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Safety and Efficacy of the Long-term Adjuvant Treatment of Primary Intermediate- to High-Risk Malignant Melanoma (UICC/AJCC Stage II and III) with a Standardized Fermented European Mistletoe *(Visum album L.)* Extract

Results from a multicenter, comparative, epidemiological cohort study in Germany and Switzerland

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Safety and Efficacy of the Long-term Adjuvant Treatment of Primary Intermediate- to High-Risk Malignant Melanoma (UICC/AJCC Stage II and III) with a Standardized Fermented European Mistletoe *(Viscum album L.)* Extract

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Summary

Background: **Mistletoe therapy is the most frequently used complementary treatment in cancer patients in Germany and Switzerland. However, its safety and efficacy were controversially discussed, also in case of malignant melanoma (MM).**

Objectives: **The present study should evaluate the therapeutic safety and efficacy of a long-term therapy with a standardized fermented European mistletoe (*Viscum album L.*) extract Iscador™ (FME) during post-surgical aftercare of primary intermediate to high-risk MM (UICC/AJCC stage II–III) patients and compare it with an untreated parallel control group from the same cohort.**

Methods: **The study was designed as a multicenter, comparative, retrolective, epidemiological cohort study with parallel groups, carried out according to the guidelines of Good Epidemiological Practice (GEP). All patients suffered from surgically treated and histopathologically confirmed primary MM in UICC/AJCC stage II–III without distant metastases. In the study group, FME was administered subcutaneously 2–3 times weekly for at least six months, while the untreated control group was merely observed ("watchful waiting"). In both groups some patients also received radio-, chemo-, and/or immunotherapy. The patients were followed until the last visit or until death. Unselected, chronologically ordered, and standardized anonymous data from medical records that satisfied the predefined eligibility criteria were included for the "per protocol" analysis. Safety was assessed by the number**

Key words

- **Iscador™**
- **Melanoma**
- **Mistletoe, comparative cohort study, efficacy, safety, survival**
- ***Viscum album L.***

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of patients with FME-associated adverse drug reactions (ADRs) and by the search for tumor enhancement. The primary endpoint of efficacy was the adjusted tumor-related survival. Secondary endpoints were the overall-, the disease-free- and the brain metastasis-free survival. The survival results were analyzed after adjustment for baseline imbalances, treatment regimens and other potential confounders by the Cox proportional hazard regression method.

Results: **686 eligible patients (329 FME vs. 357 controls) from 35 centers were observed for a median aftercare of 81 vs. 52 months. The median FME therapy duration was 30 months. At baseline, both groups were comparable concerning demography, tumor history and risk factors for progression. Additional adjuvant chemotherapy was more frequent in the study group, while immunotherapy was more frequent in the control group. Eleven patients (3.3 %) developed systemic ADRs attributed to the FME-treatment, and 42 patients (12.8 %) developed local ADRs, with mild to intermediate (WHO/CTC grade 1–2) ADR severity and spontaneous resolution in most cases. In six patients the ADRs resulted in therapy termination. Life-threatening ADRs, ADR-related mortality or tumor enhancement were not observed. On the contrary, the incidence rate of lung metastases and the adjusted hazard ratio for brain metastases were significantly lower in the FME group. In the course of the study and during aftercare a total of 212 (30.9 %) patients relapsed or progressed, and 107 (15.6 %) died. A significantly longer tumor-related survival was found in the FME group when compared with the untreated controls (unadjusted tumor-related mortality rate 8.9 % vs. 10.7 %, Kaplan-Meier estimate, Log-rank test, p = 0.017), which was confirmed after adjusting for potential confounders by the tumor-related mortality hazard ratio estimate *HR (95 % confidence intervals) = 0.41 (0.23–0.71), p = 0.002.* The adjusted HR results of the overall survival, disease-free survival, and the brain metastases-free survival were also significantly superior in the FME group.**

Conclusion: **The long-term FME treatment in patients with primary intermediate to high-risk MM appears safe. Tumor enhancement was not observed. When compared with an untreated parallel control group from the same cohort, the results of the FME treatment suggested a significant survival benefit in primary stage II–III MM patients. These results on survival warrant reconfirmation in a prospective randomized clinical trial with optimized study design and treatment conditions.**

Zusammenfassung

Wirksamkeit und Sicherheit der komplementären Langzeitbehandlung von malignem Melanom mit mittlerem bis hohem Risiko (UICC/AJCC Stadium II–III) mit einem standardisierten, fermentierten Mistelextrakt *(Viscum album L.)* / Ergebnisse einer multizentrischen, komparativen, epidemiologischen Kohortenstudie in Deutschland und der Schweiz

Hintergrund: **Misteltherapie ist die am häufigsten angewendete komplementäre Behandlung bei Krebspatienten in Deutschland und in der Schweiz. Ihre Sicherheit und Wirksamkeit wurden jedoch kontrovers diskutiert, auch im Falle vom malignen Melanom.**

Ziele: **Die vorliegende Studie sollte die therapeutische Sicherheit und Wirksamkeit einer langfristigen Therapie mit einem standardisierten, fermentierten Mistelextrakt (*Viscum album L.*, Iscador®, FME) bei Patienten mit malignem Melanom mit mittlerem bis hohem Risiko (UICC/AJCC Stadium II–III) im Rahmen der onkologischen Nachsorge, nach vollständiger Tumorresektion, im Vergleich mit einer unbehandelten parallelen Kontrollgruppe aus derselben Kohorte, untersuchen.**

Methoden: **Die Studie wurde geplant und durchgeführt als multizentrische, vergleichende, retrolektive, epidemiologische Kohortenstudie mit Parallelgruppen, entsprechend den Richtlinien für Gute Epidemiologische Praxis (GEP). Alle Patienten, hatten ein reseziertes, histopathologisch bestätigtes, primäres, malignes Melanom in UICC/AJCC Stadien II–III ohne Fernmetastasen. In der Prüfgruppe wurde FME subkutan 2- bis 3mal wöchentlich für mindestens drei Monate verabreicht, während die unbehandelte Kontrollgruppe lediglich beobachtet wurde ("watchful waiting"). In beiden Gruppen erhielten einige Patienten auch Radio-, Chemo-, und/oder Immuntherapie. Die Patienten wurden bis zum letzten Besuch oder bis zum Tod dokumentiert. Unselektierte, chronologisch geordnete, standardisierte und anonymisierte Daten aus medizinischen Aufzeichnungen, welche die vordefinierten Teilnahmekriterien erfüllten, wurden in die „per Protokoll"-Analyse eingeschlossen. Die Sicherheit wurde entsprechend der Patientenanzahl mit FME-bedingten Nebenwirkungen (UAR) und ggf. durch einen Nachweis einer therapiebedingten Tumorstimulation ("tumor enhancement") bewertet. Das primäre Zielkriterium der Wirksamkeit war das adjustierte tumorabhängige Überleben der Patienten.**

Sekundärkriterien der Wirksamkeit waren das Gesamtüberleben, tumorfreies Überleben und das Überleben ohne Gehirnmetastasen. Die Überlebensanalysen erfolgten nach Adjustierung für Unterschiede in der Ausgangslage, bei den Therapieverfahren und bei anderen potentiellen Störvariablen ("Confounder") mit der Cox Proportional Hazard Regression-Methode mit Berechnung des adjustierten "hazard ratio" (HR) mit 95 % Vertrauensintervall.

Ergebnisse: **Insgesamt 686 Patienten (329 FME vs. 357 Kontrollen) aus 35 Zentren wurden über eine mediane Zeit von 81 vs. 52 Monaten beobachtet. Die durchschnittliche Therapiedauer mit FME betrug 30 Monate. Bei der Ausgangslage waren beide Gruppen bezüglich Demographie, Tumorvorgeschichte und Risikofaktoren vergleichbar. Zusätzliche adjuvante Chemotherapie war häufiger in der Prüfgruppe, während die Immuntherapie häufiger in der Kontrollgruppe verabreicht wurde. 11 (3,3 %) der Patienten entwickelten systemische und 42 (12,8 %) lokale UAR, die auf FME Behand-**

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lung, zurückgeführt wurden. Die UAR waren überwiegend mild (WHO/CTC Klasse 1–2) mit Spontanheilung in den meisten Fällen. Bei sechs Patienten führten die UAR zum Therapieabbruch. Lebensbedrohliche UAR, UAR-bedingte Mortalität oder ein Tumor Enhancement wurden nicht beobachtet. Im Gegenteil, die Häufigkeit der Lungenmetastasen und das adjustierte HR für Gehirnmetastasen waren in der FME-Gruppe signifikant niedriger als in der Kontrollgruppe. Im Verlauf der Studie und der Nachbeobachtung kam es bei insgesamt 212 (30,9 %) der Patienten zu Rezidiven oder einer Progression und insgesamt 107 (15,6 %) starben.

Im Vergleich zur Kontrollgruppe wurde ein signifikant längeres tumorabhängiges Überleben in der FME-Gruppe beobachtet (unadjustierte tumorbedingte Mortalitätsrate 8,9 % vs. 10,7 %, Kaplan-Meier-Methode, Log-Rank-Test, p = 0,017). Dieses Ergebnis wurde nach Adjustierung für potentielle Confounder mit der Cox Proportional Hazard Regression-Methode bestätigt. Das adjustierte HR für tumorabhängiges Überleben betrug *(HR, 95 % Vertrauensintervall) 0,41 (0.23–0.71), p = 0,002.*

Die adjustierten HR-Ergebnisse für das gesamte, das tumorfreie Überleben und das Gehirnmetastasen-freie Überleben waren in der FME Gruppe ebenfalls signifikant überlegen.

***Schlußfolgerung:* Eine langfristige FME-Behandlung bei Patienten mit primärem malignem Melanom mit mittlerem bis hohem Risiko, scheint sicher zu sein. Ein Tumor Enhancement wurde nicht beobachtet. Im Vergleich mit einer unbehandelten parallelen Kontrollgruppe aus der gleichen Kohorte zeigen die Ergebnisse der FME-Behandlung einen signifikanten Überlebensvorteil in den AJCC/UICC-Stadien II-III. Diese positiven Ergebnisse bezüglich des Überlebens sollten in einer prospektiven, randomisierten klinischen Prüfung mit optimiertem Studiendesign und optimierten Behandlungsbedingungen bestätigt werden.**

1. Introduction

Malignant melanoma (MM) is a melanocytic tumor affecting mainly the skin. It is diagnosed in adults of all ages, most commonly between 50 and 60 years. The incidence of the tumor has been increasing steadily all over the world within the last 40 years [1], Australia: [2]; USA: [3, 4]; Europe: [5–10]. Prognosis of primary non-metastatic MM depends mainly on tumor stage, thickness, nodal involvement and histological level [11, 12]. Accordingly, early detection of MM is a main issue in cancer treatment. Thus, MM screening has become part of public education and prevention campaigns all over the world [2, 13, 14]. In order to increase the effectiveness and reduce the costs of the screening programs, they have been partly supplemented by educational campaigns teaching full-body self-examination in the population [14, 15]. These preventive measures have been very effective with respect to reduction of the incidence of malignant skin tumors. Moreover, more tumors are now detected at an early stage [16, 17].

Beside early tumor detection, another focus of tumor prevention is the early recognition of tumor progression in melanoma aftercare. There is evidence that early detection and surgical excision of local metastases prolongs survival time of MM patients [18]. The 10-year survival rate of MM patients without metastases is about 75 %. It decreases to 20–40 % in patients with loco-regional metastases and to about 3 % in patients with distant metastases [12, 19–21]. Hence, the aftercare of MM patients focuses on the detection of tumor progression at an early stage.

For this reason, in Germany like in most Western countries guidelines and recommendations were developed to frame the aftercare of MM patients [22, 23]. According to these guidelines, MM aftercare is recommended for a period of 10 years after primary excision or any tumor progression.

In Germany, like in many countries, aftercare is particularly intense during the first 5 years. For instance, in medium-risk MM, aftercare is recommended every 3 months. Controls in the sixth to tenth years after diagnosis are carried out at intervals of one year.

Basic treatment of any primary melanoma is surgical excision with safety margins [24–28]. The value of prophylactic lymphadenectomy is controversial [29–32]. However, there has been some evidence that sentinel lymph node dissection may be favorable for a better prognosis [33-35].

Abbreviations

ADRs, adverse drug reactions (“side effects”) attributed to the FME treatment
AJCC, American Joint Committee on Cancer
Control, untreated control group (“watchful waiting”)
Cox regression, Cox proportional hazard regression
CR, complete remission
CRF, standardized case report form
CTC, Common Toxicity Criteria (NIH, NCI, WHO)
DKG, German Cancer Society
EORTC, European Organization for Research and Treatment of Cancer
FME, standardized fermented European mistletoe extract
GCP, Good Clinical Practice guidelines
GEP, Good Epidemiological Practice guidelines
HR, hazard ratio
NED, no evidence of disease (i.e. tumor-free status)
Pts, patients
RCT, randomized controlled (clinical) trial
SD, standard deviation of the sample
SOPs, Standard Operating Procedures
UICC, International Union Against Cancer
95 % CI, 95 % confidence intervals

Since melanomas are hardly susceptible to radiation, radiation therapy is only applied as second line treatment (e.g. in inoperable brain metastases) [36].

Adjuvant chemo-immunotherapy is performed in many variants. Clear superiority over purely surgical therapy with respect to survival and recurrence rate has only been demonstrated in a few studies so far, and only in patients with lymph node metastases [31, 37–48]. More recent procedures like tumor vaccination and adoptive immune transfers are still in the probationary phase [12, 49].

Extracts from European mistletoe (*Viscum album L.*) have been in wide use for adjuvant MM treatment in alternative therapy as well as in established dermatology especially between 1970 and 1990. The chemical, biochemical, pharmacological, toxicological and clinical properties of mistletoe are summarized in [50, 51]. Although there were no conclusive clinical data published on the effectiveness of mistletoe in melanoma, some arguments for treatment were derived from in vitro studies [51] as well as from small clinical trials and case reports (summarized in [50]).

In the Freiburg University Department of Dermatology approximately 1,200 patients with MM were treated between the years 1980 and 1995 with FME in a standardized way [53]. These patients in stage II or III received injections of the FME product P ("pini") subcutaneously three times a week. No prospective data were gained on this treatment. However, preliminary reports on possible side effects of FME treatment in melanoma, especially a suspicion of an enhancement of brain metastases [54], led to a complete withdrawal of FME treatment in the Freiburg University Department of Dermatology in 1996. Although these negative reports were never confirmed by any clinical data publication [50], there remained considerable suspicion about possible tumor enhancement in melanoma patients.

In the meantime, many patients who had received FME for years were concerned about a possible worse prognosis. Thus, a first retrospective study was undertaken in 1998 in Freiburg in order to obtain evidence on the question of tumor enhancement. In this study, controlled by historical data from the melanoma database, no tumor enhancement could be detected [55]. However, there were neither indications of any effectiveness of FME.

Since there were a variety of methodological weaknesses in the retrospective study, another investigational approach was planned with a validated, optimized method of assessing controlled observational clinical data, i.e. the comparative, retrolective, epidemiological cohort study [56–58].

We decided to investigate the safety and efficacy of the complementary treatment with FME (Iscador™ [1]), in the post-surgical therapy of patients with primary, stage II–III MM in a large observational study cohort. Because FME has been licensed in Germany and Switzerland for many years, and has been frequently prescribed as complementary therapy for cancer, also in MM, the availability of a sufficient number of well-documented medical records for retrolective data acquisition and comparative analysis was expected.

The following questions were addressed in this study: (1) Are there any relevant long-term side effects of FME treatment in intermediate to high-risk MM? (2) In particular, are there any hints of a tumor enhancement? (3) In addition to the safety issue, is there any conclusive data on the efficacy of FME treatment regarding clinical outcome, particularly on survival in MM?

2. Methods

2.1. Objectives

The study objectives were to evaluate the safety and therapeutic efficacy of a long-term therapy of primary intermediate to high-risk UICC/AJCC stage II–III MM patients who were treated with FME during a post-surgical aftercare and compared with an untreated parallel control group.

2.2. Study design

The study was designed as a multicenter, comparative, retrolective, epidemiological, cohort study with parallel group design, without intervention. The data with the starting point (i.e. the primary tumor surgery) located in the past was collected forward in time, without previous knowledge of the outcome (i.e. "retrolective" cohort approach) [56–60] from anonymously made medical records in standardized case report forms (CRF). The disease course of the FME-treated patients was compared within the same cohort with the course of untreated control patients who were carefully followed ("watchful waiting") during a comparable time interval. The pre-specified study protocol was designed in accordance with Good Epidemiological Practice (GEP) guidelines [61, 62] and the IFAG standard operating procedures (SOPs) for retrolective cohort studies [57, 60]. Similar optimized comparative observational study approaches were repeatedly validated against randomized controlled (clinical) trials and successfully used for evaluation of clinical therapy [63–67] and for complementary cancer treatment [68-72]. Comparative epidemiological cohort studies can be accepted for the proof of efficacy and safety of "well established" and marketed drugs in the EC [73], and can also meet the Evidence Based Medicine (EBM) requirements (with evidence level II) [74, 75].

2.3. Selection of centers

The study centers consisted of randomly selected hospitals and medical practices in Germany and Switzerland that had been treating patients with primary MM for several years with or without adjuvant FME therapy, provided they accepted the study protocol including monitoring and data audit, as well as the participation in the study.

2.4. Selection of patients

The study evaluated a cohort of primary, histopathologically confirmed, intermediate to high-risk stage II and III MM

[1] Manufacturer: Weleda AG, Arlesheim (Switzerland).

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patients selected according to the eligibility criteria in the study protocol that had tumor surgery between 1985 and 2001, and follow-up for at least three years or until death. In each center, all eligible patients were included in the study in chronologic order without any further selection and irrespective of the study course or disease outcome, only limited by the pre-specified maximal study sample size, which was set to a total of 800 patients.

In this non-interventional study the treatment allocation to a regimen with or without FME was carried out according to the individual patients' health status and preference at the discretion of the treating physicians and the treatment ended before the study commencement. Because the treatment preference for the FME therapy varied between the centers, all endpoint results were adjusted for possible confounding center effects.

2.5. Eligibility and exclusion criteria

The eligibility criteria consisted of a histopathologically confirmed diagnosis of primary high-risk UICC/AJCC stages II–III MM in patients following tumor surgery. The AJCC stage II (UICC T3-4 N0M0) is defined as localized melanoma with a vertical lesion thickness (Breslow) of > 1.5 mm and invasion level (Clark) IV–V. Stage III (UICC any T, N1M0) is defined as any T-stage melanoma with presence of regional lymph node metastasis, but without distant metastases [12, 25, 76–78].

All completely documented cohort patients of any age or gender who met the eligibility criteria were included for the study irrespective of the kind of surgical treatment, disease outcome and treatment compliance, providing they were treated following surgery between 1985 and 2001 for at least six months with or without adjuvant FME treatment and followed up for at least three years, or until death. The only exclusion criterion was a severe protocol violation, such as the history of previous tumor disease, recurrent or metastatic melanoma at study initiation, absence from surgical treatment, therapy with mistletoe products other than FME and incomplete documentation with missing essential data.

Patients with premature study termination due to any reason were kept in the study and evaluated up to the last available data. The reason for any exclusion was documented and the excluded cases were not replaced.

2.6. Treatment

FME group patients were treated with 2-3 weekly subcutaneous injections. The choice of the FME product (i.e. P, M, or Q) (P: FME host tree "pini", M: mali, Q: quercus), the dose and the treatment regimen was carried out at the discretion of the treating physician.

The untreated parallel control group was carefully followed (by "watchful waiting"). The FME treatment regimen as well as any other drug or non-drug therapy during the study were documented and analyzed.

2.7. Endpoint criteria

The primary endpoint of FME *safety* was assessed by the incidence of systemic and local ADRs explicitly attributed by the physician to the FME treatment. Type, number, severity and outcome of all FME-attributed ADRs were evaluated according to the WHO/CTC-criteria [79]. In addition, any sign of tumor enhancement in the FME group as compared with the control group, particularly any increasing incidence of brain metastases was documented and analyzed. The safety results were reconfirmed by a sensitivity analysis of all originally acquired cohort patients, including those with severe protocol violation ("intention-to-treat" approach).

The primary endpoint criterion of *efficacy* was the melanoma-associated mortality, i.e. the tumor-related survival (TS), explicitly confirmed by the clinical investigator. The result was expressed as adjusted hazard ratio (HR). The secondary endpoints of efficacy were defined as overall survival (OS), disease-free survival (DFS), i.e. the time until the first occurrence of tumor relapse or progression (recurrence, local or distant metastasis), and brain metastasis-free survival (B-MFS) during the study and follow-up time interval. Evaluation of efficacy was performed according to the "per protocol" approach, i.e. cases with severe protocol violation and missing essential data were excluded from the analysis.

2.8. Data acquisition and quality assurance

Clinical investigators who were instructed and supervised by professional monitors according to the GEP and GCP rules transferred the anonymously made medical record data of all eligible patients to standardized CRFs. All CRF data was checked for completeness and plausibility according to the standardized SOPs.

A data quality audit was performed by an independent auditor.

2.9. Statistical analysis

The statistical analysis was performed according to the study protocol. For the analysis of safety descriptive methods were applied, while for survival analysis the calculation of the hazard ratio for outcomes adjusted for baseline imbalances, treatment regimens and confounder effects was carried out with the Cox proportional hazard regression method. An exploratory Kaplan-Meier analysis with non-adjusted data was also performed. The endpoint results were adjusted for a potential confounding effect of centers, patient's age and gender, and known prognostic factors such as the initial tumor stage (UICC/AJCC), melanoma type and localization, vertical tumor thickness (Breslow), concurrent diseases and for other concurrent adjuvant treatment regimens, such as chemo-, immuno-, or radiotherapy. The selection of covariates was based on the published prognostic relevance [11, 80] and was also reconfirmed in a sensitivity analysis by stepwise inclusion and exclusion procedures in the Cox proportional hazard regression. Only adjusted results were considered for final interpretation. The statistical calculations were performed using the software SPSS for Windows(tm), Testimate(tm), StatXact(tm), and LogXact(tm) (SPSS Software).

3. Results

3.1. Study centers

35 centers were randomly selected from German and Swiss hospitals and medical practices that were known to treat MM patients with or without adjuvant FME therapy had accepted the study protocol and agreed to participate in the study.

3.2. Patients

From the total of 738 originally collected patient data records 52 (7.0 %) were excluded due to severe protocol

Table 1: Baseline characteristics. Patients' demographic baseline criteria and prognostic factors, "control" = control group without FME ("watchful waiting"), SD = standard deviation.

Baseline demographic and prognostic criteria (sample size 329 vs. 357)	Value (SD or range) FME group	Value (SD or range) control group
Age years, mean (SD)	51.4 (15.1)	53.7 (15.5)
Gender (males/females) (%)	41.9 / 58.1	46.5 / 53.5
Advanced UICC tumor stage T (pT3/pT4) (%)	81.5 / 16.1	78.4 / 21.3
UICC/AJCC tumor stage (II/III) (%)	91.5 / 8.5	95.0 / 5.0
Melanoma localization (head-neck/ limb) (%)	15.5 / 35.9	11.8 / 37.0
Melanoma type (SSM/nodular) (%)	36.8 / 38.6	41.2 / 32.2
Melanoma lesion thickness (Breslow) mm, mean (SD)	2.7 (1.7)	2.9 (1.8)
Patients with concurrent chronic diseases (%)	36.2	42.0
Time from surgery to start of aftercare (months), median (range)	2.0 (1–4)	2.0 (1–4)
Study and follow-up duration (months), median (range)	81.0 (1–335)	52.0 (1–212)

Table 2: Treatment data. Overview of treatment regimen. "control" = control group without FME ("watchful waiting"), SD = standard deviation, FME host tree P = "pini", M = "mali", Q = "quercus".

Treatment regimen (sample size 329 vs. 357)	Value (SD or range) FME group	Value (SD or range) control group
FME: therapy duration (months), median (range)	30 (1–336)	not applicable
FME host tree: P (pini) / M, or Q, or combined) (%)	83.3 / 16.7	not applicable
Patients with any concurrent adjuvant therapy (%)	27.1	31.1
Patients with adjuvant radiotherapy (%)	7.9	5.9
Patients with adjuvant chemotherapy (%)	10.0	5.9
Patients with adjuvant immunotherapy (%)	9.1	17.6
Patients with combined chemo-immunotherapy (%)	3.0	2.2
Patients with other concurrent therapy (%)	9.7	10.6

violation, such as recurrent or metastatic disease at study onset, or missing essential data, leaving 686 (329 FME and 357 control) eligible patients for evaluation of safety and efficacy according to the "per protocol" approach.

3.3. Patient baseline characteristics

The patient characteristics are summarized in Table 1. 41.9 % males and 58.1 % females (mean age 51.4 years) were included in the FME group and 46.5 % males and 53.5 % females (mean age 53.7 years) in the control group. The demographic data, as well as the baseline values of tumor characteristics and prognostic factors were well balanced between the treatment groups.

In both groups the majority of the patients had a stage II disease (91.5 % vs. 95.0 %) and the median time from surgery to the start of aftercare (2.0 vs. 2.0 months) was identical, while the median follow-up duration was significantly longer in the FME group (81 vs. 52 months).

3.4. Treatment regimens

The test group patients received the FME therapy for a median duration of 30 months. On average, FME injections were administered 2–3 times per week. Ing the FME group 83.3 % of the patients received FME P, while 16.7 % were treated with FME extracts from other host trees, such as M, Q or a combined treatment (Table 2). During the study, 27.1 % of the FME group and 31.1 % of the control group patients were treated with at least one other adjuvant therapy course. Chemotherapy was given to 10.0 % vs. 5.9 %, immunotherapy to 9.1 % vs. 17.6 %, combined chemo-immunotherapy to 3.0 % vs. 2.2 % and radiotherapy to 7.9 % vs. 5.9 % of the patients. The endpoint results were adjusted for all adjuvant treatments.

3.5. Assessment of FME safety

3.5.1. Systemic ADRs

In the test group 11 (3.3 %) evaluable patients experienced systemic ADRs attributed to the FME treatment. The ADRs were non-specific, with predominantly mild to intermediate severity (WHO/CTC grade 1–2) reactions: headache (4), fatigue (3), fever (3), allergy (1), itching (1), Quincke's edema (1), exanthema (1), eczema (1),"sick feeling" (1), loss of hair (1), sleep disturbance (1), dyspnea (1), and pancreatitis recurrence (1). In most cases a spontaneous recovery occurred within one week, and premature treatment termination associated with systemic ADRs was observed in one case. Life-threatening ADRs did not occur.

3.5.2. Local reactions

Local ADRs at the injection site were more commonly reported. 42 (12.8 %) of FME-treated patients experienced at least one local reaction at the injection site, mainly erythema (41), edema (12), itching or local pain (3), or other local reactions (3). The local ADRs showed predominantly mild to intermediate severity (WHO/CTC grade 1–2) with spontaneous recovery in most cases. In 5 cases the FME treatment was prematurely terminated due to local reactions.

3.5.3. Tumor enhancement

In the comparative survival analysis (see below), no evidence of any form of tumor enhancement in the FME group was found. Particularly, there was no indication for an increased frequency or earlier onset of brain metastases in the FME group. In contrast, despite the longer follow-up duration the FME group showed a lower incidence rate (3.0 % vs. 4.2 %) and significantly reduced the adjusted hazard ratio for brain metastases

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(HR = 0.33 (0.13–0.86), p = 0.024), when compared with the control group. Similarly, for lung/mediastinal metastases, the incidence was significantly lower in the FME group (5.5 % vs. 10.4 %, p = 0.024).

In conclusion, the safety analysis results suggest that the FME treatment was safe and well tolerated. A tumor enhancement was not found, and there is no indication of increasing incidence of brain or any other metastases in the FME treatment group.

3.6. Assessment of FME efficacy

In the course of the study and follow-up, in a total of 212 (30.9 %) patients recurrence or progression was observed, and 107 (15.6 %) died. The total unadjusted tumor-related mortality was 9.9 %.

3.6.1. Tumor-related survival (TS)

Tumor-related survival was the primary endpoint of efficacy in this study. The unadjusted tumor-related mortality rate was 8.9 % vs. 10.7 % for the whole follow-up duration in the FME vs. control group, respectively. The exploratory Kaplan-Meier analysis of unadjusted data showed a significant survival benefit of the FME group compared with the controls (Log rank test, p = 0.017). The primary endpoint result, the estimated adjusted hazard ratio (95 % confidence limit, CI) for tumor-related mortality, was calculated as

$HR_{(TS)}$ = 0.41 (0.23–0.71), p = 0.002 (Table 3 and Fig. 1), which confirmed the survival benefit of the FME treatment. A sufficient number of cases was available "at risk" for the survival analysis in both therapy groups (242 vs. 194 at 5 years, 159 vs. 101 at 8 years and 115 vs. 66 at 10 years).

Among the prognostic factors a significantly higher adjusted tumor-related mortality hazard ratio was observed in UICC/AJCC stage III vs. stage II (HR_{TS}) = 4.11 (2.11–8.02), p < 0.001) and in males vs. females ($HR_{(TS)}$ = 2.38 (1.38-4.10), p = 0.002). The adjusted effects of age, melanoma type and localization, as well as the tumor thickness (Breslow) were not statistically significant.

In conclusion, the results suggest a significant and clinically relevant reduction of the tumor-related mortality hazard in the FME group in comparison with the control group.

Table 3: Results of the survival analysis.

Endpoint criteria of efficacy Survival analysis	Adjusted hazard ratio[a)] and 95 % CI (FME vs. control)	p-Value (Wald) Cox regression (FME vs. control)
Tumor-related survival (TS)	0.41 (0.23–0.71)	0.002
Overall survival (OS)	0.64 (0.42–0.96)	0.033
Disease-free survival (DFS)	0.73 (0.55–0.97)	0.029
Brain metastasis-free survival (B-MFS)	0.33 (0.13–0.86)	0.024

[a)] Outcome results were adjusted for patients' age and gender, study center group, tumor stage (UICC/AJCC), melanoma type and localization, vertical tumor thickness (Breslow), concurrent disease, and concurrent adjuvant radio-, chemo-, immuno-, or chemo-immunotherapy. Adjusted hazard ratio was estimated with Cox proportional hazard regression method, 95 % CI = confidence intervals.

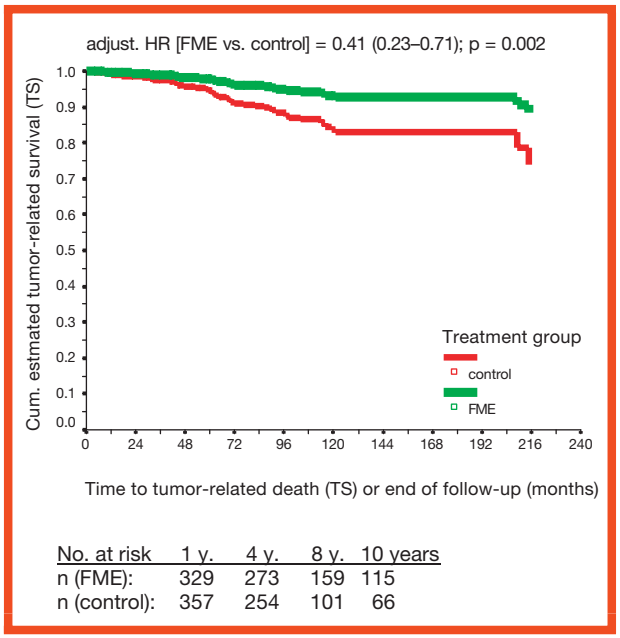

Fig. 1: Adjusted tumor-related survival (TS). Comparison of TS in patients with or without adjuvant FME treatment in the Cox proportional hazard regression. Thick green line = FME group, thin red line = control group. HR = adjusted hazard ratio, 95 % CI = 95 % confidence intervals, p = p-value, adjust. = multivariate adjusted for baseline imbalance, therapy regimen and confounders: patients' age and gender, study center group, tumor stage (UICC/AJCC), melanoma type and localization, vertical tumor thickness (Breslow), concurrent disease, and concurrent adjuvant radio-, chemo-, immuno-, or chemo-immunotherapy.

3.6.2. Secondary efficacy criteria

Among the secondary efficacy criteria, following results were obtained (adjusted hazard ratio (95 % CI)): overall survival (OS): $HR_{(OS)}$ = 0.64 (0.42–0.96), p = 0.033, disease-free survival (DFS), i.e. time interval to the first tumor event (progression): $HR_{(DFS)}$ = 0.73 (0.55–0.97), p = 0.029), and the brain metastasis-free survival (B-MFS), i.e. time interval to the first brain metastasis: $HR_{(B\text{-}MFS)}$ = 0.33 (0.13–0.86), p = 0.024.

Overall metastases rate (22.2 % vs. 24.9 %, p = 0.419) and metastases location were not significantly different between the therapy groups, except for lung/mediastinal metastases, which occurred significantly less frequently in the FME group (5.5 % vs. 10.4 %, p = 0.024).

A trend for less lymph node metastases in the FME group (11.9 % vs. 17.4 %, p = 0.052) was also observed.

These secondary endpoint criteria analysis results suggest a significant benefit in the overall survival, the disease-free- and the brain metastasis-free interval in the FME group.

4. Discussion

4.1. Study design and methods

The present comparative retrolective cohort study with 686 evaluable primary, high to intermediate risk (UICC/

AJCC stage II–III) MM patients showed a statistically significant improvement of the tumor-related and overall survival as well as a longer disease-free and brain metastasis-free interval in the FME-treated group, when compared with the control group without FME. However, these results need to be interpreted with some caution because the applied study design shares some potential risk for bias with other nonrandomized observational studies, such as the risk of bias by case selection, and treatment differences between the groups, the risk of a possibly lower quality of retrolectively documented data from medical records, the potential baseline imbalance of endpoint criteria, prognostic factors and other confounders, and the problem of missing values.

In order to effectively minimize these potential biases, we utilized the following measures [56–58]: (1) a large study cohort from randomized centers that used treatment regimens with or without FME, (2) use of a standardized parallel groups study design with strict adherence to the study protocol, (3) application of GEP rules and SOPs, (4) unselected chronologic data collection restricted only by the eligibility criteria, (5) standardized and anonymously made data documentation in CRFs, (6) integrated control, monitoring, independent auditing of data quality, and (7) multivariate adjusted endpoint criteria for baseline imbalances, treatment regimens and other potential confounders. No model- or covariate-dependent bias was detected in the present study in a large set of sensitivity analyses. The potential impact of missing data was expected to be negligible, due to the presence of only few missing values. The sensitivity analysis results on missing values largely resemble the results of the main "per protocol" analysis and thus strongly support its credibility. A sufficient quality of the standardized data acquisition from the medical records was already reported [81, 82]. The remaining limitation of this study design might be a lack of sufficient compensation for possible bias from hidden (i.e. not documented) confounders. However, the results from sensitivity analyses under various model conditions well reproduced the results of the main analysis and hence did not indicate the presence of any effective hidden confounder effect. Due to the well balanced baseline data in the therapy groups and the application of adjusted survival analyses, it seems very unlikely that the treatment effect on the endpoint criteria could be biased.

4.2. Mistletoe therapy in cancer patients

Concerning the therapy of cancer with mistletoe extracts, a large amount of empirical and clinical data from anecdotal reports and small non-controlled studies was published in the past, but the critical review of the results often revealed substantial methodical shortcomings. These early studies were often non-conclusive, or were not confirmed in other studies on clinical efficacy of mistletoe in cancer. This lack of consistency possibly resulted from an uncompensated large heterogeneity of the patients' characteristics, type, and severity of the tumor disease and the differing mistletoe as well as conventional treatment regimens. Other possible reasons for lacking conclusion were insufficient sample size, missing parallel control group, and methodological flaws [50, 83]. Regarding tumor response, a significant effect of mistletoe treatment on tumor recurrence and on survival was not found in a controlled randomized study in head and neck tumors [84]. Significantly longer survival was documented in a randomized matched pairs study nested within a prospective non-randomized study with solid tumors, particularly with breast cancer. These tumors were treated conventionally, either accompanied by mistletoe (FME) therapy or without additional mistletoe therapy [85]. In a randomized trial of glioma patients a significantly prolonged survival was found in the mistletoe treatment group, particularly in advanced stage III/IV disease [86]. Some reasons for the controversy about these studies were discussed in depth by Kienle [50]. In a multicenter, comparative, retrolective cohort study, a short-term (median one year) complementary treatment with a different standardized mistletoe extract was associated with significantly fewer recurrences in primary, non-metastatic breast carcinoma, but no effect on survival could be found when compared with controls without mistletoe treatment due to the short follow-up duration [72]. In a recent multicenter, comparative, retrolective cohort study concerning a long-term treatment of non-metastatic breast cancer patients with FME compared with untreated control group a significant improvement of quality of life as well as a survival benefit were observed [69].

Regarding the toxicity, in the present study the FME treatment was well tolerated without any life-threatening ADRs, particularly without severe allergic reactions. The frequency of systemic ADRs and local reactions at the injection site had about the same magnitude and severity as in previously published clinical trials on FME [50, 87–89].

A tumor enhancement was not observed. This is in agreement with the published clinical data (updated summary in [50]). Consequently, the complementary FME treatment in MM patients can be regarded as safe.

4.3. Mistletoe therapy in MM patients

Although there was no earlier conclusive clinical data published on the effectiveness of mistletoe in melanoma, some arguments in favor of the mistletoe treatment can be found in a few older small clinical trials and case reports that were summarized by Kienle and Kiene [50].

The present data ("cohort study") indicate that there may be a benefit of FME in the treatment of MM concerning the clinical outcome (survival, relapse). These data, however, contradict the outcomes reported in the recently published phase III EORTC/DKG RCT by Kleeberg et al. [43] ("RCT study"). In this RCT study the FME

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treatment arm was embedded in the DKG (German Cancer Society) part of a four arm study investigating also the potential efficacy of the adjuvant treatment with the recombinant interferons rIFN-α2b and rIFN-γ compared with an untreated control group. In contrast to the cohort study, the RCT study failed to show a significant effect on the disease-free interval (DFI) or survival in the interferon or FME treatment groups. The different DFI and survival outcomes could be explained by the fact that both studies substantially differ in relevant baseline prognostic factors, as well as in the therapy regimen. Thus, in both studies completely different populations of MM patients were evaluated. Consequently, the studies are not directly comparable.

(1) Both studies substantially differed in the tumor stage at the study commencement. The RCT study groups consisted of more than 50 % stage III patients compared with 5–8 % stage III patients included in the cohort study.

(2) In the RCT study more than 60 % of the patients had a tumor thickness > 3 mm (i.e. met the inclusion criterion), while in the cohort study less than 8.5 % of the patients had a tumor thickness > 3 mm.

(3) Median FME therapy duration was 2.5 years in the cohort study but only ≤ 1 year in the RCT study. In an earlier FME study in breast carcinoma [70] and also in the present cohort study on MM, the FME therapy duration appears to be an important factor influencing the therapy effect on survival in the evaluated solid tumors.

(4) At least two or three years of continuous FME therapy duration appears necessary for a significant beneficial effect on survival in intermediate to high risk MM and primary non-metastatic breast carcinoma patients, respectively.

(5) In both MM studies different FME products were administered. While in the cohort study the FME P (pini) product was used in 83.3 % of the patients, in the RCT study all patients were treated with the FME M (mali) product. As it has been shown recently [69] and also demonstrated in the present cohort study, the FME P (pini) product appears more effective than M (mali) or other FME products on survival in the evaluated solid tumors.

(6) The median follow-up was shorter (6.8 vs. 4.3 years) in the cohort study than in the RCT study (overall 8.2 years). In both studies all patients without a tumor event were followed as censored cases until the last visit or last available data and appropriate statistical methods for uni- and multivariate survival analysis with censored data were applied in both studies (i.e. Cox regression). Therefore, it seems unlikely that the follow-up time difference could contribute to the explanation of the different outcome results.

(7) Further, in the RCT study the FME group was relatively small for an assumption of general validity of the results that were based on initially 102 vs. 102 patients from 45 centers, while in the cohort study 329 vs. 357 patients from 35 centers were included. The number of patients at risk for dying decreased to 44 vs. 52 patients at 4 years and 22 vs. 20 patients at 8 years in the RCT study, while 273 vs. 254 patients at 4 years and 159 vs. 108 patients at 8 years remained at risk in the cohort study. This might be the reason for an insufficient test power at five years and later under the RCT study conditions as described in the method section, while the test power of the cohort study remained sufficient (> 80 %) for more than 5 years of follow-up.

(8) Concerning the FME safety it is important to mention that despite some remarks of suspicion in the RCT study discussion, in both studies neither a FME therapy-associated tumor enhancement nor an increased incidence of brain metastases were shown.

On the contrary, in the cohort study the number of the brain and lung metastases was lower and the time interval to their first appearance was significantly longer in the FME therapy group than in the controls. In addition, the overall toxicity of the FME treatment was found very low in both studies, indicating that this treatment is safe. A critical review of the controversial discussion concerning the suspicion of tumor enhancement allegedly associated with the FME therapy, which includes also the preliminary results from the at that time incomplete RCT study [54, 90, 91], was presented by Kienle [50].

(9) In conclusion, the above mentioned data indicate that in comparison with the cohort study the RCT study patients suffered from much more advanced disease with an a priori unfavorable prognosis, irrespective of the aftercare treatment. This baseline prognostic difference might be one reason why in the cohort study the FME therapy showed a survival benefit, while the RCT study did not. However, the cohort study subgroup analysis (data not shown) revealed similar results (i.e. adjusted HR estimates for tumor-related survival) in the intermediate risk stage II subgroup (HR = 0.38) as in cases with advanced disease and lymph node involvement (stage III disease) with a HR = 0.32, despite the small sample size in the latter subgroup. According to the present knowledge, the published data suggests that the FME treatment regimen in the RCT study was possibly suboptimal. In order to achieve a significant beneficial effect on survival in MM a continuous long-term FME treatment of more than two years and the preference of the FME P (pini) product should be considered.

The safety data in both studies did not reveal any significant toxicity, and no tumor enhancement was observed, indicating that the FME therapy is safe.

5. Conclusion

The clinical safety and efficacy of the adjuvant treatment with FME in primary, intermediate to high-risk UICC/AJCC stage II–III MM were evaluated in a multicenter, comparative, retrolective, epidemiological cohort study with 329 patients treated with FME and compared to 357 control patients of the same cohort without FME therapy (“watchful waiting”).

The FME treatment was well tolerated with mostly local and rare systemic ADRs, which were of predominantly mild to intermediate severity and finally com-

pletely resolved. FME-related mortality or tumor enhancement was not observed in the FME group.

After a median follow-up of 6.8 (FME) and 4.3 (control) years and a median FME therapy duration of 30 months, the tumor-related survival showed a statistically significant reduction of the adjusted tumor-related mortality hazard ratio in the FME group as compared with the parallel untreated control group. A significant benefit in the FME group was also found in the overall mortality, the disease-free survival, and the brain metastasis-free survival. Despite some possible methodological limitations inherent to any non-randomized design, the results of the present study suggest a significant and clinically relevant survival benefit from the adjuvant FME therapy in UICC/AJCC stage II–III MM patients.

For a final evidence-based treatment recommendation, the significant survival benefit of the long-term adjuvant FME therapy shown in the present study deserves further confirmation in a future well designed prospective controlled randomized clinical trial with optimized design and FME treatment regimen.

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