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CLINICAL TRIAL

The utility of DHL-HisZnNa, a novel antioxidant, against anticancer agent-induced alopecia in breast cancer patients: a multicenter phase II clinical trial

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Abstract

Purpose Chemotherapy-induced alopecia (CIA) is a distressing adverse effect of anticancer drugs; however, there are currently no mechanisms to completely prevent CIA. In this study, we performed a clinical trial to examine whether sodium *N*-(dihydrolipoyl)-l-histidinate zinc complex (DHL-HisZnNa), an alpha-lipoic acid derivative, prevents CIA in patients with breast cancer.

Methods Between July 2014 and May 2015, we performed a multi-center, single arm, clinical trial involving 103 breast cancer patients who received adjuvant chemotherapy at three medical institutions in Japan. During chemotherapy, a lotion containing 1% DHL-HisZnNa was applied daily to the patients' scalps. The primary endpoint was the incidence of grade 2 alopecia; the secondary endpoints were the duration of grade 2 alopecia, alopecia-related symptoms, and drug-related adverse events. Alopecia was evaluated by three independent reviewers using head photographs taken from four angles.

Results Safety analysis was performed for 101 patients who started the protocol therapy. After excluding one patient who experienced disease progression during treatment, 100 patients who received at least two courses of chemotherapy underwent efficacy analysis. All original 101 patients developed grade 2 alopecia, the median durations of which were 119 days (112–133 days) and 203 days (196–212 days) in the groups treated with four and eight courses of chemotherapy, respectively. Mild or moderate adverse events potentially related to DHL-HisZnNa were observed in 11 patients. Alopecia-related symptoms were observed in 53 patients (52%).

Conclusions The application of 1% DHL-HisZnNa to the scalp did not prevent CIA. However, this drug may promote recovery from CIA.

Trial registration number: UMIN000014840.

Keywords Alopecia · Antioxidants · Chemotherapy · DHL-HisZnNa

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Abbreviations

CIA	Chemotherapy-induced alopecia
CTCAE	Common Toxicity Criteria for Adverse Events
DHL-HisZnNa	Sodium *N*-(dihydrolipoyl)-l-histidinate zinc complex

Introduction

Chemotherapy-induced alopecia (CIA) has a substantial impact on a patient's body image and is one of the most distressing adverse effects of chemotherapy [1]. The incidence of CIA varies depending on the chemotherapy regimen, but adjuvant chemotherapy for breast cancer is a particularly more widespread cause of CIA [2]. Moreover, there are no drugs or devices available that can completely prevent CIA.

CIA is thought to be caused by chemotherapy-induced oxidative stress [3], and it has also been reported that hair follicle cell apoptosis due to chemotherapy-induced cytotoxicity also contributes to this adverse effect [4, 5] Alpha-lipoic acid derivatives, which are potent endogenous antioxidants, have been shown to have anti-apoptotic effects [6, 7]. Based on these previous data, we hypothesized that alpha-lipoic acid derivatives are effective in preventing CIA. Hence, we developed a lotion containing a stable, water-soluble alpha-lipoic acid derivative (Fig. 1) derived from the poorly-soluble and unstable alpha-lipoic acid. We previously reported the results of our in vivo model in which the application of sodium *N*-(dihydrolipoyl)-l-histidinate zinc complex (DHL-HisZnNa) to the body surface prevented cytarabine-induced alopecia in rats [8]. In this study, we performed a clinical trial to examine whether DHL-HisZnNa prevents CIA in breast cancer patients undergoing chemotherapy.

Methods

Study design and patients

This phase II, multicenter, single-arm clinical trial was performed in female breast cancer patients who received pre- and postoperative adjuvant chemotherapy between July 2014 and May 2015. Because the incidence rate of grade 2 alopecia during adjuvant chemotherapy for breast cancer is almost 100% [2], we did not use a control group.

This clinical trial was performed at three facilities in Japan: Kyushu Cancer Center, Aichi Cancer Center, and the University of Tsukuba Hospital. This study was approved by the ethics committee of Kyushu Cancer Center, the primary institution (approval number: 2014-89). Informed consent was obtained from all individual participants included in the study.

The main inclusion criteria were: (i) women between 20 and 80 years of age, (ii) diagnosed with breast cancer, (iii) treated with four or eight cycles of adjuvant therapy with taxane or anthracycline, and (iv) an Eastern Cooperative Oncology Group performance status score of 0–1 (Table 1). Patients for whom chemotherapy was contraindicated as well as those with severe hypersensitivity or an underlying disease were excluded (Table 1).

The lotion containing 1% DHL-HisZnNa was purchased from Beatrix Inc. (Chiba, Japan), who we contracted to manufacture the drug.

Intervention and evaluation

During chemotherapy, a 4-mL dose of the study drug was applied four times a day to each patient's scalp (Fig. 2). Combination therapy that included surgery, radiotherapy, hormone therapy, and the molecular targeted agent trastuzumab was allowed before, during, and after chemotherapy. The application of topical agents (e.g., other hair growth stimulators and steroids) and permanent waves (hairstyle change) was prohibited.

Alopecia was evaluated by a central judging committee of three independent evaluators (K. K., T. T., and C. S.) who performed image analysis. Photographs of each participant's

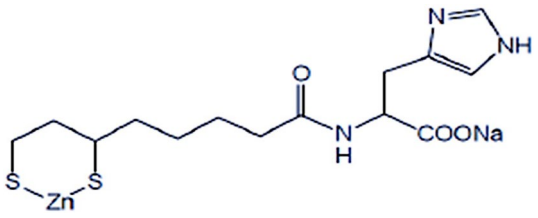

Fig. 1 DHL-HisZnNa chemical structural formula

Table 1 Patient inclusion and exclusion criteria

Inclusion criteria
Histologically proven breast carcinoma
Planned chemotherapy used anthracycline and/or taxane
Age 20–80 years
Performance status: 0, 1
Adequate organ function
Consent to join this trial
Exclusion criteria
Previous history of hypersensitivity due to drug and/or cosmetics or hair growth agent
Severe complication
Pregnancy or lactation
Male patient
No ability or intention to comply with the protocol method
Physicians think inadequate

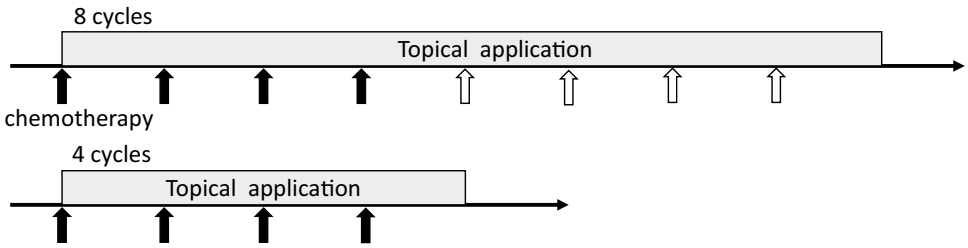

Fig. 2 Intervention schedule. Patients applied the experimental drug to their scalp daily during chemotherapy

Table 2 Alopecia score and grade (Common Toxicity Criteria for Adverse Events, version 4.0)

Alopecia category from photograph	Grade	
A: 0%	0	No hair loss
B: 1–25%	1	Hair loss of < 50% of normal
C: 26–50%		
D: 51–75%	2	Hair loss of ≧ 50% of normal
E: 76–100%		

head taken from four different angles (left and right temporal regions, the parietal region, and occipital region) were classified into five categories (A: no alopecia, B: 1–25% alopecia, C: 26–50% alopecia, D: 51–75% alopecia, and E: 76–100% alopecia). The evaluations were combined into a single assessment as follows: If all three evaluations match, that category was used; if the assessment of one evaluator differed from that of the other two, the assessment of the majority (two of three) was used; and if none of the three assessments matched, the mid-range category among the three was adopted. Alopecia was graded according to the Common Toxicity Criteria for Adverse Events version 4.0 (CTCAE v4.0) grading system as follows: (i) grade 0 (no alopecia [A]); (ii) grade 1 (1–25% alopecia [B] or 26–50% alopecia [C]); and (iii) grade 2 (51–75% alopecia [D] or 76–100% alopecia [E]) (Table 2).

The photographs were acquired before chemotherapy, during each course of chemotherapy, and at 3, 6, 9, and 12 months after chemotherapy. The treatment period was defined as that in which the study drug was applied during chemotherapy. The post-treatment follow-up period was 12 months.

The primary endpoint was the incidence of grade 2 alopecia, while the secondary endpoints were the duration of grade 2 alopecia, study drug-related adverse events, and alopecia-related symptoms.

Statistical analysis

Based on existing report that the incidence of grade 2 alopecia in patients receiving adjuvant chemotherapy for breast cancer is almost 100% [2], our null hypothesis was that the incidence of grade 2 alopecia in our patients would be 95%. We also hypothesized that the study drug would reduce the incidence of grade 2 alopecia by 10%. The expected number of enrolled patients calculated using a binomial test was 100 with 90% statistical power at $P < 0.05$ (one-tailed).

Results

A total of 103 participants were enrolled between July 2014 and May 2015. We assessed the safety for 101 participants who received one cycle of chemotherapy at least as safety and the efficacy for 100 participants who received more than 2 cycles. The median age was 50 years (range, 20–75 years). Thirty-four (34%) and 66 participants (66%) received four and eight cycles of chemotherapy, respectively. The patients' clinical and treatment characteristics are shown in Table 3. Of the 101 patients who commenced protocol therapy, 85 (84%) completed it. The reasons for discontinuing therapy in 15 patients were: (i) withdrawal of consent ($n = 10$); (ii) transfer to another institution ($n = 1$); (iii) worsening of complications ($n = 1$); and (iv) other reasons ($n = 3$) (Fig. 3).

All 101 patients developed grade 2 alopecia (i.e., the primary endpoint). The median durations of affliction with grade 2 alopecia (i.e., a secondary endpoint) in the groups treated with four and eight courses of chemotherapy were 119 days (range, 112–133 days) and 203 days (range, 196–212 days), respectively. Adverse events potentially related to the study drug were observed in 11 patients; all were grade 1 or 2 per CTCAE version 4.0 criteria. These adverse events consisted of rash/exfoliation ($n = 6$), itching ($n = 3$), pigment disorder ($n = 1$), and other symptoms ($n = 5$) (Table 4).

Alopecia-related symptoms were observed in 53 patients (52%) and consisted of scalp pain ($n = 18$ [18%]), scalp itching ($n = 28$ [28%]), or both ($n = 7$ [7%]). Alopecia grades at baseline and 12 months after chemotherapy are shown in Fig. 4. Of the 100 patients who were subjected to efficacy analysis, 71 (71%) had grade 0 or 1 alopecia at 3 months after chemotherapy, while 7 (7%) had grade 2 at that time.

Table 3 Patient characteristics

	$N = 101$ *n* (%)
Age	
Median (range)	50 (22–75)
Menstrual status	
Premenopausal	61
Postmenopausal	40
Performance status	
0	99
1	2
Stage[a]	
I	35
IIA	32
IIB	17
IIIA	9
IIIB	5
IIIC	2
IV	1
Pretreatment	
Operation	
No	32 (32)
Yes	69 (68)
Radiation (for breast)	
No	100 (99)
Yes	0
Unknown	(1)
Hormonetherapy	
No	98 (97)
Yes	2 (2)
Unknown	1 (1)
Chemotherapy	
No	100 (99)
Yes	0
Unknown	1 (1)
Trastuzumab	
No	99 (98)
Yes	1 (1)
Unknown	1 (1)

[a]Union for International Cancer Control-TNM classification, 7th edition

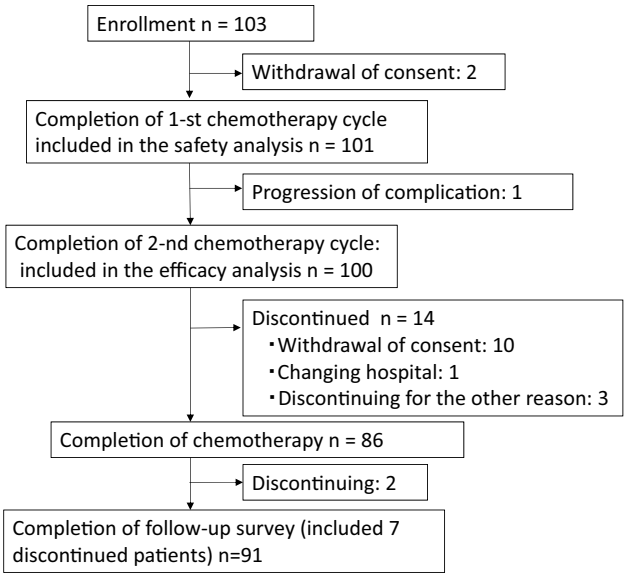

Fig. 3 CONSORT diagram. The population included in the primary efficacy analysis comprised patients who completed at least two cycles of chemotherapy; the secondary efficacy analysis was of patients who completed at least one cycle of chemotherapy

Table 4 Adverse events

Adverse event	G1,2	G3,4
Rash/exfoliation	6 (6%)	0 (0%)
Itching	3 (3%)	0 (0%)
Pigment disorder	1 (1%)	0 (0%)
Other	5 (5%)	0 (0%)

Discussion

Our trial revealed that DHL-HisZnNa failed to prevent CIA, as all patients developed grade 2 alopecia. It has been reported that CIA-induced pain and itching are observed in approximately 50% of patients [9], which is consistent with the incidence of CIA-induced pain and itching (52%) observed in our trial. Additionally, there were no study drug-related grade 3 or 4 adverse events, demonstrating the safety of DHL-HisZnNa. The incidences of grade 0/1 alopecia were 71% and 86% at 3 and 6 months after chemotherapy, respectively. Although there are some reports of patients using a wig, experiencing onset of hair growth, and recovering their quality of life during CIA abatement [10–12], there have been no reports of alopecia recovery as evaluated using an alopecia grading system. Therefore, it is difficult to compare previously published data to ours. However, breast cancer specialists at our institution noted that the recovery from alopecia was surprisingly fast. The alopecia grade is calculated based on its severity compared to the total hair volume; however, because alopecia measurement devices are not well described, the condition is generally assessed visually by the attending physicians. Therefore, evaluations of alopecia severity appear to be extremely subjective. In this study, we established a central judging committee comprising three breast cancer specialists (i.e., independent evaluators) who evaluated photographs taken from four different angles. Because the concordance rate of the three evaluators using this method was high (data not shown), the accuracy of alopecia grading in this clinical trial may be higher than

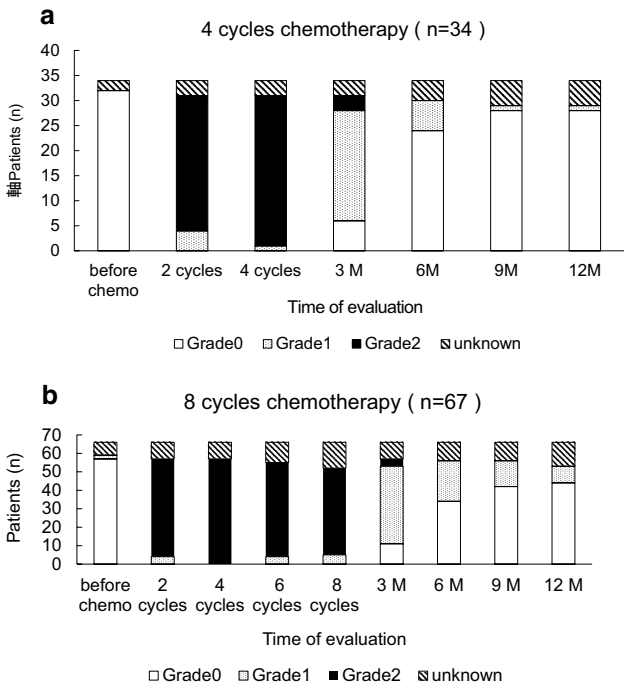

Fig. 4 **a, b** Transition of alopecia grade. The graph shows the participants' alopecia grade at each evaluation

that in previous studies that used conventional CIA evaluation methods.

On the other hand, some animal models have shown that antioxidants (e.g., N-acetylcysteine [3] and L-cysteine/vitamin B16 [13]) promote CIA prevention. We also previously found that drugs containing 1% DHL-HisZnNa reduced cytarabine-induced CIA [8]. Moreover, there are reports that, besides their use in scalp cooling, oral administration of tocopherol [14] or *Panicum miliaceum* [15] prevents CIA in humans. These results support the notion that DHL-HisZnNa may promote recovery from alopecia. While there are reports [16–18] of breast cancer patients who had not recovered from CIA 1 year after chemotherapy, the incidence of grade 0 alopecia in the groups treated with four or eight courses of chemotherapy at 1 year after the trial in our clinical study was 100% (after excluding those with unknown CIA status). Therefore, DHL-HisZnNa may be effective in preventing permanent CIA.

While there are various studies of CIA prevention, the only method currently in clinical use is scalp cooling [19–21], which reportedly attenuates damage to hair follicles by causing vasoconstriction that reduces blood flow and thus lowers exposure to reactive chemotherapeutic agents [22, 23]. Scalp cooling has been shown to reduce grade 2 alopecia [20, 21]; however, it is more time-consuming and costly and still requires the use of a wig or head cover [10], thus limiting activities of daily living [24]. DHL-HisZnNa may therefore be a more useful treatment for CIA than scalp cooling, as it is very convenient and causes less discomfort. Moreover, the drug may potentially be combined with scalp cooling agents, which may increase the efficacy of alopecia prevention.

Limitations

The study cohort comprised breast cancer patients who received adjuvant chemotherapy, but there was no control group. The reasons for this were that grade 2 alopecia occurs in almost all patients receiving anticancer adjuvant chemotherapy and that the primary endpoint was the incidence of grade 2 alopecia. However, our study did not use an objective measure to demonstrate fast recovery from alopecia during recuperation from chemotherapy. Therefore, future clinical trials that accurately examine the incidence of alopecia-related symptoms, alopecia recovery time, and the mechanism of its development are warranted.

Conclusions

Our trial showed that the application of a lotion containing 1% DHL-HisZnNa to the scalp does not prevent CIA in patients undergoing treatment for breast cancer. However, the safe and convenient drug may promote recovery from CIA.

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Compliance with ethical standards

Conflict of interest Oita University received Oita University Aderans Company Limited Collaboration Research Fee.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Lemieux J, Maunsell E, Provencher L (2008) Chemotherapy-induced alopecia and effects on quality of life among women with

breast cancer: a literature review. Psycho-Oncology 17:317–328. https://doi.org/10.1002/pon.1245
2. Moebus V, Jackisch C, Lueck HJ et al (2010) Intense dose-dense sequential chemotherapy with epirubicin, paclitaxel, and cyclophosphamide compared with conventionally scheduled chemotherapy in high-risk primary breast cancer: mature results of an AGO phase III study. J Clin Oncol 28:2874–2880. https://doi.org/10.1200/JCO.2009.24.7643
3. D'Agostini F, Bagnasco M, Giunciuglio D et al (1998) Inhibition by oral N-acetylcysteine of doxorubicin-induced clastogenicity and alopecia, and prevention of primary tumors and lung micrometastases in mice. Int J Oncol 13:217–224
4. Schilli MB, Paus R, Menrad A (1998) Reduction of intrafollicular apoptosis in chemotherapy-induced alopecia by topical calcitriol-analogs. J Invest Dermatol 111:598–604. https://doi.org/10.1046/j.1523-1747.1998.00350.x
5. Botchkarev VA (2003) Molecular mechanisms of chemotherapy-induced hair loss. J Investig Dermatol Symp Proc 8:72–75. https://doi.org/10.1046/j.1523-1747.2003.12175.x
6. Hiratsuka T, Inomata M, Kono Y et al (2013) DHLTauZnNa, a newly synthesized alpha-lipoic acid derivative, induces autophagy in human colorectal cancer cells. Oncol Rep 29:2140–2146. https://doi.org/10.3892/or.2013.2394
7. Kono Y, Inomata M, Hagiwara S et al (2012) Antiproliferative effects of a new alpha-lipoic acid derivative, DHL-HisZnNa, in HT29 human colon cancer cells in vitro. Expert Opin Ther Targets 16(Suppl 1):S103–S109. https://doi.org/10.1517/14728222.2011.640320
8. Hagiwara S, Uchida T, Koga H et al (2011) The alpha-lipoic acid derivative sodium zinc dihydrolipoylhistidinate reduces chemotherapy-induced alopecia in a rat model: a pilot study. Surg Today 41:693–697. https://doi.org/10.1007/s00595-010-4481-z
9. Chon SY, Champion RW, Geddes ER et al (2012) Chemotherapy-induced alopecia. J Am Acad Dermatol 67:e37–e47. https://doi.org/10.1016/j.jaad.2011.02.026
10. van den Hurk CJ, van den Akker-van Marle ME, Breed WP et al (2014) Cost-effectiveness analysis of scalp cooling to reduce chemotherapy-induced alopecia. Acta Oncol 53:80–87. https://doi.org/10.3109/0284186X.2013.794955
11. Duvic M, Lemak NA, Valero V et al (1996) A randomized trial of minoxidil in chemotherapy-induced alopecia. J Am Acad Dermatol 35:74–78. https://doi.org/10.1016/s0190-9622(96)90500-9
12. Cho J, Choi EK, Kim IR et al (2014) Development and validation of Chemotherapy-induced Alopecia Distress Scale (CADS) for breast cancer patients. Ann Oncol 25:346–351. https://doi.org/10.1093/annonc/mdt476
13. D'Agostini F, Fiallo P, Ghio M et al (2013) Chemoprevention of doxorubicin-induced alopecia in mice by dietary administration of L-cystine and vitamin B6. Arch Dermatol Res 305:25–34. https://doi.org/10.1007/s00403-012-1253-1
14. Martin-Jimenez M, Diaz-Rubio E, Gonzalez Larriba JL et al (1986) Failure of high-dose tocopherol to prevent alopecia induced by doxorubicin. N Engl J Med 315:894–895. https://doi.org/10.1056/NEJM198610023151416
15. Gardani G, Cerrone R, Biella C et al (2007) A case–control study of Panicum Miliaceum in the treatment of cancer chemotherapy-induced alopecia. Minerva Med 98:661–664
16. Prevezas C, Matard B, Pinquier L et al (2009) Irreversible and severe alopecia following docetaxel or paclitaxel cytotoxic therapy for breast cancer. Br J Dermatol 160:883–885
17. Kluger N, Jacot W, Frouin E et al (2012) Permanent scalp alopecia related to breast cancer chemotherapy by sequential fluorouracil/epirubicin/cyclophosphamide (FEC) and docetaxel: a prospective study of 20 patients. Ann Oncol 23:2879–2884. https://doi.org/10.1093/annonc/mds095
18. Fonia A, Cota C, Setterfield JF et al (2017) Permanent alopecia in patients with breast cancer after taxane chemotherapy and adjuvant hormonal therapy: Clinicopathologic findings in a cohort of 10 patients. J Am Acad Dermatol 76:948–957. https://doi.org/10.1016/j.jaad.2016.12.027
19. Shin H, Jo SJ, Kim DH et al (2015) Efficacy of interventions for prevention of chemotherapy-induced alopecia: a systematic review and meta-analysis. Int J Cancer 136:E442–E454. https://doi.org/10.1002/ijc.29115
20. Nangia J, Wang T, Osborne C et al (2017) Effect of a scalp cooling device on alopecia in women undergoing chemotherapy for breast cancer: the SCALP Randomized Clinical Trial. JAMA 317:596–605. https://doi.org/10.1001/jama.2016.20939
21. Friedrichs K, Carstensen MH (2014) Successful reduction of alopecia induced by anthracycline and taxane containing adjuvant chemotherapy in breast cancer—clinical evaluation of sensor-controlled scalp cooling. SpringerPlus 3:500. https://doi.org/10.1186/2193-1801-3-500
22. Bulow J, Friberg L, Gaardsting O et al (1985) Frontal subcutaneous blood flow, and epi- and subcutaneous temperatures during scalp cooling in normal man. Scand J Clin Lab Invest 45:505–508
23. Al-Tameemi W, Dunnill C, Hussain O et al (2014) Use of in vitro human keratinocyte models to study the effect of cooling on chemotherapy drug-induced cytotoxicity. Toxicol In Vitro 28:1366–1376. https://doi.org/10.1016/j.tiv.2014.07.011
24. Mols F, van den Hurk CJ, Vingerhoets AJ et al (2009) Scalp cooling to prevent chemotherapy-induced hair loss: practical and clinical considerations. Support Care Cancer 17:181–189. https://doi.org/10.1007/s00520-008-0475-4