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Final results of the EORTC 18871/DKG 80-1 randomised phase III trial: rIFN-α2b versus rIFN-γ versus ISCADOR M® versus observation after surgery in melanoma patients with either high-risk primary (thickness >3 mm) or regional lymph node metastasis

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Abstract

Between 1988 and 1996, the European Organisation for Research and Treatment of Cancer Melanoma Group (EORTC-MG) performed a prospective, randomised phase III adjuvant trial to evaluate the efficacy and toxicity of low dose recombinant interferon-alpha 2 b (rIFN-α2b) (1 MU) or recombinant interferon gamma (rIFN-γ), (0.2 mg) both given subcutaneously (s.c.), every other day (qod), for 12 months in comparison with an untreated control group. The German Cancer Society (DKG) added a fourth arm with Iscador M®, a popular mistletoe extract. High-risk stage II patients (thickness >3 mm) and stage III patients (positive lymph nodes) without distant metastasis were randomised and followed until their first progression or death. An intention-to-treat analysis was performed. From 1988 to 1996, a total of 830 patients were randomised: 423 in the three-arm EORTC 18871 trial and 407 patients in the four-arm DKG 80-1 trial. The median follow-up was 8.2 years and a total of 537 relapses and 475 deaths were reported. At 8 years, the disease-free interval (DFI) rate was 32.4% and the overall survival (OS) rate was 40.0%. In terms of the DFI, the hazard ratio estimates (95% Confidence Intervals (CI)) were: 1.04 (0.84, 1.30) for the comparison of rIFN-α2b versus control, 0.96 (0.77, 1.20) for rIFN-γ versus control, and 1.32 (0.93, 1.87) for *Iscador M®* versus control. In terms of OS, the corresponding estimates (95% CI) for the 3 treatment comparisons were: for IFN-α2b 0.96 (0.76, 1.21), for rIFN-γ 0.87 (0.69, 1.10) and for *Iscador M®* 1.21 (0.84, 1.75), respectively. The results show no clinical benefit for adjuvant treatment with low dose r*IFN*-α2b or rIFN-γ or with *Iscador M®* in high-risk melanoma patients.

Keywords: Melanoma; Stage II–III; Adjuvant therapy; IFN-α; IFNγ Iscador; Prognostic factors

1. Introduction

Patients with a primary melanoma, tumour thickness (Breslow) greater than 3 mm, have a chance of cure of approximately 50% or less if the tumour is ulcerated. Patients with regional lymph node metastases have a cure rate of approximately 30% or less depending on the number of pathologically involved regional lymph nodes [1,2]. This situation demands effective adjuvant treatment strategies. The European Organisation for Research and Treatment of Cancer (*EORTC*) *Melanoma Group* carried out a prospective, randomised controlled adjuvant trial in order to assess the value of low-dose recombinant interferon-alpha (rIFN-α2b) and interferon-gamma (rIFN-γ) versus a control group. The *German Cancer Society* added a fourth arm, *Iscador M®*, a popular mistletoe extract, to clarify whether claims of its efficacy were justified.

Recombinant interferon-alpha (rIFN-α) and rIFN-gamma (rIFN-γ) are cytokines with pleiotropic *direct* anti-proliferative/pro-differentiation, and protein synthesis-inhibiting antitumour activity, as well as *indirect*

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antitumour functions, including activation of host effector effects that may result from the augmented expression of tumour cell surface antigens, rendering the tumour more susceptible to host effector cells in general [3,4]. For such a wide range of biological activities, it has been recognised that the dose for optimal biological activity may differ greatly from the maximally-tolerated dose (MTD). High doses of rIFN-γ have been shown to suppress, and low doses to activate the cytotoxic properties of patients' monocytes [5]. In view of the differences in the biological spectrum of action and the supposed greater susceptibility of a small tumour load to therapeutic interventions, it seemed possible that adjuvant therapy with low-dose rIFN-α or rIFN-γ might be active in melanoma patients. The rationale for their use in the adjuvant setting was also supported by an overall response rate of 16% in stage IV melanoma patients treated with various doses and schedules of rIFNs [3].

Since its introduction by R. Steiner in 1920, *viscum album* has been used in the treatment of cancer [6]. Evidence has been accumulated from *in vivo* and *in vitro* studies that *Iscador*®, a viscum album extract, may exert some, although unspecific, growth inhibitory action on tumour cells [7–11]. Experiments using mistletoe-extracts in 14 established solid tumours showed some tumoricidal effect in 2 of them (14%) [12]. In addition, studies indicated that *Iscador*® may play a role in immuno-modulation [16–20]. Because of numerous reports about the clinical value of *Iscador*® [13–15,21–24], further investigations were considered necessary to prove its antitumour activity in humans. The nature of the active components of *Iscador*® is unknown, and may (at least partly) be attributed to its bacterial constituents, mainly *lactobacilla*. In addition, non-fermented compounds isolated from mistletoe might influence the immune system and tumour growth [11,16,17].

Clinical studies describing the effect of *Iscador*® in cancer therapy are numerous, often compiling data from each other [7,9–11,13–15,20–24]. They do not follow the design, follow-up and quality control that is requested by the practice of evidence-based medicine [12,25,26]. Phase-I studies with *Iscador*® do not exist and phase-II studies cannot be interpreted because of the lack of objective parameters defining histopathology, microstaging, clinical stage, response rate and duration of remissions [26–28]. In addition, *Iscador*® is usually given as an adjunct to other forms of therapy, prohibiting any reliable interpretation of its impact in the treatment of melanoma or cancer in general. All of these studies uniformly claim a benefit from *Iscador*® treatment in terms of quality of life, disease-free and overall survival [7–10,13,14,21–24,29]. Following anecdotal reports about the efficacy of the apple variation "*viscum mali*" *(M)* in patients with melanoma [30], *Hiscia Laboratories,* after initial reluctance and a prolonged period of preparation, finally decided to offer *Iscador M*®.

Controlled trials with *Iscador*® were urgently called for by the German Government *(Gesamtprogramm zur Krebsbekämpfung* 1979/1986) and the *German Cancer Society (DKG)* to scientifically prove or disprove the respective allegations of the representatives of anthroposophical and homeopathic medicine. Because of its importance in health economics the *EORTC-MG* included *Iscador M*® in their treatment protocols.

2. Patients and methods

Patients between 14 and 80 years of age after resection of a primary melanoma (stage II, Breslow thickness >3 mm) or after curative dissection of regional lymph node metastases (stage III) were eligible for this study. The primary tumour had to be resected with a >2-cm free margin, whereas regional lymph node metastasis were resected according to Karakousis as described in Refs. [31,32]. It was up to the participating institution to decide whether a clinically node-negative patient should undergo an elective lymph node-dissection (ELND). This policy could not be changed during the study. Adjuvant therapy was started within 6 weeks after definitive surgery. The diagnosis was reviewed by the EORTC Melanoma Pathology-Review-Board. On-study forms and reports had to be mailed to the EORTC Data Center every 2 months for the first year, every 3 months for the second year, and every 6 months thereafter. Initial staging included a chest X-ray, ultrasound of the regional lymph nodes and abdominal or a computerised tomography (CT-scan).

Drugs: rIFN-α2b (Intron A®), Essex, Germany): Treatment schedule: 1 MU, subcutaneously (s.c.) every other day (qod), for 1 year. *rIFN-γ* (*Imukin*®), Boeringer-Ingelheim, Germany): 0.2 mg s.c. qod for 1 year. Treatment with *Iscador M*®, (Hiscia-Laboratories, Arlesheim, Switzerland) was started at "dose level 0", and the dose was escalated from 0.01 to 1.0 mg/ml, qod, over 2 weeks. After 3 days without treatment, injections were resumed for 14 doses (28 days) of 20 mg/ml followed by 7 days of no treatment. This schedule was recommended and considered optimal by the producers of Iscador®. All treatments were given for one year or until tumour progression. The drugs were supplied free of charge.

2.1. Statistical considerations

For each study, randomisation was done centrally at the EORTC Data Center in Brussels, and patients were stratified according to several factors: institution, Breslow thickness of their primary (1.5–3 versus 3.1–4 versus >4 mm), stage of disease (IIb versus III), site of primary (limb versus head/neck/trunk) in Stage IIb, and number of positive nodes (1, 2–4, ⩾5) and initial versus consecutive Stage III patients. The primary end-point of

the study was the disease-free interval (DFI), i.e. the time from the date of randomisation until the date of first recurrence; patients who did not progress were censored at their last follow-up date. The secondary end-point was the duration of survival (i.e. time from randomisation until death, whatever the cause); patients still alive were censored at their last follow-up date. In order to detect an increase in the DFI rate at 5 years from 30% in the control group to 40% (corresponding to a hazard ratio of 0.76) in one of the IFN groups (either rIFN-α2b or rIFN-γ), it was necessary to randomise a total of 259 patients per arm, of whom 168 had to be followed until relapse (1-sided test, alpha = 5%, beta = 20%) [35]. No adjustment for multiple comparisons was done. The trials were stopped once the planned sample size was reached to provide sufficient power to answer the IFN-question. To assess the efficiacy of Iscador, it was impossible to continue the randomisation after an 8-year recruitment period. An interim evaluation at that time showed an approximately 10% lower 2-year DFI rate in the Iscador® arm compared with the control group.

The actuarial curves were computed using the Kaplan–Meier technique and standard errors (SE) of the estimates were obtained via the Greenwood formula. [33]. The differences between the curves were tested for statistical significance using the two-tailed logrank test or the logrank test stratified by a categorical factor, like the trial reported in Ref [33]. The Cox's proportional hazards model was used to obtain the estimate and the 95% Confidence Interval (CI) of the hazard ratio (HR) of the instantaneous event rate in one treatment group versus that in the control group, after adjusting for possible confounding factors; the Wald test was used to determine the prognostic importance of each variable included in the model [34]. For the IFN-question, the Cox model was stratified by study (EORTC 18871 versus DKG 80-1). The prognostic interaction between the 2 variables was tested by including the product of these 2 variables in the model. All analyses were performed according to an intent-to-treat-principle. The database was frozen on February 12, 2003. The SAS 8.2 software was used for the statistical analyses.

3. Results

Between January 1988 and March 1996, 830 patients were randomised from 45 institutions in 13 countries (see Appendix): 423 in the EORTC 18871 3-arm trial (142 patients in the control arm, 139 pts in the rIFN-alpha arm and 142 in the rIFN-gamma arm) and 407 in the DKG-80-1 4-arm trial (102 pts in the control arm, 101 pts in the rIFN-alpha arm, 102 in the rIFN-gamma arm and 102 in Iscador-M arm). Therefore, to assess the value of IFN alpha and gamma treatment, a total of 728 pts ("IFN question group") were randomised: 244 pts in the control group, 240 in the rIFN-alpha and 244 in the rIFN-gamma. 102 patients randomised in the control group of the DKG 80-1 trial were used to assess the value of the IFN arms (total of 728 pts), as well as the value of Iscador (204 pts: 102 controls and 102 pts in the Iscador group). Thus 728 were analysed to answer the "IFN question", and 204 to answer the "Iscador question".

3.1. Patient characteristics

Demographic data by treatment group in the IFN and Iscador treatment groups are summarised in Table 1. 42% of all patients were Stage IIb, 40% of the IFN and 49% of the Iscador group. Stage and the other factors were well balanced in the treatment groups, and within the IFN and Iscador groups. In the stage IIb patients, approximately 20% of the slides had a Breslow thickness <3 mm, irrespective of having been reviewed by the Pathology Board of the EORTC-MG. Among the reviewed slides, 50% of the primary tumours had a thickness between 2.75 and 3 mm. Median age for the total group was 52 years, with a range of 14–84 years.

3.2. Treatment feasibility and inclusion in analyses according to the CONSORT statement

Out of 830 patients, 37 (4.6%) were considered as ineligible: 12 due to prior or concurrent treatment, 8 because of discrepancies in the histology-reports between the local pathologist and the EORTC Melanoma Extramural and the Pathology Review Boards, 3 due to the presence of metastases, 5 due to disease stages that were part of the exclusion criteria, 3 due to concurrent disease, and 6 due to other causes. In addition, for 13 (1.6%) patients, the eligibility criteria could not been checked due to insufficient documentation.

For each treatment group, IFN and Iscador, Table 2 shows the applicability of the treatment arm allocated by randomisation, as well the quality of follow-up. The reasons for protocol violations, going off the protocol treatment, e.g. due to toxicity, and patient's ineligibility, were documented. For all patients, all possible efforts were made to follow them until their first relapse and death.

3.3. Disease-free interval (DFI): treatment comparisons

Fig. 1a and b show the DFI according to the treatment arm. Interferon and Iscador, respectively. Table 3 shows recurrence and survival data for the 2 treatment groups. None of the treatment comparisons yielded a significant difference. The results of pair-wise treatment comparisons are given in Table 4. The estimated hazard ratios for the rIFN arms were close to 1, and the lower 95% CIs did not contain 0.76, indicating that the expected improvement of 10% in terms of efficacy is

Table 1
Patient characteristics by treatment arm

	IFN group			*Iscador-M*® group	
	Control	rIFN-α2b	rIFN-γ	Control	*Iscador-M*®
Variable/total	244 (100%)	240 (100%)	244 (100%)	102 (100%)	102 (100%)
Stage					
IIb	96 (39.3)	99 (41.3)	95 (38.9)	49 (48.0)	50 (49.0)
III	148 (60.7)	141 (58.8)	149 (61.1)	53 (52.0)	52 (51.0)
Gender[a]					
Male	138 (57.3)	127 (54.0)	135 (56.5)	53 (53.5)	65 (64.4)
Female	103 (42.7)	108 (46.0)	104 (43.5)	46 (46.5)	36 (35.6)
Breslow thickness					
⩽3	104 (42.6)	101 (42.1)	97 (39.8)	37 (36.3)	40 (39.2)
3.1–4	55 (22.5)	61 (25.4)	60 (24.6)	24 (23.5)	25 (24.5)
>4	85 (34.8)	78 (32.5)	87 (35.7)	41 (40.2)	37 (36.3)
Localisation					
Limb	105 (43.0)	106 (44.2)	109 (44.7)	48 (47.1)	46 (45.1)
Elsewhere	139 (57.0)	134 (55.8)	135 (55.3)	54 (52.9)	56 (54.9)
Stage IIb	96 [100%]	99 [100%]	95 [100%]	49 [100%]	50 [100%]
Ulceration[a]					
No	45 [51.1]	45 [70.3]	44 [51.2]	21 [46.7]	26 [55.3]
Yes	43 [48.9]	19 [29.7]	42 [48.8]	24 [53.3]	21 [44.7]
ELND[a]					
No	77 [81.9]	76 [80.0]	73 [79.3]	38 [80.9]	37 [77.1]
Yes	17 [18.1]	19 [20.0]	19 [20.7]	9 [19.1]	11 [22.9]
Stage III	148 [100%]	141 [100%]	149 [100%]	53 [100%]	52 [100%]
Number of LN^+					
1	68 [45.9]	65 [46.1]	61 [40.9]	24 [45.3]	24 [46.2]
2–4	49 [33.1]	52 [36.9]	54 [36.2]	19 [35.8]	18 [34.6]
⩾5	31 [20.9]	24 [17.0]	34 [22.8]	10 [18.9]	10 [19.2]
Type of stage III					
Initial	26 [17.6]	20 [14.2]	30 [20.1]	5 [9.4]	8 [15.4]
Consecutive	122 [82.4]	121 [85.8]	119 [79.9]	48 [90.6]	44 [84.6]

Between [ ],%s were computed in the Stage IIb or Stage III patients. ELND, elective lymph node-dissection; LN^+, lymph node-positive; rIFN-α2b, recombinant interferon-α2b; rIFN-γ, recombinant interferon-γ.

[a] Some data are missing in these subgroups.

Table 2
Flow chart (cf CONSORT statement)

	IFN group			*Iscador-M*® group	
	Control	rIFN-α2b	rIFN-γ	Control	Iscador
Total randomized	244	240	244	102	102
Allocation					
Received treatment as allocated	219	205	201	91	86
Did not receive treatment as allocated or data not available	25	35	43	11	16
Follow-up					
No follow-up at all	1	2	2	1	3
Lost to follow-up	6	4	5	5	2
Off study due to protocol violation	4	2	0	1	4
Off-study due to toxicity	0	11	19	0	5
Analysis					
Included in analyses	244	240	244	102	102

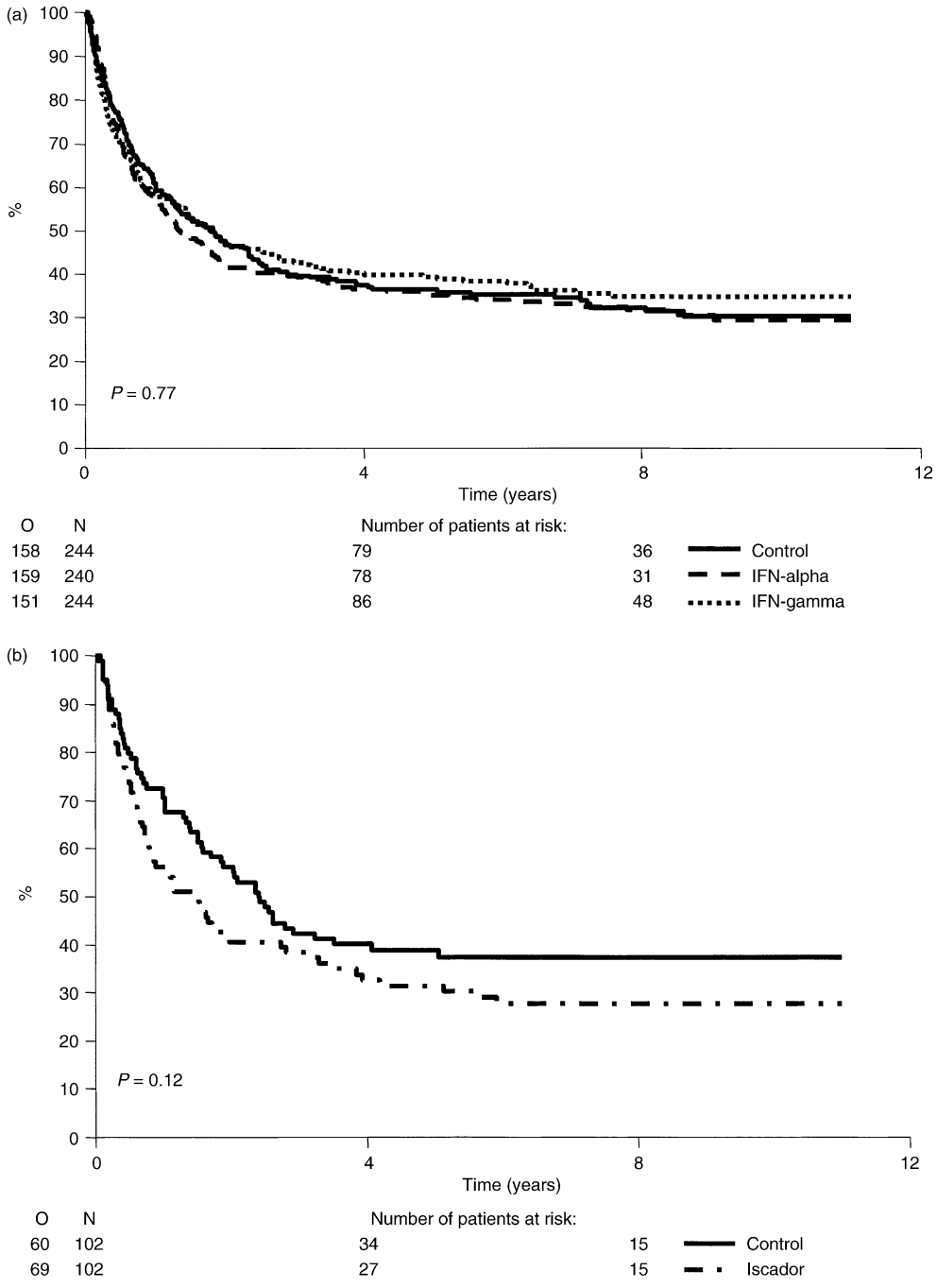

Fig. 1. (a) IFN group: disease-free interval by treatment arm. (b) ISCADOR group: disease-free interval by treatment arm. O, observed; N, number; IFN, interferon

unlikely. The treatment differences were similar in stage IIb and III patients (data not shown). For patients given Iscador M®, the estimated hazard ratio was 1.32, and the lower limit of the confidence interval was 0.93. In the 99 stage IIb patients, the estimated hazard ratio was 1.00, with a 95% CI of 0.59–1.70, and in the 105 stage III patients, the estimated hazard ratio was 1.53, with a 95% CI of 0.97–2.42.

Regarding the type of first relapse, no clear differences were observed between the treatment arms (Table 3). These data were in agreement with the results in terms of DFI (Fig. 1a and b).

3.4. Disease-free interval: prognostic factors

By combining the two studies, EORTC 18871 and DKG 80-1, and pooling data from all the randomised arms, the initial stage of disease appeared to be the most important prognostic factor ($P<0.0001$): the 8-year DFI rate for stage IIb patients was 39.3% (SE = 2.9%)

versus 27.6% (SE = 2.1%) for stage III patients (Fig. 2). In stage IIb patients, females had a better prognosis than males ($P = 0.0009$), as had patients with a limb-localised melanoma ($P = 0.0001$) versus one localised in the head, neck or trunk regions. Since more women had limb-melanomas their better prognosis may be partly due to differences in the location of the primary melanoma. As most patients had a high Breslow thickness, even those categorised in the <3 mm group, the prognostic importance of the thickness was limited ($P = 0.07$). Patients with an ELND had a longer DFI than those who did not undergo an ELND. In contrast, age did not have an impact on the DFI. In stage III patients, prognosis was significantly associated with the number of positive lymph nodes (1 versus 2–4 versus >4: $P = 0.0003$), whether a patient had an initial or consecutive stage III melanoma ($P = 0.03$), and according to the Breslow thickness of the primary (<3 versus 3–3.99 versus >4 mm: $P = 0.002$). Gender and tumour localisation were marginally significant, whereas age did not have a clear impact on the DFI in this group of patients either. Finally, the combination of stage and

Table 3
Recurrence status and type of first progression after randomisation, and survival status and cause of death by treatment arm

	IFN group			*Iscador-M*® group	
	Control	rIFN-α2b	rIFN-γ	Control	*Iscador-M*®
Variable/total	244 (100%)	240 (100%)	244 (100%)	102 (100%)	102 (100%)
No recurrences	86 (35.2)	81 (33.8)	93 (38.1)	42 (41.2)	33 (32.4)
Recurrences	158 (64.8)	159 (66.2)	151 (61.9)	60 (58.8)	69 (67.6)
Local	11 (4.5)	4 (1.7)	3 (1.2)	4 (3.9)	3 (2.9)
In transit	11 (4.5)	11 (4.6)	15 (6.1)	6 (5.9)	5 (4.9)
Regional	54 (22.1)	46 (19.2)	50 (20.5)	16 (15.7)	21 (20.6)
Distant metastases	81 (33.2)	97 (40.4)	83 (34.0)	34 (33.4)	39 (38.3)
Unknown	1 (0.4)	1 (0.4)	0 (0.0)	0 (0.0)	1 (1.0)
Alive	96 (39.3)	103 (42.9)	114 (46.7)	48 (47.1)	42 (41.2)
Dead	148 (60.7)	137 (57.1)	130 (53.3)	54 (52.9)	60 (58.8)
Melanoma	124 (50.8)	129 (53.8)	119 (48.8)	45 (44.1)	57 (55.9)
Chronic disease	5 (2.0)	2 (0.8)	2 (0.8)	1 (1.9)	1 (1.0)
Other	11 (4.5)	5 (2.1)	5 (2.0)	4 (3.9)	2 (2.0)
Unknown	8 (3.3)	1 (0.8)	4 (1.7)	4 (3.9)	0 (0.0)

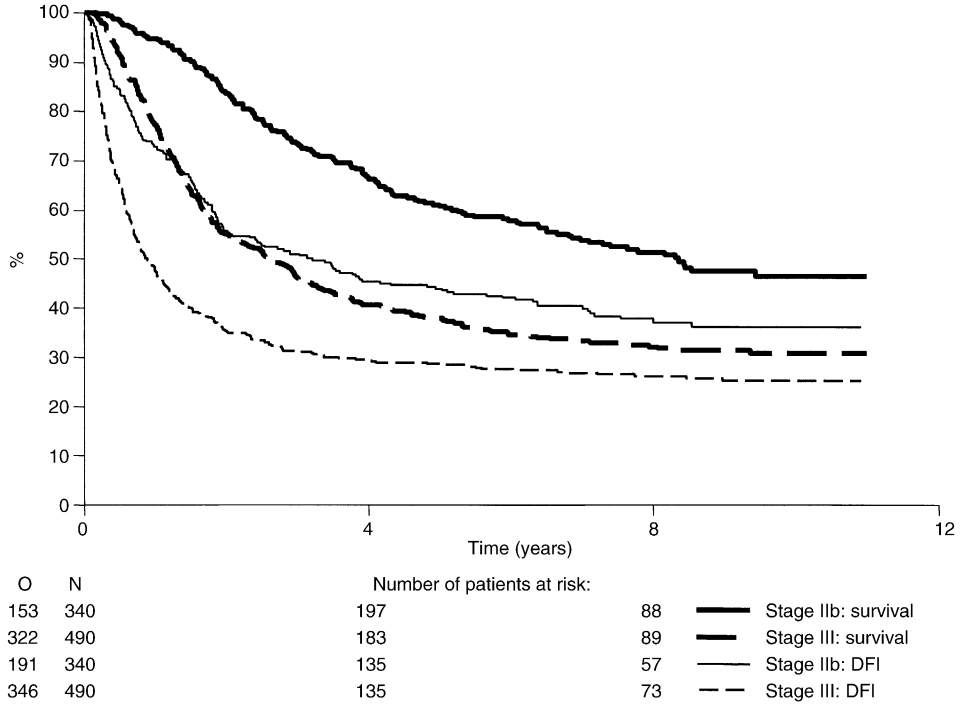

Fig. 2. EORTC 18871 and DKG 80-1: survival and DFI by stage.

the number of lymph nodes, i.e. stage IIb (no positive lymph nodes) versus 1 versus 2–4 versus >4 positive nodes, was the most important prognostic factor.

3.5. Disease-free interval: multivariate analyses

Considering all 830 patients, the following factors appeared to be of prognostic importance in the Cox multivariate analysis: stage/number of positive lymph nodes (HR = 1.41, $P < 0.00001$), localisation (HR = 1.45, $P < 0.001$) and Breslow thickness (HR = 1.22, $P = 0.0002$). Gender and ulceration have not been retained in the final model, due to their borderline independent prognostic importance and/or due to missing information.

For the Interferon group ($n = 728$), these 3 variables remained of high prognostic importance. Adjusting for these variables, the treatment effect was not significant (see Table 4). The estimated hazard ratios were quite close to the corresponding ones obtained in the univariate analyses.

For the Iscador group, comprising 204 patients, only stage/number of positive lymph nodes appeared to be of prognostic importance. The 2 other variables were only borderline significant, despite the fact that the same trends were observed as in the overall group of 830 patients. The comparison of Iscador versus control, when these 3 factors were kept in the Cox model, yielded a non-significant result ($P = 0.10$), and the estimated hazard ratio was 1.34, with a 95% CI that contained 1. The interaction between treatment and the most important prognostic variable, stage/number of positive lymph nodes, was not significant ($P = 0.71$). Considering the stage of disease (IIb versus III) only, without taking into consideration the number of positive nodes, but keeping in the model both Breslow thickness and localisation, did not change the results. The interaction between stage of disease and treatment was not significant either ($P = 0.47$).

3.6. Duration of survival: treatment comparisons

The survival curves according to treatment arm are given in Fig. 3a. No significant impact was observed for rIFNs treatment. The estimates of the hazard ratios were close to 1 and the 95% CIs contained 1 (Table 4).

The impact on survival of Iscador M® treatment is shown in Fig. 3b. The estimated hazard ratio was 1.21 and the 95% CIs contained 1 (Table 4). In stage IIb patients, the estimated hazard ratio was 0.85, with a 95% CI of 0.46–1.56, and in stage III patients, it was 1.61, with a 95% CI of 1.01–2.56.

3.7. Duration of survival: prognostic factors

Stage of disease was the strongest prognostic factor (Fig. 2). Interestingly, at 10 years, the survival curves are only slightly higher than the disease-free interval curves (approximately 10% for stage IIb and 5% for stage III), indicating that for most subjects in this high-risk population, a relapse ultimately results in death. The factors of prognostic importance for DFI were the same as for survival. In stage IIb patients, the final outcome for patients with or without an ELND was similar ($P = 0.87$).

3.8. Survival: multivariate analyses

As for the DFI, the following factors appeared to be of prognostic importance for survival in the Cox multivariate analysis: stage/number of positive lymph nodes (HR = 1.53, $P < 0.00001$), localisation (HR = 1.71, $P < 0.001$) and Breslow thickness (HR = 1.22, $P = 0.0007$).

For the Interferon group, after adjusting for these 3 factors, the treatment effect was not significant (see Table 4).

Similarly, comparing Iscador versus control groups in terms of their survival, following adjustment for these 3

Table 4
Results of the Cox Proportional Hazards model: hazard ratio estimates with the corresponding 95% Confidence Intervals, and the *P*-values given by the Wald test

	rIFN group		*Iscador-M®* question
	rIFN-α2b versus control	rIFN-γ versus control	*Iscador-M®* versus control
Univariate analyses			
Disease-free interval	1.04 (0.84, 1.30) $P = 0.71$	0.96 (0.77, 1.20) $P = 0.73$	1.32 (0.93, 1.87) $P = 0.12$
Survival	0.96 (0.76, 1.21) $P = 0.72$	0.87 (0.69, 1.10) $P = 0.25$	1.21 (0.84, 1.75) $P = 0.31$
Multivariate analyses[a]			
Disease-free interval	1.05 (0.84, 1.30) $P = 0.69$	0.96 (0.77, 1.20) $P = 0.73$	1.34 (0.95, 1.91) $P = 0.10$
Survival	0.98 (0.77, 1.23) $P = 0.85$	0.87 (0.69, 1.10) $P = 0.24$	1.27 (0.87, 1.84) $P = 0.21$

[a]: The following variables, with the following codes, have been considered in the model, in addition to treatment group: stage/number of positive lymph nodes (0 = stage IIb, 1 = 1 node, 2 = 2–4 nodes, 3 = 5+ nodes), Breslow thickness (0 = <3 mm, 1 = 3.1–4 mm, 2 = >4 mm) and localisation (0 = limb, 1 = other localisation). For the IFN group, the Cox model has been stratified by study (EORTC 18871 and DKG 80-1).

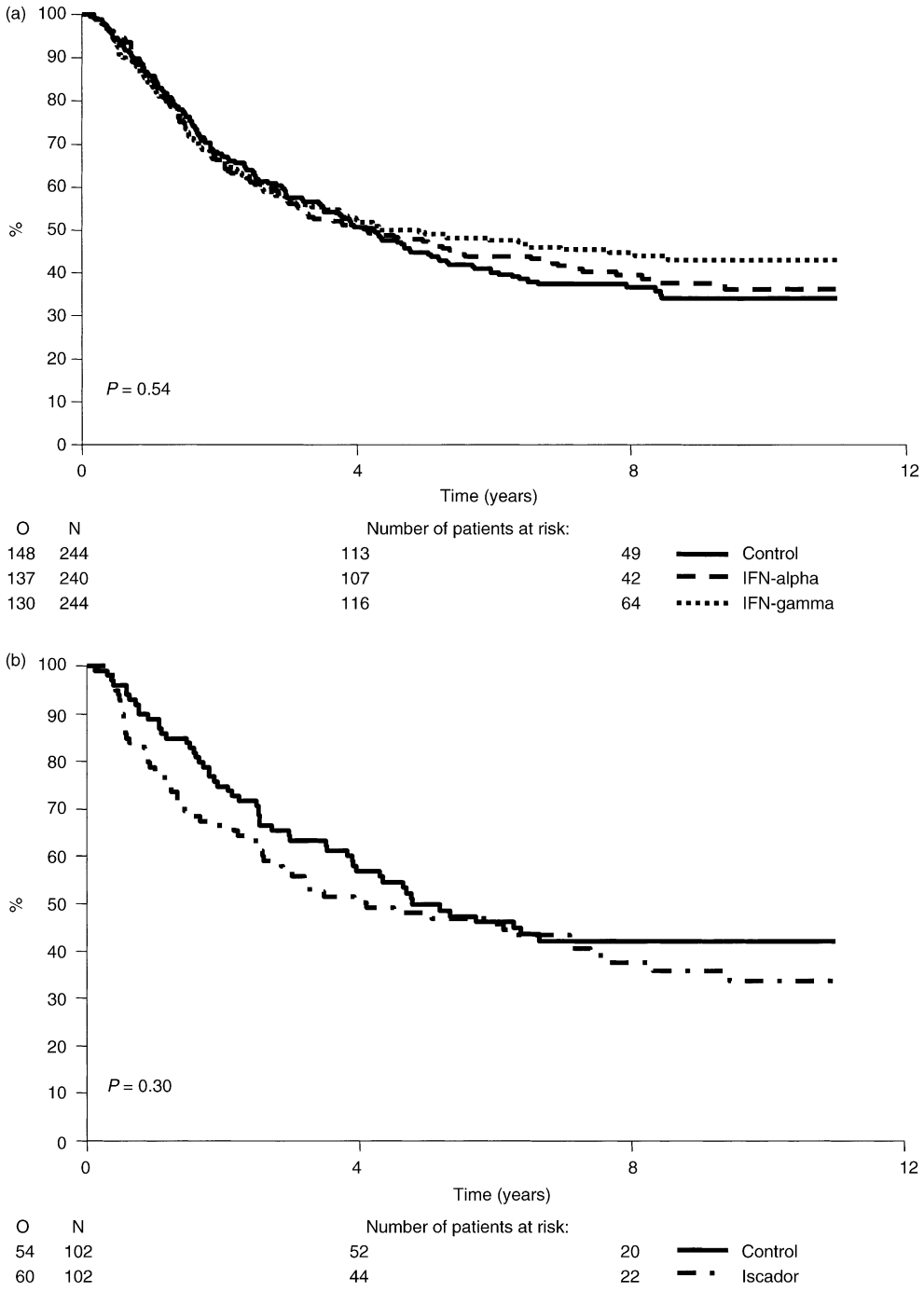

Fig. 3. (a) IFN group: survival by treatment arm. (b) ISCADOR group: survival by treatment arm.

factors, resulted in similar results to those obtained in the univariate analysis. The interaction between stage of disease and treatment was not significant ($P = 0.18$).

3.9. Toxicity

Minor side-effects (fever, chills, night-sweats, fatigue, myalgias, arthralgias and headaches) were seen in approximately a 1/3 of patients treated with rIFN and usually subsided after a few weeks. In 11 patients (4.6%) randomised in the *IFN-α2b* group, 19 (7.8%) in the *IFN-γ* and 5 (4.9%) in the *Iscador-M*® arm, treatment had to be discontinued due to World Health Organisation (WHO) grade 3-4 toxicities (e.g. anorexia, general malaise, depressive moods, fever and local skin inflammation at the site of injection) (Table 2). No organ toxicity was observed.

4. Discussion

The autonomous course of melanoma progression and our limited insight into the interactions between tumour and host require a particularly critical evaluation of new as well as historical immuno-modulatory treatments. We therefore studied the natural course of patients with high-risk melanoma, and the impact of biological response modifiers according to the concept of *good clinical practice* over a 10 year period. The actuarial rates of disease-free and overall survival in melanoma patients with stages IIb or III merge after 8–10 years, indicating that most patients who progress ultimately died. Patients who progress loco-regionally have only a 30% chance of surviving. Patients with a tumour-thickness of 3 mm or more have a similar poor prognosis to those presenting with regional lymphatic metastases. Depth of invasion remains the dominant risk factor underlining the importance of prevention and early detection strategies. Ulceration of the primary tumour has recently been demonstrated to be a very important independent prognostic factor [2]. In our study, ulceration of the primary was not a stratification factor. Furthermore, ulcerated primaries were well balanced between the various treatment arms, and so we do not think that our results had been influenced by any imbalances. Females fared better than males, even after correcting for the higher survival-rates that are associated with limb versus head, neck or trunk locations. Age was not a significant risk factor, although there was a trend for a more accelerated course in people over 50 years of age, although this did not result in differences in the mortality rates.

ELND, considered at the time to be "standard treatment" in some European hospitals, did not yield any benefit in terms of overall survival. This observation is consistent with the EORTC 18832 trial on prophylactic limb perfusion, and the literature on randomised trials regarding ELND [36]. In agreement with the literature [2], the number of involved nodes was shown to be a highly relevant prognostic marker: 5 or more positive nodes conferring a worse prognosis ($P < 0.001$) compared with 2-4 or 1 node only.

When the location of first recurrence in the 537 (64.7%) patients who progressed was analysed, it was noted that local relapses were rare: they occurred in only 21 (2.5%) patients, underlining that the so-called "safe" margins of >2 cm are of negligible importance for the course of disease. However, their 8-year survival rate after relapse was approximately 10%, which is comparable with the outcome of the 42 (5.1%) patients who had the first sign of relapse in transit metastasis (ITM) only.

Neither treatment improved disease-free or total survival rates. In an earlier randomised trial of the *American Southwest Oncology Group (SWOG)* a negative trend was noted for IFN-γ [37]. In our trial, there was no clear trend, the estimated hazard ratio being very close to 1.

Iscador-M® also appeared not to yield a better outcome (time to progression and survival) compared with the patients randomised to the control group. However, a drug-related acceleration of the course of disease might be possible. One constituent of mistletoe-extract, the galactoside-specific lectin, is a potent biological response modifier in a narrow low-dose range [38]. Its long-term administration promoted tumour cell growth in an experimental animal model [39]. It is known that immuno-modulation by lectins involves enhanced secretion of multifunctional proinflammatory cytokines such as Interleukin 6 (IL6) [40,41] and that IL6 undergoes a transition from paracrine growth inhibition to autocrine stimulation during melanoma progression [42]. There is also an inverse correlation between patient prognosis and elevated IL6 serum levels [43,44]. *In vitro* [45,46] and *in vivo* studies [47,48] have demonstrated IL6-induced stimulation of melanoma cell proliferation. Our data therefore support, but do not provide significant proof of earlier warnings about a potential negative effect of mistletoe extracts in melanoma patients, since the observations did not reach significance. In our DKG 80-1 trial, the estimated hazard ratio of the *Iscador-M®* arm versus the control was 1.32 for DFI and 1.21 for the duration of survival, indicating that there is no positive effect from *Iscador-M®* treatment. We could not detect any interaction between the stage of disease and treatment effect, most probably due to a lack of statistical power. Furthermore, the inefficacy of yet another mistletoe preparation has recently been reported in a prospective, randomised trial involving 477 head and neck cancer patients [49].

Regarding the rIFN-alpha arm, the estimated hazard ratio was close to 1, and the lower limits of the 95% intervals was above 0.76, which demonstrates that, at the chosen dose schedule, rIFN-*α2b* did not improve the DFI or survival rates.

Severe side-effects which interfered with the patient's wellbeing or led to the discontinuation of treatment occurred in 5–8% of patients, and were similar in all rIFNs and *Iscador-M®* arms.

rIFN-alpha has been given as adjuvant treatment to melanoma patients since 1984. Published prospective randomised trials have used either low doses of IFN (LDI) or high dose IFN (HDI): The *WHO-trial* 16 (3 MU *tiw* s.c. for 18 months, $n = 427$) reported initially a significant prolongation of the DFI [50], but mature data showed this to be a transient effect, with the survival curves of the treated and control populations converging ($P = 0.221$) [51]. The *Austrian Malignant Melanoma Cooperative Group* tested a similar, but shorter, regimen in stage II melanoma patients (3 MU s.c. daily for 3 weeks followed by tiw for 12 months,

$n=311$) leading to a prolonged DFI ($P=0.02$), but not a prolonged survival after 41 months of observation [52]. Similar results were obtained by the *French Melanoma Cooperative Group* (3 MU tiw, s.c., for 18 months versus controls, $n=499$), prolonging the DFI ($P=0.04$), and with a trend towards an improved survival rate being observed ($P=0.057$) [53]. Essentially similar results were reported by the *Scottish Melanoma Cooperative Group* (3 MU tiw, s.c., for 6 months versus controls, $n=95$). The temporary impact on DFS and OS in this study failed to reach significance because of the very small sample size [54].

The mature results of 3 trials using HDI have been reported. The North Central Cancer Treatment Group (NCCTG) reported in 1995 no significant impact of HDI (20 MU/m^2 i.m. for 12 weeks) on DFS or OS in a mixed population of 262 stage II (Breslow thickness >1.7 mm) and stage III melanoma patients [55]. In 1996, the *Eastern Cooperative Oncology Group (ECOG)* reported on the outcome of 1 year HDI as evaluated in trial EST 1684 [56]. A total of 287 patients with stages IIb and III were randomised to control or treatment with 20 MU/m^2/day intravenously (i.v.) for a 4 week induction course followed by 10 MU/m^2/day s.c. tiw for 48 weeks. The results gave a significantly increased relapse-free ($P=0.005$), and overall survival ($P=0.046$) with a median prolongation of life from 2.8 to 3.8 years. However, toxicity was very high: 2 patients died and in 78% of the patients grade III–IV toxic events were reported leading to multiple dosing delays and dose reductions. Following these results, rIFN alpha was introduced as 'standard' adjuvant treatment in the United States of America (USA), and approved in Europe in 1997. Approval on the basis of only one, relatively small randomised trial was criticised by many in the medical community, especially since the modest impact did not easily outbalance the high toxicity of the treatment for all patients. So, in Europe, most physicians preferred to wait for the outcome of the third HDI-trial (Intergroup E 1690) which would confirm or reject the results of the ECOG 1684 trial. The Intergroup E1690 trial compared HDI (ECOG 1684) to LDI (3 MU, sc, tiw for 2 years) with observation in 642 stage IIb–III patients. In June 2000, data from E1690 was reported and failed to confirm the results of the ECOG 1684 trial [57]: the data showed only a borderline impact on DFS for HDI ($P=0.054$), and no impact on overall survival ($P=0.995$). LDI in the Intergroup E 1690 trial showed a curve similar to HDI for DFS and a curve slightly better that HDI for OS, but not significantly better than in the observation arm. The outcome of this trial meant the end of HDI in most of the few institutions in Europe that had adopted HDI in the adjuvant setting. A fourth trial with a HDI-arm in stage IIb–III melanoma patients was reported very recently. The Intergroup E 1694 trial was unblinded and reported early because of a significant difference for both DFS ($P=0.001$) as well as OS ($P=0.046$) between the HDI arm and the Ganglioside-vaccine arm in the 774 randomised stage IIB–III patients [58]. However, these differences were reported after a median follow-up of only 1.3 years, which makes it virtually impossible to make any solid claims about the impact on overall survival. So, whether these differences in survival will continue to be significant needs to be examined further, especially in light of the Intergroup E 1690 and the ECOG 1684 experiences. In a pooled analysis of the trials 1684 and 1690, presented at the 37th meeting of the American Society of Clinical Oncology (ASCO) in 2001, the impact on OS by HDI was not so evident, when one observes that 198 patients in the observation arms of trials E1684/1690 had died thus far, whereas 201 patients had died in the HDI arms [59]. The largest trial by far in high-risk melanoma patients (stage IIb–III) is the EORTC 18952 trial involving 1418 patients. This trial evaluates the impact of intermediate doses of IFN where an induction period of 4 weeks, 5 days/week, 10MU, s.c. is followed by a maintenance period of 10MU, s.c., tiw for 1 year versus 5MU, s.c., tiw, for 2 years, versus observation. Interim analyses indicate that the duration of treatment is more important than the dose of IFNα, since the higher dose of 10 MU for 1 year had no significant impact on the Distant Metastasis Free Interval (DMFI), the primary endpoint in this trial, whilst the lower dose of 5 MU for 2 years showed a significant impact on DMFI ($P=0.0145$) [60] [FECS-EJC Awards for best ECCO-12 Abstracts, *EJC News*, 2003, **39**, 17, 2413]. Again, these data should be interpreted with the greatest caution as this is an early report. A meta-analysis of adjuvant trials with IFN-alpha reported a dose-independent consistent impact on DFS, without any significant impact on survival [61]. This, it can be concluded that the role of IFN-alpha in melanoma remains to be defined [62]. Exploring the efficacy of non-toxic long-term therapy finds its basis in the recently demonstrated anti-angiogenic effects of interferon [63] and is currently explored in the EORTC 18991 trial, comparing 5 years of treatment with Pegylated IFN-alpha2b (PEG-Intron) with observation in 1200 stage III melanoma patients.

All in all, results with biological response modifiers over the last 15 years have been, at best, very modest. We should be skeptical of curing melanoma patients with "natural methods" that unspecifically stimulate the immune system to elminate melanoma cells which may well have induced general or local immune tolerance. We have to continue our efforts to finally arrive at a scientifically and ethically sound standard for the adjuvant treatment of patients with melanoma. These standards have two aspects: one to improve patient prognosis with a sizeable increase in the cure rates, the

other one to shield the patient from costly, irrational and even harmful interventions. It is a basic requirement of our health authorities, including the insurance companies, to actively promote clinical research. This has to become an integral part of our general healthcare in the form of controlled treatment comparisons to optimise our limited therapeutic options against the background of a most efficient use of our society's increasingly limited resources.

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Appendix.

Table of Institutions/Country participating in the European Organisation for Research and Treatment of Cancer (EORTC) Trial 18871/DKG 80-1

Country	Number of centres	Number of patients
Germany	12	268
France	8	132
Switzerland	7	110
Austria	4	80
Belgium	3	55
Great Britain	2	51
Yugoslavia	2	47
Israel	2	38
Czechia	1	20
Estonia, Spain, Greece, Poland	4	29
Total of 13 countries	45	830

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