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Randomised and Non-randomised Prospective Controlled Cohort Studies in Matched-pair Design for the Long-term Therapy of Breast Cancer Patients with a Mistletoe Preparation (Iscador): A Re-analysis

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Abstract

Objective: Expanded presentation and re-analysis of previously published data of randomized and non-randomized studies on mistletoe therapy with breast cancer patients [3, 4]. The main question is: Does a re-analysis confirm the previously reported effects of prolonging the survival of patients with breast cancer under long-term application of a complementary/anthroposophic therapy with the European mistletoe preparation Iscador?

Data Sources: (1) Randomised matched-pairs study: Breast cancer patients with only lymphatic metastases (17 pairs) that had never used mistletoe therapy were matched for several prognostic factors. By paired random allocation, one patient of a pair received a suggestion of mistletoe therapy to be applied by the attending physician. (2) Non-randomised matched-pairs studies: Patients that had already received mistletoe (Iscador) therapy were matched to control patients from the same pool using the same prognostic criteria. Three groups were recruited by this procedure: breast cancer with local recurrences and no metastases (42 pairs), breast cancer with only lymphatic metastases (55 pairs), and breast cancer with distant metastases (83 pairs).

Analysis: Cox proportional hazard models and sensitivity analyses based on subsets of the original data sets according to strict or lose application of the matching criteria.

Results: The results of this re-analysis are consistent with the earlier results, even when comparing different methods and subsets. In the randomised study, the effect of long-term Iscador therapy on overall survival is significantly in favour of the Iscador therapy: Estimate of the median difference and 95 % confidence interval in years 2.5 (0.83, 4.50). The results for the non-randomised studies were also in favour of the Iscador therapy: Breast cancer with local recurrences and no metastases: estimate of hazard ratio and 95 % confidence interval 0.52 (0.23, 1.17); breast cancer with lymphatic metastases: 0.27 (0.15, 0.50); breast cancer with distant metastases: 0.53 (0.32, 0.88). As a short-term effect of this therapy, psychosomatic self-regulation noticeably increases within 3 months in the Iscador group in comparison to the control group in the randomised study: estimate of the median difference 0.90 (0, 1.75).

Conclusion: The re-analysis demonstrates that the effects shown in the previously published data are consistent despite using different analytic methods and different subsets. Overall, the survival of patients receiving mistletoe treatment with Iscador is longer in these studies. In the short term, psychosomatic self-regulation, as a measure of autonomous coping with the disease, rises more under Iscador therapy than under conventional therapy alone.

Key words: Breast cancer, overall survival, quality of life, complementary/anthroposophic therapies, mistletoe preparation (Iscador)

Introduction

The following work presents a re-analysis of four published data sets [3, 4] on mistletoe (Iscador) therapy: one randomised matched-pairs study on breast cancer patients with lymphatic metastases (*MammaLymRand*) and three non-randomised matched-pairs studies on breast cancer patients with local recurrences and no metastases (*MammaRec*), or lymphatic metastases (*MammaLym*), or distant metastases (*MammaMet*), respectively. These studies are part of a larger program of prospective randomized and non-randomized studies taken from a pool of 10226 cancer patients.

Patients and Methods

For the background information: study objectives, study setting, design, data sources, patient recruitment, initial data assessment, observed therapy and intervention, follow-up – please see the original paper [3]. For reasons of clarity, we restate the matching processes and also describe further subsets that were used in the re-analysis.

Matching Process for the Three Prospective Non-Randomised Matched-Pairs Studies (Fig. 1)

For the three non-randomised prospective studies, matching was based on a group of patients with Iscador therapy. This group was divided into three subgroups with a predetermined stage of primary breast cancer diagnosis (here called «first diagnosis»): the first with local recurrences and no metastases (*MammaRec*),

the second with lymphatic metastases (*MammaLym*) and the third with distant metastases (*MammaMet*) (Table 2).

In the initial data assessment, the latest information on cancer stage at diagnosis (*first diagnosis*) was assessed and the patients were allocated accordingly to the corresponding stage group. The difference between the year of *first diagnosis* (coinciding with the year of a corresponding operation), and the year of the initial data assessment was in most cases 3 years or less. Patient recruitment and initial assessment was from 1971 to 1988. A control patient was found for each patient who fulfilled all the inclusion criteria. The control had to be within the pool of patients already available in the data files, who had not received mistletoe therapy and belonged to one of the three stage groups. The matching process was performed during the first 12 months after each patient with Iscador therapy entered the study and had been visited for the initial data assessment (Fig. 1). Tumour events which occurred later had no influence on this primary matching process: once allocated to one of the three stage groups, patients stayed there until death. It was checked whether the control patient was still alive at the particular time of matching; the patient was asked if she is still willing to participate in a controlled cohort study and which further therapies she had received since the last contact. If no living matching partner could be found, then the Iscador patient was excluded from all of the studies. Control patients were only used once in the mistletoe studies and were never used in other studies.

The matching criteria included: tumour stage at *first diagnosis*, status of menopause, year of first diagnosis of the breast cancer stage (with up to ±3 years difference), age at first diagnosis (with up to ±3 years difference) and type of conventional therapy (Table 4). In order not to lose too many patients, deviations from the matching criteria were allowed in two criteria at most in all three data sets (Table 2). If there was more than one control patient available, the pair with the smallest age difference was included in the study.

Patient groups with "strict matching" pairs are subgroups of all matched-pairs of patients in one stage group which exactly fulfil all matching criteria. Patient groups with a "balanced set" are subgroups of all matched-pairs of patients within one stage group: pairs with prognostic factors favouring only the patients with Iscador therapy were eliminated; they lie in between the complete data sets and the sets with strict matching.

Matching and Randomisation for the Prospective Randomised Matched-Pairs Study (Fig. 2)

As new patients came into the study pool of already available breast cancer patients with only lymphatic metastases, matched-pairs were built from 1974 to 1988 (Fig. 2).

The matching criteria included: tumour stage at *first diagnosis*, status of menopause, year of first diagnosis of the breast cancer stage (with up to ±3 years difference), age at first diagnosis (with up to ±3 years difference) and type of conventional therapy (Table 3). In order not to lose too many patients, deviations from the matching criteria were allowed within each data set (Table 1).

The difference between the year of *first diagnosis* and the year of the initial data assessment was 3 years or less in most cases. The matching process was performed during the first 12 months after initial data assessment (Fig. 2). At the time of matching, it was checked whether both patients of the pair in question

Events	Time interval	Time periods for individual studies		
		MammaRec	*MammaLym*	*MammaMet*
«First diagnosis»: breast cancer with recurrences and no metastases (*MammaRec*), or lymphatic metastases (*MammaLym*), or distant metastases (*MammaMet*)		1968–1988	1969–1988	1966–1988
	Few weeks			
Operation		1968–1988	1969–1988	1966–1988
	Weeks or months			
Approximate begin of Iscador therapy in observed therapy group		1968–1988	1969–1988	1966–1988
	Up to 36 months			
Recruitment and initial data assessment		1971–1988	1971–1988	1971–1988
	Up to 12 months			
Matching		1971–1988	1971–1988	1971–1988
	Follow-up from 1 to several months			
Follow-up		1971–1998	1971–1998	1971–1998
	Until death			
Final assessment		1998	1998	1998

Fig. 1. Chronology of events for non-randomised matched-pair studies *MammaRec*, *MammaLym* and *MammaMet*.

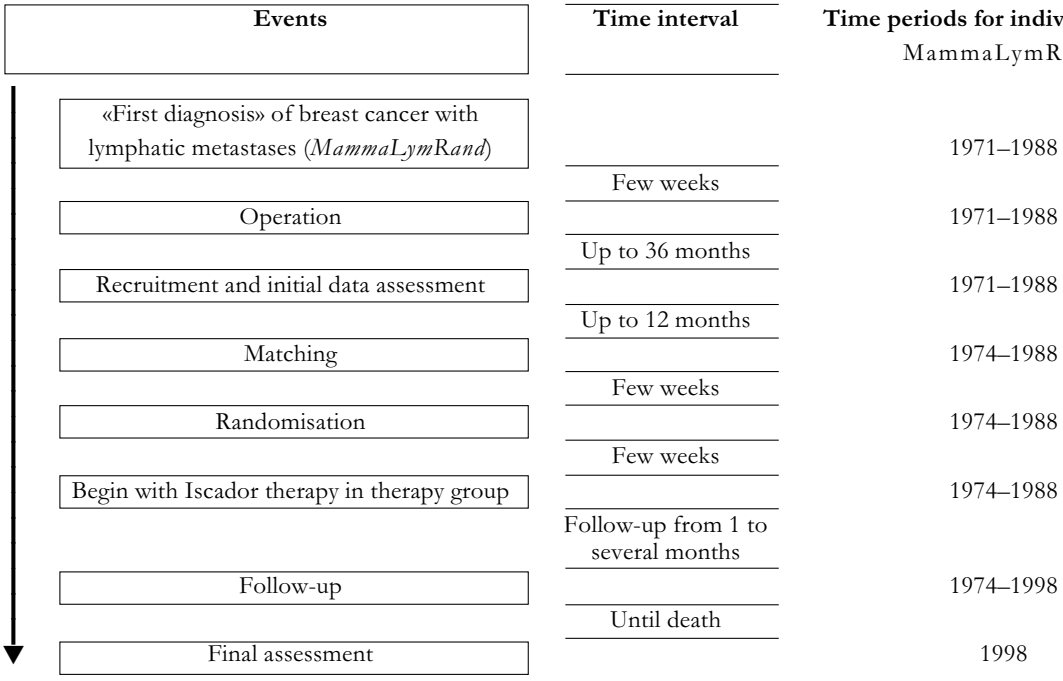


Fig. 2. Chronology of events for the randomised matched-pair study *MammaLymRand*.

were still alive and both willing to participate in a controlled cohort study. Immediately after this was confirmed, the suggestion of an intervention was randomly allocated to one of these patients by the following process: Two slips of paper with the names of the two matched partners were put into a hat by the main investigator (G.-M.), and a masked assistant drew one of the slips of paper. Beforehand, it was determined, that the patient that was selected first, has to be asked if she would be willing to ask her attending doctor for a complementary therapy with Iscador. It must be noted that the intervention not consisted of giving a therapy with Iscador, but suggesting the patient that she asks her doctor for an Iscador therapy.

Consent for study participation in this case was one-sided, as only the patient who was allocated to receive the suggestion of a therapy with Iscador was informed of this process. The other patient and their respective attending physician were not informed. Thus, this is a special case of the single randomised consent design according to Zelen [1, 14, 15].

Statistics

The analysis and presentation of the data sets reported here was made according to the suggestions made in the CONSORT statement for randomised studies [10] and its adaptation to non-randomised studies [12].

In the baseline comparisons of Iscador and control groups in the non-randomised matched-pairs studies, the Wilcoxon paired sample test (WPS) was used for the continuous variables, the marginal homogeneity test (MH) for counted data with ordered categories in paired samples and the McNemar test (MN) for binomial data in paired samples [9].

In the first stage of the analysis of overall survival, the median of the differences in survival is estimated by the nonparametric Wilcoxon paired sample test. As there are no censored survival times, this yields a reliable result. The estimate of the median difference and the 95% confidence intervals are calculated according to Hodges-Lehmann [8]. All p-values are two-sided. In order to explore the sensitivity of the matching criteria, the complete data sets are compared with the balanced sets and with the data sets according to strict matching.

In the second stage of the analysis of overall survival, the Cox proportional hazard regression model is applied to the complete data sets from the three non-randomised matched-pairs studies. The therapy with Iscador is introduced using a binary variable: either treatment or no treatment. An indicator variable for the matched-pairs is introduced and a stratified analysis based on the pairs is performed taking into account all available prognostic factors and paired interactions of the significant factors. This stratification according to matched-pairs generally results in a conservative estimate in comparison to the unmatched analysis [7, §7.1]. The model development and the assessment of model adequacy is performed according to the recommendations in the literature [6, 13]. No automatic variable selection procedure was used. No adjustment of prognostic factors, other than therapy with Iscador (binary variable), was performed in the randomised study. According to expert recommendations [13], the assumption of proportional hazards (PH) is assessed statistically and graphically; if any one but not both of these methods fail to show a positive result, we describe the PH assumption as «moderately fulfilled».

All statistical tests and confidence intervals are calculated on the basis of matched-pairs, i.e. we always used tests for two paired samples or tests with stratification according to the pairs, respectively. Confidence intervals (CI) are always 95%. CI and test results are regarded as significant if $p < 0.05$.

The statistical analyses were performed using S-Plus 6.2 for Windows Professional Edition (Insightful Corp.

Table 1. Flow chart of primary breast cancer patients from the randomised matched-pairs study *MammaLymRand*.

DATA SOURCES	**N**	
Pool of cancer patients with no mistletoe therapy [3, p. 59, Figure 1]	8475	
Pool of primary breast cancer patients with no mistletoe therapy	1882	
CHARACTERISTICS OF DATA FLOW		
Primary breast cancer patients with only lymphatic metastases and no mistletoe therapy (see Table 2)	369	
Patients used as controls in parallel non-randomised study (see Table 2)	– 64	
Patients used in another randomised study [3, p. 62, Table 3]	– 8	
Patients used as controls in another non-randomised study [not published]	– 41	
Pool of patients for building randomised matched-pairs	256	
Study	**MammaLymRand**	
	Iscador [N]	Control [N]
Resulting matched patients	17	17
Declined participation, did not receive therapy or drop-out before start of therapy in the Iscador group	0	0
Discontinued therapy, drop-out after start of therapy	0	0
Lost to follow-up	0	0
Raw data for analysis	17	17
Pairs with no deviations from the specified matching criteria	16 pairs	
Pairs with one deviation at most from the specified matching criteria	17 pairs	
Survival analysis (Cox model)	17	17
Censored	0	0
Excluded	0	0

2003, Seattle, Washington). The Wilcoxon paired sample tests, the Hodges-Lehmann estimate and confidence intervals as well as the marginal homogeneity tests were calculated with the exact procedures in StatXact 6 (Cytel Software Corporation 2004, Cambridge, Massachusetts).

Results

Flow of Patients within the Randomised Matched-Pairs Study (Table 1)

MammaLymRand (2 × 17 patients): The recruitment and initial data assessment was performed between 1971 and 1988. From the available 256 primary breast cancer patients with only lymphatic metastases and no mistletoe therapy, 17 randomized matched-pairs could be formed from 1974 until 1988. There were no non-compliers, no drop-outs and no living patients at the time of the last assessment in 1998. All 17 pairs were included in the final analysis.

Flow of Patients within the Three Non-Randomised Matched-Pairs Studies (Table 2)

The recruitment and initial data assessment was performed between 1971 and 1988 and the matched-pairs were built from 1971 until 1988. There were no living patients at the time of the last assessment in 1998.

MammaRec (2 × 42 patients): From 64 primary breast cancer patients with local recurrences of the primary tumour and no lymphatic or distant metastases that had already received mistletoe therapy, 50 non-randomized matched-pairs could be formed. 42 matched-pairs were included in the final analysis after the exclusion of 8 pairs.

MammaLym (2 × 55 patients): From 73 primary breast cancer patients with only lymphatic metastases that had already received mistletoe therapy, 64 non-randomized matched-pairs could be formed. 55 matched-pairs were included in the final analysis after the exclusion of 9 pairs.

MammaMet (2 × 83 patients): From 112 primary breast cancer patients with distant metastases that had already received mistletoe therapy, 90 non-randomized matched-pairs could be formed. 83 matched-pairs were included in the final analysis after the exclusion of 7 pairs.

Patient Characteristics for the Randomised Matched-Pairs Study (Table 3)

MammaLymRand (2 × 17 patients): The matching is perfect for stage (FIGO, TNM), status of menopause and conventional therapies [3, Table 3]. The age differences at the time of first diagnosis are 2 years and less.

Table 2. Flow chart of primary breast cancer patients from the non-randomised matched-pairs studies *MammaRec*, *MammaLym* and *MammaMet*.

CHARACTERISTICS OF DATA FLOW	**N**		**N**		**N**	
Candidates for the three non-randomised matched-pairs studies	Primary breast cancer with only local recurrences of the primary tumour and without lymphatic or distant metastases		Primary breast cancer with only lymphatic metastases		Primary breast cancer with distant metastases	
	353		483		640	
	Iscador	No Iscador	Iscador	No Iscador	Iscador	No Iscador
	69	284	114	369	144	496
Patients used in another non-randomised study [not published]	– 5	–5	–41	–41	–32	–32
Subgroup available for matching	64	279	73	328	112	464
Study	**MammaRec**		**MammaLym**		**MammaMet**	
	Iscador	Control	Iscador	Control	Iscador	Control
Resulting matched patients	50	50	64	64	90	90
Declined participation, did not receive therapy or drop-out before start of therapy in the Iscador group	1	4	2	5	0	2
Discontinued therapy, drop-out after start of therapy	0	0	0	0	0	0
Lost to follow-up	1	1	0	1	0	3
Raw data for analysis	43	43	56	56	85	85
Excluded from analysis: incomplete matching with more than 2 deviations from the specified criteria	1 pair		1 pair		2 pairs	
Matching with at most 2 deviations from the specified criteria	42 pairs		55 pairs		83 pairs	
Survival analysis (Cox model)	42	42	55	55	83	83
Censored	0	0	0	0	0	0
Excluded	0	0	0	0	0	0
Reduced data sets						
Balanced set (subgroup of complete set of matched-pairs not favouring the patients with Iscador therapy)	39	39	42	42	72	72
Strict matching (subgroup of complete set of matched-pairs of patients fulfilling exactly all matching criteria)	29	29	38	38	53	53

Differences within pairs between years of first diagnosis are 1 year at most. – Self-regulation at baseline was not matched; the difference between the therapy groups is not significant (WPS test, p = 1).

Patient Characteristics for the Three Non-Randomised Matched-Pairs Studies (Table 4)

Three sets were analysed and compared in a sensitivity analysis: (i) complete data set, (ii) balanced data set: exclusion of unbalanced pairs the risk factors of which are in favour of the mistletoe patient, (iii) reduced data set consisting of all pairs with strict matching allowing no deviations (Table 2). Details of the deviations from the strict matching within the groups (i) and (ii) are shown below.

MammaRec: For the complete set (n = 2 × 42), the matching is perfect for stage (FIGO, TNM) and status of menopause. Conventional therapies did not differ

Table 3. Patient characteristics (matching variables and other variables) in the randomised matched-pairs study *MammaLymRand* (WPS = Wilcoxon paired sample test, SD = standard deviation, NA = not available).

	Study	MammaLymRand		WPS
Prognostic variables		Iscador n = 17	Control n = 17	p
Matching variables	**FIGO TNM**			
	IIIA T2N2M0	1	1	
	T3N1-2M0	5	5	
	IIIB T4N1-4M0	10	10	
	T3N3M0	1	1	
	Menopause			
	pre	12	12	
	post	5	5	
	Year of first diagnosis			
	mean	1981.94	1981.94	
	SD	5.19	5.19	
	range	1973–1988	1973–1988	
	Age at first diagnosis			
	mean	44.47	44.59	
	SD	5.28	5.11	
	range	33–51	34–52	
	Conventional therapy			
	Operation	17	17	
	Chemotherapy	6	6	
	Radiotherapy	8	8	
	Hormone therapy	4	4	
Baseline variable	**Self-regulation**			1.00
	mean / median	2.92 / 3.10	2.87 / 3.00	
	SD	0.64	0.61	
	range	1.7–3.8	1.7–3.6	
Therapy variable	**Iscador use (years)**		NA	
	mean / median	3.51 / 5.00		
	SD	2.78		
	range	0.08–7.00		

significantly; particularly when concerning chemotherapy there were absolutely no differences. Radiotherapy was different in 7 pairs, in 5 of them only the control patient had radiotherapy and in 2 of these pairs only the Iscador patient had radiotherapy. This was judged as slightly in favour of the control group. In one case, the operation of an Iscador patient was delayed for personal reasons (refusal); this worked in favour of the control patient. The differences in the year of operation (= year of first diagnosis) was not significant (WPS test p = 0.57). The set of matched-pairs was balanced, except for 2 cases, one of which includes the above mentioned pair with the delayed operation; in the other pair, the difference of 12 years worked in favour of the Iscador group and was thus excluded. If the matching was performed too long (> 3 years) after the year of first diagnosis (first operation), then this worked in favour of the group with the bigger difference, since only surviving patients can be matched. This was the case for 5 Iscador patients. Two pairs of these had partner pairs with the reversed situation; hence 3 pairs had to be excluded. The difference in age at first diagnosis was not significant (WPS test, p = 0.99); however, for 4 pairs, the age difference was within a range of 4 and 5 years, in 3 pairs the Iscador patient was older and so no exclusion was necessary. Overall, 3 pairs were excluded in order to build a balanced set which included the above mentioned pair with a difference of 12 years in the year of operation. The exclusion resulted in a balanced set of 39 pairs, while strict matching produced 29 pairs. Self-regulation at baseline was not matched; the difference between the therapy groups at the first evaluation was not significant (WPS test, p = 0.12). The judgement of effectiveness of the therapy by the patient was slightly better in the Iscador group (MN test, p = 0.15).

Table 4. Patient characteristics (matching variables and other variables) in the non-randomised matched-pairs studies *MammaRec*, *MammaLym* and *MammaMet* (SD = standard deviation, NA = not available). Test: [1] Wilcoxon paired sample test (WPS), [2] Marginal homogeneity test (MH), [3] McNemar test (MN).

	Study	MammaRec		Test	MammaLym		Test	MammaMet		Test
	Prognostic variables	Iscador n = 42	Control n = 42	p	Iscador n = 55	Control n = 55	p	Iscador n = 83	Control n = 83	p
Matching variables	**FIGO TNM**			1.00[2]			1.00[2]			
	I T1N0M0	3	3							
	IIA T2N0M0	10	10							
	IIB T3N0M0	13	13							
	T2N1M0				3	3				
	III T4N0M0	16	16							
	IIIA T2N2M0				5	5				
	T3N2M0				5	5				
	IIIB T1-3N3M0				6	6				
	T4N1-4M0				36	36				
	IV TXNXM1							83	83	
	Menopause			1.00[3]			0.21[3]			
	pre	12	12		4	2				
	post	30	30		51	53				
	NA							83	83	
	Year of first diagnosis			0.57[1]			< 0.01[1]			< 0.01[1]
	mean	1979.83	1980.17		1979.16	1980.11		1978.89	1979.39	
	SD	5.52	4.99		5.01	4.57		5.49	5.21	
	range	1968–1987	1970–1988		1969–1987	1972–1987		1966–1988	1970–1987	
	Age at first diagnosis			0.99[1]			0.46[1]			0.73[1]
	mean	54.07	54.00		59.31	58.98		55.04	55.22	
	SD	9.64	8.97		6.20	5.55		7.01	7.36	
	range	36–70	36–68		42–70	43–70		36–68	37–69	
	Conventional therapy									
	Operation	42	42	1.00[3]	55	55	1.00[3]	83	83	1.00[3]
	Chemotherapy	3	3	1.00[3]	27	26	0.53[3]	45	42	0.10[3]
	Radiotherapy	14	17	0.29[3]	16	16	1.00[3]	53	55	0.21[3]
	Hormone therapy	10	9	0.53[3]	26	27	0.53[3]	22	23	0.53[3]
Baseline variables	**Self-regulation**			0.12[1]			0.17[1]			< 0.01[1]
	mean / median	4.02 / 4.00	3.64 / 4.00		3.87 / 4.00	3.55 / 3.00		3.76 / 4.00	3.22 / 3.00	
	SD	1.51	1.50		1.40	0.92		1.28	0.95	
	range	1.0–6.0	1.0–6.0		1.0–6.0	2.0–6.0		1.0–6.0	1.0–6.0	
	Judgement									
	Effectiveness: Yes	28	21	0.15[3]	48	37	0.02[3]	42	33	0.13[3]
	Participation RCT: Yes	NA	NA		12	18	0.25[3]	20	16	0.69[3]
Therapy variable	**Iscador use (years)**		NA			NA			NA	
	mean / median	4.05 / 2.00			3.22 / 2.00			1.87 / 1.00		
	SD	4.38			2.46			1.76		
	range	1.00–20.00			1.00–10.00			0.08–9.00		

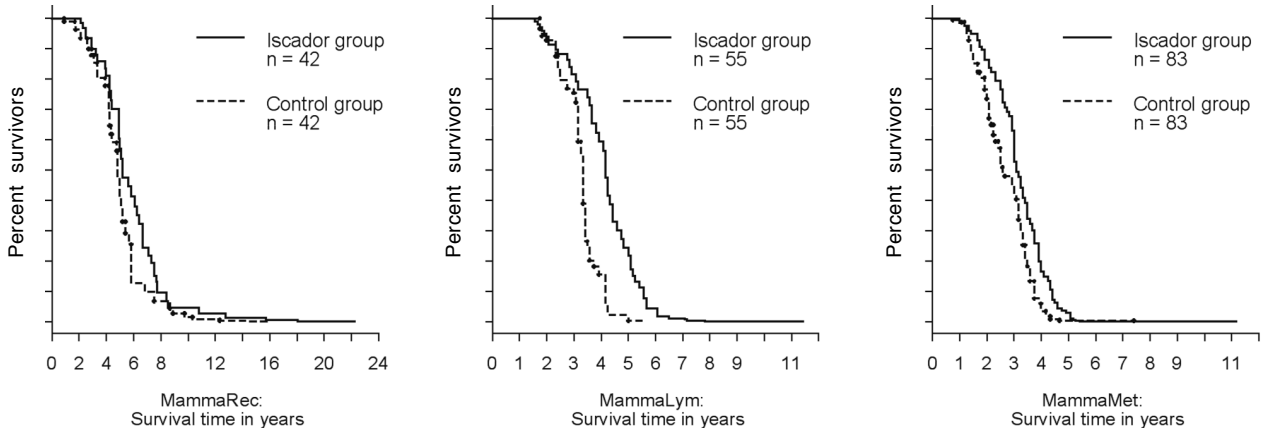


Fig. 3. MammaRec, *MammaLym* and *MammaMet* (42, 55 and 83 non-randomised matched-pairs respectively): Adjusted survival curves, showing the two groups with and without Iscador, based on the models with adjustment from Table 5.

MammaLym: For the complete set (n = 2 × 55), the matching for stage (FIGO, TNM) is perfect. The status of menopause is not significantly different within the two groups (MN test, p = 0.21). There was no significant difference in the conventional therapies. There was only one difference in chemotherapy, two in radiotherapy and one in hormone therapy. One Iscador patient had chemotherapy and her control not. This is relevant for this indication and therefore this pair was excluded. For radiotherapy, the situation is balanced: in one pair, only the Iscador patient received radiotherapy and in another pair only the control patient. The single difference in hormone therapy worked in favour of the control group: only the control patient received hormone treatment. A difference in the year of first diagnosis of > 3 years (which is significant, WPS test, p = 0.0017) in 9 pairs were judged in favour of the Iscador group and thus eliminated for balancing. The difference between the year of matching and the year of first diagnosis is clinically relevant. 3 additional pairs were judged as favouring the therapy group. The difference in age at first diagnosis was not significant (WPS test, p = 0.45); however, 4 pairs differed in more than 3 years, slightly in favour of the control group. In all, 13 pairs had to be eliminated, resulting in a balanced set of 42 pairs. Strict matching produced 38 pairs. Self-regulation at baseline was not matched; the difference between the therapy groups in the first evaluation is not significant (WPS test, p = 0.17). The judgement of effectiveness of the therapy by the patient is significantly better in the Iscador group (MN test, p = 0.02) and slightly fewer patients were prepared to participate in a double blind RCT (MN test, p = 0.25).

MammaMet: For the complete set (n = 2 × 83), the matching for stage had no exceptions. Within the 13 pairs with differences in the localizations of the metastases, 4 pairs were balanced with respect to each other and in 8 pairs the Iscador group had more localizations with metastases (data not shown); overall, the Iscador group had a worse prognosis. There were no significant differences in conventional therapies. It should be noted that there were 3 differences in chemotherapy, 2 in radiotherapy and one in each hormone therapy and other therapies. The differences in chemotherapy are relevant: in 3 pairs the Iscador patients had chemotherapy and the control patients did not, these pairs were therefore excluded. Differences in radiotherapy, hormone therapy and psychotherapy are of minor importance at this stage of cancer and were therefore judged as irrelevant. The difference in the year of first diagnosis within the matched-pairs is significant (WPS test, p = 0.0002) and these differences are relevant. For 2 pairs this worked against the control group who were therefore eliminated. Additionally, in 8 pairs (including the 2 pairs above), the difference between the year of matching and the year of operation worked against the control group and had to be eliminated. Thus, 11 pairs were eliminated, producing a balanced set of 72 pairs. The difference in age at first diagnosis was not significant (WPS, p = 0.73), however, within 16 pairs, the difference is > 3 years. Strict matching, i.e. with no exceptions in all matching variables as well as no differences in the location of distant metastases [3, Table 1], produced 53 pairs. Self-regulation at baseline was not matched; the difference between the therapy groups in the first evaluation was significant (WPS test, p = 0.0017). The judgement of effectiveness of the therapy by the patient was slightly better in the Iscador group (MN test, p = 0.13) and approximately the same number of patients were prepared to participate in a double blind RCT (MN test, p = 0.69).

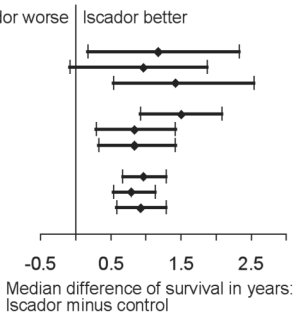
Survival

Survival was analysed for all data sets in four ways (see Table 5): (I) descriptive analysis; (II) since all patients are dead (censored values did not occur), a Wilcoxon paired sample test (WPS) was performed on the complete data sets (all data sets), and on the reduced data sets (non-randomised matched-pairs only), particularly the balanced data sets and the data sets with strict

Table 5. Overall survival for the data sets with non-randomized matched pairs: *MammaRec*, *MammaLym* and *MammaMet*. – Since all patients died within each study (no censored data), a Wilcoxon paired sample test (WPS) was performed on all data sets. "Balanced set": subgroup of complete set of matched-pairs not favouring the patients with Iscador therapy "Strict matching": subgroup of complete set of matched-pairs of patients fulfilling exactly all matching criteria. – The estimate of hazard ratio measures the Iscador vs. the control group and the p-value from the Wald test measures the significance of the estimated variable ISC (PH = proportional hazard). Adjusted variables: SR = self-regulation at baseline, DB = willingness to participate in a double-blind clinical trial. – All variables other than ISC with a significant influence on the outcome were included in the Cox model and are listed in the column 'Adjustment'.

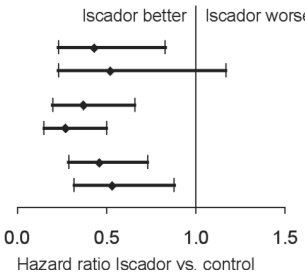
WILCOXON PAIRED SAMPLE TEST (WPS)

Non-Randomized Study	Set	# Pairs	Median Diff.	WPS p-value
MammaRec	full set	42	1.17	0.0224
	balanced set	39	0.96	0.0678
	strict matching	29	1.42	0.0011
MammaLym	full set	55	1.5	< 0.0005
	balanced set	42	0.83	0.0033
	strict matching	38	0.83	0.0022
MammaMet	full set	83	0.96	< 0.0005
	balanced set	72	0.79	< 0.0005
	strict matching	53	0.92	< 0.0005



COX PROPORTIONAL HAZARD MODEL

Non-Randomized Study	Adjustment	# Pairs	Estimate	Wald test p-value	Model fit PH assumption
MammaRec	no adjustment	42	0.43	0.012	yes
	SR	42	0.52	0.110	yes
MammaLym	no adjustment	55	0.37	0.0009	no
	SR, DB	55	0.27	< 0.0005	moderately
MammaMet	no adjustment	83	0.46	0.0009	yes
	SR	83	0.53	0.015	yes



matching; (III) a Cox proportional hazard model with no adjustment for variables other than Iscador therapy was calculated for all data sets; (IV) for all non-randomised data sets a Cox proportional hazards model was fitted to all available prognostic factors with subsequent backward elimination and assessment of model adequacy.

The results indicate a prolonging effect on survival in the Iscador therapy group. In the randomised study *MammaLymRand* the results of the descriptive statistics are significantly in favour of the Iscador therapy (Table 5). The Cox proportional hazard model, however, did not fit, because the two curves intersected (the proportional hazards assumption is thus not fulfilled); therefore the following result cannot be trusted: estimate of hazard ratio and 95 % confidence interval 0.46 (0.16, 1.31).

The results of the non-randomised studies *MammaRec*, MammaLym, *MammaMet* show positive trends in favour of the Iscador group and in most cases highly significant results (Table 5).

The Kaplan-Meier survival curves for *MammaLymRand* as well as for *MammaRec*, *MammaLym* and *MammaMet* can be found in the original paper [3]. The adjusted survival curves for *MammaRec*, *MammaLym* and *MammaMet* according to the models from Table 5 are shown in Figure 3.

Self-Regulation

Psychosomatic self-regulation was assessed twice, only for the data set *MammaLymRand*. The second assessment happened 3 months after the initial data assessment. This short-term improvement was estimated using the median of the paired differences and the 95 % confidence interval: 0.90 (0.0, 1.75); for the baseline values see Table 3.

Discussion

Design and Analysis

The aim of this analysis is explorative in nature and thus several approaches are studied. The reason for applying different types of analysis is to demonstrate (at best) the robustness of the results against different sets of constraints. Particularly non-randomised studies are susceptible to different types of biases [11] that

can be dealt with to a certain extent by comparing the results of different statistical approaches.

Randomised Matched-Pairs

When concerning survival, the conservative analysis (Table 5) yields convincing evidence in favour of Iscador for *MammaLymRand*. Since there are no censored survival times in *MammaLymRand*, and the proportional hazard assumption is definitely not fulfilled for this data set, the result of the Cox modelling is not relevant. The estimate of the median of the paired differences of the survival times Iscador vs. control is therefore appropriate: 2.5 (0.83, 4.50).

Although the matching within the study *MammaLymRand* is close to perfect, several biases might limit these results. First, there is the problem of accuracy and misclassification. The accuracy and precision of the data is in fact low: no exact dates (years instead of days and months) for diagnosis, operation, initial and follow-up data assessments and matching are available; however, this affects both therapy groups and there is no reason to assume that the consequences of these inaccuracies would affect one group more than the other.

Since the time of matching was within 12 months of the first diagnosis, the assumption of misclassifications according to shifting criteria is not plausible. Selection bias is neutralised by the randomisation process. Performance bias is not a problem, since the main co-interventions were recorded (data not shown). In addition, it is plausible to assume that the control patients often had more additional (unconventional and unrecorded) therapies, since to them these therapies were not offered by the way of study participation; this generally works in favour of the control group. Since survival is the primary endpoint, detection bias is not a problem. Attrition bias cannot happen, since no cases were lost cases in the study *MammaLymRand*.

Non-Randomised Matched-Pairs

Overall, the results for overall survival within the non-randomised studies *MammaRec*, *MammaLym*, *MammaMet* show at least positive trends in favour of the Iscador group and in most cases highly significant results.

It is important to note that in all studies the results of the unadjusted evaluations are significantly in favour of the Iscador group (Table 5). The only exception is the study *MammaRec*, where the balanced subset does not show a significant result ($p = 0.07$). This compares well with the corresponding adjusted Cox proportional hazards model (estimate of hazard ratio and 95 % confidence interval: 0.52 (0.23, 1.17) and the original paper [4]. In the other studies, all the different Cox models show significant results in favour of the Iscador therapy (Table 5).

Paired matching was used to reduce selection bias for some known prognostic factors. In all three non-randomised studies, the matching process could not be performed without exceptions in order to recruit a relevant number of patients (see Tables 2 and 4). In order to deal with the biases occurring by lose matching, with at most two deviations from the strict matching, several analytic approaches are used as a kind of sensitivity analysis. Within non-adjusted analyses, balanced subsets and sets with strict matching (see Methods section) were formed and analysed separately in order to compare results. In addition, Cox proportional hazards models were built with and without adjustments for factors other than therapy (including paired interactions, if significant).

The unadjusted analyses show comparable results for the different subsets (Table 5), proving that the original sets are fairly well balanced across the different therapy groups, at least with respect to the prognostic factors used in the matching process. This is supported by the fact that the results of the Cox proportional hazards model do not differ very much between adjusted and unadjusted analyses in most cases (Table 5).

Still, several other biases might limit these results. Using the same argument as in the case of the randomised study, biases caused by the problem of accuracy and misclassification are of minor importance. The same applies to performance and detection bias. – The single most important sources of bias for non-randomised studies are selection bias and confounding [11]. Particularly, residual bias might stem (i) from non-perfect matching, (ii) from non-matched prognostic factors and (iii) from not measured (un)known prognostic factors. The first case has already been dealt with. The second and third cases are more severe. According to the study design, several important medical prognostic factors have not been recorded (i.e. steroid receptor; histopathological type and histopathological grading). In addition, other factors were not deemed as relevant for the study objectives before the start of the study in 1971 and are therefore not available for the analysis (i.e. exact dates of first diagnosis, operation, initial data assessment and matching; socioeconomic status; social support; spirituality). The source of recruitment and the hospital were not included for reasons of anonymity. This leaves the problem of unknown factors open for speculation.

With this study design, attrition bias, which is between 5 and 14 %, is a minor problem, since with the drop-out of any study patient, the matching partner has also been excluded and hence the balance of the groups is not severely affected. There is no evidence that the reason for drop-out is related to the outcome.

Conclusion

The consistency of results with the earlier report and with different types of analyses give some evidence that in these studies the long-term therapy with the mistletoe preparation Iscador, might have a clinically relevant therapeutic effect on the survival of breast cancer patients. In the short term, in the randomized study, psychosomatic self-regulation, as a measure of autonomous coping with the disease, increases more under Iscador therapy than under conventional therapy alone. The results of this re-analysis confirm the results of the original paper [3, 4].

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Contributors
R. Grossarth-Maticek is responsible for the design and implementation of these studies as well as for the quality, reliability and documentation of the raw data. He contributed substantially to this paper with preliminary drafts and comments and gave final approval to the version to be published. Renatus Ziegler started working on this project in 2001. He proposed, executed, documented and presented the statistical analysis for this paper; wrote the first draft of this paper and gave final approval to the version to be published. For contributions to this project other than from the authors of this paper, see the papers [2, 3, 5].

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