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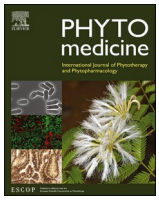

Short communication

Artesunate in glioblastoma therapy: Case reports and review of clinical studies

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ABSTRACT

Background: Artesunate, a derivative of the active ingredient artemisinin from *Artemisia annua* L. used for centuries in the traditional Chinese medicine, is being applied as front-line drug in malaria treatment. As it is cytotoxic for cancer cells, trials are ongoing to include this drug as supplement in cancer therapy. In glioblastoma cells, artesunate was shown to induce oxidative stress, DNA base damage and double-strand breaks (DSBs), apoptosis, and necroptosis. It also inhibits DNA repair functions and bears senolytic activity. Compared to ionizing radiation, DNA damages accumulate over the whole exposure period, which makes the agent unique in its genotoxic profile. Artesunate has been used in adjuvant therapy of various cancers.

Purpose: As artesunate has been used in adjuvant therapy of different types of cancer and clinical trials are lacking in brain cancer, we investigated its activity in glioma patients with focus on possible side effects.

Study design: Between 2014 and 2020, twelve patients were treated with artesunate for relapsing glioma and analyzed retrospectively: 8 males and 4 females, median age 45 years. Histology: 4 glioblastomas WHO grade 4, 5 astrocytomas WHO grade 3, 3 oligodendrogliomas grade 2 or 3. All patients were pretreated with radiation and temozolomide-based chemotherapy. Artesunate 100 mg was applied twice daily p.o. combined with dose-dense temozolomide alone (100 mg/m^2 day 1–5/7, 10 patients) or with temozolomide (50 mg/m^2 day 1–5/7) plus lomustine (CCNU, 40 mg day 6/7). Blood count, C-reactive protein (CRP), liver enzymes, and renal parameters were monitored weekly.

Results: Apart from one transient grade 3 hematological toxicity, artesunate was well tolerated. No liver toxicity was observed. While 8 patients with late stage of the disease had a median survival of 5 months after initiation of artesunate treatment, 4 patients with treatment for remission maintenance showed a median survival of 46 months. We also review clinical trials that have been performed in other cancers where artesunate was included in the treatment regimen.

Conclusions: Artesunate administered at a dose of 2 × 100 mg/day was without harmful side effects, even if combined with alkylating agents used in glioma therapy. Thus, the phytochemical, which is also utilized as food supplement, is an interesting, well tolerated supportive agent useful for long-term maintenance treatment. Being itself cytotoxic on glioblastoma cells and enhancing the cytotoxicity of temozolomide as well as in view of its senolytic activity, artesunate has clearly a potential to enhance the efficacy of malignant brain cancer therapy.

Introduction

Glioblastoma (GBM) is the most common and aggressive malignant brain tumor. To date, it remains incurable with a median survival of 15 months and a 5-year survival rate of 7.2% (Ostrom et al., 2020). Standard of care consists of surgical resection followed by radiation therapy concomitant with temozolomide (TMZ) and followed by adjuvant TMZ. This GBM gold standard treatment remained unchanged since 2005 (Stupp et al., 2005), although the therapeutic outcome is unfavorable. In the first and second recurrent situation, different protocols are used (Wen et al., 2020), but none of them is approved. The dismal prognosis of GBM calls for new and supplementary therapeutic strategies.

Abbreviations: DSBs, DNA double-strand breaks; TCM, traditional Chinese medicine; TMZ, temozolomide.

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One possible therapeutic option is based on the inclusion of natural substances with anticancer activity. A promising candidate is artesunate, which is a derivative of artemisinin, the active ingredient of *Artemisia annua* l. (sweet wormwood). Cold press extracts of this Chinese medicinal plant have been used for centuries in the traditional Chinese medicine (TCM) for the treatment of fever and infections (Efferth and Kaina, 2010). The anti-malarial activity of artemisinin has been discovered in the 1970s (Tu et al., 1981). Its semi-synthetic derivatives artesunate, dihydroartemisinin, and artemether are clinically established as components of artemisinin-combination therapies (Tu, 2016). The activity of artemisinin derivatives against trypanosomiasis, schistosomiasis, and several viral diseases has also been reported (Efferth, 2018; Nass and Efferth, 2018; Saeed et al., 2016). In the middle of the 1990s, several groups published that artemisinin and its derivatives are toxic on tumor cells (Efferth et al., 1996; Lai and Singh, 1995; Tu et al., 1981), which was later on confirmed on different cancer cell systems *in vitro* and *in vivo* (Berdelle et al., 2011; Dell'Eva et al., 2004; Efferth, 2005; Efferth et al., 2001).

The mechanism of artesunate is well described. The compound becomes activated upon uptake by the cells. The active chemical moiety is an endoperoxide bridge, which is cleaved in the presence of ferrous iron by a Fenton-type reaction, leading to the generation of reactive oxygen species (ROS) (Berman and Adams, 1997) as well as carbon-centered radical molecules (Meshnick et al., 1993). These endogenously formed reactive species attack proteins, RNA and DNA, inducing oxidative DNA lesions such as 8-oxo-guanine and DNA double-strand breaks (DSBs) (Li et al., 2008), which was confirmed in glioblastoma cells (Berdelle et al., 2011). Although the damage is subject to repair via specific pathways (Berdelle et al., 2011; Li et al., 2008), in the presence of artesunate DSBs do accumulate and finally activate the ATM/ATR axis and DNA damage dependent cellular functions that trigger cell cycle inhibition and death pathways (Berdelle et al., 2011).

As to the cellular responses, artemisinin-type drugs arrest the cell cycle (Chen et al., 2014; Steinbruck et al., 2010) and induce not only iron-dependent cell death (ferroptosis) (Eling et al., 2015; Ooko et al., 2015; Song et al., 2022) but also other modes of cell death such as apoptosis (Berdelle et al., 2011; Efferth et al., 2008, 2004; Wu et al., 2009), which was enhanced in glioblastoma cells in the presence of iron (Berdelle et al., 2011). Artesunate is also an inducer of autophagy (Chen et al., 2014). A clinically relevant aspect is that artemisinin-type drugs exert additive and synergistic interactions with many types of chemotherapeutic drugs and radiotherapy (Efferth, 2017; Reichert et al., 2012). Importantly, artesunate enhances the therapeutic effect of temozolomide, which was experimentally shown in glioblastoma cell lines, glioblastoma stem-like cells and in a mouse tumor model (Berte et al., 2016; Karpel-Massler et al., 2014). Thus, the drug downregulates RAD51 expression resulting in inhibition of homologous recombination (HR), and inhibits the TMZ-induced cellular senescence, both are key survival mechanisms (Berte et al., 2016). Artesunate was also shown to exert senolytic activity on glioblastoma cells (Beltzig et al., 2022a), which is important as senescence is the main trait triggered by temozolomide (Beltzig et al., 2022b), and senescent cells are thought to drive tumorigenesis through the senescence-associated secretory phenotype (SASP) (Demaria et al., 2017), which plays an emerging role in glioblastoma (Chojak et al., 2023). Recently it was shown that artesunate exerts antiproliferative effects by activating the ROS-triggered AMPK-mTOR axis (Zhou et al., 2020), and combined with a low dose of metformin it reduced the viability, migration and invasion capacity of glioblastoma cells (Ding et al., 2023). This is important to note as metformin induces ROS and apoptosis and ameliorates the cytotoxic effect of temozolomide in some glioblastoma cell lines (Feng et al., 2022). Metformin together with lovastatin also reduced tumor growth in a xenograft model and was associated with an extension of the overall survival of glioblastoma patients (Fuentes-Fayos et al., 2023). Therefore, artesunate might be considered as an enhancer of anticancer activity of these repurposed drugs. The anticancer effects of artemisinin-type drugs in human glioblastoma models (Berte et al., 2016; Lemke et al., 2016) and the benefit in co-exposure experiments raises the hope that this class of phytochemicals might represent a new option to improve the treatment success of glioma.

Currently, several trials are going on to assess the toxicity and therapeutic activity of artesunate in different diseases. For glioblastoma, data are lacking. Here, we report our experience obtained in 12 patients with recurrent glioma treated with artesunate, focusing on possible side effects. We show that artesunate is well tolerated and useful, together with temozolomide, for the treatment of recurrent malignant glioma grade 3 and 4.

Patients and treatments

Between 2014 and 2020, 12 patients were treated with artesunate for relapsing glioma. The median age was 45 years; 8 patients were male and 4 were female. Four patients suffered from glioblastoma WHO grade 4, five from astrocytoma WHO grade 3 and three from grade 2 or 3 oligodendroglioma. All patients were pretreated with radiation and chemotherapy with temozolomide in standard regimen and was carried out as compassionate use at the patient's request.

The treatment consisted of artesunate 100 mg twice daily p.o. (total 200 mg/day) in combination with dose-dense temozolomide alone (100 mg/m^2 day 1–5/7, 10 patients; (Strik et al., 2008) or with temozolomide 50 mg/m^2 day 1–5/7 and lomustine (CCNU) 40 mg absolute dose day 6/7 (Strik et al., 2012). Artesunate as a drug was obtained from Dafra Pharma (Turnhout, Belgium) as tablets containing 100 mg each. Patients were examined clinically at least monthly, laboratory controls including blood count, CRP, transaminases and kreatinine were taken weekly.

Results and discussion

Patients were treated for 3–72 months with artesunate, which was applied up to 12 months in combination with TMZ/CCNU based chemotherapy. Two patients with glioblastoma received long term artesunate monotherapy (20 and 72 months) as maintenance treatment after resection of the first recurrence. The median observation time was 5.5 (3 - 72) months. In all but one patient, no toxic side effects, including liver toxicity, were observed even after long-term treatment (up to 6 years). Only one female patient experienced grade 3 hematological toxicity during artesunate therapy combined with temozolomide and artesunate alone, with a leukopenia of minimal 800 leucocytes/µl that recovered well after interruption of artesunate treatment. At rechallenge with artesunate alone, white blood cell counts dropped again under 2.000, which led us in this case to discontinue artesunate treatment permanently.

The median survival time from start of treatment was 5.5 months, with a wide range of individual responses (3–81 months). Eight of the patients were treated at a late stage of the disease and had a median survival time of 4 months. Four of the patients (1 astrocytoma, 1 oligodendroglioma WHO grade 3, 2 glioblastomas WHO grade 4) were treated for remission maintenance and had a median survival of 46 (12–81+) months from start of treatment. At time of publication, one patient suffering from a grade 3 astrocytoma is still alive (105 month after diagnosis) and in good clinical condition, but with histologically proven low-grade recurrence opposite to the original manifestation. The median survival of the glioblastoma patients cotreated with artesunate in the present study was 13.1 months after relapse. For comparison, in a previous series of consecutive patients treated with dose-dense temozolomide alone, the median survival of patients was 9.1 months after relapse (Strik et al., 2008). These patients were treated mainly at first relapse, and no long-term survival was observed. Of note, the low number of patients and heterogeneous trials does not make statistical analysis useful.

Overall, the data presented in this compilation of case reports indicates that artesunate is well tolerated. Only one case of pronounced

hematotoxicity was observed, which normalized rapidly after interruption of treatment. In contrast to a previous case report (Efferth et al., 2017), no liver toxicity was observed even if treatment was applied concomitantly with temozolomide ± CCNU. It should be noted that in the aforementioned report the patient received TMZ together with a cocktail of other drugs (including anticonvulsive medication) and Chinese herbs (*Coptis-Kush*) that were reported to cause severe hepatic toxicity also in monotherapy (Lu et al., 2017). In another report, liver and bone marrow toxicity were observed following combination treatment of dichloroacetate and artesunate in a glioblastoma patient and antiepileptic medication including valproic acid (Uhl et al., 2016), which can be toxic for liver and bone marrow. Nevertheless, care is advised if the drug is combined with dichloroacetate, which inhibits transketolase-like-1 enzyme. Moreover, brainstem encephalopathy was reported after treatment of breast cancer with artemisinin (Panossian et al., 2005). These case reports prompted us to investigate the tolerability of artesunate in compassionate use in glioma patients, with a favorable outcome. Thus, no liver and neurotoxicity were observed in our group of patients. We should note that artesunate is available over the counter as food supplement, which is consumed worldwide by a large human population and obviously well tolerated. In malaria therapy, initial artesunate is given intravenously at a dose of 2.4 mg/kg BW twice daily, followed by oral administration. It is also used in malaria prophylaxis (Zou et al., 2020).

It is important to note that artesunate administered orally is well absorbed and achieves high bioavailability (>80%), with serum peak concentrations within 1 h. It is eliminated with a half-life of 20–45 min. After oral administration of 100 and 200 mg artesunate, blood peak concentrations in healthy volunteers were measured of 114 and 256 ng/ml, respectively (Morris et al., 2011). After administration of radioactively labeled artesunate, the drug was clearly found in the brain of rats (Li et al., 2006; Zhao and Song, 1989). The concentration that can be achieved in humans in the brain and notably in the tumor tissue is, to our best knowledge, unknown. We should note that artesunate is highly effective in the treatment of cerebral malaria (Clemmer et al., 2011), indicating that it penetrates the human brain well. It is conceivable that daily and long-term administration leads to an accumulation of artesunate-induced critical lesions (such as DNA damage) in the tumor tissue resulting in an antitumor response.

We are aware that our study is limited by the small number of patients, different histologies and WHO grades and the retrospective character of the investigation. Therefore, no valid analysis regarding the efficacy of the treatment is possible. The primary intention was ruling out the amicability and tolerance of treatment together with alkylating drugs. Interestingly, we observed some clinical courses with maintenance therapy that were more favorable than expected with a median survival of up to 46 months from start of treatment (WHO grade 4).

Conclusions

Artesunate has been investigated in a panel of clinical phase 1 and 2 trials in various tumor types other than glioblastoma during the past years, e.g., metastasized breast cancer, cervical carcinoma, and non-small cell lung cancer (Table 1), all of which are difficult to manage by conventional chemotherapy. In all cases, artesunate was administered together with other chemotherapeutics, which makes it difficult to assess possible side effects of artesunate precisely. Furthermore, a series of case reports on other tumor entities, stating that artemisinin

Table 1
Clinical phase 1 and phase 2 trials with artesunate.

Tumor type	No. of patients	Drug	Application	Results	Reference
Clinical phase 1 trials:					
Metastatic breast cancer	23	Artesunate	oral, 100–300 md/day for >3 weeks	Pharmacokinetics suggesting the use of saliva sampling for therapeutic drug monitoring of dihydroartemisinin	(Ericsson et al., 2014)
Metastatic breast cancer	23 verum, 11 placebo	Artesunate	200 mg/day for 14 days preoperatively	Single-center, randomized, double-blind, placebo-controlled, phase 2 trial. Follow up of 42 months: 1 relapse in the ART-treated group (= 4%) vs. 6 relapses in the placebo group (= 55%).	(Krishna et al., 2014)
Metastatic breast cancer	23	Artesunate	100–200 mg/day for 4 weeks as add on to guideline-based therapy	Single-center, randomized, double-blind, placebo-controlled phase 1 trial. 4 auditory system events, 4 vertigo (1 severe but reversible)	(König et al., 2016)
Metastatic breast cancer	23	Artesunate	100–200 mg/day for 4 weeks as add on to guideline-based therapy	3 patients experienced 6 dose-limiting adverse events altogether (leucopenia, neutropenia, asthenia, anemia) possibly related to ART (<33% at any dose level)	(Von Hagens et al., 2017)
		Artesunate	8–45 mg/kg	Phase 1 dose-escalation trial. Dose-limiting toxicities at 12 mg/kg (1 of 6 patients), 18 mg/kg (1 of 6) and 25 mg/kg (2 of 2): neutropenic fever (Gr 4), hypersensitivity reaction (Gr 3), liver function test abnormalities (Gr 3/4) along with neutropenic fever, and nausea/vomiting (Gr 3) despite supportive care. Maximal tolerated dose: 18 mg/kg. Efficacy: 4 stable diseases (including 3 with prolonged stable disease for 8, 10, and 11 cycles, for a disease control rate of 27%).	(Deeken et al., 2018)
Metastatic breast cancer	23	Artesunate	100–200 mg/day for 4 weeks as add on to guideline-based therapy	25 adverse events grade ≥ 2 at least possibly related to ART long-term add-on therapy	(Von Hagens et al., 2019)
Clinical phase 2 trials:					
Advanced non-small cell lung carcinoma	120	Artesunate	120 mg, once-a-day i.v., 1st −8th day for 8 days with/without vinorelbine (25 mg/m^2) and cisplatin (25 mg/m^2)	Median survival time: no difference; disease-control rate: ART-treated group (88.2%) vs. control group (72.7%) ($p < 0.05$); time-to-prgression: ART-treated group (24 weeks) vs. control group (20 weeks) ($p < 0.05$). Toxicity (myelosuppression, gastrointestinal side effects): no difference	(Zhang et al., 2008)
Cervical carcinoma	10	Artesunate	Oral, 100 mg/day for 2 weeks, 200 mg/day for further 3 weeks	Clincial symptoms (vaginal discharge, pain) disappeared with a median time of 7 days within a period of 3 weeks. The survival times was ina range of 2–24 months (average survival in the African context: 4 months)	(Jansen et al., 2011)
Advanced cervial carcinoma	28	Artesunate	Topical, intravaginal. 1–3 5-day treatment cycles at study weeks 0, 2, and 4	19/28 (67.9%) histological tumor regressions; 9/19 (47.4%) HPC genotype clearances	(Trimble et al., 2020)

treatment was beneficial in improving the patient's quality of life, can be taken as supportive information for a possible clinical potential of artemisinin-type drugs (Berger et al., 2005; Michaelsen et al., 2015; Singh and Panwar, 2006).

We should note that, like humans, animals also develop spontaneous cancer. Therefore, veterinary tumor studies may serve as valuable comparisons for human oncology (Kwon et al., 2023; Oh and Cho, 2023). Artemisinin-type drugs have been studied to cure spontaneously occurring tumors in dogs and cats (Kwon et al., 2023; Rutteman et al., 2013). In general, the toxicities observed were rather mild and severe adverse events were rare. The therapeutic efficacy against tumors was moderate to strong and deserves closer observations on more cases in the future. To our opinion, a synopsis of the clinical data published in the literature (Table 1) and the data reported in the present investigation suggest that artesunate is worth to be investigated in large clinical 2–3 trials, including dose escalation, to come to more decisive conclusions as to the potential as chemotherapeutic drug to fight cancer.

Overall, our small series of compassionate use points out that artesunate treatment of malignant glioma is feasible and well tolerated by the patients. The observed single favorable clinical courses indicate a certain efficacy that appears interesting enough to be proved in a larger, prospective study. Such a study should take into account the findings that the apoptotic effect of alkylating agents is enhanced by the drug's ability to downregulate HR (Berte et al., 2016), that artesunate itself is cytotoxic and, moreover, has senolytic activity in temozolomide-induced senescent glioblastoma cells (Beltzig et al., 2022a). These preclinical findings favor the use of artesunate combined with radiotherapy plus temozolomide, or in intervals (hit-and-run) during/after alkylating agent therapy, harnessing its senolytic potential. Thus, administering artesunate together with temozolomide or as monotherapy in an adjuvant setting in the primary and recurrent situation might be a reasonable, beneficial therapeutic option.

CRediT authorship contribution statement

Herwig Strik: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – review & editing. **Thomas Efferth:** Formal analysis, Methodology, Validation, Writing – review & editing. **Bernd Kaina:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Validation, Writing – original draft.

Declaration of Competing Interest

Authors declare there is no conflict of interes

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References

Beltzig, L., Christmann, M., Kaina, B., 2022a. Abrogation of cellular senescence induced by Temozolomide in Glioblastoma cells: search for Senolytics. Cells 11.
Beltzig, L., Schwarzenbach, C., Leukel, P., Frauenknecht, K.B.M., Sommer, C., Tancredi, A., Hegi, M.E., Christmann, M., Kaina, B., 2022b. Senescence is the main trait induced by Temozolomide in Glioblastoma cells. Cancers 14.
Berdelle, N., Nikolova, T., Quiros, S., Efferth, T., Kaina, B., 2011. Artesunate induces oxidative DNA damage, sustained DNA double-strand breaks, and the ATM/ATR damage response in cancer cells. Mol Cancer Ther 10, 2224–2233.
Berger, T.G., Dieckmann, D., Efferth, T., Schultz, E.S., Funk, J.O., Baur, A., Schuler, G., 2005. Artesunate in the treatment of metastatic uveal melanoma–first experiences. Oncol Rep 14, 1599–1603.
Berman, P.A., Adams, P.A., 1997. Artemisinin enhances heme-catalysed oxidation of lipid membranes. Free Radic. Biol. Med 22, 1283–1288.
Berte, N., Lokan, S., Eich, M., Kim, E., Kaina, B., 2016. Artesunate enhances the therapeutic response of glioma cells to temozolomide by inhibition of homologous recombination and senescence. Oncotarget 7, 67235–67250.
Chen, K., Shou, L.M., Lin, F., Duan, W.M., Wu, M.Y., Xie, X., Xie, Y.F., Li, W., Tao, M., 2014. Artesunate induces G2/M cell cycle arrest through autophagy induction in breast cancer cells. Antican. Drugs 25, 652–662.
Chojak, R., Fares, J., Petrosyan, E., Lesniak, M.S., 2023. Cellular senescence in glioma. J. Neurooncol 164, 11–29.
Clemmer, L., Martins, Y.C., Zanini, G.M., Frangos, J.A., Carvalho, L.J., 2011. Artemether and artesunate show the highest efficacies in rescuing mice with late-stage cerebral malaria and rapidly decrease leukocyte accumulation in the brain. Antimicrob. Agents Chemother 55, 1383–1390.
Deeken, J.F., Wang, H., Hartley, M., Cheema, A.K., Smaglo, B., Hwang, J.J., He, A.R., Weiner, L.M., Marshall, J.L., Giaccone, G., Liu, S., Luecht, J., Spiegel, J.Y., Pishvaian, M.J., 2018. A phase I study of intravenous artesunate in patients with advanced solid tumor malignancies. Cancer Chemother. Pharmacol. 81 (3), 587–596.
Dell'Eva, R., Pfeffer, U., Vene, R., Anfosso, L., Forlani, A., Albini, A., Efferth, T., 2004. Inhibition of angiogenesis *in vivo* and growth of Kaposi's sarcoma xenograft tumors by the anti-malarial artesunate. Biochem. Pharmacol 68, 2359–2366.
Demaria, M., O'Leary, M.N., Chang, J., Shao, L., Liu, S., Alimirah, F., Koenig, K., Le, C., Mitin, N., Deal, A.M., Alston, S., Academia, E.C., Kilmarx, S., Valdovinos, A., Wang, B., de Bruin, A., Kennedy, B.K., Melov, S., Zhou, D., Sharpless, N.E., Muss, H., Campisi, J., 2017. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. Cancer Disc. 7, 165–176.
Ding, W., Liao, L., Liu, J., Zhao, J., Tang, Q., Liao, Y., 2023. Lower dose of metformin combined with artesunate induced autophagy-dependent apoptosis of glioblastoma by activating ROS-AMPK-mTOR axis. Exp Cell Res 430, 113691.
Efferth, T., 2005. Mechanistic perspectives for 1,2,4-trioxanes in anti-cancer therapy. Drug Resist Updat 8, 85–97.
Efferth, T., 2017. Cancer combination therapies with artemisinin-type drugs. Biochem. Pharmacol 139, 56–70.
Efferth, T., 2018. Beyond malaria: the inhibition of viruses by artemisinin-type compounds. Biotechnol. Adv. 36, 1730–1737.
Efferth, T., Dunstan, H., Sauerbrey, A., Miyachi, H., Chitambar, C.R., 2001. The anti-malarial artesunate is also active against cancer. Int. J. Oncol 18, 767–773.
Efferth, T., Fabry, U., Osieka, R., 1996. Anti-Fas/Apo-1 monoclonal antibody CH-11 depletes glutathione and kills multidrug-resistant human leukemic cells. Blood Cells Mol Dis 22, 2–9 discussion 10.
Efferth, T., Kahl, S., Paulus, K., Adams, M., Rauh, R., Boechzelt, H., Hao, X., Kaina, B., Bauer, R., 2008. Phytochemistry and pharmacogenomics of natural products derived from traditional Chinese medicine and Chinese materia medica with activity against tumor cells. Mol. Cancer Ther 7, 152–161.
Efferth, T., Kaina, B., 2010. Toxicity of the antimalarial artemisinin and its dervatives. Crit. Rev. Toxicol 40, 405–421.
Efferth, T., Ramirez, T., Gebhart, E., Halatsch, M.E., 2004. Combination treatment of glioblastoma multiforme cell lines with the anti-malarial artesunate and the epidermal growth factor receptor tyrosine kinase inhibitor OSI-774. Biochem. Pharmacol 67, 1689–1700.
Efferth, T., Schottler, U., Krishna, S., Schmiedek, P., Wenz, F., Giordano, F.A., 2017. Hepatotoxicity by combination treatment of temozolomide, artesunate and Chinese herbs in a glioblastoma multiforme patient: case report review of the literature. Arch. Toxicol 91, 1833–1846.
Eling, N., Reuter, L., Hazin, J., Hamacher-Brady, A., Brady, N.R., 2015. Identification of artesunate as a specific activator of ferroptosis in pancreatic cancer cells. Oncoscience 2, 517–532.
Ericsson, T., Blank, A., von Hagens, C., Ashton, M., Äbelö, A., 2014. Population pharmacokinetics of artesunate and dihydroartemisinin during long-term oral administration of artesunate to patients with metastatic breast cancer. Eur. J. Clin. Pharmacol. 70 (12), 1453–1463.
Feng, S.W., Chang, P.C., Chen, H.Y., Hueng, D.Y., Li, Y.F., Huang, S.M., 2022. Exploring the mechanism of adjuvant treatment of glioblastoma using temozolomide and metformin. Int. J. Mol. Sci 23, 8171.
Fuentes-Fayos, A.C., ME, G.G., Perez-Gomez, J.M., Montero-Hidalgo, A.J., Martin-Colom, J., Doval-Rosa, C., Blanco-Acevedo, C., Torres, E., Toledano-Delgado, A., Sanchez-Sanchez, R., Peralbo-Santaella, E., Ortega-Salas, R.M., Jimenez-Vacas, J.M., Tena-Sempere, M., Lopez, M., Castano, J.P., Gahete, M.D., Solivera, J., Luque, R.M., 2023. Metformin and simvastatin exert additive antitumour effects in glioblastoma via senescence-state: clinical and translational evidence. EBioMedicine 90, 104484.
Jansen, F.H., Adoubi, I., J C, K.C., Cnodder, T.DE., Jansen, N., Tschulakow, A., Efferth, T., 2011. First study of oral Artenimol-R in advanced cervical cancer: clinical benefit, tolerability and tumor markers. Anticancer Res. 31, 4417–4422.
Karpel-Massler, G., Westhoff, M.A., Kast, R.E., Dwucet, A., Nonnenmacher, L., Wirtz, C. R., Debatin, K.M., Halatsch, M.E., 2014. Artesunate enhances the antiproliferative effect of temozolomide on U87MG and A172 glioblastoma cell lines. Anti-Cancer Agents Med. Chem 14, 313–318.
König, M., von Hagens, C., Hoth, S., Baumann, I., Walter-Sack, I., Edler, L., Sertel, S., 2016. Investigation of ototoxicity of artesunate as add-on therapy in patients with metastatic or locally advanced breast cancer: new audiological results from a prospective, open, uncontrolled, monocentric phase I study. Cancer Chemother. Pharmacol. 77 (2), 413–427.
Krishna, S., Ganapathi, S., Ster, I.C., Saeed, M.E., Cowan, M., Finlayson, C., Kovacsevics, H., Jansen, H., Kremsner, P.G., Efferth, T., Kumar, D., 2014. A Randomised, Double Blind, Placebo-Controlled Pilot Study of Oral Artesunate Therapy for Colorectal Cancer. EBioMedicine 2 (1), 82–90.

Kwon, J.Y., Moskwa, N., Kang, W., Fan, T.M., Lee, C., 2023. Canine as a comparative and translational model for human mammary tumor. J. Breast Cancer 26, 1–13.
Lai, H., Singh, N.P., 1995. Selective cancer cell cytotoxicity from exposure to dihydroartemisinin and holotransferrin. Cancer Lett 91, 41–46.
Lemke, D., Pledl, H.W., Zorn, M., Jugold, M., Green, E., Blaes, J., Low, S., Hertenstein, A., Ott, M., Sahm, F., Steffen, A.C., Weiler, M., Winkler, F., Platten, M., Dong, Z., Wick, W., 2016. Slowing down glioblastoma progression in mice by running or the anti-malarial drug dihydroartemisinin? Induction of oxidative stress in murine glioblastoma therapy. Oncotarget 7, 56713–56725.
Li, P.C., Lam, E., Roos, W.P., Zdzienicka, M.Z., Kaina, B., Efferth, T., 2008. Artesunate derived from traditional Chinese medicine induces DNA damage and repair. Cancer Res 68, 4347–4351.
Li, Q., Xie, L.H., Haeberle, A., Zhang, J., Weina, P., 2006. The evaluation of radiolabeled artesunate on tissue distribution in rats and protein binding in humans. Am J. Trop. Med. Hygiene 75, 817–826.
Lu, H., Sheng, R., Zhang, C., Lee, T.Y., 2017. Comments regarding "Hepatotoxicity by combination treatment of temozolomide, artesunate and Chinese herbs in a glioblastoma multiforme patient: case report review of the literature". Arch. Toxicol 91, 2493–2494.
Meshnick, S.R., Yang, Y.Z., Lima, V., Kuypers, F., Kamchonwongpaisan, S., Yuthavong, Y., 1993. Iron-dependent free radical generation from the antimalarial agent artemisinin (qinghaosu). Antimicrob. Agents Chemother 37, 1108–1114.
Michaelsen, F.W., Saeed, M.E., Schwarzkopf, J., Efferth, T., 2015. Activity of Artemisia annua and artemisinin derivatives, in prostate carcinoma. Phytomedicine 22, 1223–1231.
Morris, C.A., Duparc, S., Borghini-Fuhrer, I., Jung, D., Shin, C.S., Fleckenstein, L., 2011. Review of the clinical pharmacokinetics of artesunate and its active metabolite dihydroartemisinin following intravenous, intramuscular, oral or rectal administration. Malaria J. 10, 263.
Nass, J., Efferth, T., 2018. The activity of Artemisia spp. and their constituents against Trypanosomiasis. Phytomedicine 47, 184–191.
Oh, J.H., Cho, J.Y., 2023. Comparative oncology: overcoming human cancer through companion animal studies. Exp. Mol. Med 55, 725–734.
Ooko, E., Saeed, M.E., Kadioglu, O., Sarvi, S., Colak, M., Elmasaoudi, K., Janah, R., Greten, H.J., Efferth, T., 2015. Artemisinin derivatives induce iron-dependent cell death (ferroptosis) in tumor cells. Phytomedicine 22, 1045–1054.
Ostrom, Q.T., Patil, N., Cioffi, G., Waite, K., Kruchko, C., Barnholtz-Sloan, J.S., 2020. CBTRUS Statistical Report: primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2013-2017. Neuro. Oncol 22 iv1-iv96.
Panossian, L.A., Garga, N.I., Pelletier, D., 2005. Toxic brainstem encephalopathy after artemisinin treatment for breast cancer. Ann. Neurol 58, 812–813.
Reichert, S., Reinboldt, V., Hehlgans, S., Efferth, T., Rodel, C., Rodel, F., 2012. A radiosensitizing effect of artesunate in glioblastoma cells is associated with a diminished expression of the inhibitor of apoptosis protein survivin. Radiother. Oncol 103, 394–401.
Rutteman, G.R., Erich, S.A., Mol, J.A., Spee, B., Grinwis, G.C., Fleckenstein, L., London, C.A., Efferth, T., 2013. Safety and efficacy field study of artesunate for dogs with non-resectable tumours. Anticancer Res 33, 1819–1827.
Saeed, M.E.M., Krishna, S., Greten, H.J., Kremsner, P.G., Efferth, T., 2016. Antischistosomal activity of artemisinin derivatives *in vivo* and in patients. Pharmacol. Res 110, 216–226.
Singh, N.P., Panwar, V.K., 2006. Case report of a pituitary macroadenoma treated with artemether. Integr. Cancer Ther 5, 391–394.
Song, Q., Peng, S., Che, F., Zhu, X., 2022. Artesunate induces ferroptosis via modulation of p38 and ERK signaling pathway in glioblastoma cells. J. Pharmacol. Sci 148, 300–306.
Steinbruck, L., Pereira, G., Efferth, T., 2010. Effects of artesunate on cytokinesis and G (2)/M cell cycle progression of tumour cells and budding yeast. Cancer Genom. Proteom. 7, 337–346.
Strik, H.M., Buhk, J.H., Wrede, A., Hoffmann, A.L., Bock, H.C., Christmann, M., Kaina, B., 2008. Rechallenge with temozolomide with different scheduling is effective in recurrent malignant gliomas. Molec. Med. Rep 1, 863–867.
Strik, H.M., Marosi, C., Kaina, B., Neyns, B., 2012. Temozolomide dosing regimens for glioma patients. Curr. Neurol. Neurosci. Rep 12, 286–293.
Stupp, R., Mason, W.P., van den Bent, M.J., Weller, M., Fisher, B., Taphoorn, M.J., Belanger, K., Brandes, A.A., Marosi, C., Bogdahn, U., Curschmann, J., Janzer, R.C., Ludwin, S.K., Gorlia, T., Allgeier, A., Lacombe, D., Cairncross, J.G., Eisenhauer, E., Mirimanoff, R.O., 2005. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl. J. Med 352, 987–996.
Trimble, C.L., Levinson, K., Maldonado, L., Donovan, M.J., Clark, K.T., Fu, J., Shay, M.E., Sauter, M.E., Sanders, S.A., Frantz, P.S., Plesa, M., 2020. A first-in-human proof-of-concept trial of intravaginal artesunate to treat cervical intraepithelial neoplasia 2/3 (CIN2/3). Gynecol Oncol. 157 (1), 188–194.
Tu, Y., 2016. Artemisinin-A gift from traditional Chinese medicine to the world (Nobel Lecture). Angew. Chem. Int. Ed. Engl 55, 10210–10226.
Tu, Y.Y., Ni, M.Y., Zhong, Y.R., Li, L.N., Cui, S.L., Zhang, M.Q., Wang, X.Z., Liang, X.T., 1981. Studies on the constituents of Artemisia annual. Acta Pharm. Sin 16, 366–370.
Uhl, M., Schwab, S., Efferth, T., 2016. Fatal liver and bone marrow toxicity by combination treatment of dichloroacetate and artesunate in a glioblastoma multiforme patient: case report and review of the literature. Front Oncol 6, 204.
Von Hagens, C., Walter-Sack, I., Goeckenjan, M., Osburg, J., Storch-Hagenlocher, B., Sertel, S., Elsässer, M., Remppis, B.A., Edler, L., Munzinger, J., Efferth, T., Schneeweiss, A., Strowitzki, T., 2017. Prospective open uncontrolled phase I study to define a well-tolerated dose of oral artesunate as add-on therapy in patients with metastatic breast cancer (ARTIC M33/2). Breast Cancer Res. Treat 164 (2), 359–369.
Von Hagens, C., Walter-Sack, I., Goeckenjan, M., Storch-Hagenlocher, B., Sertel, S., Elsässer, M., Remppis, B.A., Munzinger, J., Edler, L., Efferth, T., Schneeweiss, A., Strowitzki, T., 2019. Long-term add-on therapy (compassionate use) with oral artesunate in patients with metastatic breast cancer after participating in a phase I study (ARTIC M33/2). Phytomedicine 54, 140–148.
Wen, P.Y., Weller, M., Lee, E.Q., Alexander, B.M., Barnholtz-Sloan, J.S., Barthel, F.P., Batchelor, T.T., Bindra, R.S., Chang, S.M., Chiocca, E.A., Cloughesy, T.F., DeGroot, J. F., Galanis, E., Gilbert, M.R., Hegi, M.E., Horbinski, C., Huang, R.Y., Lassman, A.B., Le Rhun, E., Lim, M., Mehta, M.P., Mellinghoff, I.K., Minniti, G., Nathanson, D., Platten, M., Preusser, M., Roth, P., Sanson, M., Schiff, D., Short, S.C., Taphoorn, M.J. B., Tonn, J.C., Tsang, J., Verhaak, R.G.W., von Deimling, A., Wick, W., Zadeh, G., Reardon, D.A., Aldape, K.D., van den Bent, M.J., 2020. Glioblastoma in adults: a society for neuro-oncology (SNO) and European society of neuro-oncology (EANO) consensus review on current management and future directions. Neuro Oncol 22, 1073–1113.
Wu, Z.P., Gao, C.W., Wu, Y.G., Zhu, Q.S., Yan, C., Xin, L., Chuen, L., 2009. Inhibitive effect of artemether on tumor growth and angiogenesis in the rat C6 orthotopic brain gliomas model. Integr. Cancer Ther 8, 88–92.
Zhang, Z.Y., Yu, S.Q., Miao, L.Y., Huang, X.Y., Zhang, X.P., Zhu, Y.P., Xia, X.H., Li, D.Q., 2008. Artesunate combined with vinorelbine plus cisplatin in treatment of advanced non-small cell lung cancer: a randomized controlled trial. J. Chinese Int. Med 6, 134–138.
Zhao, K.C., Song, Z.Y., 1989. Distribution and excretion of artesunate in rats. Proc. Chinese Acad. Medical Sci. Peking Union Medical College 4, 186–188.
Zhou, X., Chen, Y., Wang, F., Wu, H., Zhang, Y., Liu, J., Cai, Y., Huang, S., He, N., Hu, Z., Jin, X., 2020. Artesunate induces autophagy dependent apoptosis through upregulating ROS and activating AMPK-mTOR-ULK1 axis in human bladder cancer cells. Chem Biol Interact 331, 109273.
Zou, Y., Tuo, F., Zhang, Z., Guo, J., Yuan, Y., Zhang, H., Xu, Z., Pan, Z., Tang, Y., Deng, C., Julie, N., Wu, W., Guo, W., Li, C., Huang, X., Xu, Q., Song, J., Wang, Q., 2020. Safety and efficacy of adjunctive therapy with artesunate in the treatment of severe malaria: a systematic review and meta-analysis. Front. Pharmacol 11, 596697.