Cancer Chemother Pharmacol (2016) 77:413–427
DOI 10.1007/s00280-016-2960-7

CLINICAL TRIAL REPORT

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

Miriam König[1] · Cornelia von Hagens[2] · Sebastian Hoth[1] · Ingo Baumann[1] · Ingeborg Walter-Sack[3] · Lutz Edler[4] · Serkan Sertel[1]

Received: 21 October 2015 / Accepted: 5 January 2016 / Published online: 21 January 2016

Abstract
Purpose Artesunate (ART) has been used for a long time in the treatment of *Plasmodium falciparum* malaria and has been considered safe. The present phase I study aimed to determine the daily dose of ART that is well tolerated as add-on therapy in patients with breast cancer for 4 weeks of therapy. Ototoxicity could be a potential safety concern in settings different from malaria. Therefore, comprehensive audiological assessment was essential.
Methods The ARTIC M33/2 study was a prospective, open, uncontrolled, monocentric phase I dose-escalation study to evaluate the safety and tolerability of ART in patients with advanced breast cancer. Patients received either 100, 150 or 200 mg oral ART daily for a test phase of 4 weeks as add-on therapy to their ongoing oncological treatment. For the investigation of the safety of ART for hearing, an audiological assessment was performed with each patient before the intake of ART and after 4 weeks of therapy.
Results Twenty-three female patients were included in the study. During the test phase, four patients had adverse events (AEs) of the auditory system possibly related to the intake of ART. However, none of these AEs was classified as severe AE (SAE) and did not require treatment interruption. Four patients had AEs concerning the vestibular system (vertigo) during the test phase, one of which was classified as SAE. However, the SAE was fully reversible after discontinuation of ART.
Conclusion None of the audiological results after 4 weeks of therapy with ART showed any dose-limiting auditory toxicity. However, audiological monitoring in further clinical studies with prolonged use of oral ART in doses up to 200 mg daily is warranted.
The ARTIC M33/2 study is registered at eudract.ema.europa.eu with the Number 2007-004432-23 and at clinicaltrials.gov with the Number NCT00764036.

Keywords Artesunate · Ototoxicity · Breast cancer · Metastasis

Introduction

Artesunate (ART) is a semisynthetic derivate of artemisinin (ARM), which is isolated from the Chinese plant *Artemisia annua*. In the 1970s, ARM and its derivates, such as ART, artemether and dihydroartemisinin, were characterized as effective and well-tolerated drugs against malaria. Since then, their importance in medicine has considerably increased as well as their use worldwide [1]. In 2006, the World Health Organization (WHO) recommended the artemisinin-based combination therapy (ACT) as first-line treatment against *Plasmodium falciparum* malaria as drug resistance to most other antimalarial drug classes increased and therefore reduced their effectiveness [2].

✉ Serkan Sertel
serkan.sertel@med.uni-heidelberg.de

1 Department of Otolaryngology, Head and Neck Surgery, University of Heidelberg, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany

2 Naturopathy and Integrative Medicine, Department of Gynecological Endocrinology and Reproductive Medicine, University Women's Hospital, Heidelberg, Germany

3 Department of Clinical Pharmacology and Pharmacoepidemiology, University Hospital, Heidelberg, Germany

4 Department of Biostatistics, German Cancer Research Center (DKFZ), Heidelberg, Germany

At present, most of the research on ARM and its derivates is based on the use as antimalarial drugs. However, in vitro studies showed that ARM and its derivates also have potent anticancer activity [3–6]. Moreover, ARM has been shown to have a chemosensitization effect on cancer cells which were resistant to conventional chemotherapy [7].

As several in vivo studies [8–11] and human case reports [12, 13] also suggested an anticancer potential of artemisinin derivatives, the systematic evaluation of the safety and tolerability of artemisinin derivatives in cancer patients was justified.

Prior to our investigation, concerns had been raised that ARM and its derivatives might have neurotoxic effects impairing among others the auditory and vestibular system mainly because neuroauditory toxicity had been reported in animal studies [14, 15].

In view of the concerns that had been raised about the potential damaging effect of ARM and its derivates on the brainstem and other neuroauditory structures, another study was performed in Vietnam on 242 patients who had received up to 21 cycles of antimalarial treatment within 24 months with either ART or ARM (corresponding to a median of 168 mg/kg up to >1000 mg/kg in 6.7–7.2 % of those patients) [16]. Patients underwent clinical examinations, audiometry and brainstem evoked response audiometry (BERA) and were compared to a control group of 108 patients from the same village in Vietnam, who had not been treated with these drugs. The study could not show any evidence of neuroauditory toxicity that could be attributed to the treatment with ARM or ART. Similar results using audiometry and early latency auditory brainstem response (ABR) tests came from another case–control study from 79 patients having more than two treatment courses of artemether or artesunate within the previous 3 years and 79 untreated controls from the same malaria endemic area of Thailand [17]. However, the promising results of these studies need to be re-evaluated carefully as they did not consider the neuroauditory function of the subjects before and during the intake of ARM or ART. As there are no pre-treatment data to compare with, no statement can be made about the hearing status of the patients before the intake of the antimalarial medication. Hearing data were only obtained after the medical treatment that had already been completed. In the study of Van Vugt et al. [17] the most recent treatment cycle was finished at least 31 days before the hearing assessment. Thus both studies cannot evaluate possible reversible hearing impairments that might have occurred during or directly following ARM or ART treatment.

This limitation was eluded by a study from 2006, with fifteen healthy volunteers aged eighteen to twenty-three who underwent an artificial malaria infection and were treated with artemether–lumefantrine (AL) [18]. Audiological examinations at baseline confirmed normal hearing in all participants and were repeated during the illness (day 8 after infection, but before beginning of medical treatment) and after the end of the treatment (day 21 after infection). Two of the participants had subclinical negative hearing threshold changes in the two lowest frequencies tested (250 Hz and 500 Hz) on day 8 and 21. However, these alterations were not judged to be drug-related because they appeared after infection with malaria but before the intake of the medication. This suggests that malaria itself could be responsible for the deterioration so that overall, no clear drug-related hearing impairment could be detected in this study.

In 2004, a case–control study was performed on workers at a construction site in Mozambique. Air conduction audiometry was performed before beginning of the employment and repeated once after termination of the contract. The 150 cases were workers who had been treated with AL during their employment for uncomplicated *P. falciparum* malaria. They showed a statistically significant difference in the hearing assessment compared to 150 control workers. The intra-individual assessment of hearing threshold changes showed mostly subclinical hearing losses between the two assessments. In contrast, the hearing assessments of the healthy untreated controls did not show the same extent of threshold changes [19]. However, since the control workers had not suffered from malaria and had not been treated with AL, due to the design of the study, a structural bias cannot be excluded. Besides, the study only used air conduction audiometry for the audiological assessment, the cases and controls were not matched, and the study does not inform on possibly relevant confounders such as age or known pre-existent diseases of the subjects. Moreover, it is not sure whether the observed hearing loss might be attributed to the lumefantrine constituent of AL, to malaria itself or to other important risk factors such as intensity and duration of noise exposure at the construction site or use of other possibly ototoxic drugs as Mehta et al. [20] discuss in their comment on the report of Toovey and Jamieson.

Another study from Thailand in 68 patients treated for malaria with AL in the previous 5 years and 68 untreated controls found no evidence of auditory brainstem toxicity attributable to treatment with AL [21]. These findings are consistent with the comment of Reinhart et al. [22] following the publication of Toovey and Jamieson. Their opinion is based on 2318 patients treated with artemether–lumefantrine (AL) as antimalarial therapy in 16 clinical trials with 16 cases (0.7 %) of hearing loss. It is also based on their search of the Novartis safety database where they did not find spontaneous reports of adverse events for their marketed product co-artemether (AL) for any irreversible neuro- or auditory toxicity. Therefore, the results of Toovey

and Jamieson need to be re-evaluated carefully and in context with other clinical studies on different artemisinin compounds.

In 2005, Panossian et al. reported the case of a 42-year old female breast cancer patient after right mastectomy having been treated with tamoxifen, fluoxetine and an herbal therapy consisting among others of 200 mg artemisinin twice a day. As ARM is approximately five times less potent than ART in malaria therapy [16], this would be equivalent to <100 mg of ART per day. After 2 weeks of this medical treatment, she presented with diplopia, dysarthria and ataxic gait. In an MRI of her brain, symmetric punctual signal prolongations could be shown in the T2 sequence that improved 7 days after discontinuation of all her medical treatment as well as did the neurological symptoms. Tamoxifen, fluoxetine and the herbal treatment apart from ARM were ruled out as trigger for her disorders by the literature research, and re-exposure to tamoxifen and fluoxetine was without re-occurrence of the previously presented symptoms [23]. Considering these findings of most probably artemisinin-caused neurotoxicity in a case where cerebral malaria manifestations were no confounder for neurological symptoms, it is necessary to carefully examine the side effects, especially their dose dependence of an artemisinin-based therapy in a context other than antimalarial treatment.

As there had never been a study investigating the effects of prolonged use of ARM and its derivates in human cancer patients, the multidisciplinary ARTIC M33/2 phase I study aimed to assess the safety and tolerability of ART in patients with metastatic or locally advanced breast cancer. This article presents the results of the extensive investigation of the auditory and vestibular system within this phase I study.

Materials and methods

Study design and participants

The ARTIC M33/2 study was a prospective, open, uncontrolled, monocentric phase I dose-escalation study, and it was performed at the University Hospital of Heidelberg.

Female patients with histologically confirmed metastatic breast cancer having no safety concerns of the responsible oncologist were eligible for participation in this study on the safety of artesunate as oral add-on therapy to their respective oncological treatment.

If no dose-limiting adverse events (DL-AEs) were detected after 4 weeks, patients could give a second informed consent and continue the add-on therapy during an extension phase, which was limited up to the second progression under the add-on therapy.

Study medication regimen

The study drug was administered as an add-on therapy to the individual conventional therapy over a period of 4 weeks. Patients received one of three different doses (consecutively, dose group I: 100 mg, dose group II: 150 mg, dose group III: 200 mg) of orally administered ART (Arinate™, Dafra Pharma, Turnhout, Belgium) daily.

The first dose of the study medication was administered in the presence of one of the investigating physicians (U0), and thereafter patients were monitored for at least 4 h. On the next days, patients took the medication at home and a pill count was carried out at every following examination in the hospital.

Outcome measures

Safety monitoring before, during and after the intake of ART included DL-AEs as primary outcome as well as laboratory assessments, neurological, cardiological and audiological examinations as secondary outcomes. Details of the data collection and primary safety data not related to hearing will be presented elsewhere.

Audiological measures

To evaluate the safety and tolerability of oral ART for hearing, an audiological assessment was performed for each patient before the beginning of the intake of ART (BL; baseline) and after 4 weeks of therapy (U2).

For participants of the extension phase audiological assessments were repeated every 3 months (U3, 4 and 5). In case of occurrence of clinically relevant AEs of the auditory system, the audiological assessment of the respective patient was repeated four to 8 weeks following the withdrawal of the study medication. Further audiological assessments could be scheduled if judged clinically necessary.

The audiological assessment included otoscopy, pure-tone audiometry, tympanometry, stapedius reflex measurement, transitory evoked otoacoustic emissions (TEOAE), distortion product otoacoustic emissions (DPOAE) and brainstem evoked response audiometry (BERA), which is a synonym for early auditory brainstem responses (ABR); both terms are used in this paper. All audiological examinations were carried out in the Department of Otolaryngology, Head and Neck Surgery at the University Hospital of Heidelberg.

Pure-tone audiometry was performed with an Auritec AT 335 computer audiometry system (Auritec Medizindiagnostische Systeme GmbH, Hamburg, Germany). Hearing levels were determined via air conduction for frequencies from 125 hertz (Hz)–8 kHz.

Impedance measurement, including tympanometry and stapedius reflex measurement, was also performed with the Auritec AT 335 audiometer. For tympanometry, the pressure in decapascal (daPa), at which the eardrum has the greatest mobility and the compliance of the eardrum in milliliters were measured. The stapedius reflex was determined for the frequencies 500, 1000, 2000 and 4000 Hz. The reflex was measured in both ears separately and was determined for the ipsilateral as well as the contralateral ear.

Otoacoustic emissions were recorded in an acoustically shielded room. TEOAE were measured with an ILO88 device (Otodynamics Ltd., Hatfield, UK). Click noises with a duration of 100 μs and a repetition rate of 50 Hz were used for acoustical stimulation. The determination of the TEOAE was carried out under the following measuring conditions: Stimulus levels ranged from 80.3 to 85.4 dB and the residual noise varied from −5.6 to 3.3 dB. Criteria for the assessment of physiological stimulus responses were the visual evaluation by an experienced examiner, the amplitudes of the signal and the residual noise as well as the reproducibility of the responses.

DPOAE were determined with an ILO92 device (Otodynamics Ltd., Hatfield, UK). They were measured at frequencies from 1 to 4 kHz for both ears separately. Primary tone levels ranged from 58.5 to 80.8 dB. For each frequency, the primary tone level L2 was determined in decibel (dB) as well as the residual noise in dB and the signal to noise ratio (SNR), which is the ratio of the mean signal amplitude and the standard deviation of the residual noise. The actual amplitude should be 6 dB above the noise floor to be considered a true distortion product.

BERA was measured in an acoustically and electrically shielded room. Ag-/AgCl-electrodes were attached to the vertex, mastoid and forehead. Each ear was stimulated separately with biphasic click sounds of two times 100 μs duration while the other ear was masked with white noise. The responses were recorded with the ERA 3.7 Software (ZLE Systemtechnik, Munich, Germany) and Beyer DT-48 dynamic headphones (Beyerdynamic GmbH & Co. KG, Heilbronn, Germany) served as electric-acoustical converters. Stimulus levels varied from 80 to 90 dB and were varied in steps of 5 or 10 dB until the responses were sufficiently exact, which was normally achieved at 80 dB. Artifacts were suppressed through variable amplitude control by the examiner. By means of computer-assisted averaging of 4000 artifact-free EEG (electroencephalogram) sections, the actual auditory evoked responses could be filtered and were plotted as average curves. To evaluate the quality of the measurement, the residual noise was determined by dividing the average curves into two parts and calculating the difference between the two curves.

The latency of wave V, the amplitude of wave V and the interpeak latency between wave I and wave V were chosen for our analysis.

Screening for hearing and vestibular problems

In addition, every week each patient completed the questionnaire BN20+ for frequent screening for neurological and possible other symptoms. The BN20+ contains the Brain module BN20 of the European Organization for Research and Treatment of Cancer (EORTC), items 8–19 from the quality of life (QLQ) questionnaire QLQ C30 [24] and 8 additional unvalidated questions covering possible side effects known from malaria therapy with ART.[1] Possible audiometric and vestibular changes were monitored with the following questions:

Question no. 51: Did you have any noises, ringing or buzzing in the ears?
Question no. 52: Did you perceive any hearing loss?
Question no. 53: Did you have dizziness or vertigo?
The answers to the questionnaire represent subjective assessments of the patients and were descriptively analyzed.

Statistical analysis

The statistical analysis was done as intention to treat (ITT) analysis. In other words, each patient who received the study drug at least once was included in the analysis.

The secondary endpoints reported here investigate possible ototoxic effects of ART analyzed through the audiological assessment. The results are displayed in this paper together with a description of all AEs of the auditory and vestibular system. In general, the analysis was done for each ear separately. Since the audiological measurements were of continuous or ordinal scale and the assumption of a normal distribution was not justified, the Wilcoxon signed rank test was applied to test for differences between the baseline (BL) values and the values after 4 weeks (U2, using the notation of the study protocol) of ART therapy. The null hypothesis of no differences between BL and U2 was tested one-sided versus the alternative hypothesis of impairment of hearing, which indicated an adverse event. A p value of <0.05 would indicate statistical significance for the respective audiological endpoint. The results of the audiological measurements were described in detail by reporting the median (Med), minimum (Min) and maximum (Max) for the right and the left ear for BL and U2.

[1] This use of the tool BN20+ as screening instrument for frequent screening for safety monitoring was permitted by the EORTC.

Furthermore, we calculated the median of the changes between BL and U2 values as well as between the right and left ear (see Table 3) on which the statistical test (Wilcoxon signed rank) is based.

Since the individual dose groups contained only a small number of patients and since no clear dependency on dose was observed, we combined the data of all 23 patients both at baseline (BL) and after 4 weeks of ART therapy (U2).

The analysis of pure-tone audiometry was done for each ear separately at the 250, 500, 750, 1000, 1500, 2000, 3000, 4000 and 6000 Hz frequencies. Here, we compared the audiometric threshold value of the left ear from the screening assessment at 250 Hz with the value at 250 Hz of the left ear after 4 weeks of therapy with ART. This was done for each frequency in each of both ears. The thresholds at 125 and 8000 Hz were not taken into account in the statistical analysis since, due to practical reasons, many values were missing and thus the comparability with the other frequencies was not given.

In the impedance measurement we had two values for each ear to analyze—the middle ear pressure in daPa and the compliance in milliliters.

The stapedius reflex registration was done for frequencies of 500, 1000, 2000 and 4000 Hz for each ear separately, and the reflex was measured ipsi- and contralateral each time.

The analysis of TEOAE was restricted to the two values for True OAE level (total emission amplitude corrected for residual noise) and for reproducibility in each ear.

DPOAE were analyzed by comparison of BL and U2 values of the True DP levels at the 1000, 1500, 2000, 3000 and 4000 Hz frequencies.

To analyze the results of BERA, three baseline values were compared to the values after 4 weeks of ART intake—the latency of wave V, the amplitude of wave V and the interpeak latency between wave I and wave V. In accordance with the other hearing tests, the values were determined for both ears separately.

Results

Between 2008 and 2011 a total of 23 patients with metastatic breast cancer completed the study; they took either 100, 150 or 200 mg ART as add-on therapy to their oncological treatment regimen. Dose group I contained six patients (1-01 to 1-06), dose group II seven patients (2-01 to 2-07) and dose group III ten patients (3-01 to 3-10). Two patients who received <75 % of their planned dose without experiencing a dose-limiting adverse event were replaced.

Patient characteristics and audiological function before the beginning of the add-on therapy with ART are provided in Table 1. Audiological assessments after 3, 6 and 12 months of the add-on therapy with artesunate were available from nine, seven and three patients, respectively. A flow chart of the study is depicted in Fig. 1.

In den BN20+ questionnaire, five patients had AEs concerning the auditory system (subclinical hearing loss, tinnitus)—four during the first 4 weeks and another one after 11 months of the add-on therapy. None of those was a dose-limiting AE. Six patients presented with vertigo that could not certainly be ruled out to originate from the vestibular system, four during the first 4 weeks and two others after 2 and 10 months. One of these AEs was classified DL-AE and was documented 5 days after stopping ART. A causal association with the add-on therapy with ART could not definitely be excluded. The DL-AE healed without any residues as well as did the other vestibular AEs including two patients in whom vertigo reappeared after 2 months. A detailed listing of auditory or vestibular adverse events possibly related to the intake of ART is given in Table 2.

The statistical analysis of the hearing data provided the following results:

For pure-tone audiometry no statistical significance was observed ($p > 0.1$). To compare the baseline and follow-up values after 4 weeks, we also illustrated the pure-tone air conduction thresholds in Fig. 2.

There was a statistically significant difference in the impedance measurement from baseline to the values after 4 weeks for the pressure in Pascal in the left ear ($p = 0.01$), whereas changes of the pressure in the right ear as well as of the compliance in both ears were not significant ($p > 0.05$).

In the stapedius reflex measurement no significant changes were seen for all the contralateral measurements (right and left ear) of the reflex as well as for the ipsilateral reflex at 500, 1000 and 4000 Hz in both ears and the ipsilateral reflex in the left ear ($p > 0.05$). However, the ipsilateral stapedius reflex in the right ear at 2000 Hz showed a statistically significant difference ($p = 0.01$).

No statistical significance was observed for TEOAE (p values > 0.1) as well as for DPOAE (p values > 0.05) and BERA (p values > 0.05).

A detailed synopsis of the results of the audiological assessments is presented in Table 3.

Discussion

As most common chemotherapeutics still have a large number of undesirable side effects, the request for new drugs with high anticancer activity and low incidence of adverse effects remains a prevailing issue in clinical oncology. Considering the promising and still growing in vitro and in vivo data for anticancer activity of artemisinin derivatives [3, 4, 25–28], the investigation of the safety and

Table 1 Patient characteristics and audiological functions before the beginning of artesunate therapy

	Dose group 1	Dose group 2	Dose group 3	Total study population
Ethnic origin				
Caucasian	$n = 6$ (100 %)	$n = 7$ (100 %)	$n = 10$ (100 %)	$n = 23$ (100 %)
Age at initial diagnosis				
Mean ± SD	44.2 ± 6.2	47.7 ± 10.5	46.7 ± 13.5	46.3 ± 10.8
Minimum, median, maximum	35, 45, 51	31, 48, 61	31, 42, 72	31, 44, 72
Age at beginning of the study				
Mean ± SD	51.8 ± 8.1	58.7 ± 10.4	56.0 ± 12.3	55.7 ± 10.6
Minimum, median, maximum	39, 53, 61	44, 60, 71	41, 54, 73	39, 57, 73
Smoking habits				
Smoker	1 (17 %)	1 (14 %)	0 (0 %)	2 (9 %)
Former smoker	3 (50 %)	3 (43 %)	5 (50 %)	11 (48 %)
Non-smoker	2 (33 %)	3 (43 %)	5 (50 %)	10 (43 %)
Postmenopausal	5 (83 %)	7 (100 %)	10 (100 %)	22 (96 %)
ECOG performance status				
0	5 (83 %)	7 (100 %)	3 (40 %)	16 (70 %)
1	1 (17 %)	0 (0 %)	6 (60 %)	7 (30 %)
2–4	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
NYHA				
No heart failure	6 (100 %)	6 (86 %)	10 (100 %)	22 (96 %)
I	0 (0 %)	1 (14 %)	0 (0 %)	1 (4 %)
II–IV	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Oncological standard therapy (several possible)				
Endocrine[a]	2	6	7	15
Monochemotherapy[b]	4	1	3	8
Bisphosphonates[c]	3	5	8	16
Others[d]	2	3	3	8
Chronic accompanying diseases of hearing	3 (50 %)	2 (28 %)	2 (20 %)	7 (30 %)
Audiological assessment normal and age appropriate at baseline[e]				
Yes	5 (83 %)	7 (100 %)	10 (100 %)	22 (96 %)
No	1 (17 %)	0 (0 %)	0 (0 %)	1 (4 %)

[a] Tamoxifen, letrozole, anastrozole, exemestane, fulvestrant, megestrol acetate, GnRH analogues and others

[b] Gemcitabine, doxorubicin, capecitabine and others

[c] Clodronate, pamidronate, ibandronate, zoledronate and others

[d] e.g., Trastuzumab in patients with overexpression of HER2/neu

[e] Before starting add-on therapy with artesunate

tolerability of prolonged use of these drugs in human cancer patients was justified, because their short-time use for malaria treatment has only few adverse effects. Therefore, our phase I study aimed to investigate the safety and tolerability of ART as oral add-on therapy in twenty-three breast cancer patients for 4 weeks. It showed that ART generally was well tolerated for hearing and the vestibular system in the doses examined. All AEs were only possibly caused by the add-on therapy with artesunate. Five days after the last intake of the add-on therapy a dose-limiting vertigo was observed, but the patient fully recovered thereafter. Additionally, two ongoing subclinical abnormalities in the hearing assessment and an ongoing tinnitus without clear causality were detected while pre-existing hearing or vestibular impairments in other patients did not deteriorate.

In the statistical analysis of the hearing data, only two parameters of hearing impairment showed a statistically significant difference (p values lower than 0.05) between baseline and the 4 weeks measurement. Those were the impedance measurement of the left ear and the stapedius reflex measurement at 2000 Hz in the right ear. All the other p values did not exceed the significance level of 0.05. When discussing these two significances from a statistical point of view, two counteracting issues were noted: on the

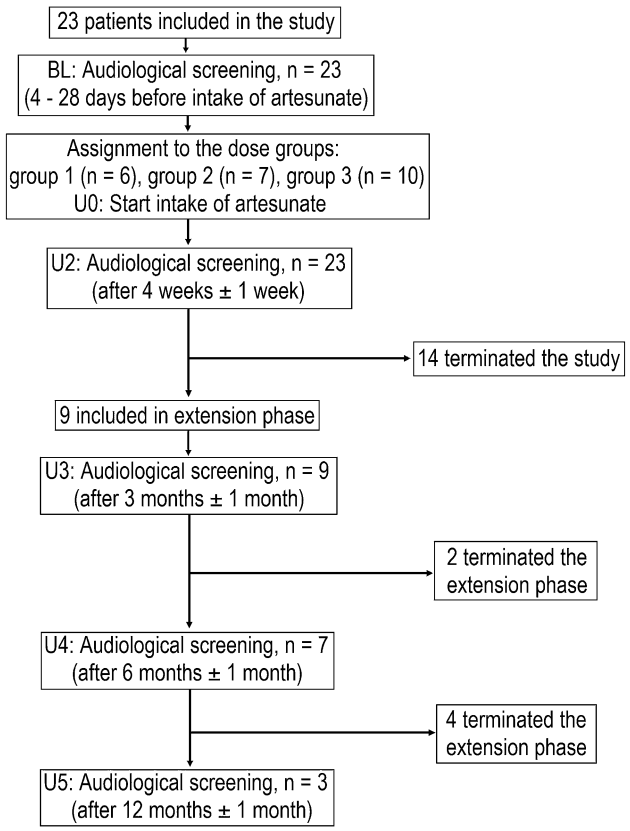

Fig. 1 Study flow chart giving an overview over the enrolment, dose groups, follow-up dates and numbers of patients in each follow-up

one hand the sample size of this study was small ($n = 23$) so that the power to detect relevant differences was not high. On the other hand, as we implemented a broad battery of audiometric examinations and many parameters were examined, the type 1 error was inflated so that actually a lower threshold value than $p < 0.05$ might have been implemented. Therefore a clinical assessment of the two parameters with $p < 0.01$ appeared indicated to judge the relevance on ototoxicity.

The two noticeable parameters were not registered in the same test and thus are not directly consecutive values. Moreover, they were observed only in one of the two ears although the study drug was administered systemically and not locally. Therefore, a causal association to the study drug appears to be very unlikely. Consequently, we could maintain the null hypothesis stating that there was no difference between the BL and U2 values and reject the alternative hypothesis.

Results from a randomized placebo controlled clinical study in twenty-three colorectal cancer patients [29] who received 200 mg oral artesunate or placebo daily for 2 weeks prior to surgery did not report any AE from the auditory or vestibular system. However their cumulative doses were lower, audiological assessments were missing and the number of patients taking ART was much smaller.

Our results concerning the safety of ART for hearing are consistent with other clinical studies performed since 2007 in larger numbers of patients after short-time treatments for malaria with ART or other derivatives of ARM.

A randomized, prospective, three-armed study of Carrasquilla et al. [30] comparing the auditory safety and efficacy of the treatment of 265 patients with uncomplicated *P. falciparum* malaria from Columbia either with artemether–lumefantrine (AL), atovaquone-proguanil (AP) or with artesunate-mefloquine (AM) for 3 days did

Table 2 Adverse events possibly related to artesunate affecting the auditory and vestibular system

Category/symptom (patient number)	Dose (mg/day)	CTCAE Vs. 3.0	Causality	Day of artesunate	Duration of AE (days)	Outcome
Auditory/ear						
Temporary hearing loss (1-01)	100	1	Possible	328	135	Fully recovered
Tinnitus (2-02)	150	2	Possible	8	1	Fully recovered
Tinnitus (2-03)	150	2	Possible	14	>4 years	Ongoing
Subclinical hearing loss (3-04)	200	1	Possible	28		Ongoing
Subclinical hearing loss (3-10)	200	1	Possible	29		Ongoing
Dizziness from neurology						
Vertigo (1-01)	100	1	Possible	306	26	Fully recovered
Vertigo (2-01)	150	1	Possible	1	10	Fully recovered
Vertigo (2-02)	150	3	Possible	FU day 5	17	Fully recovered
Vertigo (2-04)	150	1 2	Possible	12 56	30 21	Fully recovered
Vertigo (2-05)	150	2	Possible	66	1	Fully recovered
Vertigo (3-08)	200	1	Possible	2 64	14 29	Fully recovered

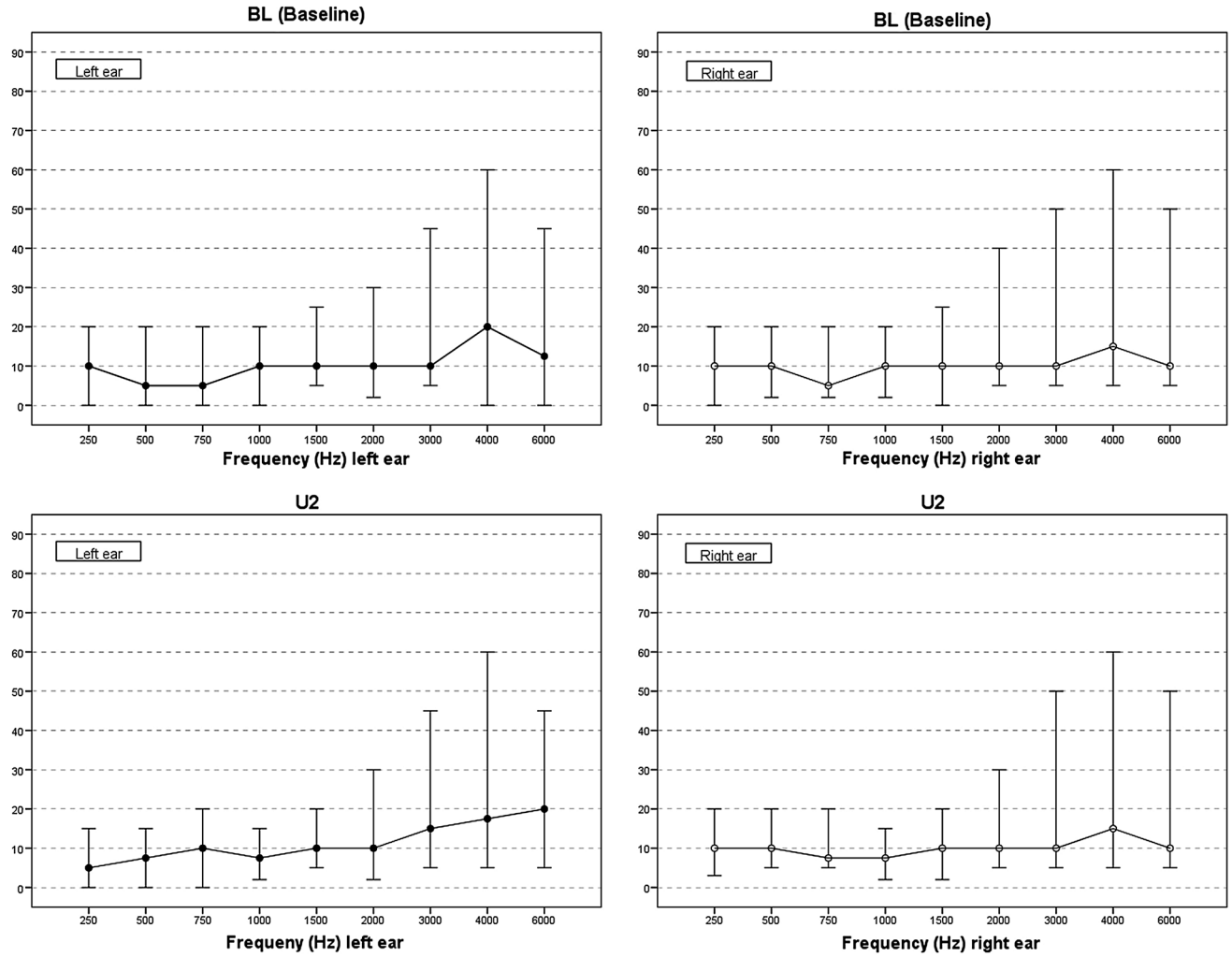

Fig. 2 Pure-tone air conduction audiometric thresholds at baseline and after 4 weeks of therapy with artesunate. Left and right ear are depicted separately. Audiometric thresholds are given as mean values of all 23 patients with range in dB; *x-axis* frequency in Hz, *y-axis* hearing thresholds in dB

not show any adverse effect on brainstem auditory pathways or pure-tone threshold measurements related to the drug exposure in neither of the three study groups. Carrara et al. [31] found that there was no toxic effect of ART on the auditory pathways in patients with acute uncomplicated *P. falciparum* malaria from Thailand who had been treated with a standard 3-day dose of ART combined with mefloquine. Gurkov et al. [32] examined 97 patients with uncomplicated *P. falciparum* malaria from Ethiopia who were treated with a standard 3-day regimen of AL, AP or quinine. Their comprehensive audiological monitoring including a follow-up after 90 days could not detect any toxic effect of AL or AP on peripheral hearing or brainstem auditory pathways either. Other experimental and clinical antimalarial studies also included another important factor in their examinations: Hearing levels were tested in a standardized murine cerebral malaria model or before beginning of the medical treatment and at different stages of the malaria disease, allowing to make a statement about the effects of acute malaria infection on hearing. In 2010, Schmutzhard et al. [33] examined the effects of malaria infection for hearing in twenty mice compared to a control group of nine healthy mice. Auditory brainstem responses were measured before infection with malaria and at its peak. The study revealed significant hearing impairments in the malaria group compared to the baseline values, especially in ten mice that developed cerebral malaria. The control group did not have any hearing alterations. This leads us to the conclusion that the malaria infection itself is an important factor in the development of hearing impairments and therefore needs to be taken into account in the evaluation of malaria treatments. These findings are consistent with another study performed in 2012 on 58 children in Ghana suffering from uncomplicated malaria

Table 3 Detailed synopsis of the results of the audiological assessment at baseline and after 4 weeks of artesunate therapy presented with values at baseline and after 4 weeks for right and left ear, changes between the values at baseline and after 4 weeks of therapy, differences between right and left ear and *p* values of the statistical analysis determined with the Wilcoxon signed rank test of the values at baseline and after 4 weeks of artesunate therapy; n.s. = not specified, n.d. = not determined; Min = Minimum, Max = Maximum, Med = Median; ipsi = ipsilateral, contra = contralateral

Otoscopy	Baseline (BL) $n = 23$		4 weeks (U2) $n = 23$		Changes BL–U2	Differences right/left	*p* values BL–U2
	Right ear	Left ear	Right ear	Left ear			
Tympanic membrane							
No pathology	18	18	21	21	3	n.s.	n.d.
Pathology	1	1	0	0	1		
Missing values	4	4	2	2			
Tubal dysfunction	0	1	0	0	1	1	n.d.
Missing values	0	0	1	1			
Chronic secretory otitis media	0	0	0	0	0	0	n.d.
Missing values	0	0	1	1			
Chronic mesotympanic otitis media	0	0	0	0	0	0	n.d.
Missing values	0	0	1	1			
Chronic epitympanic otitis media	0	0	0	0	0	0	n.d.
Missing values	0	0	1	1			

Pure-tone audiometry	Baseline (BL)		4 weeks (U2)		Changes[b] BL–U2		Differences[c] right/left		*p* values BL–U2	
(normal range ~0 dB)	Right ear (dB)	Left ear (dB)	Right ear (dB)	Left ear (dB)	R	L	BL	U2	R	L
250 Hz median	10	10	10	5	0	0	0	0	0.96	0.95
Min, max	0, 20	0, 20	3, 20	0, 15						
Number of patients[a]	$n = 23$	$n = 23$	$n = 22$	$n = 22$						
500 Hz median	10	5	10	7.5	0	0	0	0	0.48	0.93
Min, max	2, 20	0, 20	5, 20	0, 15						
Number of patients[a]	$n = 23$	$n = 23$	$n = 22$	$n = 22$						
750 Hz median	5	5	7.5	10	0	0	0	0	0.58	0.58
Min, max	2, 20	0, 20	5, 20	0, 20						
Number of patients[a]	$n = 23$	$n = 23$	$n = 22$	$n = 22$						
1000 Hz median	10	10	7.5	7.5	0	0	0	0	0.26	0.33

Table 3 continued

Pure-tone audiometry	Baseline (BL)		4 weeks (U2)		Changes[b] BL–U2		Differences[c] right/left		*p* values BL–U2	
(normal range ~0 dB)	Right ear (dB)	Left ear (dB)	Right ear (dB)	Left ear (dB)	R	L	BL	U2	R	L
Min, max	2, 20	0, 20	2, 15	2, 15						
Number of patients[a]	$n = 23$	$n = 23$	$n = 22$	$n = 22$						
1500 Hz median	10	10	10	10	0	0	0	0	0.48	0.37
Min, max	0, 25	5, 25	2, 20	5, 20						
Number of patients[a]	$n = 23$	$n = 23$	$n = 22$	$n = 22$						
2000 Hz median	10	10	10	10	0	0	0	0	0.19	0.71
Min, max	5, 40	2, 30	5, 30	2, 30						
Number of patients[a]	$n = 23$	$n = 23$	$n = 22$	$n = 22$						
3000 Hz median	10	10	10	15	0	0	0	0	0.71	0.76
Min, max	5, 50	5, 45	5, 50	5, 45						
Number of patients[a]	$n = 23$	$n = 23$	$n = 22$	$n = 22$						
4000 Hz median	15	20	15	17.5	0	0	0	0	1.00	1.00
Min, max	5, 60	0, 60	5, 60	5, 60						
Number of patients[a]	$n = 23$	$n = 23$	$n = 22$	$n = 22$						
6000 Hz median	10	12.5	10	20	0	0	0	0	0.13	0.17
Min, max	5, 50	0, 45	5, 50	5, 45						
Number of patients[a]	$n = 22$	$n = 20$	$n = 21$	$n = 19$						

Tympanometry	Baseline (BL)		4 weeks (U2)		Changes[b] BL–U2		Differences[c] right/left		*p* values BL–U2	
	Right ear	Left ear	Right ear	Left ear	R	L	BL	U2	R	L
Pressure (daPa) med	−20	−16	−20	−20	4	4	−12	0	0.82	0.01
Min, max	−188, 192	−52, 80	−96, 48	−148, 68						
Number of patients[a] (normal range ~0 daPa)	$n = 23$	$n = 23$	$n = 23$	$n = 23$						
Compliance (ml) med	1.6	1.8	1.5	1.6	0	0.1	−0.2	0	0.73	0.50
Min, max	0.4, 2.8	1.0, 2.6	0.9, 2.9	0.8, 3.1						
Number of patients[a] (normal range 1–4 ml)	$n = 23$	$n = 23$	$n = 22$	$n = 22$						

Table 3 continued

Stapedius reflex (normal range 70–90 dB)	Right ear (dB)	Left ear (dB)	Right ear (dB)	Left ear (dB)	R	L	BL	U2	R	L
ipsi 500 Hz med	80	75	75	75	0	0	0	0	0.17	0.07
Min, max	75, 90	75, 85	75, 90	75, 90						
Number of patients[a]	$n = 19$	$n = 20$	$n = 20$	$n = 19$						
ipsi 1000 Hz med	85	82.5	85	85	5	0	5	0	0.07	0.81
Min, max	75, 100	75, 95	75, 95	75, 100						
Number of patients[a]	$n = 22$	$n = 20$	$n = 21$	$n = 20$						
ipsi 2000 Hz med	85	85	85	87.5	0	0	0	−5	0.01	0.32
Min, max	75, 100	75, 105	75, 95	75, 100						
Number of patients[a]	$n = 21$	$n = 21$	$n = 21$	$n = 20$						
ipsi 4000 Hz med	92.5	85	87.5	80	0	0	0	0	0.68	0.46
Min, max	75, 95	75, 95	75, 95	75, 95						
Number of patients[a]	$n = 14$	$n = 14$	$n = 14$	$n = 13$						
contra 500 Hz med	85	85	85	90	0	0	0	−5	0.83	0.17
Min, max	75, 100	75, 100	75, 100	75, 100						
Number of patients[a]	$n = 16$	$n = 15$	$n = 18$	$n = 16$						
contra 1000 Hz med	85	85	85	85	0	0	0	0	0.28	0.20
Min, max	75, 95	75, 100	75, 100	75, 95						
Number of patients[a]	$n = 18$	$n = 18$	$n = 19$	$n = 18$						
contra 2000 Hz med	87.5	90	85	90			0	−5	0.38	0.26
Min, max	75, 100	75, 100	75, 100	75, 100	0	−5				
Number of patients[a]	$n = 18$	$n = 17$	$n = 19$	$n = 17$						
contra 4000 Hz med	85	85	80	85	5	0	0	0	0.43	0.71
Min, Max	75, 95	75, 95	75, 95	75, 95						
Number of patients[a]	$n = 11$	$n = 12$	$n = 14$	$n = 10$						

TEOAE	Baseline (BL) $n = 23$		4 weeks (U2) $n = 23$		Changes[b] BL–U2		Differences[c] right/left		*p* values BL–U2	
	Right ear	Left ear	Right ear	Left ear	R	L	BL	U2	R	L
True OAE level (dB) med	11.8	11.5	11.5	11.7	0.7	0.6	0	1.2	0.30	0.15
Min, max	4.6, 21.8	−5.5, 23	1.5, 20.9	0.2, 22.4						

Table 3 continued

TEOAE	Baseline (BL) $n = 23$		4 weeks (U2) $n = 23$		Changes[b] BL–U2		Differences[c] right/left		p values BL–U2	
	Right ear	Left ear	Right ear	Left ear	R	L	BL	U2	R	L
Number of patients[a] (normal range 8–25 dB)	$n = 21$	$n = 19$	$n = 22$	$n = 20$						
Reproducibility (%) med	90.8	88.8	92.5	88.2	0.4	1	2.7	5.6	0.15	0.88
Min, max	14.2, 99.2	4.0, 99.3	26.4, 98.8	14.1, 99.1						
Number of patients[a] (normal range >60 %)	$n = 23$	$n = 23$	$n = 23$	$n = 23$						
DPOAE	Baseline (BL) $n = 23$		4 weeks (U2) $n = 23$		Changes[b] BL–U2		Differences[c] right/left		p values BL–U2	
(normal range >5 dB over noise in 3 of 5 frequencies)	Right ear (dB)	Left ear (dB)	Right ear (dB)	Left ear (dB)	R	L	BL	U2	R	L
True DP 1000 Hz med	3.3	4.5	−0.2	−1.3	0	1.9	0	0	0.12	0.06
Min, max	−6.7, 11.8	−4.9, 10.3	−7.7, 9.6	−8.5, 4.1						
Number of patients[a]	$n = 13$	$n = 10$	$n = 14$	$n = 13$						
True DP 1500 Hz med	6.2	4.4	5.3	6.7	−1.6	−0.6	2.2	1.3	0.95	0.51
Min, max	−10.3, 14.7	−10.2, 19.4	−9.7, 16.4	−10.5, 14.4						
Number of patients[a]	$n = 22$	$n = 21$	$n = 22$	$n = 19$						
True DP 2000 Hz med	8.0	6.1	7.8	4.3						
Min, max	−13.3, 15.3	−5.9, 14.7	−6.9, 16.5	−6.5, 14.4						
Number of patients[a]	$n = 21$	$n = 20$	$n = 20$	$n = 23$	−0.4	−0.7	0.2	1.8	0.41	0.82
True DP 3000 Hz med	7.1	4.8	9.8	5.3	0	−1.3	2.4	2.4	0.86	0.71
Min, max	−2.6, 17.4	−9.5, 11.7	−4.0, 15.1	−11.6, 16.7						
Number of patients[a]	$n = 21$	$n = 19$	$n = 21$	$n = 20$						
True DP 4000 Hz med	5.7	3.7	5.2	3.3	1.4	−0.9	3.8	4.1	0.09	0.86
Min, max	−17.7, 9.1	−16.4, 15.8	−17.6, 17.2	−16.9, 18.1						
Number of patients[a]	$n = 20$	$n = 21$	$n = 23$	$n = 20$						
BERA	Baseline (BL) $n = 23$		4 weeks (U2) $n = 23$		Changes[b] BL–U2		Differences[c] right/left		p values BL–U2	
	Right ear	Left ear	Right ear	Left ear	R	L	BL	U2	R	L
Latency t5 (ms) med	6.1	6.1	6.1	6.0	0.05	0.02	0.11	0.1	0.051	0.24

Table 3 continued

BERA	Baseline (BL) $n = 23$		4 weeks (U2) $n = 23$		Changes[b] BL–U2		Differences[c] right/left		p values BL–U2	
	Right ear	Left ear	Right ear	Left ear	R	L	BL	U2	R	L
Min, max	5.7, 6.7	5.6, 6.	5.7, 6.5	5.6, 6.						
Number of patients[a] (normal range 5.5–6.0 ms)	$n = 23$	$n = 23$	$n = 22$	$n = 23$						
Amplitude A5 (nV) med	319	267	289	321	−6.5	−13	23	13.5	0.88	0.47
Min, max	74, 538	123, 566	79, 554	153, 533						
Number of patients[a] (normal range 220–540 nV)	$n = 23$	$n = 23$	$n = 22$	$n = 23$						
Interpeak latency t5–t1 (ms) med	4.1	4.1	4.1	4.1	0.02	0	−0.01	0.02	0.51	0.55
Min, max	3.8, 4.5	3.7, 4.4	3.8, 4.4	3.5, 4.4						
Number of patients[a] (normal range 3.7–4.5 ms)	$n = 22$	$n = 21$	$n = 21$	$n = 21$						

[a] Number of patients can vary due to undetectable values for some frequencies in several patients (e.g., in the stapedius reflex measurement at 4000 Hz ipsilateral and contralateral) or because in some patients not all the tests from the hearing assessment were completed (e.g., otoscopy and search for tympanic membrane abnormalities)

[b] Changes BL–U2 calculated as median of differences between BL and U2 threshold values for right (R) and left (L) ear

[c] Differences right/left calculated as median of differences between right and left ear thresholds for baseline (BL) and U2 (U2)

and treated with either artesunate-amodiaquine (AA), AL or amodiaquine [34]. Hearing assessment was performed at baseline—before beginning the drug intake—and at days 3, 7, 28 and after 9 months and compared to a randomly selected age- and sex-matched control group of 57 healthy children from the same area in Ghana who were not treated with the study medication. The study showed elevated hearing thresholds in all three treatment groups, especially throughout the acute phase of the disease that were, however, fully reversible after a follow-up period of 9 months. Therefore it is most probable that these hearing impairments are owed to malaria itself and not to the artemisinin compound of the medication.

The findings from our study do not confirm the results of the retrospective study of Toovey [19] about small but irreversible hearing impairment after treatment for malaria with AL although the cumulative doses of ART used in our study were considerably higher than the doses administered in the above-mentioned malaria studies. Therefore we can confirm that oral ART was well tolerated for hearing in the majority of our breast cancer patients, but ongoing subclinical hearing losses or tinnitus cannot be excluded after long-term exposure.

Conclusion

Our results show that the continuous intake of ART for 4 weeks in doses up to 200 mg daily was well tolerated concerning neuro-audiological function at all three doses tested in patients with metastatic or locally advanced breast cancer. However, a temporary dose-limiting vertigo was observed: Two patients experienced ongoing subclinical hearing loss and another one an ongoing tinnitus. Therefore regular audiological assessments should be included in clinical studies investigating oral ART in the treatment of cancer patients to increase the database on ototoxicity.

Acknowledgments We thank Anthony Dayan for his preclinical toxicology assessment allowing the conduct of this study. This work was supported by H.W. and J. Hector Stiftung, Weinheim, Germany, HEIFAN (Heidelberger Förderverein der Ambulanz für Naturheilkunde) e. V., and Monika-Kutzner-Stiftung, Berlin, Germany. Study medication was kindly provided by Dafra Pharma International, Research and Development, Turnhout, Belgium.

Author contributions M.K. analyzed data and wrote the main paper; C.v.H. designed and planned the study, was the principal investigator of the study, recruited the patients, as well as critically revised the paper; I.W-S designed and planned the study and critically revised the paper; S.H. collected and analyzed data; I.B. designed experiments and collected data; L.E. planned, consulted and reviewed biostatistical analysis; S.S. designed and performed experiments, analyzed data as well as wrote and critically revised the paper. All authors discussed the results and implications and commented on the manuscript at all stages as well as read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest No conflicts of interest are declared.

Ethical standards The ARTIC M33/2 study obtained approval by the competent federal authority (BfArM Submission No. 4033804) and by the Ethical Committee of the Faculty of Medicine, Heidelberg University, Germany, registered as AFmu-495/2007 before start of recruitment. Written informed consent was obtained from each participant prior to screening. The study was performed in accordance with the Declaration of Helsinki (1964) and its later amendments and was registered with ClinicalTrials.gov number NCT00764036 and with EudraCT-Number 2007-004432-23.

References

1. Davis TM, Karunajeewa HA, Ilett KF (2005) Artemisinin-based combination therapies for uncomplicated malaria. Med J Aust 182(4):181–185
2. World Health Organization (2006) Guidelines for the treatment of malaria. World Health Organization, Geneva
3. Sun WC, Han JX, Yang WY, Deng DA, Yue XF (1992) Antitumor activities of 4 derivatives of artemisic acid and artemisinin B in vitro. Zhongguo Yao Li Xue Bao 13(6):541–543
4. Efferth T, Dunstan H, Sauerbrey A, Miyachi H, Chitambar CR (2001) The anti-malarial artesunate is also active against cancer. Int J Oncol 18(4):767–773
5. Li Y, Shan F, Wu JM, Wu GS, Ding J, Xiao D, Yang WY, Atassi G, Leonce S, Caignard DH, Renard P (2001) Novel antitumor artemisinin derivatives targeting G1 phase of the cell cycle. Bioorg Med Chem Lett 11(1):5–8
6. Singh NP, Lai H (2001) Selective toxicity of dihydroartemisinin and holotransferrin toward human breast cancer cells. Life Sci 70(1):49–56
7. Reungpatthanaphong P, Mankhetkorn S (2002) Modulation of multidrug resistance by artemisinin, artesunate and dihydroartemisinin in K562/adr and GLC4/adr resistant cell lines. Biol Pharm Bull 25(12):1555–1561
8. Disbrow GL, Baege AC, Kierpiec KA, Yuan H, Centeno JA, Thibodeaux CA, Hartmann D, Schlegel R (2005) Dihydroartemisinin is cytotoxic to papillomavirus-expressing epithelial cells in vitro and in vivo. Cancer Res 65(23):10854–10861. doi:10.1158/0008-5472.CAN-05-1216
9. Chen HH, Zhou HJ, Wang WQ, Wu GD (2004) Antimalarial dihydroartemisinin also inhibits angiogenesis. Cancer Chemother Pharmacol 53(5):423–432. doi:10.1007/s00280-003-0751-4
10. 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(12):2359–2366. doi:10.1016/j.bcp.2004.08.021
11. Moore JC, Lai H, Li JR, Ren RL, McDougall JA, Singh NP, Chou CK (1995) Oral administration of dihydroartemisinin and ferrous sulfate retarded implanted fibrosarcoma growth in the rat. Cancer Lett 98(1):83–87
12. Berger TG, Dieckmann D, Efferth T, Schultz ES, Funk JO, Baur A, Schuler G (2005) Artesunate in the treatment of metastatic uveal melanoma–first experiences. Oncol Rep 14(6):1599–1603
13. Singh NP, Verma KB (2002) Case report of a laryngeal squamous cell carcinoma treated with artesunate. Arch Oncol 10(4):279–280
14. Genovese RF, Newman DB, Brewer TG (2000) Behavioral and neural toxicity of the artemisinin antimalarial, arteether, but not artesunate and artelinate, in rats. Pharmacol Biochem Behav 67(1):37–44

15. Genovese RF, Newman DB, Li Q, Peggins JO, Brewer TG (1998) Dose-dependent brainstem neuropathology following repeated arteether administration in rats. Brain Res Bull 45(2):199–202
16. Kissinger E, Hien TT, Hung NT, Nam ND, Tuyen NL, Dinh BV, Mann C, Phu NH, Loc PP, Simpson JA, White NJ, Farrar JJ (2000) Clinical and neurophysiological study of the effects of multiple doses of artemisinin on brain-stem function in Vietnamese patients. Am J Trop Med Hyg 63(1–2):48–55
17. Van Vugt M, Angus BJ, Price RN, Mann C, Simpson JA, Poletto C, Htoo SE, Looareesuwan S, White NJ, Nosten F (2000) A case–control auditory evaluation of patients treated with artemisinin derivatives for multidrug-resistant *Plasmodium falciparum* malaria. Am J Trop Med Hyg 62(1):65–69
18. McCall MB, Beynon AJ, Mylanus EA, van der Ven AJ, Sauerwein RW (2006) No hearing loss associated with the use of artemether–lumefantrine to treat experimental human malaria. Trans R Soc Trop Med Hyg 100(12):1098–1104. doi:10.1016/j.trstmh.2006.02.017
19. Toovey S, Jamieson A (2004) Audiometric changes associated with the treatment of uncomplicated falciparum malaria with co-artemether. Trans R Soc Trop Med Hyg 98(5):261–267; discussion 268-269. doi:10.1016/j.trstmh.2003.11.001
20. Mehta U, Barnes KI, Kathard H, Vugt M, Durrheim D (2005) Comment on: audiometric changes associated with the treatment of uncomplicated falciparum malaria with co-artemether. Trans R Soc Trop Med Hyg 99(4):313–314; author reply 314-315. doi:10.1016/j.trstmh.2004.11.006
21. Hutagalung R, Htoo H, Nwee P, Arunkamomkiri J, Zwang J, Carrara VI, Ashley E, Singhasivanon P, White NJ, Nosten F (2006) A case–control auditory evaluation of patients treated with artemether–lumefantrine. Am J Trop Med Hyg 74(2):211–214
22. Reinhart H, Hughson C, de Palacios PI (2005) Comment on: audiometric changes associated with the treatment of uncomplicated falciparum malaria with co-artemether. Trans R Soc Trop Med Hyg 99(4):315–316; author reply 316-317. doi:10.1016/j.trstmh.2004.11.008
23. Panossian LA, Garga NI, Pelletier D (2005) Toxic brainstem encephalopathy after artemisinin treatment for breast cancer. Ann Neurol 58(5):812–813. doi:10.1002/ana.20620
24. Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, Filiberti A, Flechtner H, Fleishman SB, Dehaes JCJM, Kaasa S, Klee M, Osoba D, Razavi D, Rofe PB, Schraub S, Sneeuw K, Sullivan M, Takeda F (1993) The European-Organization-for-Research-and-Treatment-of-Cancer Qlq-C30—a quality-of-life instrument for use in international clinical-trials in oncology. J Natl Cancer I 85(5):365–376. doi:10.1093/jnci/85.5.365
25. Sertel S, Eichhorn T, Sieber S, Sauer A, Weiss J, Plinkert PK, Efferth T (2010) Factors determining sensitivity or resistance of tumor cell lines towards artesunate. Chem Biol Interact 185(1):42–52. doi:10.1016/j.cbi.2010.02.002
26. Sertel S, Eichhorn T, Simon CH, Plinkert PK, Johnson SW, Efferth T (2010) Pharmacogenomic identification of c-Myc/Max-regulated genes associated with cytotoxicity of artesunate towards human colon, ovarian and lung cancer cell lines. Molecules 15(4):2886–2910. doi:10.3390/molecules15042886
27. Krishna S, Bustamante L, Haynes RK, Staines HM (2008) Artemisinins: their growing importance in medicine. Trends Pharmacol Sci 29(10):520–527. doi:10.1016/j.tips.2008.07.004
28. Firestone GL, Sundar SN (2009) Anticancer activities of artemisinin and its bioactive derivatives. Expert Rev Mol Med 11:e32. doi:10.1017/S1462399409001239
29. Krishna S, Ganapathi S, Ster IC, Saeed ME, Cowan M, Finlayson C, Kovacsevics H, Jansen H, Kremsner PG, Efferth T, Kumar D (2015) A randomised, double blind, placebo-controlled pilot study of oral artesunate therapy for colorectal cancer. EBioMedicine 2(1):82–90. doi:10.1016/j.ebiom.2014.11.010
30. Carrasquilla G, Baron C, Monsell EM, Cousin M, Walter V, Lefevre G, Sander O, Fisher LM (2012) Randomized, prospective, three-arm study to confirm the auditory safety and efficacy of artemether–lumefantrine in Colombian patients with uncomplicated *Plasmodium falciparum* malaria. Am J Trop Med Hyg 86(1):75–83. doi:10.4269/ajtmh.2012.11-0192
31. Carrara VI, Phyo AP, Nwee P, Soe M, Htoo H, Arunkamomkiri J, Singhasivanon P, Nosten F (2008) Auditory assessment of patients with acute uncomplicated *Plasmodium falciparum* malaria treated with three-day mefloquine-artesunate on the north-western border of Thailand. Malar J 7:233. doi:10.1186/1475-2875-7-233
32. Gurkov R, Eshetu T, Miranda IB, Berens-Riha N, Mamo Y, Girma T, Krause E, Schmidt M, Hempel JM, Loscher T (2008) Ototoxicity of artemether/lumefantrine in the treatment of falciparum malaria: a randomized trial. Malar J 7:179. doi:10.1186/1475-2875-7-179
33. Schmutzhard J, Kositz CH, Lackner P, Dietmann A, Fischer M, Glueckert R, Reindl M, Stephan K, Riechelmann H, Schrott-Fischer A, Schmutzhard E (2010) Murine malaria is associated with significant hearing impairment. Malar J 9:159. doi:10.1186/1475-2875-9-159
34. Adjei GO, Goka BQ, Kitcher E, Rodrigues OP, Badoe E, Kurtzhals JA (2013) Reversible audiometric threshold changes in children with uncomplicated malaria. J Trop Med 2013:360540. doi:10.1155/2013/360540