Complementary materials: Ronald Grossarth-Maticek, Renatus Ziegler: Prospective Controlled Cohort Studies on Long-Term Therapy of Breast Cancer Patients with a Mistletoe Preparation (Iscador®). *Forschende Komplementärmedizin* 13(2006)(5).

Tables

Table 1
Flow chart of primary breast cancer patients from the randomized matched-pair study 'MammaRand'

DATA SOURCES	**N**	
Pool of cancer patients with no mistletoe therapy [19, p. 59, fig. 1]	8475	
Pool of primary breast cancer patients with no mistletoe therapy (fig. 1)	1882	
CHARACTERISTICS OF DATA FLOW		
Primary breast cancer patients without recurrences, lymphatic or distant metastases and no mistletoe therapy (see Table 2)	733	
Patients used as controls in parallel non-randomized study (see table 2)	– 105	
Patients used in another randomized study [19, p. 62, table 3]	– 4	
Patients used in another randomized study [not published]	– 96	
Patients used as controls in another non-randomized study [not published]	– 63	
Pool of patients for building randomized matched-pairs	465	
Study	**'MammaRand'**	
	Iscador [N]	*Control [N]*
Resulting matched patients	59	59
Declined participation, not received therapy or dropout before start of therapy in Iscador group	5	14
Discontinued therapy, drop-out after start of therapy	0	0
Lost to follow-up	1	1
Raw data for analysis	38	38
Pairs with 3 deviations from the specified matching criteria	5 pairs	
Pairs with no deviations from the specified matching criteria	10 pairs	
Survival analysis (Cox model)	38	38
Censored	8	8
Excluded	0	0

Table 2
Flow chart of primary breast cancer patients from the non-randomized matched-pairs study 'Mamma'

CHARACTERISTICS OF DATA FLOW	**N**	
Pool of primary breast cancer patients (fig. 1)	2451 = 1882 + 569	
Candidates for the non-randomized matched-pair study: Primary breast cancer without recurrences, lymphatic or distant metastases	975	
	Iscador 242	No Iscador 733
Patients used in another non-randomized study [not published]	–63	–63
Subgroup available for matching	179	670
Study	**'Mamma'**	
	Iscador	*Control*
Resulting matched-pairs	105	105
Declined participation, not received therapy or drop-out before start of therapy in Iscador group	0	2
Discontinued therapy, drop-out after start of therapy	0	0
Lost to follow-up	2	4
Raw data for analysis	97	97
Excluded from analysis: incomplete matching with more than 2 deviations from specified criteria	13 pairs	
Matching with at most 2 deviations from the specified criteria	84 pairs	
Survival analysis (Cox model)	84	84
Censored	6	4
Excluded (missing SR)	1	0
Reduced data sets		
Balanced set	73	73
Strict matching	24	24

SR	Self-regulation
Balanced set	Subgroup of complete set of matched-pairs *not* favoring patients with Iscador therapy.
Strict matching	Subgroup of complete set of matched-pairs of patients exactly fulfilling all matching criteria.

Table 3
Patient characteristics (matching variables and other variables) in the randomized matched-pair study 'MammaRand'

Prognostic variables			'MammaRand' *Iscador* n = 38	'MammaRand' *Control* n = 38	WPS p
Matching variables	**FIGO**	**TNM**			
	I	T1aN0M0	23	23	
	IIA	T2N0M0	7	7	
	IIB	T3N0M0	8	8	
	Grading				
	1		26	26	
	2		3	3	
	3		4	4	
	NA		5	5	
	Age at first diagnosis				
	mean		52.79	52.87	
	SD		7.03	7.29	
	range		36–63	36–62	
	Conventional therapy				
	Operation		38	37	
	Chemotherapy		18	19	
	Radiotherapy		17	19	
	Hormone therapy		5	12	
Baseline variables	**Co-therapy**				
	Non-Iscador CAM therapy		0	8	
	Psychotherapy		1	7	
	Self-regulation				0.44
	mean / median		3.95 / 3.90	3.82 / 3.80	
	SD		0.65	0.62	
	range		2.6–5.5	2.5–5.5	
Therapy variable	**Iscador use (years)**			NA	
	mean / median		10.69 / 10.04		
	SD		5.48		
	range		1.75–20.83		

WPS Wilcoxon paired sample test
SD Standard deviation
NA Not available

Patient characteristics of non-randomized study 'Mamma': Building balanced pairs

Concerning the patient characteristics of the study 'Mamma' (Table 4), the difference in the stages between the two groups is not significant (MH test, p = 1). Particularly, the matching concerning stage produced one pair where the control had a worse stage than the Iscador patient (T3 vs. T2). Since these stages are not much different and the grading is equal (G3), we judged this difference as not relevant. In two pairs, the grading of the Iscador patients is worse (G3 vs. G2 or G1) and in two pairs the grading of the Iscador patients is slightly better (G1 vs. G2). We judged this situation as slightly in favor of the control group. Overall, grading in the Iscador group and in the control group is not significantly different (MH test, p = 1). The status of menopause is perfectly matched. Concerning therapies, differences in chemotherapy are judged as relevant which is also significant (MN test, p = 0.04). Apart from the 57 perfectly matched-pairs, there are 27 pairs with differences in chemotherapy treatment; in 8 pairs, only Iscador patients had chemotherapy and in 8 pairs – all other parameters being equal or with small differences – only the control patients had chemotherapy. In addition, the Iscador patients in 11 pairs received chemotherapy, but not the controls; this was judged as relevant, since it favors the Iscador group. For radiotherapy, the situation is judged as balanced (MN test, p = 0.69). Concerning age at first diagnosis, the difference is not significant (WPS test, p = 0.69). Hence, for building a balanced set, 11 pairs where only the Iscador patient received chemotherapy are eliminated, yielding a balanced set of 73 pairs. Strict matching, i.e. with no exceptions in all matching variables produced 24 pairs. – Self-regulation at baseline was not matched; the difference between the therapy groups in the first evaluation is significant (WPS test, p = 0.002).

Table 4
Patient characteristics (matching variables and other variables) in the non-randomized matched-pairs study 'Mamma'

Prognostic variables			'Mamma' *Iscador* n = 84	'Mamma' *Control* n = 84	Test p
Matching variables	**FIGO**	**TNM**			1.00[2]
	I	T1N0M0	50	50	
	IIA	T2N0M0	15	16	
	IIB	T3N0M0	19	18	
	Grading				1.00[2]
	1		29	29	
	2		21	22	
	3		21	19	
	NA		13	14	
	Menopause				1.00[3]
	prae		12	12	
	post		40	40	
	NA		32	32	
	Age at first diagnosis				0.69[1]
	mean		52.15	52.18	
	SD		9.13	9.86	
	range		32–66	29–69	
	Conventional therapy				
	Operation		84	84	1.00[3]
	Chemotherapy		58	47	0.04[3]
	Radiotherapy		50	47	0.69[3]
	Hormone therapy		34	31	0.69[3]
Baseline variables	**Co-therapy**				
	Non-Iscador CAM therapy		6	18	0.01[3]
	Psychotherapy		16	18	0.76[3]
	Self-regulation				< 0.01[1]
	mean / median		3.91 / 3.80	3.60 / 3.70	
	SD		0.66	0.78	
	range		2.2–5.5	1.6–5.5	
Therapy variable	**Iscador use (years)**			NA	
	mean / median		8.53 / 8.13		
	SD		5.29		
	range		1.00–23.83		

SD Standard deviation
NA Not available
[1] Wilcoxon paired sample test (WPS)
[2] Marginal homogeneity test (MH)
[3] McNemar test (MN)

Statistics

The analysis and presentation of the data sets reported here is made as close as possible to the suggestions of the CONSORT statement for randomized studies [32] and its adaptation to non-randomized studies [33].

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, ignoring the censoring of the survival times. Since there are at least as many censored survival times (if any) in the group with Iscador therapy as in the control group, this generally yields a conservative result with respect to the Iscador group. The estimate of the median difference and the 95% confidence intervals are calculated according to Hodges-Lehmann [34]. Given censored event times, the log-rank statistic is used, including stratification according to the matched-pairs. All p-values are two-sided.

In the baseline comparisons of Iscador and control groups in the non-randomized matched-pair study, the Wilcoxon paired sample test (WPS) is used for continuous variables, the marginal homogeneity test (MH) for count data with ordered categories in paired samples and the McNemar test (MN) for binomial data in paired samples [35].

In the second stage of the analysis of overall survival, the Cox proportional hazard regression model is applied to the complete data set from the non-randomized matched-pair study. The therapy with Iscador is introduced through a binary variable: either therapy or no therapy. 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 interactions of the significant factors. This stratification according to matched-pairs generally results in a conservative estimate with respect to the unmatched analysis [36, § 7.1]. The model development and the assessment of model adequacy is performed according to the suggestions in [37, 38]. No automatic variable selection procedure is used. Concerning the randomized study, no adjustment of prognostic factors is performed. According to the suggestions in [38], the assumption of proportional hazards (PH) is checked statistically *and* graphically; if any one but not both of these methods fail to show a positive result, we say that the PH assumption is «moderately fulfilled».

The comparison of the time to recurrences, lymphatic metastases, distant metastases and death by cancer between the groups with or without Iscador therapy is based on an analysis of multiple events per subject [39]. In order to compare the results of different statistical models, two options are analyzed: (i) for the case of non-ordered events it is assumed that the multiple events can happen in any order of time, which is consistent with general clinical experience; (ii) for the case of ordered events, we assumed that recurrences occur first, then lymphatic metastases and finally distant metastases before death, since this sequence is the most common.

All statistical tests and confidence intervals are calculated on the basis of the 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 judged as significant, if $p < 0.05$.

The statistical analyses are perfomed using S-Plus 6.2 for Windows Professional Edition (Insightful Corp. 2003, Seattle, Washington). The Wilcoxon paired sample tests, the Hodges-Lehmann estimate and confidence intervals and the marginal homogeneity tests are calculated for $n < 100$ with the exact procedures from StatXact 6 (Cytel Software Corporation 2004, Cambridge, Massachusetts).

Table 5
Descriptive statistics of survival for the randomized and non-randomized matched-pair study

Study	**Set of pairs**	**Survival: Range in years**		**Survival: Median in years**		**Hodges-Lehmann Estimates**		**WPS**	**Stratified Log-rank Test**
		Iscador	*Control*	*Iscador*	*Control*	Median difference in survival in years	CI for median difference in survival in years		p-value
'MammaRand'	complete set 2×38	7.00 – 21.50	2.08 – 23.08	14.63	13.83	1.04	–0.63, 2.63	0.214	0.194
'Mamma'	complete set 2×84	2.75 – 25.08	2.08 – 24.17	11.75	10.13	1.46	0.79, 2.21	< 0.0001	0.0002
	balanced set 2×73	2.75 – 25.08	2.08 – 24.17	11.75	10.00	1.63	0.92, 2.42	< 0.0001	< 0.0001
	strict matching 2×24	4.00 – 23.00	2.67 – 16.17	10.75	10.08	1.33	0.12, 2.79	0.0198	0.221

Balanced set subgroup of complete set of matched-pairs *not* favoring patients with Iscador therapy
Strict matching subgroup of complete set of matched-pairs of patients exactly fulfilling all matching criteria

On the assumption that all patients are dead, a Wilcoxon paired sample test (WPS) is performed on the complete data sets (both data sets), and on the reduced data sets (non-randomized matched-pairs only), particularly, the balanced data sets and the data sets with strict matching (positive median differences are in favor of Iscador therapy); to take account of the censored survival times, a stratified log-rank test is calculated also on the basis of the matched-pairs.

Table 6
Survival for the data sets with non-randomized matched-pairs 'Mamma'

Study	**Statistics**	**Fitted variables**	**Model adequacy**	**Set of pairs**	**Value**	**Result**
'Mamma'	Cox model	–	PH assumption **not** fulfilled	complete set 2×84	Estimate and CI for hazard ratio p-value	0.42 0.27, 0.68 0.0003
	Cox model	SR, Th3, Th6	PH assumption moderately fulfilled; 1 pair with missing values from self-regulation	2×83	Estimate and CI for hazard ratio p-value	0.43 0.27, 0.68 0.0003
	Cox model* with interaction	SR, Th3, Th6, ISC : SR	PH assumption fulfilled: 1 pair with missing values from self-regulation	2×83	Estimate and CI for hazard ratio p-value	0.0023 0.00005, 0.104 0.0017

* Model used in adjusted survival curves in Figure 3
CI 95 %-confidence interval
PH Proportional hazard
ISC Iscador therapy
SR Self-regulation at baseline
Th2 Chemotherapy
Th3 Radio therapy
Th6 Psychotherapy
DB Willingness to participate in a double-blind clinical trial

The hazard ratio estimate measures the Iscador vs. the control group and the p-value from the Wald test measures the significance of the estimated variable ISC.

All variables other than ISC with a significant influence on the outcome were included in the Cox model and are listed in the column 'Fitted variables'.

Table 7
'MammaRand' (38 randomized matched-pairs): Numbers of events and time to the event of local recurrences, lymphatic metastases and distant metastases

Type of analysis	Set of pairs	Statistics	Fitted variables	Indicator	Local recurrences		Lymphatic metastases		Distant metastases	
I					*Iscador*	*Control*	*Iscador*	*Control*	*Iscador*	*Control*
	complete set 2×38	count	–	number	6	9	17	22	23	28
II	complete set 2×38	WPS	–	Estimate and CI for median difference in survival in years	1.63 –0.71, 4.00		1.88 –0.21, 4.17		1.46 –0.46, 3.38	
		WPS		p-value	0.147		0.063		0.126	
		SLR		p-value	0.16		0.003		0.055	
III	complete set 2×38	Cox model	–	Estimate and CI for hazard ratio	0.44 0.14, 1.44		0.27 0.11, 0.67		0.50 0.24, 1.03	
				p-value	0.18		0.0048		0.061	
				model adequacy	PH assumption fulfilled		PH assumption **not** fulfilled		PH assumption moderately fulfilled	
Type of analysis	**Set of pairs**	**Statistics**	**Fitted variables**	**Indicator**	**All events (including death)**					
IV	complete set 2×38	extended Cox model for unordered events	–	Estimate and CI for hazard ratio	0.47 0.30, 0.76					
				p-value	0.0017					
				model adequacy	PH assumption **not** fulfilled					
V	complete set 2×38	extended Cox model for ordered events	–	Estimate and CI for hazard ratio	0.65 0.47, 0.91					
				p-value	0.012					
				model adequacy	PH assumption moderately fulfilled					

WPS Wilcoxon paired sample test
SLR Stratified log-rank test
CI 95 %-confidence interval
PH Proportional hazard [see Statistics section]

The hazard ratio estimate measures the Iscador vs. the control group and the p-value from the Wald test measures the significance of the estimated variable ISC (= Iscador therapy).

Type of analysis: (I) descriptive analysis, (II) WPS tests on the assumption that all patients had their events and stratified SLR tests taking account of censored event times and matched pairs, (III) traditional Cox proportional hazards model with assessment of model adequacy, (IV) on the clinical plausible assumption that the time to the event of local recurrences, lymphatic or distant metastases do not necessarily happen in an ordered fashion, an extended Cox model with unordered events is set up according to [39, section 8.4], (V) for reasons of comparison, a model according to Anderson and Gill for ordered events (first recurrence, then lymphatic and distant metastases before death) that are independent within subjects is constructed as outlined in [39, section 8.5].

Table 8
'Mamma' (84 non-randomized matched-pairs): Numbers of events and time to the event of local recurrences, lymphatic metastases and distant metastases

Type of analysis	Set of pairs	Statistics	Fitted variables	Indicator	Local recurrences		Lymphatic metastases		Distant metastases	
I					*Iscador*	*Control*	*Iscador*	*Control*	*Iscador*	*Control*
	complete set 2×84	count	–	number	19	23	60	64	76	72
	balanced set 2×73	count		number	16	22	53	58	67	64
	strict matching 2×24	count		number	5	7	20	22	22	21
II	complete set 2×84	WPS	–	Estimate and CI for median difference in survival in years	1.75 0.75, 2.83		2.17 1.29, 3.04		1.13 0.50, 1.88	
		WPS		p-value	0.0006		< 0.0001		0.0014	
		SLR		p-value	0.17		< 0.0001		0.0001	
	balanced set 2×73	WPS	–	Estimate and CI for median difference in survival in years	2.25 1.17, 3.29		2.38 1.46, 3.38		1.38 0.75, 2.13	
		WPS		p-value	< 0.0001		< 0.0001		0.0001	
		SLR		p-value	0.048		< 0.0001		< 0.0001	
	strict matching 2×24	WPS	–	Estimate and CI for median difference in survival in years	1.75 0.12, 3.42		1.67 0.54, 3.25		1.21 –0.33, 2.25	
		WPS		p-value	0.028		0.012		0.078	
		SLR		p-value	0.206		0.007		0.016	
III	2×83 / 2×80 / 2×81	Cox model	SR, Th2	Estimate and CI for hazard ratio	0.42 0.21, 0.83		0.22 0.10, 0.47		0.36 0.21, 0.62	
				p-value	0.012		< 0.0001		0.0002	
				model adequacy	PH assumption fulfilled; 1 pair with missing values from self-regulation		PH assumption fulfilled; 4 pairs with missing values		PH assumption moderately fulfilled; 3 pairs with missing values	
	2×81	Cox model with interactions	SR, Th2, ISC : SR, ISC : Th2	Estimate and CI for hazard ratio					0.0056 0.00013, 0.24	
				p-value					0.007	
				model adequacy	no significant interaction		no significant interaction		PH assumption fulfilled; 3 missing values: 1 in Iscador group	
Type of analysis	**Set of pairs**	**Statistics**	**Fitted variables**	**Indicator**	**All events (including death)**					
IV	complete set 2×84	extended Cox model for unordered events	SR, Th2	Estimate and CI for hazard ratio	0.36 0.24, 0.54					
				p-value	< 0.0001					
				model adequacy	PH assumption **not** fulfilled					
	complete set 2×84	extended Cox model for unordered events with interactions	SR, Th2, ISC : SR	Estimate and CI for hazard ratio	0.039 0.0059, 0.26					
				p-value	0.0008					
				model adequacy	PH assumption moderately fulfilled					
V	complete set 2×84	extended Cox model for ordered events (Andersen-Gill)	SR, Th2	Estimate and CI for hazard ratio	0.66 0.55, 0.79					
				p-value	< 0.0001					
				model adequacy	PH assumption moderately fulfilled, no significant interactions					

WPS	Wilcoxon paired sample test
SLR	stratified log-rank test
CI	95 %-confidence interval
PH	proportional hazard
ISC	Iscador therapy
SR	self-regulation at baseline
Th2	chemotherapy.
Balanced set	subgroup of complete set of matched-pairs *not* favoring the patients with Iscador therapy
Strict matching	subgroup of complete set of matched-pairs of patients fulfilling exactly all matching criteria

The hazard ratio estimate measures the Iscador vs. the control group and the p-value from the Wald test measures the significance of the estimated variable ISC.

Type of analysis: (I) descriptive analysis, (II) WPS tests on the assumption that all patients had their events and stratified SLR tests taking account of censored event times and matched pairs, (III) traditional Cox proportional hazards model with assessment of model adequacy, (IV) on the clinical plausible assumption that the time to the event of local recurrences, lymphatic or distant metastases do not necessarily happen in an ordered fashion, an extended Cox model with unordered events is set up according to [39, section 8.4], (V) for reasons of comparison, a model according to Anderson and Gill for ordered events (first recurrence, then lymphatic and distant metastases before death) that are independent within subjects is constructed as outlined in [39, section 8.5].

All variables other than ISC with a significant influence on the outcome were included in the Cox model and are listed in the column 'Fitted variables'.

Table 9

Improvement of self-regulation within 12 months for the data sets with randomized matched-pairs 'MammaRand' and non-randomized matched-pairs 'Mamma'

Study	Set of pairs	Median difference	CI	WPS, p-value
'MammaRand'	complete set, 2×38	0.35	0.05, 0.60	0.034
'Mamma'	complete set, 2×83 (missing value in 1 pair)	0.20	0.00, 0.35	0.031
	balanced set, 2×72 (missing value in 1 pair)	0.15	0.00, 0.35	0.055
	strict matching, 2×24	0.30	0.05, 0.60	0.014

WPS	Wilcoxon paired sample test
CI	95 %-confidence interval).
Balanced set	Subgroup of complete set of matched-pairs *not* favoring patients with Iscador therapy
Strict matching	Subgroup of complete set of matched-pairs of patients exactly fulfilling all matching criteria

For the baseline values see Tables 3 and 4.