Molecular Mistletoe Therapy: Friend or Foe in Established Anti-Tumor Protocols? A Multicenter, Controlled, Retrospective Pharmaco-Epidemiological Study in Pancreas Cancer

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Abstract: Mistletoe is often used as complementary therapy in oncology. The anti-tumor effects of mistletoe (Iscador®) are well documented in-vitro in respect to inhibition of cell proliferation, induction of apoptosis, segmental activation of immune competent cells and trapping of chemotherapeutic drugs within cancer cells by modulating the inhibitory potential of P-glycoprotein (P-gp)-mediated transport of cell toxifying substances (cytotoxic drugs). However, the clinical activity of mistletoe treatment remains still controversial. Implementation of mistletoe therapy as supportive care into anti-cancer programs should be based on the best evidence and must continually be evaluated to ensure safety, efficacy, collection of new data, and cost-effectiveness. Useful domains that can be evaluated include symptom control, adherence to conventional treatment protocols, quality of life, individual outcome and potential advantages of a whole-system health approach. Here we report the results of a multicenter, controlled, retrospective and observational pharmaco-epidemiological study in patients suffering from a pancreatic carcinoma. After surgery the patients were treated by adjuvant chemotherapy with gemcitabine supported by Iscador®, or with gemcitabine alone, or any other best of care, but not including Iscador®. Using a novel methodological pharmaco-epidemiological design and statistical approach it could be shown that Iscador® offers benefits - symptom control, overall survival - as supportive care within gemcitabine protocols of patients with surgically resected pancreatic carcinoma.

Keywords: Pancreatic carcinoma, pharmaco-epidemiological study, gemcitabine, Iscador®, supportive care, integrative oncology, symptom control, overall survival, mistletoe.

BOTANICALS AND CANCER – A QUESTION OF CLINICAL TRIALS

Mainstream oncologists often face the situation that numerous cancer patients use treatments in addition to other than those recommended and proven in clinical studies. Unconventional approaches to decrease disease- and therapy-related symptoms, to sustain tumor remission or halt the spread of cancer include botanicals, nutritional supplementation and off-label use of pharmaceuticals. A systematic review of clinical trials with unconventional anticancer agents has been published in the year 2006 [1] by identfying 198 different clinical trials, whereby 20 trials were Phase I, three were Phase I and II, 70 were Phase II and 105 were Phase III. The authors conclude that future research on unconventional therapies should involve Phase I (dose-finding) and Phase II studies to determine the suitability of definitive trials. Vickers AJ [2,3] suggested Phase I and Phase II designs for anticancer botanicals and supplements, because anticancer botanicals and supplements are unlikely to bring about rapid tumor regression, even if they do extend survival, compared to conventional Phase II trials, which use tumor response as an end point, often defined as a 50% or greater decrease in tumor size. Furthermore, also botanicals might be regarded by patients who are emphatic for complementary and alternative medicine as safe, but safety is not guaranteed when the botanicals are used in combination with other agents in the complex medical setting of oncology. It is well documented that plant extracts are active in-vitro against different tumor cell lines [4,5], the standard approach however is to isolate, (semi-) synthesize and administer the single chemical compound in Phase I studies after having demonstrated the proof-of-principle in the test tube and/or animal models. Botanical medicine does not stick to the paradigm of molecular dissection of a medicinal herb and purifying one active compound but rather favours the idea that different components in a botanical may have synergistic activities (holistic approach). There is also some evidence that the presence of multiple compounds in a botanical extract can buffer the toxic effects of a single, anti-tumor constituent [6]. With the increasing use of botanicals by cancer patients it is most important to address the issue in the context of methodological approaches of clinical studies. It is well documented [7] that cancer patients participating in Phase I trials in conventional oncology prior botanical use is common and associated with age, stated acknowledgement of prognosis, and

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quality of life. Botanicals are biologically active agents that can be useful under certain circumstances, but they may be counterproductive when used by patients in early-phase cancer trial by threatening the accuracy of the clinical data and clinical outcome [8]. Therefore, it is mandatory for the introduction of botanicals in oncology to develop new methodological approaches of both molecular targeted and clinically oriented therapies [9,10]. Awareness of pharmacokinetic interactions due to concomitant use of botanicals and conventional anti-tumor agents is one prerequisite in early-phase trials. Within Phase II and III studies the design depends on the aims of the clinical questions which should be answered. Botanicals can be used in chemoprevention studies to prevent or delay the onset of a disease, in palliative care studies to reduce symptoms of pain and fatigue and to meet the needs at the end of the life, and in conventional chemo-/radiotherapy studies to increase response rates and to decrease therapy-related adverse reactions and in adjuvant settings to manage cancer symptoms and decreasing the risk of metastases formation.

Here, we present the results of a medicinal herb (Viscum album L.) as supportive care in an adjuvant chemotherapy setting with Gemcitabine or 5-Fluorouracil (5-FU) in patients undergoing curative-intent resection of pancreatic cancer.

MISTLETOE EXTRACT IN CANCER PATIENTS

Mistletoe extracts are commonly used in Europe in cancer patients. The evidence from randomized clinical studies to support the view that the application of mistletoe extracts has impact on survival or leads to an improved ability to fight cancer or to withstand anticancer treatment is weak [11]. However, using a novel methodological pharmaco-epidemiological design it could be recently shown that mistletoe extracts may offer benefits as supportive treatment within chemo- and/or radiotherapy protocols in non-metastatic colorectal carcinoma [12]. The results suggested a beneficial effect of mistletoe extract treatment (Iscador^R, Weleda, Arlesheim, Switzerland) within adjuvant chemotherapy in stage I – III of colorectal tumor patients by improving disease- and therapy-related symptoms and possible extension of disease-free survival.

In order to reconfirm a clinical benefit of mistletoe treatment in adjuvant and supportive settings, a second study was initiated in patients suffering from pancreatic tumors. Adenocarcinoma of the pancreas carries a grim prognosis. Surgery is currently the only curative option, but even the few patients undergoing complete resection of early localised disease run a high risk for relapse and death. The relatively few randomized trials available have not established a definite standard of care due to the study limitations [13]. The published Charite´-Study [14] has demonstrated a definitive advantage of adjuvant gemcitabine therapy after complete, curative-intent resection of pancreatic cancer compared to observation.

By means of a novel, non-interventional, multicenter, controlled, retrolective pharmaco-epidemiological cohort study with parallel groups we tried to confirm that adjuvant chemotherapy (gemcitabine) and mistletoe extract (Iscador^R) is clinically superior to observation/gemcitabine alone.

AIM AND THE DESIGN OF THE STUDY

The present study was designed i) to evaluate with standardized epidemiological methods safety and effectiveness of the mistletoe product Iscador^R (ISC), a fermented extract from Viscum album L., as supportive therapy with chemo- and/or radiotherapy protocols in university and community hospitals and community medical practices in surgically treated pancreas carcinoma patients compared to chemotherapy (gemcitabine) alone or together with best supportive care, however without ISC and ii) to generate a well founded working hypothesis for a randomized, prospective clinical trial.

A multicenter, controlled, retrolective, observational cohort study with parallel groups was carried out in agreement with Good Epidemiological Practice, the details of which have already been presented [15]. In short, this cohort study design is characterized by sampling anonymous data of eligible patients from original medical records in standardized case report forms (CRF), irrespective of the outcome, and by a follow-up forwarded, starting from a defined origin, e.g. time of diagnosis or time of primary surgery, located in the past, with pre-specified endpoints.

Treatments are usually finalized before study commencement. A similar design is successfully used in pharmacological-epidemiological studies and is compared with randomized clinical trials [16,17].

CENTERS

The centers from a published list of oncological departments and clinics within hospitals as well as community based oncology practices, which were trained to treat pancreatic tumor patients in the course of oncology aftercare by adjuvant chemotherapy, mostly gemcitabine, either with or without additional supportive ISC, were contracted in random order and included in the study. The randomly selected centers, not the patients, accepted the study protocol and signed an informed consent form for participation. From each center, the eligible patients were enrolled in the study in chronological order, without any further selection until the predetermined maximum of cases was reached. The procedure was controlled by the study monitor. Owing to the retrolective, non-interventional study design, informed consent from the patients was not required. The center investigators ensured that all patients´ data were strictly anonymous that local legal and regulatory requirements were obeyed before transferring the data into standardized CRFs.

PATIENTS

The eligible anonymous patients´ data were extracted from the medical records according to the study protocol until a pre-determined number of 400 patients was achieved. The data were transferred by study investigators from the medical records into the standardized CRFs under data quality and plausibility check by the study monitors. Eligible were all data from a cohort of surgically treated patients of both sexes, any age, and any stage of the disease, irrespective of the disease outcome and treatment compliance, who were treated in Germany or Switzerland between 1993 and 2002 after surgery by adjuvant chemotherapy with gemcitabine, and a few cases with 5-FU, supported by ISC or chemotherapy alone or in addition other best of care, but not including ISC; the patients´ records were followed for at least three years or until death. Data from patients with other malignant tumors in their medical records, other mistletoe extracts, different from ISC, and missing essential data, were excluded from the analysis. All efforts were made to ensure the proper transfer of the data, the accuracy, completeness, and reliability of the acquired data as specified in the protocol. All essential aspects of the protocol, including the completion of the CRFs were discussed in detail with the center investigators, and if necessary, the centers were provided with written informations.

ENDPOINT CRITERIA

Following pre-defined confounder-adjusted outcome endpoints were evaluated: i) rate and adjusted risk (odds) of documented adjuvant/palliative care therapy attributed to adverse drug reactions assessed by the WHO/NIH-CTC criteria in oncology; ii) pre-defined quality of life surrogate criteria adapted from the symptom scales of the EORTC QLQ-C30 questionnaire, consisting of rate and adjusted risk

Table 1. Baseline Demographics, Tumor Stage and Clinical Status

Baseline demographic and prognostic criteria initial sample size= 396 (201 vs. 195)		Value ISC group % or mean (± SD)	Value control group % or mean (± SD)	P-value (Fisher's exact test or exact Mann-Whitney test)
Age at onset of aftercare; mean, (SD), years		58.2 (10.7)	63.7 (9.8)	< 0.001
Body weight at aftercare onset; mean, (SD), kg:		69.0 (11.7)	69.9 (13.8)	0.073
Gender	males %	55.7	49.7	0.268
	females %	44.3	50.3	
Tumor stage	early (T1 - T2, Tx) %	45.3	28.7	0.001
	advanced (T3 - T4) %	54.7	71.3	
Tumor stage	node negative (N0, Nx) %	33.4	62.6	< 0.001
	node positive (N1 - N2) %	66.6	37.4	
Tumor grade	low (G1 - G2) % high (G3 - G4) %	83.5 16.5	73.8 26.2	0.020
Tumor stage UICC	I %	11.4	13.3	< 0.001
	IIa %	12.4	12.8	
	IIb %	49.8	24.6	
	III %	11.4	19.5	
	IV %	15.0	29.8	
Tumor localization-pancreas	head %	74.1	69.2	0.023
	body %	13.9	10.8	
	tail %	7.5	10.8	
	others %	4.5	9.2	
Tumor multiplicity	solitary %	87.6	93.3	0.061
	multiple %	12.4	6.7	
Tumor post-surgical status	CR/NED %	30.8	40.5	< 0.001
	residual tumor %	69.2	59.5	
Comorbidity (concurrent diseases)	yes %	55.2	61.5	0.222
Aftercare / follow-up duration:				
	median (range), months	15.2 (0-159)	10.1 (0-122)	0.001

SD, standard deviation, CR, complete remission, NED, non evident disease, ISC, Iscador.

(odds) of persistence of pre-specified disease- and treatment associated symptoms, particularly fatigue, pain, skin and mucosal reactions, gastrointestinal and CNS symptoms; iii) adjusted overall survival (OS) calculated by the Cox proportional hazard regression method.

The pharmaco-epidemiological, two-arm study was designed to reject the null hypothesis that adjuvant chemotherapy protocols with gemcitabine supported by ISC, did not improve tumor- and therapy-related symptoms and overall survival, defined as the time from surgical intervention until death from any cause.

The overall control group represents the total number of patients, who received only gemcitabine or who received gemcitabine and/or other supportive care, but not ISC. Qualified survival analyses were also performed in subpopulations stratified by UICC staging and for those patients receiving only gemcitabine without any other documented best supportive care treatment.

SAFETY

Safety was assessed by the number of patients with documented systemic and local adverse drug reactions attributed to the mistletoe therapy. The number and severity (range 0-6) of adverse drug reactions were evaluated according to CTC. Any evidence of possible tumor enhancement in the ISC group was also documented.

STATISTICAL ANALYSIS

The statistics is based on established design and methods for observational cohort studies adapted to pharmaco-epidemiological research [18-21]. The analysis was performed according to the study protocol with the original data set. Missing values were not replaced. The tests of hypotheses concerned the adjusted endpoint criteria difference between the gemcitabine/ISC group and gemcitabine alone or in addition to best supportive care without ISC. Two-sided statistical tests were performed at $\alpha = 0.05$, using 95% confidence interval method whenever possible.

The test power has to be not less than 80%, and α-adjusting for multiple testing was not performed. The SPSS^R and GraphPad^R softwares were applied.

BIAS MANAGEMENT

In order to minimize a possible bias inherent in a non-randomized study due to baseline imbalance, different therapy regimens and other confounders ("confounding bias") the adjuvant/palliative care related adverse drug reactions and quality of life surrogate endpoint results were adjusted for confounder effects by multivariable logistic regression analysis, using the adjusted odds ratio (OR) with 95% confidence intervals (95% CI). In the survival analysis, multivariable Cox proportional hazard regression was used with adjusted hazard ratio (HR) and its 95% CI. Only confounder-adjusted endpoint criteria results were considered for final interpretation. The pre-defined confounders used for adjusting have been: age, gender, comorbidity, tumor surgery (R0, R1), UICC/AJCC tumor stage, post-surgery staging (complete remission (CR)/non-evident disease (NED) vs. biochemical remission vs. residual disease), chemo- and/or radiotherapy, and concurrent treatment with high-dose vitamins or trace elements (see also Table **1** and Table **2**). The primary results were re-confirmed by sensitivity analysis using pre-defined multivariable models and adjusting procedures, such as stepwise elimination and forward selection procedures, stratification, and the application of propensity scores for adjusting [18].

THERAPY

Patients of the gemcitabine/ISC group (n = 201) received adjuvant chemotherapy with 6 cycles of gemcitabine on day 1, 8, and 15 every 4 weeks and commercially available batches of Iscador^R (Weleda, Arlesheim, Switzerland) according to the producer´s recommendations by 2 to 3 weekly subcutaneous injections. The ISC choice of treatment regimen for the particular patient was left at the discretion of the treating physician. The ISC treatment was administered with the intention of supportive care therapy.

Table 2. Treatment Regimen

Treatment regimen initial sample size= 396 (201 vs. 195)	Value ISC group % or mean (± SD)	Value control group % or mean (± SD)	P-value (Fisher's exact test or exact Mann-Whitney test)
Radiation therapy received (%)	4.5	18.5	< 0.001
Chemotherapy received % (mainly Gemcitabine)	71.6	43.6	< 0.001
Chemotherapy duration: mean (SD), months	6.9 (7.3)	4.7 (6.3)	n/a
Other supportive therapy %	54.7	32.8	< 0.001
Vitamins (high-dosed), trace elements etc. %	39.8	0.0	< 0.001
Analgesic therapy %	80.6	71.8	0.045
ISC therapy duration: median (range), months	15.0 (1-87)	n/a	-

n/a, not applicable, SD, standard deviation, ISC, Iscador.

Most of the patients in the control group (n = 195) received also regularly gemcitabine chemotherapy cycles, but without ISC. A small group of patients got other forms of best supportive care (see also subgroup analysis). Any other treatment options concerning best supportive care devoted to complementary and alternative medicine (CAM) or any medications for co-morbidity were left at the discretion of the treating physician and accepted for inclusion without restriction. The treatment regimens are summarized in Table **2**.

RESULTS

A total of 396 patients with histologically verified pancreatic tumor who had macroscopic complete resection and not prior radiation or neoadjuvant chemotherapy were eligible for the study. The records of the 201 pts in the chemotherapy/ISC group and 195 pts (overall control) in the chemotherapy and/or best of care group were obtained from 17 oncological departments of university and community hospitals as well as from private practices. The anthropomorphic data, the tumor staging, the stages of the disease according to UICC and baseline characteristics of the tumors are summarized in Table **1**. Some baseline imbalances were observed and statistically significant. Within the overall control group (chemotherapy without ISC but with/without best of care) more patients were at high risk (T3/T4 tumor stage: 71.3 % of the patients), but less patients in this group had regional lymphnode involvement (37.4% vrs. 66.7%). However according to the UICC staging significantly more patients were categorized in stage II (IIa and IIb) disease in the chemotherapy/ISC group (UICC stage II 62.2% vrs. 37.4%) that means that most of the patients in the chemotherapy/ISC group had extended disease in respect to tumor size (more than 2cm in diameter), involving extrapancreatice structures, including regional lymphnodes (66.7% vrs. 37.4%) but not affecting the Truncus coeliacus and/or superior mesenteric arteria. More patients in the control group were diagnosed with UICC stage IV disease and the tumor has already infiltrated the Truncus coelicus and/or superior mesenteric arteria (29.8% vrs. 15.0%). There is also a statistically significant difference between the median follow-up of the patients in the chemotherapy group with ISC (mean 15.2 months) vrs. the chemotherapy group without ISC/best of care (mean 10.1 months). This is mostly due to the post surgically baseline data of the tumor - tumor and disease staging - at the begining of aftercare, the severity and aggressiveness of the tumor, although patients in this group had standard gemcitabine chemotherapy treatment, if applicable. As shown in Table **2**, significantly less patients received chemotherapy in this overall control group (43.6.% vs. 71.6%) and the total time of application (4.7 months vrs. 6.9 months) was also decreased, because of the advanced morbidity of their disease (Table **1**: UICC stage of the disease IV, 29.8%). Concomitantly, significantly more patients got radiotherapy in the overall control group (18.5%) whereas signficantly more patients within the chemotherapy/ISC group took high-dose vitamins and/or other supportive therapies (CAM-related therapies). Therefore, only multivariable adjusted outcome results, confirmed in sensitivity analysis, were subjected for interpretation and subgroup evaluation was mandatory (e.g. total number of patients in a gemcitabine/ISC protocol vs. total number of patients treated only by gemcitibine).

Systemic adverse drug reactions attributed to ISC was documented in 3 pts (1.5%). All systemic ISC-related adverse drug reactions were mild to medium (toxicity grade 1-2) and clinically relevant as fatigue,

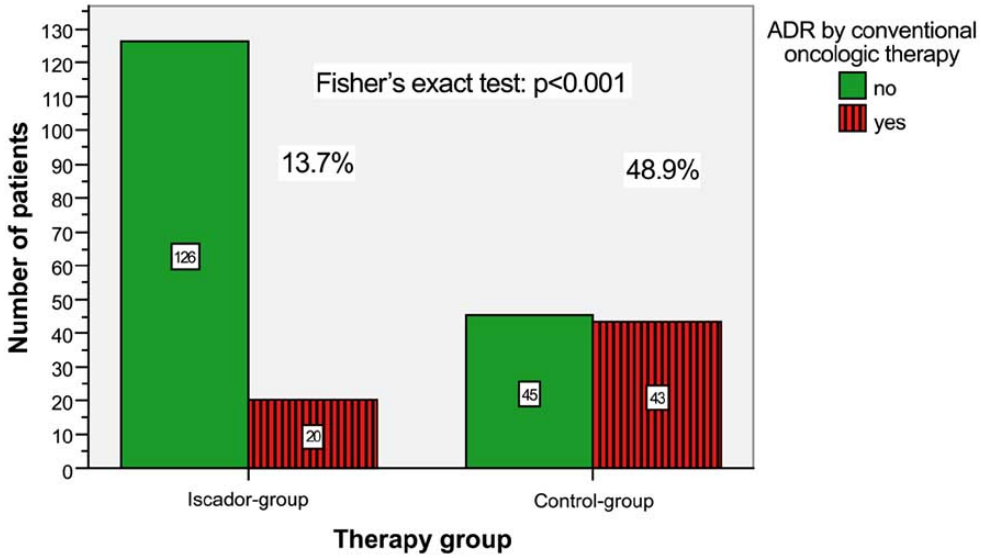

Fig. (1). Number of patients which experienced adverse drug reactions ADR. In 126 pts no ADR were seen, when the gemcitabine protocol was supplemented with Iscador®, 20 pts reported ADR.

In the control group including patients mostly treated by gemcitabine alone, in 45 pts no ADR were documented; in 43 pts ADR were clinically manifested. The incidence was calculated in contingency tables using Fisher´s exact test.

low-grade fever and ISC immune intolerance. Local ISC-related adverse drug reactions at the site of subcutaneous injection were of toxicity grade 1-3, like induration, edema, erythema, itching and local pain and occurred in 45 pts (22.4%). Life-threatening or persisting ISC related adverse drug reactions, clinically relevant interations between ISC and other medications, or even tumor enhancement were not observed.

Within the gemcitabine/ISC group signifcantly fewer patients than in the overall control group experienced tumor-associated symptoms or cytotoxic drug related adverse reactions (incidence 13.7% vrs. 48.9%); particularly, common adverse drug reactions, such as nausea/vomiting, diarrhea, leucopenia and fever/infections were remarkably lower which, on the other hand, improved quality of life. The adjusted odds ratio (OR) to develop adverse drug reactions during the chemotherapy was OR (95% CI) = 0.26 (Fig. **1**). The persistence of most individual symptoms – either disease- or therapy-related – was decreased at the end of the first therapy cycle (Fig. **2**). The adjusted total symptom scale revealed an OR = 0.43, statistically not significant, but a prominent trend to more symptom-free patients in the gemcitabine/ISC group.

Among 396 evaluable patients a total number of 315 pts (79.5%) died during the study period. The adjusted relative hazard to die from any cause during the onset of aftercare and within the follow-up period was significantly lower in the gemcitabine/ISC group than in the overall control group. The adjusted hazard ratio (HR, 95% CI) was HR = 0.52 (0.40 – 0.68), p < 0.001, suggesting a relevant overall (OS) survival benefit for patients treated concomittantly with a gemcitabine/ISC protocol (Fig. **3**).

A subgroup analyis for OS stratified according to the UICC staging was performed and the results expressed in:

i) number of patients,

ii) adjusted hazard ratio with 95% CI and

iii) reduction of hazard ratio in percentages in the gemcitabine/ISC group.

UICC stage I: 49 pts, HR = 0.80 (0.3 –1.91), risk reduction 20%

UICC stage II: 198 pts HR = 0.49 (0.29–0.81), risk reduction 51%

UICC stage III: 61 pts HR = 0.80 (0.32-1.97), risk reduction 20%

UICC stage IV: 88 pts HR =0.65 (0.35-1.20), risk reduction 35%

The adjusted OS results showed a consistent trend for OS prolongation in favor of the gemcitabine/ISC group, which was significant (p = 0.006) for the patients in UICC stage II.

DISCUSSION

The development of gemcitabine, a difluorinated analogue of the nucleoside deoxycytidine, was an advance in the treatment of pancreatic cancer. The first

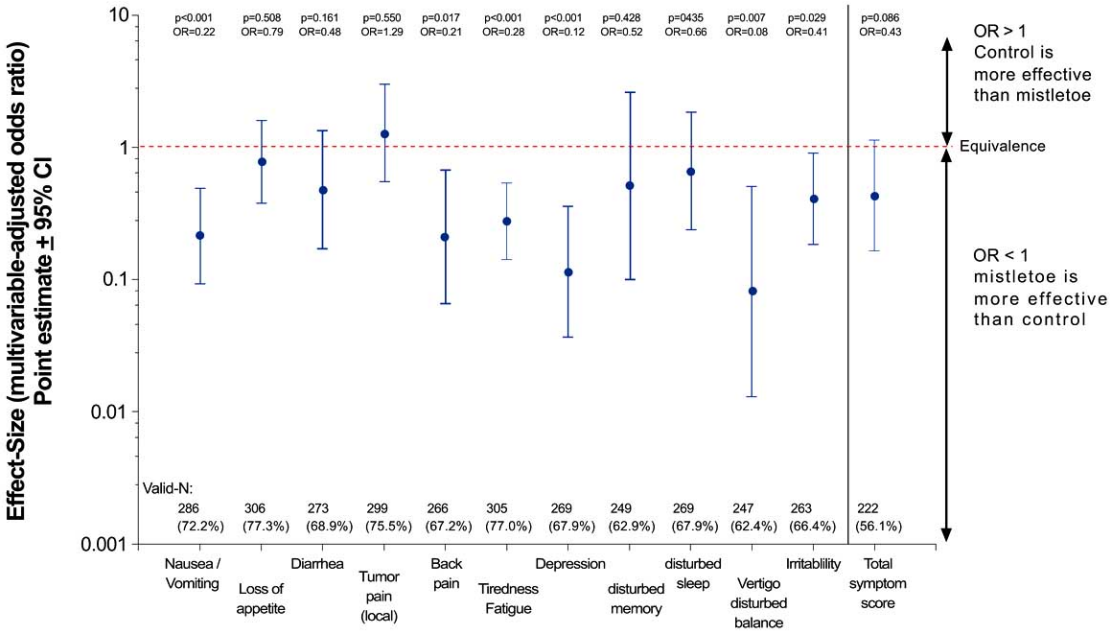

Fig. (2). Symptom persistence risk estimates adjusted for single symptom persistence and total symptom score calculated by logistic regression and Wald test. The x-axis nominates the single symptoms and the total number of patients and percentages who experienced the different symptoms. The logarithmic y-axis denominates the multi-variable adjusted odds ratios (OR). OR > 1 means treatment in the control group (gemcitabine alone) is more effective, OR < 1 means gemcitabine plus Iscador® is superior. The bars show the 95% confidence intervals of OR and the statistical significance is listed (p-values) above. The fully lined black bars indicate those symptoms which are statistically signifiantly controlled by the gemcitabine/Iscador® protocol.

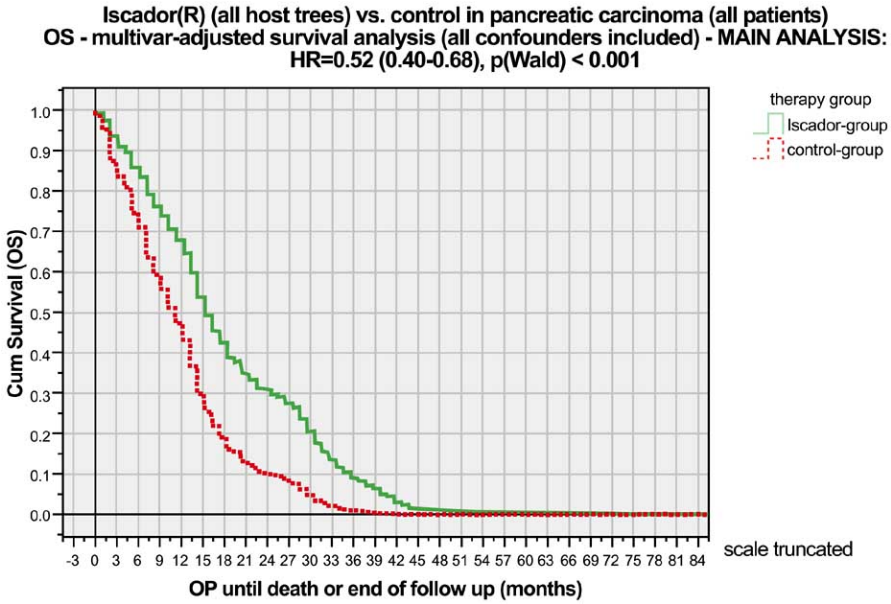

Fig. (3). Multivariable-adjusted survival analysis, including all confounders showing the cumulative overall survival (OS) in the gemcitabine/Iscador® (green line) and the control group (red line). The adjusted OS hazard ratio (HR) was calculated by Cox proportional hazard regression method (Wald test) and confirmed in sensitivity analysis.

choice of treatment is, however, total or partial pancreatectomy, such as pancreaticoduodenectomy and distal pancreatectomy, whenever oncologically appropriate [22]. The role of adjuvant chemoradiation therapy for pancreatic adenocarcinoma remains controversial [23]. Benefit of adjuvant chemoradiation therapy is seen only in patients with LN-positive disease, regardless of resection margin status. Chemoradiation therapy in patients with LN-negative disease may contribute to reduced disease-free survival. Clinical evidence does not support the use of adjuvant radiotherapy in a chemoradiation protocol in pancreatic carcinoma, possibly because it delays sequential chemotherapy [24]. Five major randomized trials (GITSG, EORTC, ESPAC-1, RTOG 9704 and CONKO-1) have shaped world opinion on adjuvant therapy regimens among resected patients with pancreatic cancer. Adjuvant chemotherapy is superior to observation following surgery and gemcitabine is superior to 5-FU as adjuvant chemotherapy [25]. As these trials have failed to show improvements in survival using gemcitabine in combination with other chemotherapeutic agents, although the gemcitabine/oxaliplatin combination has shown some promise, this single chemotherapeutic agent remains the basic standard to treat pancreatic carcinoma after surgical intervention [26,27]. As the survival advantage is still small, multiple approaches with other agents in combination with gemcitabine or on the way in clincal settings in order to improve clinical outcome, either in disease-free and/or overall survival rates [28] or in quality of life [29].

In Europe many cancer patients use mistletoe preparations as a complementary therapy regimen nested in in mainstream anti-tumor strategies. Mistletoe therapy is one therapeutic essential of anthroposophic medicine and often subjected to critical discussion in respect to safety and effectiveness [30,31]. However, there is ample convincing experimental evidence that mistletoe lectins, viscotoxins and other components, alone or in combination, show anti-tumoral properties by induction of apoptosis [32], by interaction with the cell cycle machinery causing a substantial delay through S-phase progression [33], by affecting tumor angiogenesis [34] and by modulating gene signatures of tumor cells, a result, which might lead to mistletoe specific targeted gene therapy, e.g. navigated by different concentrations of lectins within a mistletoe preparation [35]. Very recently, it has been shown that the mistletoe (Viscum album L.) preparation Iscador^{R} contains molecular compounds, which are inhibitory substrates of the transmembrane efflux pump P-glycoprotein (P-gp) which is part of the multiple efflux transporter proteins ABC (ABCB1) [36].

The effect of inhibiting intentionally these transporters in tissues exposed to chemotherapy agents might result in an increased cytoplasmatic drug concentration and cytotoxicity. The challenge is to find an appropriate dose and time schedule to apply such modulators of the ABC drug transporters, like verapamil [37], flavonoids [38] and mistletoe [36], to increase drug absorption and to modulate a more open therapeutic window of drugs (chemotherapeutic agents), which otherwise exhibit a narrow therapeutic window. Interestingly, it was recently suggested that cancer stem-like cells expanded during the acquisition of gemcitabine developed resistance and in therapeutic application, targeted therapy against ABC transporters by timely inhibition could be applied to overcome drug resistance in the treatment of pancreatic cancer [39]. Mistletoe extracts, like Iscador^{R}, because of the safe application and the explored mode of action, are first

candidates, as shown in this pharmaco-epidemiological study, to be included into chemotherapy protocols to increase response rates, concomittantly do not augment adverse drug reactions, but improve clinical outcome and quality of life as demonstrated very recently in colorectal cancer patients [12]. In colorectal cancer patients with resected tumors it is well established that 5-FU based chemotherapy protocols are the first line chemotherapy; in pancreatic cancer, the difluorinated nucleoside analogue gemcitabine is the standard protocol for adjuvant therapy. In both studies, the colorectal study [12] and the study presented here, fluorinated nucleoside analogues seem to support chemotherapy at its best, if the clinical parameters i) adverse drug reaction, ii) quality of life and iii) overall survival are defined as endpoints of a trial.

We present here clinical data of a retrolective pharmaco-epidemiological non-interventional study, therefore, the possible effects of unknown hidden confounders had to be minimized and lessions have to be learned from improving the methodological and ethical validity of such best supportive care studies [40]. In this study, significantly more patients in the gemcitabine/ISC group received additional high-dose vitamins and trace elements than in the control group. Such type of inhomogeneity is quite common in non-randomized chemotherapy studies, because many patients use antioxidant supplements during chemotherapy. However, the evidence is currently insufficient to inform clinician and patient guidelines on the use of antioxidant supplements during cancer treatment. Well designed clinical trials and observational studies are needed to determine the short- and long-term effects of such supplements [41]. Possible confounding effects on the outcome measures were included as adjusting factors (covariates) in the multivariable logistic regression and the Cox proportional hazard regression (survival analysis). Owing to this model, the possible confounding effects of such additional therapies were minimized, and the mistletoe therapy results became largely unbiased estimates. These results were confirmed in sensitivity analyses, too. Therefore, in this study, the adjusted outcomes could be interpreted as free of possible effects of the vitamins or trace element therapies or as adjusted results accounting for possible confounding effects of group inhomogeneity of any other therapy. Also, we are fully aware that our analysis may be flawed by the reliability of the staging and the pathological data sets, by the surgical expertise and the patients´ compliance, the timely schedule of chemotherapy - in short, by the stringency of the adjuvant therapy regimens, because surgery and aftercare were carried out in community hospitals and community-based oncology practices, which are often under the pressure of managed care of cost containment, budget deficits and have to deal effectively with improved health care quality. On the other hand it is reasonable to assume that this study represents a patients´ population seen in routine clinical practice, and the quality of patient care, outside of clinical trials and therefore not governed by stringent protocol rules which are mandatory in prospective clinical trials, may mirror the current day-to-day standard in Germany and Switzerland. The comparison of survival data, obtained in this study with the CONKO-001 study reveals an emerging problem of modern medicine that highly efficacious treatments do not show significant effectiveness in real world systems of care. There is common sense that patients enrolled in clinical trials in comprehensive cancer centers show a better outcome of their disease. „Certified Cancer Centers“ (CCC), the designation of „National Cancer Institute“ (NCI) are often viewed by patients and referring providers as an indication of clinical excellence. These designations are often associated with lower risk of enigmatic diagnoses, with lower risk of postoperative death and improve long-term survival. Possible factors responsible for these benefits include surgeon training, multidisciplinary care, and adherence to treatment guidelines [42].

The overall survival outcome of this study clearly advocates for the treatment of cancer patients in university-/community- and/or private practice-based comprehensive cancer centers.

The median overall survival time of the patients in the control group of this retrolective study presented here, and either receiving gemcitabine alone, or in combination with complementary medical care, but excluding medical mistletoe supplemention (ISC), was 14.2 months which is within the range of reported survival data for locally advanced disease (stage I – III) [43], but does not reach the overall survival data of the gemcitabine group of the so far largest and well designed CONKO-001 trial, irrespectively of the resection status (R0: 21.7 months vrs. R1: 22.1 months) [14]. This might be due to a selected pancreas tumor population, as discussed above, not so often seen up-to-now in single private practices. However, and this is most remarkable, if the single agent chemotherapy (gemcitabine) regimen was supplemented by ISC, the overall survival was similar to the gemcitabine group of the CONKO-001 trial (20.3 months vrs. 21.7/22.1 months) and the quality of life was significantly improved because symptom control was effectively increased.

Gemcitabine can be still considered as the best single agent in the treatment of locally advanced and metastatic pancreatic cancer. The frustrating lack of significant clinical achievements in the treatment of pancreatic cancer remains one of the medical oncology´s biggest disappointments. However, the results of this pharmaco-epidemiological study that ISC is i) clinically safe, ii) decreases adverse drug reactions and tumor- and therapy-related symptoms and iii) improves prognosis for overall survival in R0 and/or R1 resected tumor patients when applied as adjuvant therapy with gemcitabine warrants prospective randomized clinical trials. This study is the first study which reports results in pancreatic cancer patients who

received a combination of chemo-/botanical therapy. These results strongly suggest that within a prospective two-arm study, using the gemcitabine/ISC protocol vs gemcitabine alone, the overall survival might be significantly prolonged beyond the median survival of roughly 22 months, due to the discussed stringency of prospective clinical trials and because of the possibility to administer the intended cycles of chemotherapy without declining dose-limiting biochemical and laboratory parameters and quality of life.

Accumulating experimental and clinical evidence suggest that many botanicals interfer with a variety of molecular targets and processes involved in cancer. Sometimes, like for curcumin, a polyphenolic compound from the dietary spice turmeric, a delivery platform was developed [44] to use the strong potential of a botanical anti-cancer agent in the clinical arena. Furthermore, it is mandatory to search for new therapeutic targets [45] which might – in addition to conventional therapeutic procedures - be modulated by botanicals, too, in order to improve the prognosis of an otherwise uniformly lethal disease.

ISC has shown now in numerous preclinical studies anti-tumor activity and in two retrolective pharmaco-epidemiological, non-interventional studies improv-ements in quality of life and clinical outcome. ISC exhibits a pharmacological supportive activity for the mode of action of mono- (5-FU) [12] and difluorinated nucleoside analogues (gemcitabine) leading to a better prognosis in colorectal [12] and pancreatic cancer.

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CONFLICT OF INTEREST

The corresponding author (K.S.Z.) declares no competing interest.

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