarial agents derived from Sweet wormwood (*Artemisia annua L*). Artesunate, artemether and arteether are derivatives of artemisinin that are wholly or partially converted into the active metabolite dihydroartemisinin (DHA)[1]. Sweet wormwood (qinghao) has been used in traditional Chinese medicine for two millenia to treat fevers and a variety of inflammatory conditions. Humanity has probably wrestled with the life-threatening global epidemic of malaria since as far back as 770 BC with a detailed description of symptoms of malaria described in the *Inner Canon of the Yellow Emperor*, written around the time of the Chun Qiu and Qin Dynasties (770–207 B.C.)[12]. Renowned

Chinese physician Ge Hong (284-363) listed qinghaosu as an essential remedy for fevers in *Emergency Prescriptions Kept up One's Sleeve*. In his treatise "On Airs, Waters, and Places" 400 B.C, Hippocrates described "agues" and "tertian fevers" frequently affecting populations living close to swamps and marshlands[13]. Although to date, there are still over 200 million cases of malaria a year worldwide, the majority of these are now curable and will be treated with artemisinin combination therapy. This discovery represented a significant milestone in the history of medicine and infectious disease, a triumphant example of how scientific collaboration, commitment and traditional wisdom can converge to provide a breakthrough cure for a once lethal disease[14].

The global cancer pandemic

Whilst one epidemic may now have an elixir, another provides a global challenge for which we need an equally urgent response – the global pandemic of cancer. Cancer is now the second leading cause of death globally and accounts for an estimated 9.6 million deaths annually. Globally, cancer accounts for 1 in 6 deaths, with 70% of cancer related deaths occurring in low- and middle-income countries (LMICs)[15]. In many LMICs cancer is a neglected disease with the majority of patients unable to afford novel diagnostics and effective therapeutics available in more affluent countries. Drug discovery and novel drug development takes on average 13-15 years to go from bench to bedside at an average cost of USD1-2 billion[16]. Repurposing of 'old' drugs for new indications can shorten this pathway substantially with significant cost savings. Interestingly, drug repurposing/repositioning is not a new concept. The antimalarial quinine was used in the early 20th century as an anti-arrythmic agent to treat atrial fibrillation. Difluoromethylornithine (DFMO) which was originally developed as an anti-

cancer therapy has been repurposed as a treatment for sleeping sickness. To date there are over 100 candidate drugs undergoing drug repurposing clinical trials for cancer.

The cancer burden crisis in LMICs is a humanitarian crisis that requires an urgent paradigm shift for which we (as a global community) need to utilise similar principles of multidisciplinary scientific collaboration, political commitment and research innovation that resulted in a malaria cure four decades ago. A global commitment to a dedicated programme of drug repurposing for cancer underpinned by the highest quality of robust scientific evidence and well designed clinical translational trials must form part of our roadmap to tackle this pandemic. In recent times the COVID-19 global pandemic has demonstrated how inter-reliant and connected we are as a global community. Highly coordinated, open and collaborative efforts to advance clinical translation research rapidly for affordable therapeutics through efforts such as the SOLIDARITY trial for COVID-19 have demonstrated the ability to ensure potentially life saving research in a humanitarian crisis is accessible globally (http://www.isrctn.com/ISRCTN83971151).

Artemisinins: multiple modes of action against cancer

Carcinogenesis in humans is a complex process and 8 key hallmarks have been well described[17]. These include sustained proliferative signalling, evasion of cell death and growth suppression, induction of angiogenesis, invasion and metastasis, reprogramming energy metabolism and evasion of immune destruction [17]. In the last three decades, artemisinins have shown potent and broad anticancer properties in a range of cell lines and animal models, supporting the hypothesis that artemisinins have

the potential to be developed as an effective anti-cancer therapy. Several comprehensive reviews have examined the evidence for the anti-cancer effects of artemisinins[1,3,10]. Multiple potential mechanisms of action include anti-proliferative effects through cell-cycle disruption, reactive oxygen species (ROS) -induced DNA damage, induction of apoptosis, anti-angiogenesis, immunomodulation and induced radiosensitivity.

Studies of artemisinins in *in vitro* experiments and animal models have demonstrated broad anti-cancer activity including pro-apoptotic, anti-proliferative, anti-angiogenesis and anti-metastatic effects[1,9,10]. Artesunate displays cytotoxic effects against numerous cancer cell lines including colon, breast, leukaemia, melanoma, central nervous system, ovarian, renal and prostate cancers[18,19,20,21,22]. The active metabolite of artemisinins, dihydroartemisinin (DHA), has demonstrated antineoplastic effects in breast, glioma, colon, lung, ovarian, pancreatic, renal cell and leukaemia cancer cell lines[23,24,25,26,27,28,29,30,31,32,33].

Artesunate has been shown to promiscuously target multiple critical biological pathways and over 300 specific artesunate targets using a chemical proteomics approach with artemisinin-based activity probes[33]. Cellular functions associated with these target proteins include growth and proliferation, cell death and survival, protein synthesis, fatty acid metabolism, cellular movement, free radical scavenging and energy metabolism. Pleiotropic alkylation targets in different cell lines raise questions about which ones are key to anticancer effects of artemisinins, and which ones may be bystanders to the main efficacy pathways. In depth mechanism of action studies in various cancer models are needed to better understand the nature of specific molecular targets in different cellular environments.

Artemisinins: effects on the cell cycle

Cell growth and repair require progression through the cell cycle which is controlled by a series of cyclins and cyclin-dependent kinases. Inhibitors of cell division include the cip/kip family of inhibitory proteins p16, p21, p27 and p57. Studies have shown that artemisinins induce cell cycle arrest via a number of pathways. Artesunate was shown to markedly impede the growth of human breast cancer cell and nasopharyngeal cancer cell lines by inducing G1 cell cycle arrest[34] as well as impeding proliferation in colon, small cell lung cancer, leukemia and glioma cell lines by inducing cell cycle arrest at G2/M phase[35]. Artesunate induced cell cycle arrest at the G2/M phase and induced oncosis-like cell death in renal cell cancer[36]. Decreased levels of cyclin B, cyclin D1 and transcription factor E2F1 were also observed.

In human nasopharyngeal cancer cells, artemisinin upregulated p16 and p27 and suppressed the level of cyclin D1, cyclin E, CDK2, CDK4 and CDK6[37]. Artemisinins also induce G1 cell cycle arrest by deactivating the retinoblastoma protein (pRb), a mediator of cell cycle progression and disrupting transcription of the cyclin CDK4 promoter in prostate cancer lines[38]. DHA was found to inhibit cell cycle progression from G0/G1 into S phase in pancreatic cell lines via reduction of CDK2, CDK4 and CDK6, cyclin E and NF-κB. Levels of the p27 inhibitory protein were amplified[39]. Artesunate induced radiosensitivity in cervical cancer cell lines by inducing apoptosis and G2/M cell cycle arrest[40].

Artemisinins: effects on cancer cell death pathways

Endoperoxide bridge cleavage and the formation of alkylating radicals are thought to be key mechanisms underlying the anti-cancer effects of artesunate[19,20,33]. Cancer

cells are highly proliferative, requiring a heavy iron load which acts as a cofactor in the synthesis of deoxyriboses prior to cell division[41]. Artemisinins induce cellular damage via the formation of reactive oxygen species (ROS) such as hydroxyl and superoxide anion radicals. When free iron is available, artemisinins are converted into a highly potent alkylating radical, capable of inducing direct oxidative damage in cancer cells. Other studies in cancer model cell lines also implicate overexpression of the transferrin receptor and enhanced anti-cancer effects of artemisinins[19,20,42]. Artemisinin–transferrin conjugates have higher anti-cancer efficacy than artemisinins alone[43,44], for example, a DHA–transferrin conjugate has demonstrated at least 280 times more potent anti-cancer activity in breast cancer cells compared to normal breast cells[45].

Apoptosis (programmed cell death) is maintained by a complex balance between a family of pro-apoptotic proteins (BAX, BAK, BAD, Bid) and anti-apoptotic proteins (Bcl-2 and Bcl-xl)[17]. When cells detect DNA damage, tumour suppressor protein TP53 is upregulated, leading to increased levels of pro-apoptotic proteins and cytochrome *c* (caspase activator) leading to programmed cell death. Artemisinins can induce apoptosis in lung adenocarcinoma and prostate cancer cells lines via activation of BAX[46,47]. Artesunate can also induce apoptosis in breast cancer cell lines and leukemic T cells via iron dependent ROS formation resulting in cytochrome c release and cleavage of procaspases-2, 3, 8 and [48,49]. Artesunate can also inhibit colorectal cancer cell proliferation via activation of mitochondrial apoptosis by suppressing the fatty acid biosynthetic pathway and nuclear factor kappa-light-chain-enhancer of activated beta (NF-κB) pathway[50]. NF-κB is a family of inducible transcription factors that play a pivotal role in DNA transcription, cell survival, cytokine production and inflammation.

Artesunate has also been shown to induce oncosis in renal cancer cell lines[36]. Increased levels of calpain-1, calpain-2 expression and decreased levels of α-tubulin expression were exhibited in cell lines treated with artesunate. Calpains are a family of calcium-dependent cysteine proteases that exert proteolytic cleavage on a number of cellular substrates, including cytoskeletal proteins. In oncosis, ion pumps are affected by ATP depletion, leading to the collapse of mitochondrial potential resulting in cytomembrane destruction and the accumulation of reactive oxygen species (ROS)[36].

Ferroptosis is a novel mode of iron-dependent oxidative cell death characterized by the lethal accumulation of lipid-based reactive oxygen species (ROS). In a head and neck squamous cell cancer model, artesunate induced ferroptosis by decreasing cellular glutathione (GSH) levels and increasing lipid ROS levels[51].

Artemisinins have also been shown to regulate the process of autophagy[52]. Autophagy is a degradative process of recycling organelles and cytoplasmic content in order to maintain cellular haemostasis and involves multiple signaling pathways. Cancer cells dysregulate autophagy as a tumour adaptation mechanism in nutrient-deplete tumour microenvironments[52]. DHA has been shown to switch off activity of NF-κB, leading to upregulation of autophagy in several cancer cell lines. Accumulation of reactive oxygen species (ROS) through inhibition of NF-κB further stimulates autophagy. These various modes of cancer cell death are interlinked, for example, DHA can promote autophagic independent degradation of ferritin, resulting in free iron accumulation and dysgregulation of iron homeostasis that in turn can sensitise cancer cells to ferroptosis[53].

Artemisinins: effects on critical cell signalling pathways

Artemisinins target several critical cell signal transduction pathways in cancer including inhibition of the Wnt/β-catenin pathway[54,55]. The Wnt/beta-catenin cell signalling pathway has been identified as a critical pathway in colorectal carcinogenesis[70,71]. β-Catenin is a multifunctional protein that plays a vital role in physiological homeostasis and unregulated expression has been implicated in cancer development. β-Catenin has a role as a transcriptional co-regulator as well as an adaptor protein for intracellular adhesion. The chief regulator of β-catenin is Wnt, a family of 19 glycoproteins that regulate both β-catenin-dependent (canonical Wnt) and -independent (non-canonical Wnt) cell signaling pathways[44]. Artesunate was shown to inhibit the hyperactive Wnt/β-catenin pathway in colorectal cancer cell lines, thereby attenuating cancer cell growth[54]. Artesunate also suppressed proliferation and promoted apoptosis of colorectal cancer cells in a dose-dependent manner[55]. Immunohistochemical staining showed membranous translocation of regulator protein beta-catenin and inhibition of unrestricted activation of the Wnt/β-catenin pathway. *In vivo* studies showed that artesunate significantly slowed the growth of colorectal human tumor xenografts and delayed the development of liver metastases[55]. Artesunate has also been shown to induce apoptosis via inhibition of the Wnt/β-catenin pathway in myelodysplastic syndrome (MDS) cells[73].

Artemisinins also inhibit epidermal growth factor receptor (EGFR) as well as BCR/ABL signaling. Downregulation of cyclin D1, c-MYC/MYX, mTOR, survivin, c-Met, EGFR, Src, FAK, and α-tubulin expression have also been observed[3,10,26,56,57]. Artesunate was found to induce oxidative deoxyribonucleic acid (DNA) damage, sustained DNA double-strand breaks and Ataxia telangiectasia mutated/Ataxia telangiectasis

(ATM/ATR) and Rad-3 related protein damage response in glioblastoma cell lines[58]. Artesunate was shown to be a powerful inducer of oxidative DNA damage, which was dose dependent and paralleled by cell death via apoptosis and necrosis. Artesunate was also shown to provoke a DNA damage response (DDR) with phosphorylation of ATM, ATR, Chk1 and Chk2 proteins. In an ovarian cancer cell line model, artesunate induced oxidative stress resulted in DNA double-strand breaks (DSBs) and downregulation of RAD51 foci and homologous recombination repair (HRR), thereby impairing DSB repair[59].

Artemisinins: anti-angiogenic effects

Angiogenesis plays a vital role in cancer cell growth and development. Isolated tumour cells without an adequate nutrition and oxygen supply display a growth restriction of about 1–2 mm^3. Cancer cells require pro-angiogenic stimuli such as metalloproteinase (MMP) and vascular endothelial growth factor (VEGF) and a reduction in anti-angiogenic factors such thrombospondin and tissue inhibitor of metalloproteinase (TIMP) to maintain a viable blood supply required for growth[17]. Under hypoxic conditions, hypoxia induced factor (HIF-1α) and NF-κB are activated resulting in transcription of VEGF and angiogenesis. Artemisinins were shown to reduce the levels of HIF-1α and VEGF in mouse embryonic stem cells[60]. In a mouse lung carcinoma model, administration of artemisinin resulted in a reduction in VEFG-C and lymphangiogenesis[61]. Artesunate also suppressed levels of VEGF and KDR/flk-1 resulting in reduced tumour growth in BALB/c nude mice implanted with human ovarian cancer cells[62], in Kaposi's sarcoma (KS-IMM) xenograft mice[63] and in a rat glioma model[64]. Artesunate was also shown to exert anti-angiogenic effects on renal cancer cells in vitro in a dose-dependent manner[36]. Following treatment with

artesunate, renal cell tumours in a subcutaneous xenograft model showed decreased levels of proliferation marker Ki-67 and reductions in mean microvessel density compared to controls.

Artemisinins: effects on tumour cell migration, invasion and metastasis

A key hallmark of cancer cells is their ability to detach from the primary tumour, degrade the extracellular matrix and metastasise through the bloodstream[17]. Metalloproteinases (MMPs) play a critical role in tumour migration, invasion and metastases via degradation of the extracellular matrix. E-cadherin is an important cell adhesion molecule. One study in human melanoma cells, showed that artemisinin was able to reduce MMP2 levels thereby blocking cell migration[65]. In human pancreatic and ovarian cancer cells lines, DHA suppressed the levels of MMP2, inhibiting NF-κB and metastases[66,67]. Similarly, artesunate was shown to abrogate MMPs and NF-κB activity, thereby blocking metastases in non-small cell lung cancer lines. Artemisinins downregulated the levels of MMP2 and TIMP-2 whilst up-regulating E-cadherin in hepatocarcinoma cells lines[68]. Artesunate has been shown to upregulate the expression of adhesion molecules integrin β1 and neural cell adhesion molecule (NCAM) in embryonal rhabdomyosarcoma cells, thereby reducing migration and invasion[69].

Artemisinins: modulation of key inflammatory pathways

Inflammatory cytokines such as Interleukin-6 (IL-6), Interleukin-1β (IL-1β), Nuclear factor kappa B (NF-κB), Nitric Oxide (NO) and Tumour Necrosis Factor alpha (TNF—α) are key regulators of cancer development[74,75]. Chronic inflammation is a key

characteristic of carcinogenesis and inflammation in the tumour microenvironment which leads to the release of pro-inflammatory cytokines and triggers oxidative stress, DNA damage and uncontrolled cell proliferation[75,76,77,78].

Around a quarter of cancers are attributed to infectious agents that cause chronic inflammation including hepatocellular, bladder, gastric and cervical cancer[75]. Tumour-promoting inflammation is also induced by chemical and environmental human carcinogens, such as tobacco, alcohol, asbestos, aflatoxins and nitrosamines. It is increasingly recognized that these carcinogens can also block the resolution of inflammation resulting in cancer development and progression[75,79,80].

Inflammation-induced cell proliferation has been linked to stimulation of carcinogen-induced mutations[80], demonstrating that inflammation and DNA damage can act synergistically to induce mutations that further drive carcinoma progression and recurrence[80]. Oxidative stress and chronic inflammation can lead to pro-tumorigenic feedback loops between inflammation, DNA damage and apoptosis which then contribute to cancer progression[81]. Inflammation enhances the production of cytokines that drive carcinogenesis such as IL-6, IL-1β and NF-κB.

NF-κB plays a vital role in driving inflammation, oxidative stress, regulation of apoptosis, cancer cell invasion, migration and metastasis[75,82,83]. Toll-like receptors (TLR) signaling microbes, tissue damage, or primary cytokines activate NF-κB that then triggers the production of multiple pro-inflammatory cytokines including nitric oxide (NO) synthase, prostaglandin synthesis enzymes as well as pro-angiogenic and

pro-tumorigenic mediators. TNF-α stimulates anti-apoptotic signals resulting in the avoidance of cell death[84].

IL-6-induced chronic inflammation also triggers STAT3/NF-κB signaling which plays an important role in various cancer related inflammatory processes, oxidative stress and evasion of apoptosis[85]. In addition to inflammation and oxidative stress, NF-κB plays a larger role in the avoidance of apoptosis, which permits cancer cells to evade death while also allowing non-cancer cells to accumulate DNA damage, mutations, and increased compensatory proliferation[86].

A recent study in a mouse model showed that artesunate attenuated the haemorrhagic shock-induced activation of NF-κB and reduced the expression of pro-inflammatory proteins including NO, TNF-α and IL-6, protecting against multi-organ failure[5]. This may also have implications for the management of oncological patients in the pre and post surgical setting.

The anti-inflammatory effects of artesunate have also been investigated in animal models of myocardial ischaemia, acute lung injury and nephritis[87,88,89,90]. In a rat model of transient myocardial ischemia, artesunate conferred myocardial protection via inhibition of glycogen synthase kinase- 3β, inhibition of NF-κB, activation of endothelial NOS, activation of the STAT3 (SAFE) pathway and activation of the PI3K/Akt/ERK 1/2 (RISK) pathway[87]. In a lung sepsis model, artesunate inhibited release of lipopolysaccharide/endotoxin(LPS)-induced IL-6 and TNF-α from bone marrow-derived monocytes, peritoneal macrophages and a RAW264.7 mouse cell line[68]. In a rat model of nephritis, artesunate conferred protection against renal failure by attenuating levels of IL-6, TNF-α, TGF-β1, TLR4, and NF-κB expression[89]. In

another rat model, artesunate inhibited renal reperfusion-stimulated lung inflammation by attenuating serum and pulmonary IL-6, MIP-2, PGE_2, NO and MDA levels and activating the HO-1 pathway[90].

Findings from these pump priming studies exploring the anti-inflammatory effects of artesunate in a variety of clinical conditions should be used to inform the careful design of robust mechanism of action studies for cancer.

Future perspectives: strategies to enhance artesunate cytotoxicity

A number of research groups have explored the potential for synergy between artesunate and other molecular compounds and drugs as part of novel combination therapy. As previously discussed, artesunate has been shown to promiscuously target multiple critical biological pathways and over 300 specific artesunate targets using a chemical proteomics approach with artemisinin-based activity probes[33]. Cellular functions associated with these target proteins include growth and proliferation, cell death and survival, protein synthesis, fatty acid metabolism, cellular movement, free radical scavenging and energy metabolism. Differential cytotoxicity of artesunate against colorectal cancer (CRC) cells compared to normal colon epithelial cells has been linked to the increased capacity of cancer cells to synthesise heme. The addition of heme synthesis precursor aminolevulinic acid (ALA) to artesunate treatment was found to dramatically enhance cytotoxicity in a mouse xenograft CRC model[33]. The ability of artemisinins to target multiple cancer pathways may allow this class of molecules to target various cancer types as well as evade chemoresistance[52].

Artesunate combination therapy with other existing established anticancer therapies has also shown synergistic effects in a number of cancer cell line models[52]. Artesunate synergistically enhanced the inhibitory effect of tyrosine kinase inhibitor sorafenib on cell growth in HepG2 and Huh7 hepatocellular cancer cells lines[91]. Artesunate in combination with selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor erlotinib lead to additive or synergistic inhibition of cell growth across a range of glioblastoma multiforme cancer cell lines[92]. Combination therapy with paclitaxel and artesunate have also shown therapeutic synergy in breast cancer cell lines[93]. Artesunate has also shown ability to restore sensitivity of castrate resistant prostate cancer cell lines and mouse models to anti-androgen therapy[94]. Tamoxifen–artemisinin and estradiol-artemisinin based hybrids have also shown anticancer activity against breast and prostate cancer cell lines[95]. Artemisinin compounds have displayed anticancer activity in cancer cell lines resistant to conventional chemotherapies such as doxorubicin, hydroxyurea and methotrexate without developing cross resistance[96]. Artemisinin activity does not appear to be influenced by drug resistance related genes such as P-gp, multidrug resistance-associated protein 1 (MRP1) and breast cancer resistance protein (BCRP)[97]. In cisplatin-resistant ovarian cancer cells, combination therapy with cisplatin and DHA induced cancer cell autophagy and resulted in an enhanced antiproliferation[98].

Target versatility combined with a high specificity for cancer cells, a low toxicity profile and the absence of cross-resistance to conventional chemotherapeutic agents suggests artesunate as a suitable candidate for novel combination anticancer therapy.

Clinical translational studies of artemisinins as anti-cancer therapies

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One artemisinin in particular, artesunate, has been deployed in a number of clinical translational studies. Artesunate has a hemisuccinate group which confers substantial water-solubility and high oral bioavailability and therefore a convenient oral route of administration. Artesunate is extensively hydrolysed by plasma esterases and possibly CYP2A6. Its main metabolite, DHA is metabolised through glucuronidation. The plasma elimination half-life of artesunate is 3-29 minutes whilst its active metabolite DHA has a plasma elimination half-life of 40 to 95 minutes[99].

In a Phase II trial in advanced non-small cell lung cancer, patients were treated with standard combination vinorelbine and cisplatin chemotherapy +/- intravenous artesunate (120 mg OD IV) for 8 days[100]. Each treatment group consisted of 60 patients. The rate of disease control rate in the trial group (88.2%) was significantly higher than that of the control group (72.7%) ($p<0.05$). Progression free survival was 24 weeks in patients treated with artesunate compared to 20 weeks in the control arm ($p<0.05$). Artesunate was well tolerated and significant differences in toxicity was observed between the two groups.

In a Phase I pilot study of advanced cervical cancer, 10 patients with Stage III or IV cervical cancer were treated with dihydroartemisinin 200mg once a day for 28 days[101]. Patients reported symptomatic clinical benefit in terms of alleviation of vaginal discharge and pain and treatment was well tolerated. On immunohistochemical staining, tumour blocks showed a reduction in expression of p53, Epidermal growth factor receptor (EGFR) and antigen Ki-67.

A phase I study of intravenous artesunate in patients with advanced solid tumor malignancies enrolled 19 patients in an accelerated titration dose escalation study with planned dose levels of 8, 12, 18, 25, 34 and 45 mg/kg given on days 1 and 8 of a 21-day cycle[102]. The maximum tolerated dose (MTD) in this study was determined to be 18 mg/kg. Four patients had stable disease, including three with prolonged stable disease for 8, 10, and 11 cycles, with a disease control rate of 27%.

A Phase I dose-escalation study of artesunate vaginal inserts in biopsy-confirmed Cervical Intraepithelia Neoplasia (CIN) 2/3 conducted in 28 patients showed that treatment was safe and well tolerated[103]. Patients with CIN 2/3 received 1, 2, or 3 five-day treatment cycles at study weeks 0, 2, and 4, respectively, prior to a planned, standard-of-care resection at study week 15. No serious adverse reactions were reported. In the modified intention-to-treat analysis histologic regression was observed in 19/28 (67.9%) subjects. In patients whose lesions underwent histologic regression, clearance of HPV genotypes detected at baseline occurred in 9 of the 19 (47.4%) subjects.

The ARTIC M33/2 reported long term results from compassionate open-label use of oral artesunate 100, 150 or 200 mg daily as add-on therapy to individual patients guideline-based oncological therapy for breast cancer[104]. In Phase I of the trial, 23 patients received artesunate therapy for 4 ± 1 weeks. Following this, thirteen patients continued the add-on therapy as compassionate use. A cumulative total of 3825 treatment days were reported as a result. In individual patients up to 1115 cumulative treatment days (37 months) and cumulative artesunate doses up to 167.3 g were reached. Twenty five adverse events graded ≥ 2 and at least ‘possibly related’ to

artesunate long-term add-on therapy were documented. However no major safety concerns were reported.

We conducted the first pilot randomised, double-blind, placebo-controlled trial in 20 patients with operable colorectal cancer and demonstrated that a 2 week course of neoadjuvant artesunate was safe, well tolerated, resulted in a reduction in Ki67 tumour proliferation index and conferred a potential survival advantage[105]. Two patients experienced Grade 3 neutropenia. Both cases were uncomplicated, with 1 case resolving spontaneously and 1 resolving with Granulocyte Colony Stimulating Factor (GCSF). Of note, both patients were of low body weight limit 50kg in the pilot study (dose limiting side effects of artesunate on bone marrow suppression seen at doses above 4mg/kg). These results provided the basis for a Phase II randomised, double blind, placebo controlled trial of neoadjuvant artesunate versus placebo for 14 days in 200 patients (NeoART) (https://clinicaltrials.gov/ct2/show/NCT02633098) which is currently recruiting. Our primary end point is recurrence free survival 2 years after surgery. In the current Phase II NeoART study, we have set the lower limit in terms of body weight at 52kg to reduce the risk of neutropenia. We also review patients on Day 7 and Day 14 with a toxicity check and full blood count test to monitor for neutropenia. A mirror study is also open to recruitment in Vietnam to test the potential effects of artesunate in a different ethnic and genetically diverse population (NeoART-V) (https://clinicaltrials.gov/ct2/show/NCT03093129).

Conclusion

In summary, artemisinins have an excellent safety and tolerability profile, having been used to treat tens of millions of adults and children globally for malaria. This class of

drugs are off patent and affordable at USD1 per dose, thereby representing a feasible treatment option for patients in LMICs. An artemisinin drug repurposing programme for a variety of cancer types and clinical settings as well as combination therapies from pump priming window studies to Phase III clinical trials is urgently needed. This includes neoadjuvant, adjuvant and combination therapy, strategies for re-sensitisation of drug resistant cancers and radiosensitisation. Mechanism of action studies deploying proteomics, metabolomics, genomics and immunology studies will be central to understanding how artemisinins exert their anticancer effects in different cellular environments and enable optimal deployment from bench to bedside. Once new anti-cancer indications for existing drugs are confirmed through robust clinical trials established drug production pipelines must then be ready for licensing, technology transfer and local manufacturing in partner LMICs.

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Figure

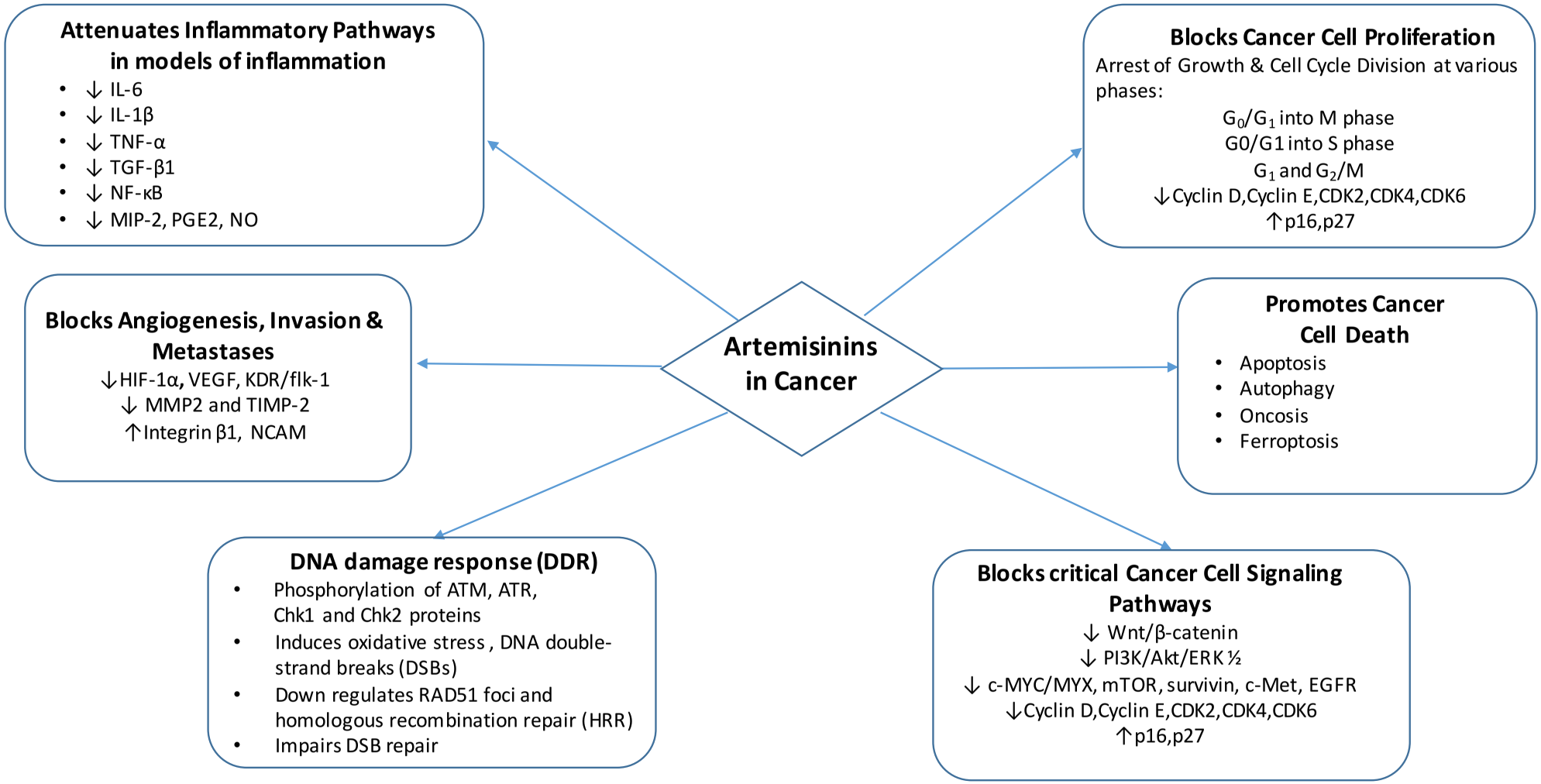

Figure 1: Artemisinins: multiple modes of action against cancer

CDK2/4/6; Cyclin dependent kinase 2/4/6:mTOR; mammalian target of rapamycin: EGFR;epidermal growth factor: HIF-1α; Hypoxia Inducible Factor 1 alpha: VEGF; Vascular endothelial growth factor: KDR/flk-1;kinase insert domain receptor MMP2; Matrix Metallopeptidase 2: TIMP-2; Tissue inhibitor of metalloproteinases 2: NCAM; Neural Cell Adhesion Molecule: IL-6;interleukin-6: IL-1β; Interleukin-1β: TNF-α; Tumour necrosis factor-α: TGF-β1; Transforming growth factor beta 1: NF-κB; nuclear factor kappa-light-chain-enhancer of activated B cells: MIP-2; Macrophage Inflammatory Protein 2: PGE2; Prostaglandin E_2 : NO;Nitric Oxide

Table

Country	Study Design	Dosing Regimen	Study Population	Outcome
China	Phase II open label randomised controlled trial	Vinorelbine and cisplatin chemotherapy +/- intravenous artesunate (120 mg OD IV) for 8 days[100]	• Metastatic Non Small cell Lung Cancer • 120 patients (60 in each arm)	The rate of disease control rate in the trial group (88.2%) vs control group (72.7%) ($p<0.05$). Progression free survival 24 weeks in artesunate treatment group compared to 20 weeks in the control arm ($p<0.05$).
Ivory Coast	Phase I open label pilot study	In a Phase I pilot study of advanced cervical cancer, 10 patients with were treated with Dihydroartemisinin (DHA) for 28 days[101]	• Stage III or IV cervical cancer • 10 patients	Patients reported symptomatic clinical benefit in terms of alleviation of vaginal discharge and pain. No G3/4 toxcities related to treatment recorded. Immunohistochemistry - tumour blocks showed a reduction in expression of p53, Epidermal growth factor receptor (EGFR), and antigen Ki-67.
United States	Phase I open label dose escalation study	Accelerated titration dose escalation study of IV artesunate with planned dose levels of 8, 12, 18, 25, 34 and 45 mg/kg given on days 1 and 8 of a 21-day cycle[102]	Stage IV solid tumour malignancies	The maximum tolerated dose (MTD) in this study was determined to be 18 mg/kg. Four patients had stable disease, including three with prolonged stable disease for 8, 10, and 11 cycles, with a disease control rate of 27%.
United States	A Phase I dose-escalation study	Patients with CIN 2/3 received 1, 2, or 3 five-day treatment cycles (artesunate vaginal inserts) at study weeks 0, 2, and 4, respectively, prior to a planned, standard-of-care resection at study week 15[103]	• Cervical Intraepithelia Neoplasia (CIN)2/3 • 28 patients	No serious adverse reactions were reported. Modified intention-to-treat analysis histologic regression 19/28 (67.9%) subjects. In patients whose lesions underwent histologic regression, clearance of HPV genotypes detected at baseline occurred in 9 of the 19 (47.4%) subjects.
Germany	Phase I open-label study	Oral artesunate 100, 150 or 200 mg daily as add-on therapy to 23 individual patients guideline-based oncological therapy for breast cancer for 4 ± 1 weeks. Following this, 13 patients continued the add-on therapy as compassionate use [104]	• Metastatic Breast Cancer • 23 patients	No safety concerns. Cumulative total of 3825 treatment days were reported as a result. In individual patients up to 1115 cumulative treatment days (37 months) and cumulative artesunate doses up to 167.3 g were reached.
United Kingdom	Phase I randomised, double blind, placebo controlled trial	Neoadjuvant artesunate versus placebo for 14 days prior to surgery[105]	• Stage 1- 3 Colorectal Cancer • 20 patients	Neoadjuvant artesunate was safe, well tolerated, resulted in a reduction in Ki67 tumour proliferation index and conferred a potential survival advantage

Table 1: Clinical Trials of Artemisinins in Cancer