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Artesunate in the treatment of metastatic uveal melanoma - first experiences

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Abstract. Artesunate (ART) is a derivative of artemisinin, the active principle of the Chinese herb *Artemisia annua* L. Artesunate is approved for the treatment of multidrug-resistant malaria and has an excellent safety profile. It has been shown that Artesunate, apart from its anti-malarial activity, has cytotoxic effects on a number of human cancer cell lines, including leukemia, colon cancer and melanoma. We report on the first long-term treatment of two cancer patients with ART in combination with standard chemotherapy. These patients with metastatic uveal melanoma were treated on a compassionate-use basis, after standard chemotherapy alone was ineffective in stopping tumor growth. The therapy-regimen was well tolerated with no additional side effects other than those caused by standard chemotherapy alone. One patient experienced a temporary response after the addition of ART to Fotemustine while the disease was progressing under therapy with Fotemustine alone. The second patient first experienced a stabilization of the disease after the addition of ART to Dacarbazine, followed by objective regressions of splenic and lung metastases. This patient is still alive 47 months after first diagnosis of stage IV uveal melanoma, a situation with a median survival of 2-5 months. Despite the small number of treated patients, ART might be a promising adjuvant drug for the treatment of melanoma and possibly other tumors in combination with standard chemotherapy. Its good tolerability and lack of serious side effects will facilitate prospective randomized trials in the near future.

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Abbreviations: AJCC, American Joint Committee of Cancer; DTIC, Dacarbazine; ART, Artesunate

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Introduction

Artesunate (ART) is a semi-synthetic derivative of artemisinin, a compound extracted from *Artemisia annua* L., also known as 'sweet wormwood' or 'qinhao' (Chinese for 'from green herb'). Artemisinin was isolated by Chinese researchers in 1972 and its structure elucidated in 1979 (1) (Fig. 1). The plant *Artemisia annua* L., a perennial herb from the family of composite flowers, has been used in traditional Chinese medicine as a remedy for chills and fevers for more than 2000 years. Originally from Northern China, the plant now grows wild in many countries, although the amount of artemisinin can vary considerably, depending on plant material and growing conditions (2). The World Health Organization recommends the use of artemisinin and its derivatives in geographical areas with multidrug-resistant malaria (3). Propitious features of ART are the activity against otherwise multidrug-resistant *Plasmodium* strains, its good tolerability and the lack of serious side effects. ART has been used for the treatment of more than 1 million cases of malaria and is considered a safe drug with no obvious adverse reactions or noticeable side effects, even when given to children (3).

It was recently demonstrated that ART, apart from its anti-malarial activity, inhibits the growth of leukemic cells (4) and acts in an antiviral manner (5). Subsequently, it was shown that ART is also active against a variety of human cancer cells lines (6). In addition, the molecular modes of anticancer activity have been elucidated (7). Importantly, a comparison between the cytotoxicity of ART and that of other standard cytostatic drugs showed that ART was active in molar ranges comparable to those of established anti-tumor drugs. Of note, the concentration of ART applied in these *in vitro* studies was significantly lower compared to serum levels usually accomplished in anti-malaria therapy. Other independent studies have confirmed these findings and additionally demonstrated anti-angiogenic effects of ART (8,9). The mode of action in cancer cells is similar to the cytotoxicity against malaria *Plasmodia* and becomes apparent in the presence of free iron. Fe^{2+} catalyzes the opening of the endoperoxide bridge in artemisinin, leading to the formation

of very reactive free radicals, which cause extensive damage to either parasites or cancer cells (10,11). Since tumor cells sequester relatively large amounts of iron compared to normal cells, they are more susceptible to the cytotoxic effects of ART. It is therefore conceivable to further increase the activity of ART by loading cancer cells with iron, e.g. co-administration of iron-supplementing medication.

Malignant melanomas constitute 1-3% of all malignant tumors, but the global incidence is rising dramatically at a rate of 6-7% per year. The striking upward trend has been evident for several decades, and the incidence of melanoma continues to increase more rapidly than any other cancer (12). While the skin is the most common site for melanoma development, this neoplasm can occur in any tissue that contains melanocytes. The uvea is the second most common site for melanoma development, and uveal melanoma constitute approximately 13% of all malignant melanomas (13). With an incidence of 0.79/100000, uveal melanoma is the most common malignant intraoccular tumor of adults (13). At least 30% of patients with uveal melanoma experience distant metastasis within 10 years, usually to the liver, but also to other organs. With distant metastasis, stage IV according to AJCC 2002 (14), the prognosis drops dramatically. Only 13% survive >1 year, and the mean survival time after occurrence of distant metastasis is only 2-5 months (15,16). Metastatic uveal melanomas usually demonstrate a far worse response to therapy compared to cutaneous melanomas. While Dacarbazine (DTIC), the only chemotherapeutic agent approved for the treatment of advanced cutaneous melanoma, yields overall response rates of about 15% (17), it is far less active in uveal melanoma with response rates of <1% (18). A four-drug chemoimmunotherapy regime has been reported to induce a considerable objective response and may have contributed to prolonged survival. However, the anti-tumor activity of this regime was associated with severe toxicity, and therefore a large multicenter trial was terminated (19). Fotemustine is yet another chemotherapeutic agent for the treatment of uveal melanoma. Based on a case report of three uveal melanoma patients (20) who experienced a rather favorable outcome and a prolonged survival after treatment with Fotemustine in combination with interferon-α and interleukin-2, a larger trial has been subsequently initiated, demonstrating a prolonged survival of treated patients compared to historical controls (21). However, there is not a randomized prospective study yet available that has proven the beneficial effects of any therapy for metastatic uveal melanoma compared to the best supportive care regarding overall survival.

Here, we report the first experiences with ART in the treatment of advanced uveal melanoma in two patients that had disease progression despite standard chemotherapy.

Patients and methods

Patient characteristics. The clinical course of the disease and treatment modalities are depicted in detail in Fig. 2a and b.

Patient one, a female born in 1932, was diagnosed with uveal melanoma in August 1996. Her left eye was enucleated, and subsequently it was under control without pathologic findings until February 2001, when distant metastases were

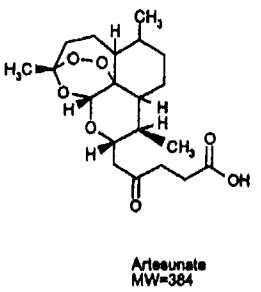

Figure 1. Chemical structure of Artesunate.

first diagnosed. Upon examination, she was in good general health. Two small skin tumors were found on the scalp and the left axillary region. A CT-scan revealed multiple lung metastases on both sides, as well as multiple liver metastases. Chemotherapy with Dacarbazine (DTIC) was initiated at a concentration of 850 mg per m^2 body surface. After 3 months and 4 courses, a CT-staging showed progressive disease, therefore DTIC was stopped and chemotherapy with Fotemustine at a concentration of 100 mg per m^2 body surface was started (Fig. 2a). After 3 months and 4 courses, the disease was moderately progressing, although a CNS metastasis was diagnosed and had to be treated by stereotactic irradiation, and 3 new skin metastases on the scalp were detected and surgically removed. After 3 additional courses, the known metastases were slightly increasing in size. Since no new organs became involved, we proceeded with Fotemustine therapy. However, after another 3 courses, a further progression was diagnosed in February 2002 with known metastases increasing in size and number, as well as the involvement of another organ, the pancreas. Due to a lack of standard regimens after 2 chemotherapies had failed, we started a compassionate treatment with daily ART (2 times daily, 50 mg p.o.) in combination with Fotemustine after receiving the informed consent of the patient. The first staging after 3 courses of Fotemustine in combination with ART showed a mixed response 3 months after the initiation of this therapy mode. Although there were no significant changes in the liver, lung metastases were decreasing in number and there was no evidence of new metastases in visceral organs or on the skin. However, two new CNS tumors were diagnosed by MRT and subsequently irradiated. After this phase of relative stabilization of disease progression, the subsequent staging demonstrated significant progress. We therefore changed the chemotherapy to DTIC, but continued with ART. However, the disease continued to progress and the patient died in January 2003, 23 months after entry into stage IV as defined by AJCC (14). Throughout the combination therapy, there were no additional side effects other than those caused by chemotherapy alone, such as nausea, vomiting and bone marrow insufficiency leading to anemia, leukopenia and thrombopenia with the expected latency after the chemotherapy courses.

Patient two, a male born in 1926, had a left eye uveal melanoma diagnosed in November 1987, which was treated by enucleation and subsequent radiotherapy. Without regular follow-up, the patient was healthy until November 2000 when round structures were seen in a routine chest X-ray. The histopathological examination of a tumor biopsy revealed

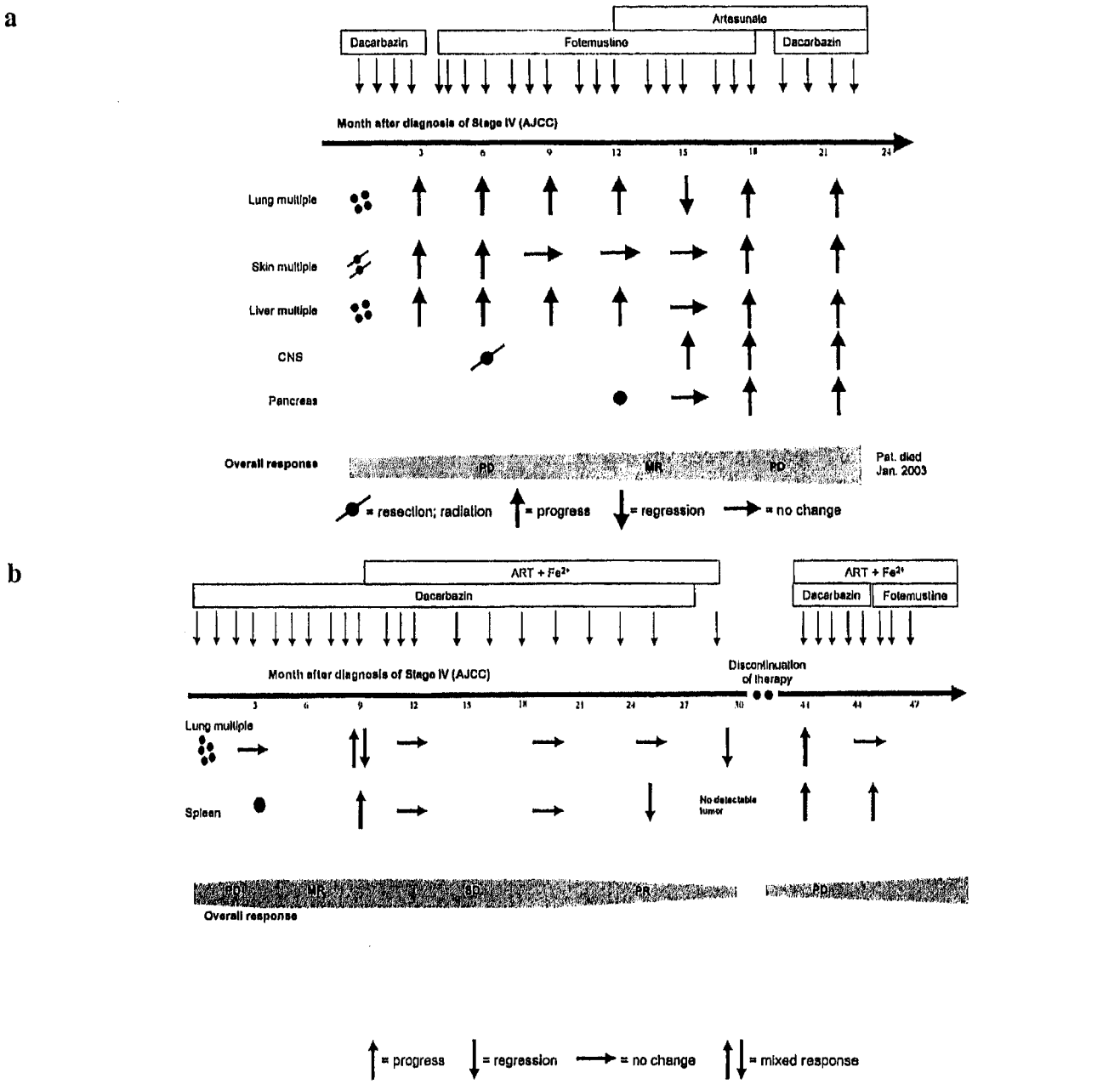

Figure 2. The clinical course and therapy after diagnosis of stage IV uveal melanoma. The upper horizontal bars depict the general anticancer drug regimen (Dacarbazine vs. Fotemustine ± Artesunate). Each vertical arrow symbolizes a single chemotherapy cycle with either Dacarbazin or Fotemustine. Below is the time axis in months. The bold arrows below demonstrate the clinical response to therapy, as indicated for each organ system. The grey horizontal bar at the bottom gives a quick overview of the general response to therapy. MR, mixed response; PD, progressive disease; PR, partial remission; SD, stable disease.

melanoma metastases. At that time, there were no other detectable metastases. Chemotherapy with DTIC at a concentration of 850 mg per m^2 body surface was initiated. After a phase of relatively stable or only slowly progressing disease for 9 months (Fig. 2b), a marked enlargement of a previous, barely detectable spleen metastasis was observed. Because lung metastases showed a mixed response and no other organs became involved, we decided to continue with DTIC, but started a concomitant therapy with 100 mg ART (50 mg, twice daily p.o.) after the patient gave his informed consent. In addition, the patient received iron medication (40 mg Fe^{2+}, 3 times daily) to potentially increase the efficacy of ART. In the following months, the disease first became

of DTIC (the last 10 of which were in combination with ART) when all but one lung and the spleen metastases completely disappeared. In April 2003, the patient was free of symptoms and temporarily had no follow up or anti-cancer medication. When we saw the patient again in March 2004, new splenic tumors and progressing intrapulmonal metastases were detected. Therefore, a therapy with Fotemustine was considered. However, since the treatment with DTIC and ART was previously successful, we decided to resume that regimen. After 6 more courses of DTIC and ART, the splenic metastases were slightly increasing in size, therefore we changed the protocol to Fotemustine and ART. As of October 2004, the patient was still in good clinical condition, 47 months

Discussion

We report on the first experiences of long-term treatment of uveal melanoma with ART under close clinical surveillance. Firstly, ART treatment was without measurable toxicity. Secondly, one of the treated patients exhibited a favorable clinical course of disease with an actual survival of 47 months after entry into stage IV, which is most remarkable considering the extremely poor prognosis. Both patients were suffering from metastatic uveal melanoma and progressing, despite standard chemotherapy with DTIC or Fotemustine. Metastatic uveal melanoma is extremely difficult to treat, despite reports on some promising chemo-(immuno-) therapy regimens (20), and complete responses are the exception and mostly anecdotal (18,22). The largest prospective trial to date, performed by Becker *et al*, reported only one CR in 48 patients treated, although some beneficial effects of Fotemustine were discussed regarding overall survival compared to historical controls (21). However, there is no convincing evidence that any therapy leads to an increased overall survival of patients with metastatic uveal (N.B. or even metastatic cutaneous) melanoma. Thus, novel treatment approaches are clearly needed.

Regarding our patients with metastatic uveal melanoma, it was not possible to include them in the mentioned clinical trials, as they were not available at that time. We therefore proposed, based on the *in vitro* evidence of cytotoxic activity of ART against melanoma cells (6) and the excellent safety record of ART, a 'compassionate-use treatment' with ART in combination with ongoing standard chemotherapy.

The therapy was well tolerated in both patients with no detectable additional side effects. In patient one, the combination of ART with Fotemustine was followed by a temporary mixed response with the regression of some lung metastases, but the development of new CNS tumors. The patient survived 11 months after start of the ART/Fotemustine therapy, which is significantly longer than the median survival time. The second patient had only moderate progress of his metastatic disease under DTIC-monotherapy, however, he showed a stabilization of the disease after the addition of ART and subsequently achieved an objective response with the regression of the splenic metastasis and all but one lung metastasis. After the discontinuation of therapy, the disease was again progressing. However, this patient is still alive and in good clinical condition, under current treatment with Fotemustine and ART.

Besides numerous reports describing the *in vitro* activity of ART against cancer cells (4,6,23,24), there is no report to date in which ART was applied to treat human cancer. However, ART is distributed worldwide via the Internet, and probably taken by many cancer patients outside of study protocols and not under the close supervision of physicians. It is therefore mandatory to obtain more information regarding the clinical relevance of its anti-tumor activity. The combination of ART with classical chemotherapeutic agents (e.g. anthracyclins) is particularly promising, since ART provides a second independent mechanism that might augment the anti-cancer activity without increasing toxicity (25). From the *in vitro* studies (26), it is further conceivable that loading tumor cells with bivalent iron, by potentially and simply providing Fe^{2+} in tablet form, might increase the susceptibility to the action of ART. It is tempting to speculate that, in the case of patient two, the addition of Fe^{2+} had an actual clinical impact and resulted in an improved response to therapy.

In summary, we hope that this report will stimulate further clinical research to delineate the anti-cancer activity of ART. To this end, we have planned a prospective double-blind placebo controlled trial applying ART as an additional drug together with either Dacarbazine for cutaneous melanoma or Fotemustine for uveal melanoma. If ART holds the promise of augmenting the activity of established chemotherapies, this might be a safe and inexpensive modality to improve treatment options for these currently incurable diseases.

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