A Pooled Analysis of Eastern Cooperative Oncology Group and Intergroup Trials of Adjuvant High-Dose Interferon for Melanoma

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ABSTRACT

Purpose: **Nearly 2000 patients with stage IIB and III melanoma have participated in four multicenter, randomized trials, conducted by the Eastern Cooperative Oncology Group and the Intergroup, investigating adjuvant high-dose IFN-α 2b therapy. The objectives of this study were to update the analyses of each individual trial and to analyze prognostic factors and treatment effects based on pooled data.**

Experimental Design: **Survival and disease status were updated to April 2001. Analysis of prognostic factors using optimized statistical models was based on data from patients in E1684, E1690, E1694, and E2696. Analysis of treatment effects *versus* observation (Obs) was based on data from 713 patients randomized to high-dose IFN-α 2b (HDI) or Obs in Trials E1684 and E1690.**

Results: **Updated analysis of E1684, E1690, and E1694 confirmed their original conclusions, now at median follow-up intervals of 2.1–12.6 years. Based on two-sided univariate log-rank analysis of pooled data from E1684 and E1690 (median follow-up, 7.2 years), relapse-free survival (RFS)—but not overall survival (OS)—was significantly prolonged (two-sided log-rank *P* value = 0.006) for patients treated with HDI *versus* Obs. Among all patients, prognostic factors that significantly negatively impacted RFS and OS included ulceration, recurrent disease at entry, enrollment in E1684, and age > 49 years. Multivariate statistical models adjusting for these factors confirmed the statistically significant RFS benefit of HDI *versus* Obs but did not demonstrate a significant OS benefit in the pooled populations.**

Conclusions: **In patients with high-risk resected melanoma, HDI is effective adjuvant therapy with strong evidence for improved RFS and evidence for moderate improvement in OS based on two prospective randomized studies but not the pooled analysis. Analyses of predictors of relapse and response are now needed to improve the therapeutic value of this modality.**

INTRODUCTION

The Eastern Cooperative Oncology Group (ECOG) and Intergroup trials of adjuvant treatment of melanoma conducted over the past 15 years have examined the effect of treatment on relapse-free survival (RFS) and overall survival (OS) in patients with American Joint Committee on Cancer (AJCC) stage IIB or III melanoma. In these trials, patients were randomized to receive high-dose IFN-α 2b (HDI), low-dose IFN-α 2b (LDI), observation (Obs), or the ganglioside GM2/keyhole limpet hemocyanin vaccine (GMK). The ECOG Trial E1684 was a randomized comparison of HDI *versus* Obs; Intergroup Trial E1690 was a randomized comparison of HDI and LDI *versus* Obs; and Intergroup Trial E1694 was a randomized comparison of the GMK vaccine *versus* HDI. The ECOG Trial E2696 was a randomized, Phase II trial of GMK alone *versus* GMK with either concurrent or sequential HDI. Based on the data from these trials, it has been shown that HDI (20 million International Units/m^2/day i.v. × 4 weeks; 10 million International Units/m^2 3 times a week s.c. × 48 weeks) is the most effective and rigorously evaluated adjuvant therapy for high-risk melanoma.

A total of 287 patients were accrued to E1684 between 1984 and 1990, and the results of this trial were first reported in 1996 with a median follow-up interval of 6.9 years (range, 0.6 to 9.6 years; Ref. 1). Eligible patients randomized to 52 weeks of HDI had significantly improved RFS and OS compared with Obs. The median RFS was 1.72 years in the HDI arm *versus* 0.98 year in the Obs arm [stratified log-rank one-sided *P* value (P_1) = 0.0023], and the median OS was 3.82 *versus* 2.78 years (P_1 = 0.0237), respectively. These data supported regulatory approval of the HDI regimen for adjuvant therapy in patients with stage IIB and stage III melanoma.

E1690 accrued 642 patients between 1991 and 1995 and was first reported in 2000 with a median follow-up interval of 4.3 years (2). This trial was designed using a cure-rate model derived from E1684 data, and the impact of treatment with HDI and LDI *versus* Obs was estimated by comparing the hazard

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ratio (HR) for relapse or death between treatment groups. In the intent-to-treat analysis of RFS, treatment with HDI was associated with a statistically significant benefit compared with Obs (HR = 1.28; P_1 = 0.025). Moreover, this benefit was maintained after adjusting for significant prognostic factors, including stage of disease and number of positive lymph nodes (P_1 = 0.015). In contrast, LDI was not associated with a significant RFS benefit compared with Obs. Neither the HDI nor LDI regimen had an apparent impact on OS compared with Obs in this trial. However, a retrospective analysis of salvage therapy suggested a disproportionate crossover of patients from the Obs arm to HDI therapy off protocol at the time of regional recurrence (stage IIB patients in this trial were not required to undergo lymphadenectomy), which may have confounded the survival analysis.

Intergroup trial E1694 accrued 880 patients between 1996 and 1999 and was designed to test whether GMK was superior to HDI. This trial was closed early, based on an interim analysis in April 2000 indicating that GMK was significantly inferior to HDI for both relapse and mortality end points. The results of this trial were reported in 2001 based on a final analysis in June 2000, with a median follow-up interval of 16 months (3). Among eligible patients in this trial, HDI provided a statistically significant RFS benefit (HR = 1.47; P_1 = 0.0015) and OS benefit (HR = 1.52; P_1 = 0.009) compared with GMK. A similar benefit was observed in the intent-to-treat analysis of RFS (HR = 1.49) and OS (HR = 1.38). Cox regression analysis adjusting for gender, ECOG performance status, nodal status, and age also demonstrated a statistically significant RFS and OS benefit for HDI *versus* GMK.

Finally, ECOG Trial E2696 was a randomized, Phase II trial that enrolled 107 patients with resected stage IIB, stage III, and stage IV disease, (including patients with resectable in-transit metastases or extracapsular extension of nodal disease formerly AJCC designated stage IV, M1 disease but currently classified as AJCC stage IIIC disease; Ref. 4). Patients were randomized to GMK plus concurrent HDI (Arm A), GMK plus sequential HDI (Arm B), or GMK alone (Arm C). The RFS analysis at a median follow-up interval of 24 months demonstrated that the combination of GMK plus HDI reduced the risk of relapse compared with GMK alone (HR = 1.75 for Arm C *versus* Arm A; HR = 1.96 for Arm C *versus* Arm B; Ref. 5). This treatment benefit was statistically significant after adjusting for gender, performance status, time to resection, nodal status, and age [two-sided log-rank *P* value (P_2) = 0.016 for Arm A *versus* Arm C; P_2 = 0.03 for Arm B *versus* Arm C].

The relapse and survival status of patients enrolled in these four studies have been recently updated to April 2001, and the original end points have been reanalyzed. The updated data from these studies also formed the basis of a pooled analysis to identify factors prognostic for clinical outcome based on the aggregate experience and to perform a multivariate analysis of the RFS and OS benefits of adjuvant HDI in patients with stage IIB and stage III melanoma. One immunological analysis of potential markers of dose and response to IFN has been completed (6), and additional studies are ongoing to establish additional prognostic factors as well as predictors of antitumor response with HDI.

PATIENTS AND METHODS

The survival and disease status of patients enrolled in four randomized trials of adjuvant treatment of melanoma conducted or coordinated by ECOG (E1684 and E2696) and Intergroup (E1690 and E1694) between 1985 and 2000 continue to be actively followed. The most current relapse and survival data as of April 2001 were extracted from the ECOG database, and these updated results were then pooled and analyzed to identify factors with prognostic significance and to assess treatment effects, adjusting for significant prognostic factors.

Patients. Eligibility criteria for the four studies were relatively consistent. Patients were required to have deep primary tumors (>4 mm) in the absence of lymph node involvement (AJCC stage IIB disease) or regional lymph node involvement (AJCC stage III disease) at either presentation or recurrence. Patients entered on E2696, which was open concurrently with E1694, could also have had in-transit skin or s.c. lesions beyond the site of the primary or extracapsular extension of lymph node metastases (AJCC stage IIIC or stage IV disease; Ref. 4). For all studies, there was complete pathology review in the ECOG Melanoma Committee Pathology Office at the University of Pittsburgh. Patients must have had definitive wide excision with adequate margins, and in E1684, all patients were required to undergo complete regional lymphadenectomy. Surgery, lymphadenectomy, and randomization must have been performed within a reasonable time period (56–70 days). Patients entered on E2696 must have been ineligible for E1694, so they were required to be at least 70 days but no more than 365 days after surgery. Patients were required to have adequate hematological and end organ function. For the vaccine studies, patients could not have evidence of autoimmune disorders or history of a severe reaction to shellfish. Patients could have had no prior chemotherapy, radiotherapy, or immunotherapy and were required to have an ECOG performance status of ≤1 and to be at least 18 years of age. Early studies excluded patients over 70 years of age.

All patients randomized to any treatment were considered for inclusion in the analysis of prognostic factors. For the multivariate analysis of treatment effects associated with HDI, only patients randomized to HDI or Obs in E1684 and E1690 were included in the analysis.

Statistical Methods. Median RFS and OS times were computed. The log-rank test was used to analyze differences in RFS and OS between the different categories for each prognostic factor. Two-sided tests at the 0.05 level were considered statistically significant. The method of Kaplan and Meier (7) was used to estimate survival functions over time. Proportions of patients in each category for all prognostic factors were computed, along with the proportion of patients with missing information.

From information available at baseline, important patient characteristics available for at least 85% of patients were selected. Most of these were treated as dichotomous variables. Continuous variables were split at the median to generate a dichotomous variable. To categorize patients' serum lactate dehydrogenase as normal or abnormal, the upper limits of relevant laboratory normal values for serum lactate dehydrogenase were used when available. When the upper limits of normal

were unknown, the median known value of 225 units/ml was used. An indicator variable for each clinical protocol was incorporated. Cox proportional hazard models (8) were developed to examine prognostic factors for RFS and OS and treatment effects on RFS and OS, adjusting for significant prognostic factors. To ensure that models would be comparable, only those patients with nonmissing values for all variables were included in the variable selection algorithm. Because it was not feasible to evaluate all possible models, a forward selection method of automatic variable selection using the likelihood ratio statistic (Statistical Analysis System package, version 8.1) was used as the measure of optimal model fit. Backward elimination as an alternative to forward selection and an examination of Akaike's Information Criterion and the Schwartz Bayesian Criterion, in addition to the likelihood ratio statistic, were also used to assess model fit. In all cases, the optimal model satisfied multiple criteria. Optimal models were selected from among the candidates provided by these statistical methods, favoring the more parsimonious models. From the optimal models, the HR for each term and its 95% confidence interval and the P_2 for statistical significance are reported. Final models were calculated with data from those patients with nonmissing values for the selected covariates. This was a somewhat larger subset than the subset used for variable selection.

RESULTS

A total of 1916 patients were randomized in these four cooperative group trials. Three patients, who represented duplicate registrations, were excluded along with one patient whose survival status was unknown. Therefore, 1912 patients were potentially available for the analysis of prognostic factors. Patient outcome within each trial is summarized in Table 1 (1–3, 5). Patient status is shown as either Failed/Died, thus counting as an event in the analysis of RFS, or Died, thus counting as an event in the analysis of OS. These data represent a survival update at a median follow-up of 12.6 years for E1684, 6.6 years for E1690, 2.1 years for E1694, and 2.8 years for E2696.

Table 1 Patient outcome by protocol based on updated survival data

Trial	E1684	E1690	E1694	E2696	Total
Median follow-up (yrs)	12.6	6.6	2.1	2.8	3.5
Observation, *N*	140	212			352
Failed/died	106	128			234
Died	95	103			198
High-dose IFN-α2b, *N*	146	215	438		799
Failed/died	95	120	157		372
Died	93	108	102		303
GMK,[a] *N*			439	35	474
Failed/died			202	20	222
Died			127	9	136
Other,[b] *N*		215		72	287
Failed/died		129		33	162
Died		104		16	120
Total	286[c]	642	877[c]	107	1912

[a] GMK, GM2/keyhole limpet hemocyanin vaccine.
[b] Low-dose IFN α-2b or GMK + concurrent or sequential high-dose IFN α-2b.
[c] One patient in E1684 provided no follow-up; 3 patients in E1694 were duplicate registrations.

Table 2 Patient baseline and disease characteristics ($N = 1912$)

		N	%
Gender	Female	700	36.6
	Male	1211	63.3
	Unknown	1	0.1
Race	White	1871	97.8
	Black	14	0.7
	Other/unknown	15	0.5
	Missing	12	0.5
ECOG[a] performance status	0	1580	82.6
	1	329	17.2
	Unknown	3	0.2
Site of primary tumor	Head/neck	232	12.1
	Upper limb	265	13.8
	Lower limb	396	20.7
	Trunk	828	43.3
	Other	80	4.2
Pigmentation	Amelanotic	132	6.9
	Melanotic	1417	74.1
	Unknown	363	19.0
Ulceration	No	1046	54.7
	Yes	601	31.4
	Unknown	265	13.9
Disease at study entry	Primary	994	51.9
	Recurrent	913	47.8
	Unknown	5	0.3
Micrometastases	No	312	16.3
	Yes	24	1.3
	Unknown	1576	82.4
Satellite metastases	No	396	20.7
	Yes	29	1.5
	Unknown	1487	77.8
Extranodal extension	None/microscopic	512	26.8
	Yes/extensive	132	6.9
	Unknown	1268	66.3
Tumor thickness	<3 mm	859	44.9
	≥3 mm	864	45.2
	Unknown	189	9.9
Age	≤49 yrs	962	50.3
	>49 yrs	948	49.5
	Unknown	2	0.2
Primary tumor stage	T1	254	13.3
	T2	405	21.1
	T3	358	18.7
	T4	706	36.9
	Unknown	189	9.9
Nodal stage	N0	117	6.1
	N1	547	28.6
	N2	229	12.0
	N3	182	9.5
	Unknown	837	43.8
Size of primary tumor	<1.44 mm	721	37.7
	≥1.44 mm	726	38.0
	Unknown	465	24.3
Size of largest lymph node	<0.2 mm	202	10.6
	≥0.2 mm	368	19.2
	Unknown	1342	70.2
WBC count	$<6.9 \times 10^9$/liter	885	46.3
	$\geq 6.9 \times 10^9$/liter	936	49.0
	Unknown	91	0.5
Lactate dehydrogenase	Normal	1453	76.5
	Abnormal	278	14.5
	Unknown	181	8.9

[a] ECOG, Eastern Cooperative Oncology Group.

Patient Characteristics

Patient baseline and disease characteristics are summarized in Table 2 (1–3, 5). Because the studies span a significant time period, the characteristics collected at baseline varied some-

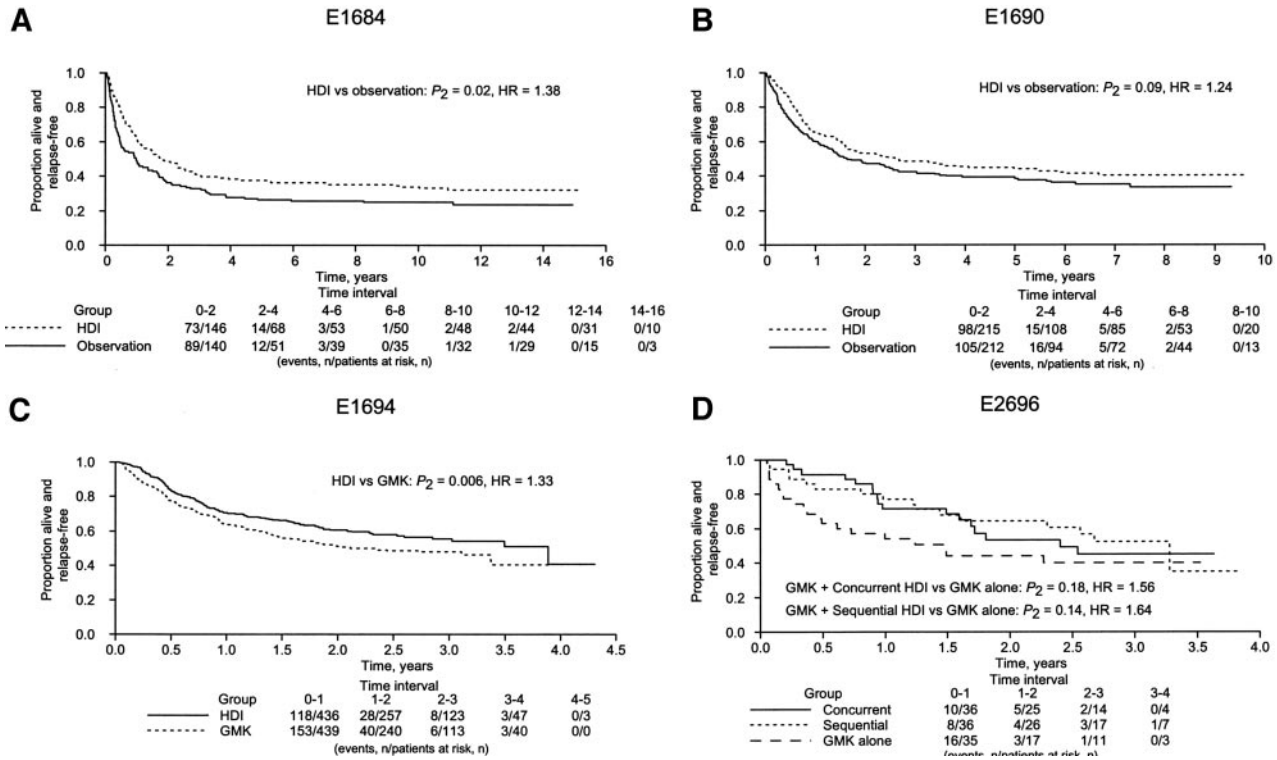

Fig. 1 Kaplan-Meier estimates of relapse-free survival for patients treated on (*A*) E1684 at median follow-up of 12.6 years, (*B*) E1690 at a median follow-up of 6.6 years, (*C*) E1694 at a median follow-up of 2.1 years, and (*D*) E2696 at a median follow-up of 2.8 years. *HDI*, high-dose IFN-α 2b; *HR*, hazard ratio; *GMK*, GM2/keyhole limpet hemocyanin.

what. This was the source of most missing data. Median age was 49 years (range, 17–85 years). Most patients (63%) were male, and almost all (98%) were white. The most common site of primary disease was the trunk (43%). Approximately half of patients had recurrent disease at study entry, and 31% had ulcerated primary tumors. Most patients (83%) had an ECOG performance status of 0.

Outcome by Clinical Trial

Results of the individual clinical trials included in this pooled analysis have been published previously (1–3, 5). Results reported here are based on the survival and disease status update.

E1684. The updated Kaplan-Meier estimate of RFS for patients enrolled in E1684 at a median follow-up of 12.6 years is shown in Fig. 1*A*. The significant clinical benefit of HDI *versus* Obs with respect to RFS is still evident (HR = 1.38; P_2 = 0.02). It bears note that because all patients entering E1684 were required to undergo regional lymphadenectomy, this outcome also represents "distant disease-free survival," which has been adopted as a surrogate for OS in some cooperative groups. With additional follow-up, the improvement in OS associated with HDI *versus* Obs (Fig. 2*A*) has diminished somewhat (HR = 1.22; P_2 = 0.18), which may be caused by deaths from competing causes in an aging study population that is now well into the seventh decade of life (median age is now >60 years).

E1690, E1694, and E2696. At a median follow-up of 6.6 years for patients treated in E1690, the RFS benefit associated with HDI (Fig. 1*B*) shows a trend toward statistical significance (HR = 1.24; P_2 = 0.09), and there continues to be no apparent OS benefit for HDI *versus* Obs as originally reported (Fig. 2*B*). In E1694, at a median follow-up of 2.1 years, HDI continues to demonstrate superiority to the GMK vaccine in terms of both RFS (HR = 1.33; P_2 = 0.006) and OS (HR = 1.32; P_2 = 0.04) as shown in Figs. 1*C* and 2*C*, respectively. The Kaplan-Meier estimates for RFS and OS in E2696 are shown in Figs. 1*D* and 2*D*, respectively. The aggregate mature data from these four trials provide the highest level of evidence demonstrating the clinical benefit of HDI.

Log-Rank Assessment of Treatment Effect (Pooled Analysis)

In the two-sided univariate log-rank comparison of HDI *versus* Obs based on 713 patients enrolled in the Obs-controlled trials E1684 and E1690 (median follow-up of 7.2 years), HDI was superior to Obs with respect to RFS (HR = 1.30; P_2 < 0.006). However, this analysis did not demonstrate a significant OS benefit associated with HDI (HR = 1.08; P_2 = 0.42). The Kaplan-Meier analysis of these pooled data are shown in Fig. 3.

Univariate Analyses of Prognostic Factors

Table 3 (1–3, 5) shows the number of patients who were alive and alive without relapse based on important prognostic

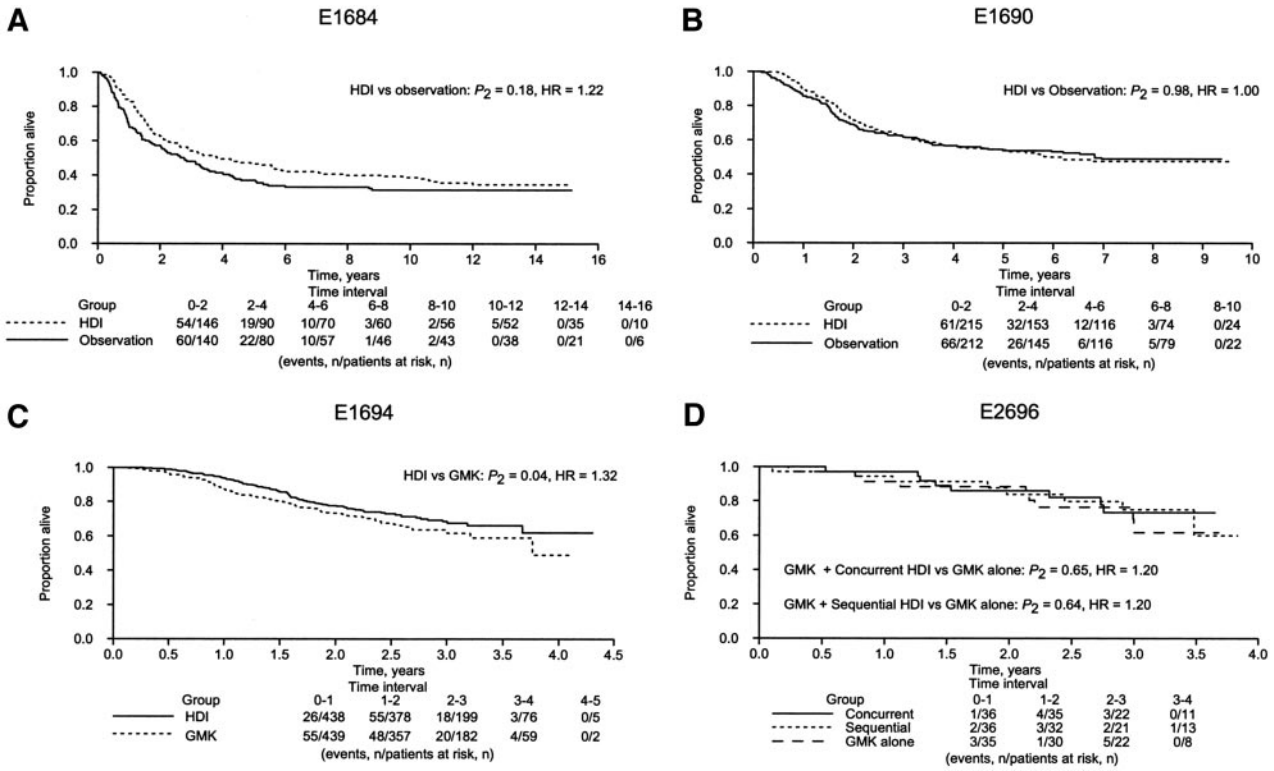

Fig. 2 Kaplan-Meier estimate of overall survival for patients treated on (*A*) E1684 at median follow-up of 12.6 years, (*B*) E1690 at a median follow-up of 6.6 years, (*C*) E1694 at a median follow-up of 2.1 years, and (*D*) E2696 at a median follow-up of 2.8 years. *HDI,* high-dose IFN-α 2b; *HR,* hazard ratio; *GMK,* GM2/keyhole limpet hemocyanin.

factors. Also shown are median RFS and OS with two-sided log-rank *P*s, indicating which variables were significantly correlated with clinical outcome. Median RFS for all patients was 2.3 years (95% confidence interval, 1.9–2.7 years). Median OS for all patients was 6.2 years (95% confidence interval, 5.1–8.8 years). In univariate analyses, the following factors were significant predictors of improved RFS: enrollment in a protocol other than E1684; primary tumor of the upper limb; absence of ulceration; primary disease at study entry; absence of micrometastases; absence of microscopic extranodal extension; Breslow thickness < 3 mm; age ≤ 49 years; absence of nodal disease; size of primary tumor < 1.44 mm; largest tumor focus of an involved lymph node < 0.2 mm; and normal lactate dehydrogenase at study entry. Similarly, in univariate analyses, the following factors were significant predictors of improved OS: enrollment in a protocol other than E1684; female gender; upper limb as primary site; absence of ulceration; primary disease at study entry; absence of micrometastases; absence of extranodal extension; age ≤ 49 years; absence of nodal disease; and tumor size < 1.44 mm.

Multivariate Models of Prognostic Factors

Based on the proportions of patients with missing values and the prognostic significance of factors observed in the univariate analysis, 11 factors were selected for testing in the multivariate models. Of the 1912 patients available for inclusion, 1418 patients had no missing values for these 11 candidate variables and thus contributed information. Table 4 (1) shows the model of prognostic factors for RFS. Patients whose primary site was the upper limb had improved RFS compared with patients whose primary site was elsewhere (HR = 0.73). The following factors (in order of significance) were prognostic for decreased RFS: recurrent disease at study entry; ulceration of the primary tumor; enrollment in E1684; abnormal lactate dehydrogenase; Breslow thickness ≥ 3 mm; and age > 49 years. Table 5 (1) shows the model of factors prognostic for OS. The following factors (in order of significance) were prognostic for decreased OS: ulceration of the primary tumor; enrollment in E1684; recurrent disease at study entry; and age > 49 years.

Effect of Treatment Adjusting for Prognostic Factors

The effect of treatment with HDI *versus* Obs was evaluated in these multivariate models based on information from 713 patients enrolled in E1684 and E1690 and randomized to HDI or Obs. These models adjusted for ulceration, recurrent disease at study entry, enrollment in E1684, and age >49 years, which were the most highly significant prognostic factors predictive of worse clinical outcome.

The model assessing the effects of treatment on RFS, adjusting for ulceration (HR = 1.44), enrollment in protocol E1684 (HR = 1.37), and recurrent disease at study entry (HR = 1.25),

Table 3 Univariate analysis of prognostic factors

		Alive and relapse free	Median RFS (yrs)[a]	2-sided log-rank *P*	Alive	Median OS (yrs)	2-sided log-rank *P*
Total patients, *N*	1912	920	2.3		1155	6.2	
Trial[b,c]	E1684	85	1.4	0.0001	98	3.2	<0.0001
	E1690	265	2.3	0.84	327	7.0	0.60
	E1694	516	3.1	0.003	648	3.8	0.008
	E2696	54	2.5	0.84	82	3.5	0.01
Gender[b]	Female	356	3.0	0.04	446	11.0	0.02
	Male	564	2.0		709	5.7	
Race	White	907	2.4	0.38	1,131	6.5	0.87
	Black	6	1.3		8	2.3	
	Other/unknown	4	1.7		11	4.1	
ECOG PS[b]	0	758	2.4	0.57	944	6.2	0.84
	1	162	2.3		211	7.1	
Site of primary tumor[b,c]	Head/neck	120	3.4	0.17	157	8.7	0.01
	Upper limb	145	5.0	0.003	170	11.0	0.06
	Lower limb	171	1.6	0.06	229	5.8	0.42
	Trunk	379	2.1	0.33	471	5.0	0.09
	Other	32	1.3	0.13	38	2.8	0.06
Pigmentation	Amelanotic	66	3.4	0.21	79	6.8	0.43
	Melanotic	661	2.1		829	6.0	
Ulceration[b]	No	532	3.1	<0.0001	653	9.2	<0.0001
	Yes	238	1.4		315	3.5	
Disease status at entry[b]	Primary	548	3.4	<0.0001	672	8.0	<0.0001
	Recurrent	372	1.6		481	4.4	
Micrometastases	No	134	2.8	<0.0001	152	6.2	0.007
	Yes	2	0.9		6	2.7	
Satellite metastases	No	217	2.9	0.62	278	3.8	0.40
	Yes	15	2.0		18	3.0	
Extranodal extension	None/microscopic	256	3.0	0.001	325	7.7	0.0003
	Yes/extensive	47	0.9		58	2.9	
Tumor thickness[b]	<3 mm	428	2.5	0.13	526	8.8	0.20
	≥3 mm	386	2.0		494	5.2	
Age[b]	≤49 yrs	484	2.7	0.02	604	9.2	0.004
	>49 yrs	435	1.9		550	4.7	
Tumor stage[b,c]	T1	128	2.4	0.56	161	6.3	0.27
	T2	206	2.5	0.27	245	7.7	0.78
	T3	158	1.8	0.34	204	6.2	0.32
	T4	322	2.3	0.56	410	5.5	0.82
Nodal stage[c]	N0	68	7.0	0.0005	79	12.0	0.003
	N1	282	3.4	<0.0001	355	10.8	<0.0001
	N2	77	1.6	0.003	103	3.6	0.002
	N3	48	0.9	<0.0001	67	2.3	<0.0001
Size of primary tumor	<1.44 mm	369	3.2	<0.0001	441	7.1	0.004
	≥1.44 mm	284	1.6		379	3.8	
Size of largest lymph node	<0.2 mm	107	3.4	0.01	133	8.8	0.11
	≥0.2 mm	165	1.9		218	6.2	
WBC count[b]	$<6.9 \times 10^9$/liter	438	2.5	0.18	555	7.3	0.11
	$\geq 6.9 \times 10^9$/liter	441	2.1		549	6.2	
LDH[b]	Normal	714	2.6	0.02	879	6.8	0.35
	Abnormal	124	1.7		173	5.2	

[a] RFS, relapse-free survival; OS, overall survival; ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase.
[b] Variable tested in the multivariate model.
[c] Each category tested against all others.

demonstrated a statistically significant increased risk of relapse or death associated with Obs compared with HDI (HR = 1.28; P_2 = 0.01). The model assessing the effects of treatment on OS, adjusting for enrollment in E1684 (HR = 1.68), ulceration (HR = 1.64), age > 49 years (HR = 1.32), and recurrent disease at study entry (HR = 1.22), demonstrated an increased risk of death with Obs compared with HDI that was not statistically significant (HR = 1.07; *P* = 0.52).

DISCUSSION

The updated analysis of each individual, prospectively randomized trial has confirmed the results as they were originally reported. This is the highest level of evidence supporting the clinical benefit of HDI with respect to both RFS and OS. Moreover, the significant RFS benefit observed in E1684, now at a median follow-up of 12.6 years, can be interpreted as distant disease-free survival benefit because all patients received com-

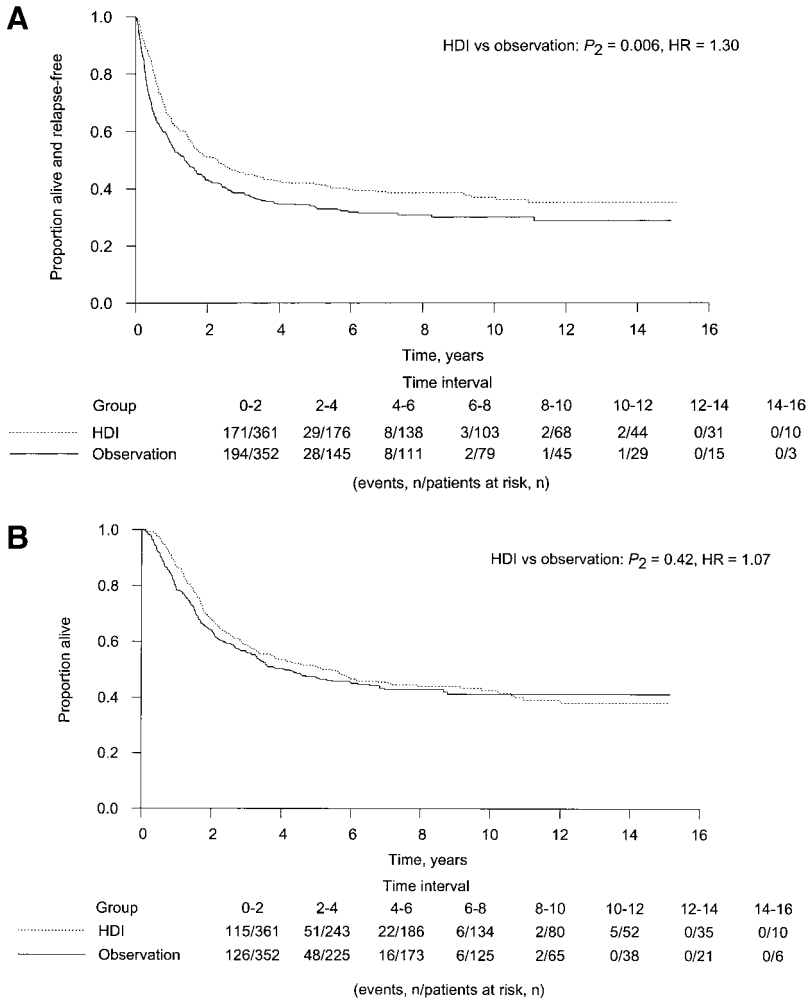

Fig. 3 Kaplan-Meier estimate of (*A*) relapse-free survival and (*B*) overall survival by treatment for patients on E1684 and E1690 based on the updated and pooled analysis (n = 713). *HDI*, high-dose IFN-α 2b; *HR*, hazard ratio.

Table 4 Proportional hazards model of prognostic factors for relapse-free survival

Variable	Hazard ratio	95% confidence interval	2-sided log-rank *P*
Recurrent disease at study entry	1.57	1.33–1.84	<0.0001
Ulceration	1.54	1.32–1.79	<0.0001
E1684	1.34	1.10–1.62	0.004
Abnormal LDH[a]	1.26	1.04–1.53	0.02
Tumor thickness ≥ 3 mm	1.23	1.04–1.46	0.02
Age > 49 yrs	1.20	1.04–1.39	0.01
Primary site = upper limb	0.73	0.59–0.91	0.005

[a] LDH, lactate dehydrogenase.

plete lymphadenectomy. Distant disease-free survival has been adopted as a surrogate for OS in trials conducted by other cooperative groups. In the univariate analysis of treatment effects based on the updated and pooled data, patients treated with HDI exhibited significantly improved RFS compared with Obs. This was observed in univariate analyses and confirmed in multivariable analyses after adjusting for significant covariates. However, neither the univariate nor the multivariate analysis of the pooled data demonstrated a statistically significant OS benefit of HDI *versus* Obs. This is not surprising, given that the larger of the two trials included in this analysis (E1690) did not show an OS benefit for HDI *versus* Obs in the primary analysis of that trial. As discussed previously, the OS analysis of E1690 was confounded by the unusually prolonged postrelapse survival of patients in the Obs arm (2), which is likely also a factor in the OS analysis reported here and potentially attributable to crossover use of IFN in patients originally assigned to Obs. Patients treated with HDI in E1694 have not been included in

Table 5 Proportional hazards model of prognostic factors for overall survival

Variable	Hazard ratio	95% confidence interval	2-sided log-rank *P*
Ulceration	1.73	1.45–2.05	<0.0001
E1684	1.71	1.39–2.11	<0.0001
Recurrent disease at study entry	1.48	1.25–1.76	<0.0001
Age > 49 yrs	1.28	1.09–1.51	0.003

the pooled analysis because the comparator in that trial was the GMK vaccine.

Several patient and disease characteristics were found to be important prognostic factors for RFS and OS. Patients who presented with ulceration of the primary tumor, patients enrolled on protocol E1684, and patients with recurrent disease at study entry did more poorly in terms of RFS and OS in every model. The significance of enrollment in E1684 as a prognostic factor may indicate that patients treated in the late 1980s did uniformly more poorly than those treated more recently. Evidence in support of this hypothesis includes a statistically significant difference in RFS and OS when comparing the Obs arm of E1684 with the Obs arms of E1690 and the vaccine arm of E1694. Enrollment in E1684 might also be an indicator of a shift in staging. It may reflect growing sophistication in characterizing the population of patients intended to be eligible for clinical trials. To ensure that this factor did not render the multivariable models otherwise inapplicable, models were rerun without this factor, and the results and conclusions were similar.

In conclusion, multivariate models, adjusting for important prognostic factors, have confirmed the durable RFS benefit of HDI compared with Obs based on mature data from this large aggregate database. In contrast, the multivariate models have not provided further evidence of an OS benefit associated with HDI. The most stringent evidence for clinical decisions emerges from properly constructed and appropriately powered randomized, controlled trials. Two separate randomized controlled trials of HDI have demonstrated a significant survival benefit associated with HDI *versus* Obs in E1684 and with HDI *versus* GMK in E1694. Although the results of the aggregate experience with HDI are instructive in relation to prognostic factors that bear on clinical outcome, the outcomes of the individual trials provide the most rigorous evidence of treatment effect. Translational studies have recently identified markers of immune response that appear to be dose-related (6), and additional studies are now under way to define more precise predictors of relapse and response that will enable us to improve the therapeutic value of this modality.

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