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ARTICLE

Interferon Alpha Adjuvant Therapy in Patients With High-Risk Melanoma: A Systematic Review and Meta-analysis

Simone Mocellin, Sandro Pasquali, Carlo R. Rossi, Donato Nitti

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Correspondence to: Simone Mocellin, MD, PhD, Clinica Chirurgica Generale 2, Department of Oncological and Surgical Sciences, University of Padova, via Giustiniani 2, 35128 Padova, Italy (e-mail: simone.mocellin@unipd.it).

Background Based on previous meta-analyses of randomized controlled trials (RCTs), the use of interferon alpha (IFN-α) in the adjuvant setting improves disease-free survival (DFS) in patients with high-risk cutaneous melanoma. However, RCTs have yielded conflicting data on the effect of IFN-α on overall survival (OS).

Methods We conducted a systematic review and meta-analysis to examine the effect of IFN-α on DFS and OS in patients with high-risk cutaneous melanoma. The systematic review was performed by searching MEDLINE, EMBASE, Cancerlit, Cochrane, ISI Web of Science, and ASCO databases. The meta-analysis was performed using time-to-event data from which hazard ratios (HRs) and 95% confidence intervals (CIs) of DFS and OS were estimated. Subgroup and meta-regression analyses to investigate the effect of dose and treatment duration were also performed. Statistical tests were two-sided.

Results The meta-analysis included 14 RCTs, published between 1990 and 2008, and involved 8122 patients, of which 4362 patients were allocated to the IFN-α arm. IFN-α alone was compared with observation in 12 of the 14 trials, and 17 comparisons (IFN-α vs comparator) were generated in total. IFN-α treatment was associated with a statistically significant improvement in DFS in 10 of the 17 comparisons (HR for disease recurrence = 0.82, 95% CI = 0.77 to 0.87; $P < .001$) and improved OS in four of the 14 comparisons (HR for death = 0.89, 95% CI = 0.83 to 0.96; $P = .002$). No between-study heterogeneity in either DFS or OS was observed. No optimal IFN-α dose and/or treatment duration or a subset of patients more responsive to adjuvant therapy was identified using subgroup analysis and meta-regression.

Conclusion In patients with high-risk cutaneous melanoma, IFN-α adjuvant treatment showed statistically significant improvement in both DFS and OS.

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Although cutaneous malignant melanoma is the least common form of skin cancer, it accounts for 75% of skin cancer deaths (1–5). During most of the 20th century, the incidence of melanoma in populations of European origin rose faster than any other solid cancer, barring lung cancer. An estimated 160 000 new cases and 41 000 deaths were reported worldwide in 2002. In the United States, the American Cancer Society reported approximately 59 940 new cases of melanoma (with an estimated lifetime risk of one in 49 for men and one in 73 for women), leading to an expected 8110 deaths in 2007. In comparison, the incidence in 2001 was approximately 47 700 new cases. This underscores that melanoma is a current and important public health concern.

The therapeutic management of cutaneous melanoma is one of the most challenging issues for oncologists (1–3). Because melanoma is among the solid malignancies most refractory to medical therapy, it makes early diagnosis and surgical removal of the primary tumor virtually the only curative approach currently available. For metastatic melanoma, no conventional or molecularly targeted drug is better than dacarbazine (DTIC); however, there is no convincing evidence that DTIC is better than best supportive care (4–6).

In patients with high-risk melanoma, that is, with American Joint Committee on Cancer (AJCC) TNM stage II (T2-4N0M0) and stage III (TanyN+M0) disease, the rate of disease recurrence ranges between 20% and 60%, with 5-year overall survival (OS) varying between 45% and 70% (7). The only agent currently approved for such patients with apparently radical surgery (ie, adjuvant setting) is interferon alpha (IFN-α) (8), which is a type I interferon mainly produced endogenously by macrophages (9). The IFN-α cluster region of chromosome 9p22 encodes 13 different IFN-α genes: Among them, the IFN-α2 gene presents three polymorphic variants, known as IFN-α(2a), IFN-α(2b), and IFN-α(2c) (10). IFN-α has anticancer effects both in the preclinical models and in the clinical setting, although the mechanism of action is still unclear (11). Regarding treatment of melanoma, only the proteins encoded by the IFN-α(2a) and IFN-α(2b) genes—which differ for

CONTEXT AND CAVEATS

Prior knowledge

Interferon-α (IFN-α) adjuvant therapy improves disease-free survival of high-risk cutaneous melanoma patients, but it is uncertain whether it also improves overall survival.

Study design

Systematic review and meta-analysis of 14 randomized controlled trials conducted between 1990 and 2008. The trials evaluated the benefit of IFN-α adjuvant treatment by comparing IFN-α with observation or any other regimen other than IFN-α.

Contribution

Statistically significant improvements in disease-free survival and overall survival of high-risk melanoma patients treated with IFN-α vs comparator regimen or observation were shown.

Implications

These results support the use of IFN-α for the treatment of high-risk cutaneous melanoma patients.

Limitations

The efficacy of different IFN-α doses was not clear from the study. The overall survival benefit was observed only when trials that used low or intermediate IFN-α doses were considered. Anticancer efficacy was limited, and the need to identify more effective agents remains.

From the Editors

a single amino acid at position 23 (lysine > arginine)—have been tested as therapeutic agents in the clinical setting, and only human recombinant IFN-α(2b) is approved for the adjuvant treatment of this deadly type of skin cancer. Despite several randomized controlled trials (RCTs) conducted on the use of IFN-α as an adjuvant treatment for melanoma, the findings are conflicting in terms of therapeutic efficacy (12–35). Most importantly, no clear OS benefit has been demonstrated so far, even after adjustment for quality of life incorporating patient values (utilities) for the toxic effects of IFN-α2b treatment and melanoma recurrence (36). According to two meta-analyses published more than 6 years ago on the results of 12 and nine RCTs, respectively, IFN-α appears to provide a statistically significant disease-free survival (DFS) advantage (mainly over observation) in patients with high-risk cutaneous melanoma, whereas no impact on OS was demonstrated (37,38). Since then, other two large RCTs enrolling 1700 patients have been published, the findings being conflicting in terms of OS benefit (24,25). This unsatisfactory situation is fostering a continuous debate among oncologists on whether the routine use of IFN-α, which is accompanied by clinically relevant toxic effects and represents a substantial economic burden for the health-care system, is justified (39–47). To quantitatively summarize the currently available clinical findings on this debated issue, we performed a formal systematic review and meta-analysis of the RCT comparing IFN-α with any comparator for the adjuvant treatment of high-risk cutaneous melanoma. We intended to answer if IFN-α treatment was associated with any survival benefit (DFS or OS), compared with observation (or any regimen other than IFN-α), in patients with high-risk cutaneous melanoma. Because no previous meta-analyses included all the currently available RCTs, our work provides the readers with the most updated quantitative review on this subject.

Methods

Eligibility Criteria

All RCTs that compared IFN-α with observation (or any regimen other than IFN-α) for the adjuvant treatment of skin melanoma were considered eligible. An important criterion for all patients enrolled in these trials was to have high-risk cutaneous melanoma, that is, radically resected TNM stages II–III disease. No drug dose, language, or publication date restriction was applied.

Information Sources and Search Strategy

A systematic review of the RCTs meeting the above-reported eligibility criteria was performed by searching the MEDLINE, EMBASE, Cancerlit, Cochrane, ISI Web of Science, and ASCO databases. The database search included various combinations of the following keywords: melanoma, interferon alpha, IFN, adjuvant, high-risk, randomized, and trial. Reference lists of original articles and review articles served as additional resources in the search strategy.

Quality Assessment and Data Extraction

For each RCT, the following data were extracted: number of patients, disease stage (as defined by the TNM system), IFN type, IFN dose, IFN schedule, randomization ratio, and results in terms of survival benefit (DFS and OS). Data were independently extracted by two investigators (S. Mocellin and S. Pasquali) to ensure homogeneity of data collection and to rule out any subjective influence in data gathering and entry. Disagreements between the investigators were resolved by iteration, discussion, and coming to a consensus. To unravel potential systematic biases, the other two investigators (C. R. Rossi and D. Nitti) did a concordance study by independently reviewing all eligible RCT. We reached a complete concordance for all variables assessed.

Authors of included published studies were contacted whenever we found that data essential for the meta-analysis were missing or unclear.

Statistical Analysis

Meta-analysis was performed following the Quality of Reporting of Meta-analyses (QUOROM) guidelines (48). Standard meta-analysis methods (49,50) were applied to evaluate the overall effect of IFN-α on the DFS and OS of patients based on reported survival data analyzed according to an intention-to-treat principle. We considered the IFN-α group as the treatment group, and any comparator (observation or any regimen not including IFN-α) was considered the control.

Hazard ratios (HRs) were always calculated as IFN-α to comparator ratios. Time-to-event outcomes were appropriately analyzed using hazard ratio. The methods to combine hazard ratio–related summary data have been extensively described elsewhere (51,52). Briefly, if the hazard ratio and log-rank variance (V) or lnHR and its variance (V^*) were presented in a trial report, they were used directly using the method of Peto (53) or the inverse variance method (51). Similarly, if the coefficient of the treatment

effect and the variance from a Cox model were provided, which correspond to the lnHR and V^*, they were used directly by using the inverse variance method (52). However, when the coefficients were not reported, we estimated the log-rank observed minus expected events (O − E) and V, or the lnHR and V* for each trial, to combine them in a meta-analysis (51,52). In cases when these methods could not be applied, Kaplan–Meier survival curves were used to generate the necessary statistics by adopting a hierarchical series of steps, as per Parmar et al. (51).

We used the reported hazard ratio and 95% confidence interval (CI) (when available) in the meta-analysis; otherwise, the hazard ratios (and their variances) were indirectly extrapolated based on the information provided by the authors (13,14,17,18). The summary effect was then computed as the mean of the effect sizes of the included trials, each of them being weighted by the inverse of its variance.

We performed a meta-analysis by first using the fixed-effects model, which assumes that all the studies share the same common (fixed or nonrandom) effect. Only within the study, variance is used to calculate the weight of each study. The consistency of results (effect sizes) among studies was assessed using the standard heterogeneity tests—the χ^2-based Cochran Q test and the I^2 statistic, $I^2 = [Q - df]/Q \times 100$ (Q being the Cochran statistic and df being the degrees of freedom [number of studies minus one], which indicates the percentage of the variability in effect estimates because of true between-study variance rather than sampling error [within-study variance]). To be more conservative, we considered that heterogeneity was statistically significant when the Cochran Q test P value was less than .1. In addition, inconsistency across studies was quantified by I^2 statistic, with heterogeneity being considered substantial for values equal to or greater than 50%.

In case of heterogeneity, meta-analysis was performed applying the random-effects model, which assumes that studies do not share the same common effect and assigns a weight to each study taking into account both within- and between-study variance. We applied the method of DerSimonian and Laird (54) in this case.

For multiple-arm trials, in which two IFN-α arms were compared with the same control arm, within-study correlation was taken into consideration. As described by Borenstein et al. (55), we first calculated a composite effect size for the comparison of any-IFN-α vs control. Next, we calculated the correlation factor (r) based on the number of cases in each arm, which allowed us to compute the variance (V) of the composite effect size according to the following formula: $V = 1/4\ [(V1 + V2) + (2r\sqrt{V1}\sqrt{V2})]$, where $V1$ and $V2$ are the variances of the original comparisons between each treatment arm and the control arm.

The extent to which the combined risk estimate might be affected by individual studies was assessed by consecutively omitting every study from the meta-analysis (leave-one-out procedure). Subgroup analyses, which considered more homogeneous studies (enrolled patients with the same TNM stage, adopted the same IFN-α regimen/type, designed to compare the same regimens, provided data adjusted by multivariable survival analysis, and ones that enrolled an adequate number of patients), were performed to identify subsets of patients more likely to benefit from this treatment and to summarize the evidence from the highest quality RCT. The mixed-effect model was applied to obtain summary effects within and across subgroups (56), whereas subgroups were compared by means of heterogeneity Q test (56).

Furthermore, to identify factors influencing the treatment effect, random-effects meta-regression (which is used when the tested covariates are not expected to explain all the variation in the effect estimates) was also implemented, as per Thompson and Sharp (57). For this purpose, we considered the following predictors: year of publication, length of follow-up, planned treatment duration, percentage of enrolled patients with lymph node metastatic disease, and percentage of patients who discontinued the treatment because of toxicity (if this information was unavailable, we considered the percentage of patients who had IFN-α dose reduction or delay in treatment because of toxicity).

Funnel plot was used to detect publication bias (58). Funnel plot asymmetry was formally investigated with the Egger linear regression approach (59) and the Begg rank correlation test (60); the impact of publication bias on the summary effects was assessed by the trim-and-fill method described by Duval and Tweedie (61).

Because the TNM staging system has evolved over the past 20 years, the single reports use different TNM versions. Therefore, for the sake of homogeneity and clarity, we have chosen to classify enrolled patients as stage II if they had T2-4 primary tumor without lymph node metastasis and as stage III if they had lymph node metastatic disease (irrespective of primary tumor thickness), as per the last AJCC TNM staging system (7).

Meta-analysis was conducted using the Comprehensive Meta-Analysis software version 2.2.046 (Biostat, Englewood, NJ). Ninety five percent confidence intervals were calculated as estimates of precision for hazard ratio. The statistical tests were two-sided, and P values less than .05 were considered statistically significant.

Results

Eligible Trials

Twenty-four trials were published between 1990 and 2008, which tested the effectiveness of adjuvant IFN-α, vs a comparator, for the treatment of high-risk cutaneous melanoma (12–35). Eight were excluded from subsequent analysis because they were not designed to answer the question of whether IFN-α was associated with any survival benefit when compared with observation alone or with any regimen other than IFN-α (28–35). Three of the eight RCTs compared different IFN-α regimens (33–35), and the remaining five tested various combination regimens like IFN-α plus interleukin-2 (30), isotretinoin (31), DTIC (28,29), or an allogeneic melanoma vaccine (32) to assess their therapeutic value against IFN-α alone (ie, they tested the hypothesis that therapeutic agents other than IFN-α increased the efficacy of IFN-α).

Two other studies (26,27), which were theoretically eligible because they compared IFN-α with observation alone, were not included in the meta-analysis for the following reasons. In one case (26), the randomization of the trial was unclear; even though the authors mentioned that patients were randomly selected to receive IFN-α therapy, there was no mention of a corresponding control arm and it seemed that surgically treated melanoma patients who were diagnosed at the time of the study were considered as a

nonrandomized control group. In addition, patients with both stage II and stage III melanoma were enrolled without mentioning any stratification of the randomization, and the results were represented separately as Kaplan–Meier curves for DFS, with no log-rank *P* values, for the two stages. Aside from these issues, the other problem was that the authors claimed that both stage I and stage II patients benefited from IFN-α therapy, although no difference in DFS or OS was detected between treatment and observation groups. The claim seems to be based on the fact that the rate of recurrence was statistically significantly different. All attempts to contact the primary investigators of this trial were unsuccessful. The randomized design was also unclear in the other study (55), but we contacted the primary investigators of this trial and confirmed the randomized design. Nonetheless, the article only reported the comparison of recurrence rates between the study groups, which prevented us from including it in the present meta-analysis based on survival data.

Thus, 14 RCTs met the inclusion criteria (Table 1). A total of 8122 patients were included in these trials, and 4362 (53.7%) patients were randomly assigned to the IFN-α group (12–25). All patients underwent radical surgery for stage II (n = 2226; 27.5%), stage III (n = 5693; 70.1%), or stages II–III (n = 192; 2.4%)

Table 1. Characteristics of randomized controlled trials (RCT) included in the meta-analysis*

RCT (first author, year [reference])	No. of patients (follow-up†)	TNM stage	% Node positive	IFN-α regimen	Arms: No. of patients
NCCTG (Creagan, 1995 [12])	264 (73)	II–III (T2-4N0M0/ TanyN+M0)	61	IFN-α(2a): 20 MU/m² × 3/wk for 4 mo (route: i.m.)	IFN-α: 132 Observation: 132
E1684 (Kirkwood, 1996 [13])	287 (83)	II–III (T4N0M0/ TanyN+M0)	89	IFN-α(2b): 20 MU/m² × 5/wk (1 mo, route: i.v.) + 10 MU/m² × 3/wk (48 wk, route: s.c.)	IFN-α: 147 Observation: 140
AMCG (Pehamberger, 1998 [14])	311 (41)	II (T2-4N0M0)	0	IFN-α(2a): 3 MU × 7/wk (3 wk, route: s.c.) + 3 MU s.c. x 3/wk (12 mo, route: s.c.)	IFN-α: 154 Observation: 157
FCGM (Grob, 1998 [15])	499 (60)	II (T2-4N0M0)	0	IFN-α(2a): 3 MU × 3/wk (18 mo, route: s.c.)	IFN-α: 253 Observation: 246
E1690 (Kirkwood, 2000 [16])	642 (52/79)	II–III (T4N0M0/ TanyN+M0)	74	IFN-α(2b) (high): 20 MU/m² × 5/wk (1 mo, route: i.v.) + 10 MU/m² × 2/wk (48 wk, route: s.c.). IFN-α(2b) (low): 3 MU × 2/wk (2 y, route: s.c.)	IFN-α (high): 215 IFN-α (low): 215 Observation: 212
SMG (Cameron, 2001 [17])	96 (78)	II–III (T3-4N0M0/ TanyN+M0)	NR	IFN-α(2b): 3 MU × 3/wk (6 mo, route: s.c.)	IFN-α: 47 Observation: 49
E1694 (Kirkwood, 2001 [18])	880 (16/25)	II–III (T4N0M0/ TanyN+M0)	77	IFN-α(2b): 20 MU/m² × 5/wk (1 mo, route: i.v.) + 10 MU/m² × 2/wk (48 wk, route: s.c.)	IFN-α: 440 GMK: 440
WHO (Cascinelli, 2001 [19])	444 (88)	III (TanyN+M0)	100	IFN-α(2a): 3 MU × 3/wk (36 mo, route: s.c.)	IFN-α: 225 Observation: 219
E2696 (Kirkwood, 2001 [20])	107 (24/34)	II–III–IV (stage IV: resectable meta static disease)	NR	IFN-α(2b) (day 1): IFN-α (from day 1) 20 MU/m² × 5/wk (1 mo, route: i.v.) + 10 MU/m² × 3/wk (48 wk, route: s.c.). IFN-α(2b) (day 28): IFN-α as above (from day 28)	IFN-α (day 1) + GMK: 36 IFN-α (day 28) + GMK: 36 GMK: 35
UKCCCR (Hancock, 2004 [21])	674 (37)	II–III (T4N0M0/ TanyN+M0)	70	IFN-α(2a): 3 MU × 3/wk (2 y, route: s.c.)	IFN-α: 338 Observation: 336
EORTC18871 (Kleeberg, 2004 [22])	830 (NR)	II–III (T3-4N0M0/ TanyN+M0)	58	IFN-α(2b): 1 MU every other day (12 mo, route: s.c.)	IFN-α: 240 IFN-γ: 244 Iscador(R): 102 Observation: 244
EORTC18952 (Eggermont, 2005 [23])	1388 (56)	II–III (T4N0M0/ TanyN+M0)	74	IFN-α(2b) (1 y): 10 MU × 5/wk (4 wk, route: s.c.) + 10 MU × 3/wk (12 mo, route: s.c.). IFN-α(2b) (2 y): 10 MU × 5/wk (4 wk, route: s.c.) + 5 MU × 3/wk (24 mo, route: s.c.)	IFN-α (1 y): 553 IFN-α (2 y): 556 Observation: 279
DeCOG (Garbe, 2008 [24])	444 (47)	III (TanyN+M0)	100	IFN-α(2a): 3 MU × 3/wk (2 y, route: s.c.)	IFN-α: 148 IFN-α + DTIC: 148 Observation: 148
EORTC18991 (Eggermont, 2008 [25])	1256 (46)	III (TanyN+M0)	100	Pegylated IFN-α(2b): 6 μg/kg/wk (8 wk, route: s.c.) + 3 μg/kg/wk (5 y, route: s.c.)	IFN-α: 627 Observation: 629

* TNM = TNM stage according to the American Joint Committee on Cancer (AJCC) melanoma classification system (9). DTIC = dacarbazine; GMK = anticancer vaccine GM2-KLH/QS-21; i.m. = intramuscular; IFN-α = interferon alpha; i.v. = intravenous; MU = mega units (1×10^6); m^2 = squared meter (of body surface); NR = not reported; s.c. = subcutaneous.

† Median follow-up (months; when available, data from trials updates are also reported).

cutaneous melanoma. In one study (20), 11 patients (0.1%) had radically resected stage IV disease.

Seventeen comparisons (IFN-α vs comparator) were generated in these trials that were included in the meta-analysis (Table 1). For four of these RCTs (13,16,18,20), an updated version has been published (62). Therefore, we performed a meta-analysis of the updated data after performing a meta-analysis of the original reports to ascertain whether more mature data influence the overall effect of meta-analysis.

In one RCT (20), the treatment arm regimen consisted of IFN-α combined with GMK (a ganglioside-based anticancer vaccine). Because GMK was also the comparator treatment, this study would allow the evaluation of any therapeutic benefit of IFN-α treatment and was included in the meta-analysis. In addition, the leave-one-out procedure was used as a sensitivity analysis to determine whether this trial had any impact on the overall effect estimated by the meta-analysis (see "Meta-analysis").

In a three-arm RCT (24), the observation group was compared with both IFN-α alone and a combination of IFN-α and DTIC. In this case, we considered only the IFN-α alone vs the observation comparison. The IFN-α plus DTIC arm was designed specifically to answer whether DTIC adds any therapeutic advantage to IFN-α and not whether IFN-α adds any therapeutic advantage to a comparator.

In one case, the findings of the two trials, EORTC18871 and DKG80-1, were reported in a single article as pooled results (and consequently as pooled HR) (22). Therefore, we have included these two trials as a single study in the present meta-analysis.

IFN-α regimens varied in terms of dosage (high dose [20 MU/m^2], intermediate dose [10 MU/m^2], and low dose [1–3 MU/m^2]), administration route (subcutaneously [s.c.], intramuscularly [i.m.], and intravenously [i.v.]), and duration of treatment (4 months to 5 years), as detailed in Table 1. IFN-α treatment was interrupted by disease progression or toxic effects; in the latter case, rates of treatment discontinuation (or dose reduction/delay) ranged from 0% to 58% (median 15%).

All patients received surgery in both IFN-α and control arms, which consisted of radical resection of the primary melanoma. Radical lymph node dissection was performed upon clinical and pathological evidence of lymph node metastasis in all RCTs, except for the E1684 trial (13), in which the enrolled patients underwent elective lymphadenectomy. Moreover, in trial EORTC18871 (22), elective lymph node dissection was performed at the discretion of the physician in charge. The spread to regional lymph nodes was assessed mainly by means of physical examination (clinically evident metastatic disease) until 2000. Thereafter, sentinel node biopsy for the detection of subclinical metastatic disease was allowed by trial protocols. Although lymph node–related data were not used for subgroup meta-analysis (because of the low number of RCTs), this information is useful to provide the reader with clues on the potential sources of between-study heterogeneity.

Meta-analysis

Disease-Free Survival. Our meta-analysis included all 14 RCTs that assessed the impact of IFN-α treatment on DFS (32–35,44–53) and showed a statistically significant benefit for patients who underwent IFN-α treatment (HR for disease recurrence = 0.82, 95% CI = 0.77 to 0.87; $P < .001$) (Figure 1). Considering the results of each single RCT, 10 of the 17 comparisons (n = 5046) found a statistically significant advantage in the IFN-α arm over the comparator arm (32,34,35,45,46,48,51–53). There was no statistically significant between-study heterogeneity ($P = .19$; $I^2 = 24.0\%$). Almost identical results were observed by substituting the data of the original reports with those described in the updated reports (HR for disease recurrence = 0.83, 95% CI = 0.78 to 0.88; $P < .001$). The absence of a "dominant" study driving the results of meta-analysis was demonstrated by the "leave-one-out" procedure that generated overall hazard ratio estimates (range = 0.81–0.83) very similar to those obtained with all comparisons ($P < .001$).

Upon subgroup analysis, we did not identify statistically significant differences in overall hazard ratio estimates according to IFN-α regimen or type, TNM disease stage, and study design (Table 2). Similarly, meta-regression did not show any statistically significant relationship between overall effect and the following predictors: year of publication, length of follow-up, planned treatment duration, percentage of enrolled patients with lymph node metastatic disease, and percentage of patients who discontinued the treatment (or had dose reduction or delay in treatment) because of toxicity.

Overall Survival. We used original data from all 12 of the 14 RCTs that assessed the impact of IFN-α on OS (33–35,44–47,49–53).

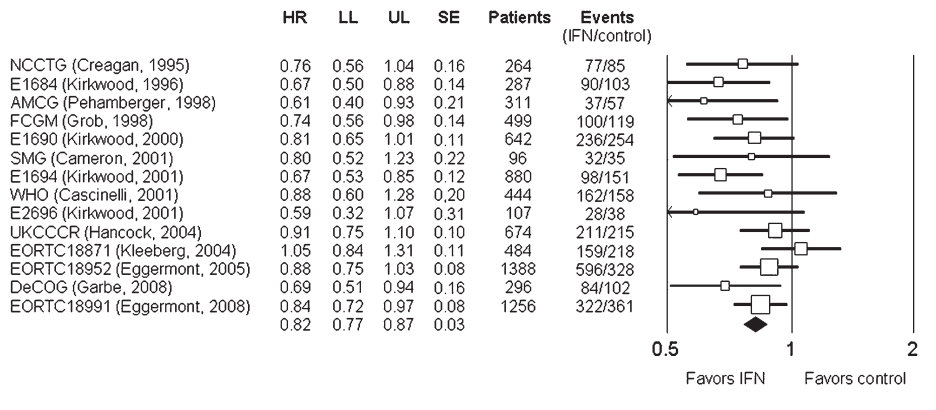

Figure 1. Forest plot of hazard ratios (HRs) (interferon alpha [IFN-α] vs control) for disease-free survival. **Squares** represent the hazard ratio of each single randomized controlled trial (RCT): The area is proportional to the weight in the meta-analysis according to the fixed-effect method, and the **horizontal line** represents the 95% confidence interval (CI). The **diamond** represents the estimated overall effect based on the fixed-effect meta-analysis of all RCTs (the **width of diamond** represents the 95% CI of the HR). LL = 95% confidence interval lower limit; UL = 95% confidence interval upper limit.

Table 2. Meta-analysis of subgroups*

Subgroups	Disease-free survival				Overall survival			
	No. of trials/ patients	HR (95% CI)	I^2 statistic	Subgroup comparison, *P*	No. of trials/ patients	HR (95% CI)	I^2 statistic	Subgroups comparison, *P*
High-dose IFN-α	6/3221	0.75 (0.68 to 0.83)	3.0	.05	5/3114	0.89 (0.77 to 1.02)	26.8	.99
Low- or intermediate-dose IFN-α	8/4901	0.85 (0.78 to 0.93)	17.1		7/4590	0.89 (0.81 to 0.98)	10.6	
Combined	14/8122	0.81 (0.75 to 0.86)	73.0	—	12/7704	0.89 (0.83 to 0.96)	0	—
IFN-α(2a)	6/2636	0.79 (0.71 to 0.89)	0	.67	5/2325	0.83 (0.71 to 0.98)	39.9	.31
IFN-α(2b)	8/5486	0.82 (0.77 to 0.88)	27.3		7/5379	0.92 (0.85 to 0.99)	0	
Combined	14/8122	0.81 (0.76 to 0.87)	0	—	12/7704	0.90 (0.84 to 0.97)	4.4	—
Comparator: observation	12/7135	0.83 (0.78 to 0.89)	6.3	.03	11/6824	0.91 (0.84 to 0.97)	4.8	.18
Comparator: GMK	2/987	0.65 (0.53 to 0.81)	0		1/880	0.73 (0.53 to 0.99)	0	
Combined	14/8122	0.82 (0.77 to 0.87)	78.7	—	12/7704	0.90 (0.84 to 0.96)	44.7	—
TNM stage II	2/810	0.70 (0.55 to 0.88)	0	.42	1/499	0.70 (0.50 to 0.98)	0	.36
TNM stage III	3/2144	0.82 (0.72 to 0.93)	26.3		3/2144	0.87 (0.68 to 1.11)	69.4	
TNM stages II–III	9/5168	0.82 (0.75 to 0.90)	0		8/5061	0.90 (0.83 to 0.98)	0	
Combined	14/8122	0.81 (0.75 to 0.87)	0	—	12/7704	0.89 (0.82 to 0.96)	1.2	—
HR reported	10/6548	0.85 (0.79 to 0.91)	0	.01	9/6441	0.92 (0.85 to 0.99)	5.6	.06
HR calculated	4/1574	0.68 (0.58 to 0.79)	0		3/1263	0.75 (0.62 to 0.92)	0	
Combined	14/8122	0.82 (0.77 to 0.87)	86.0	—	12/7704	0.90 (0.83 to 0.95)	70.4	—

* The mixed-effect model was applied to obtain summary effects within and across subgroups. TNM = TNM stage according to the American Joint Committee on Cancer (AJCC) melanoma classification system (9). All *P* values were calculated using two-sided tests. CI = confidence interval; GMK = ganglioside-based anti-melanoma vaccine; HR = hazard ratio; IFN-α = interferon alpha; — = not applicable.

Singularly taken, four of the 14 comparisons (n = 2110) found a statistically significant OS advantage in favor of patients allocated to the IFN-α arm (34,35,45,52). Our meta-analysis revealed a statistically significant reduction in the risk of death for patients undergoing IFN-α treatment (HR for death = 0.89, 95% CI = 0.83 to 0.96; $P = .002$), and we observed no statistically significant between-study heterogeneity ($P = 27$; $I^2 = 17.8\%$) (Figure 2). Almost identical results were observed by substituting the data of the original publications with those reported in the updated version (HR for death = 0.90; 95% CI = 0.83 to 0.96; $P = .003$). This also included the OS analysis of two more comparisons from RCT ECOG2696, whereas the original report analyzed only the effect on DFS.

The absence of a "dominant" study driving the results of meta-analysis was demonstrated by the "leave-one-out" procedure that generated overall hazard ratio estimates (range = 0.87–0.91) very similar to those obtained with all 14 comparisons ($P = .001$ to $P = .01$).

Upon subgroup analysis, RCTs that enrolled exclusively TNM stage III patients did not show a statistically significant OS benefit for the IFN-α arm (Table 2). We also did not detect any OS advantage for the IFN-α arm in RCTs that used high-dose IFN-α. Meta-regression did not show any statistically significant relationship between the overall effect and any of the parameters already listed in the previous paragraph.

Additional Analyses

The statistical power of the heterogeneity test is usually low in meta-analysis (63). Therefore, we also performed a meta-analysis using the random-effects model, which did not change the estimate of the risk for OS (HR for death = 0.89, 95% CI = 0.82 to 0.96; $P = .004$).

We also investigated publication bias, which was statistically significant (Begg rank correlation test $P = .04$; Egger regression test $P = .02$). Consequently, we calculated the number of potentially "missing" trials according to the above-mentioned trim-and-fill method. Two potentially missing studies were found to be necessary to obtain the funnel plot symmetry, but their inclusion in the meta-analysis did not change the overall value of the results (eg, for OS, HR for death = 0.92, 95% CI = 0.86 to 0.98; $P = .012$).

Discussion

In this meta-analysis, which is based on the largest number of patients ever considered, we found that IFN-α statistically significantly improves both DFS (risk reduction = 18%) and OS (risk reduction = 11%) of patients with high-risk cutaneous melanoma.

Two previous meta-analyses on the same subject reported a DFS advantage for high-risk melanoma patients receiving IFN-α; however, a statistically significant OS benefit was not detected (37,38). Pirard et al. (38) in their study of nine trials included the two trials with severe drawbacks in design (see Eligible Trials section), which we excluded from the present meta-analysis. These investigators also measured the risk of both disease recurrence and death by calculating odds ratios (ie, ratios between number of events), which is a notoriously inappropriate method to study survival data, which describe the time to an event of interest. This is especially

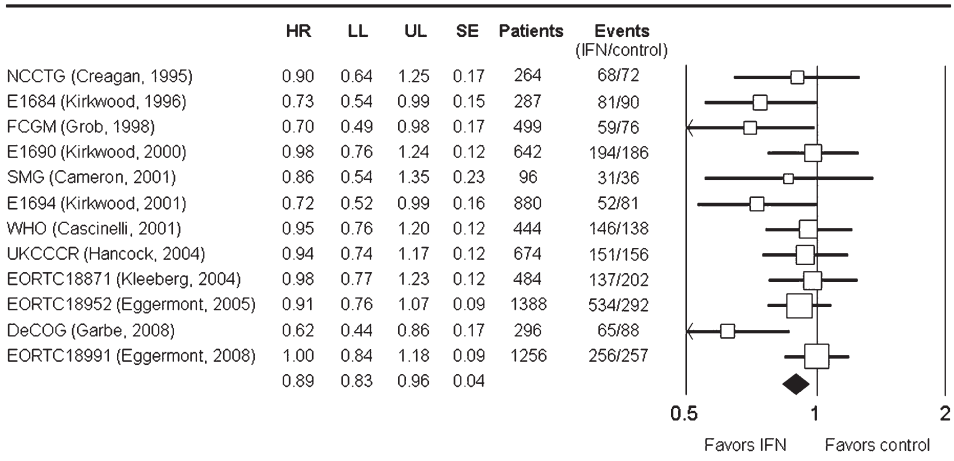

Figure 2. Forest plot of hazard ratios (HRs) (interferon alpha [IFN-α] vs control) for overall survival. **Squares** represent the hazard ratio of each single randomized controlled trial (RCT): The area is proportional to the weight in the meta-analysis according to the fixed-effect method, and the **horizontal line** represents the 95% confidence interval (CI). The **diamond** represents the estimated overall effect based on the fixed-effect meta-analysis of all RCTs (the **width of diamond** represents the 95% CI of the HR). LL = 95% confidence interval lower limit; UL = 95% confidence interval upper limit.

true when considering the OS data because in the long term, all patients of both comparison arms will die of disease or any other cause and thus no therapy-induced benefit could ever be detected using a statistics based exclusively on the number of events.

In another full-text meta-analysis, Wheatley et al. (37) used time-to-events data and found a DFS (but not OS) advantage using 12 RCTs that neither included the two more recent RCTs (24,25), nor the two trials we judged ineligible (26,27): Of note, for three included RCTs (21–23), the authors could not use the definitive data because the articles reporting them were published after the meta-analysis. The same authors have recently published an abstract describing an individual patient's data meta-analysis on the same subject (64): Considering 13 RCTs and 6067 patients (individual patient's data available for 85% of patients), these investigators could detect an IFN-α–mediated benefit in terms of both DFS (OR = 0.87, 95% CI = 0.81 to 0.93) and OS (OR = 0.9, 95% CI = 0.84 to 0.97), which supports the findings of the present study that is based on a larger sample size (n = 8122).

Although this meta-analysis shows and quantifies for the first time the OS benefit associated with IFN-α administered to patients with high-risk melanoma, our findings must be interpreted in the light of some limitations. For instance, the above-reported positive findings cannot be considered satisfying in terms of anticancer efficacy. Considering a 5-year OS rate of 60% for patients with stages II–III cutaneous melanoma (7), the number needed to treat (65) is approximately 29 patients (95% CI = 18–81 patients). Therefore, much more effort is needed to identify patients who are most likely to benefit from IFN-α adjuvant therapy and to identify more effective agents. Nevertheless, given the current lack of any other effective antimelanoma therapy, these data lend support to the use of IFN-α in the routine clinical setting to provide patients with the best chance of survival. In this regard, we must remember that other well-established adjuvant treatments, such as those routinely administered to patients with breast, colorectal, and ovarian carcinomas, are associated with analogous risk reductions (42).

Although between-study heterogeneity was not statistically significant, we cannot overlook the fact that RCTs with apparently identical design have produced different results. This discrepancy could be explained by taking into account that the number of patients enrolled by a single RCT was not always sufficient to unravel the relatively small OS advantage shown by this meta-analysis. Moreover, some comparisons might be undermined by the crossover phenomenon, as hypothesized for RCT E1690, where 31% patients randomly assigned to observation received IFN-α upon disease recurrence (16). Finally, most RCTs analyzed the effect of IFN-α on the OS and not on the DFS. This implies that longer the follow-up, lower is the likelihood of detecting a statistically significant difference between treatment and observation groups because of deaths by competing causes (62).

Randomized clinical trials demand that we adopt a regimen for the use IFN-α in the adjuvant treatment of melanoma. Another limitation in our analysis was it could not answer whether one regimen (among the ones tested thus far within the frame of RCTs) was better than the other. Also, with respect to the IFN-α dosage, findings from the subgroup analysis do not indicate a clear advantage or disadvantage of high vs low or intermediate IFN-α dose. Although the impact on OS remains statistically significant only when considering trials that used low or intermediate IFN-α dose, this is contradictory to the results of the only RCT in this direction (46), which compared high vs low IFN-α dose, and showed that low or intermediate IFN-α regimen was associated with advantage in DFS, but not in OS, over observation (16).

Concomitantly, we could not define whether the duration or total dose of IFN-α treatment affects its efficacy. A report by Eggermont et al. (23) showed that an intermediate dose of IFN-α prolonged DFS only when administered for 2 years, compared with 1 year, but had no effect on OS. However, the other three RCTs did not detect any statistically significant difference between the following: 1) the original high-dose IFN-α full regimen described by Kirkwood et al. (13) vs an intermittent schedule (34), 2) the high-dose full regimen (induction plus maintenance) vs the induction phase alone (6), and 3) a low-dose IFN-α regimen for 18 months vs the same regimen for 60 months (35). Therefore, in this regard, our findings were in agreement with three out of the four available RCTs that tested different IFN-α regimens. However, we must remember that these additional analyses (ie, subgroup analysis and meta-regression) are just exploratory in nature and that only direct comparisons within the frame of RCT (designed in the light of the current knowledge on this subject) will be able to appropriately address these issues.

There is clearly an urgency to identify more patients who are likely to benefit from the IFN-α adjuvant therapy. However, we would like to comment that the quality of some of the trials retrieved while performing this systematic review was disappointing. We have already mentioned that two trials were excluded from the meta-analysis because of poor quality of data analysis and presentation. We believe that the peer-review system should not allow the publication of articles dealing with survival data without an appropriate survival analysis, which makes the unfortunate experience of enrolled patients virtually of no value for the development of more effective therapies. The eligible RCTs, although sound in study design, did not always have a satisfactory method of reporting. As a matter of fact, multivariable data analysis (HR, 95% CI, *P* value) was not always reported in full, which did not allow us to directly incorporate these adjusted values in the meta-analysis, and in such cases, unadjusted values from univariate analysis were used. The lack of standardization of clinical trial reports was underscored in a recent survey (66). This not only restricts the full comprehension of the practical relevance of the trial itself but also compromises the accuracy of the pooled data summary while performing a meta-analysis.

To address this issue, Journals should make it mandatory for the authors to report an easy-to-obtain set of minimum required data, such as the number of events and the number of patients at risk at different time points; hazard ratio, confidence interval, and *P* values for both univariate and multivariable survival analyses; study design parameters (eg, sample size calculation, statistical power, and minimum detectable survival difference), and patients characteristics [eg, among the trials considered in this meta-analysis, the number of patients per TNM stage was missing in two reports (17,20)].

Despite a few problems and limitations, we were able to demonstrate a statistically significant benefit of IFN-α adjuvant treatment, in terms of both DFS and OS, by pooling the summary data of 14 RCTs (n = 8122) in this meta-analysis. This provides the physicians with the evidence necessary to routinely consider IFN-α in the treatment of high-risk melanoma patients. These positive findings further justify any effort to identify the subset of patients who would most benefit from this treatment to maximize its therapeutic index. It is also essential to understand the underlying molecular mechanisms responsible for sensitivity to IFN-α, and recent reports in this direction are opening new avenues to the patient-tailored adjuvant therapy of melanoma (1,67–69).

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Affiliation of authors: Department of Oncological and Surgical Sciences, Meta-analysis Unit, University of Padova, Padova, Italy (SM, SP, CRR, DN).