

Adjuvant treatment of malignant melanoma with human leukocyte interferon after radical surgery: I. general analysis

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In our randomized prospective study, patients with malignant melanoma were treated with human leukocyte interferon (HLI) after surgical removal of primary tumor (Clark level of invasion IV, V and/or thickness exceeding 1.5 mm). They were randomized in two groups: (1) those treated with HLI and (2) a control group with no immediate treatment. HLI was applied through 30 weeks in cumulative dose 6×10^7 U, and 2×10^6 U weekly. Both arms of the study included altogether 321 patients. The results of 5-year analysis showed significant differences in disease-free interval as well as in survival between both groups in favour of HLI treated patients ($p < 0.005$). In the treated group the rate of NED patients was significantly higher than in the control group. According to the stratification by sex, the difference was significant also between female as well as male patients of both groups ($p < 0.005$). In a majority of patients HLI application caused a flu-like syndrome, whereas adverse effects on blood count and chemistry could not be established. The treatment (given in the reported dose) was not toxic and could be applied on an out-patients basis.

Key words: melanoma-therapy; surgery, operative; interferons

Introduction

In the world, patients with malignant melanoma of the skin represent approximately 1% of all cancer patients. The incidence of melanoma has been rapidly increasing, reaching its double value every 6-10 years, and likewise, also melanoma-related mortality has been exhibiting a trend of constant increase. Also in Slovenia, the yearly incidence of cutaneous melanoma by

sex shows tendency of increase.¹ In the survival analysis study² of malignant melanoma in Slovenia, overall 5-year survival was 57.5%, and median survival 108 months; 5-year survival by sex was 66.4% for females and 38.5% for males. Using univariate analysis of the sex and other clinical and pathohistological variables on the survival a statistical significant difference was established so for sex as well as for the extent of the invasion by Clark levels. Irrespective of the sex, a statistically significant better survival was found in the group of patients with thinner melanoma.

Considering the high mortality rates observed in patients with malignant melanoma (with deep level of invasion) as well as ineffective treat-

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ment of advanced disease, many studies have been investigating the potential of various treatment modalities.

Since these results of malignant melanoma treatment are still unsatisfactory, especially in advanced stages of disease, an effort should be directed to earlier treatment. Unfortunately, the results of adjuvant treatment in the early stage of the disease with chemotherapy³ have also not confirmed the effectiveness of treatment so far.

During the last decade a number of clinical studies have been performed to investigate the therapeutic potential of interferons in the treatment of various malignant diseases.⁴ Although partial and occasional complete regressions have been observed in some cancer patients⁵ the overall results of single-agent interferon treatment point out the need for further clinical and laboratory research in order to establish the role of interferon in cancer treatment, particularly in solid tumors. Besides exerting a direct antiproliferative effect on mammalian cells, interferons have proved to be potent activators of natural killer cells and macrophages.⁶ These cells have also been involved as effectors in host resistance to tumor development and in tumor control processes.^{7, 8}

In view of the previously mentioned facts, we decided to establish the role of interferon as an adjunct to surgical treatment of primary malignant melanoma. A prospective randomized trial⁹ was commenced in 1988 in patients with malignant melanoma stage IIA and B according to the AJCC classification.¹⁰

Patients and methods

Three-hundred and twenty-one patient with malignant melanoma entered the study. In the protocol only patients with histologically proven primary tumor after radical surgery were included. As mentioned previously, all the patients were in Stage IIA and IIB of the disease which means that the primary tumors were classified as Clark IV, V level of invasion and/or tumor thickness exceeding 1.5 mm. The patients were randomized in two protocol arms – a group

treated with human leukocyte interferon (HHLI) and control group with no immediate treatment after radical surgery (HCON) as shown in the protocol summary (Figure 1).

All patients in both groups were on regular clinical follow-up. Complete blood counts, blood chemistry, renal and liver function tests were taken each check; these were performed monthly in the first 2 years, and later on in 2 month intervals. Complete evaluation of patients was done before and after the treatment. Patients with relapse (in both groups) were further treated as necessary (with surgery, radiotherapy, chemotherapy) and were afterward also on regular follow-up.

Treatment

Treatment consisted of i/m application of crude human leukocyte interferon (Imunološki zavod, Zagreb, Croatia) and started within the first month after surgical excision. Interferon was applied for 30 weeks in cumulative dose of 6×10^7 units. Each patient received 2×10^6 units of interferon weekly. Since at the start of the study only human leukocyte interferon was available, this analysis refers only to the application of this agent, while later in the study the additional group of patients treated with recombinant interferon alpha was introduced and the results will be published separately.

HLLI group

A total of 160 patients, 70 males and 90 females, have been entered in the HHLI group. The mean age of patients was 48 years (48 ± 14 years, range 20 – 78 years). Patients were distributed according to the primary tumor site as follows: head and neck region (HN) – 15 ; trunk (T) – 79; limbs (L) – 66 . Primary tumors were determined as superficial-spreading type (SSM) in 31 cases, nodular type (NM) in 127 cases and *lentigo maligna* type (LMM) in two cases. The level of invasion was Clark IV in 109 cases, and Clark V in 12 cases. In 39 cases the level of invasion was Clark III, but tumor thickness exceeded 1.5 mm, which was in accordance with protocol criteria.

CON group

The control group comprised 161 randomly selected patients (71 males and 90 females) in the mean age of 52 years (52 ± 13 years, range 21-84 years). As to the primary tumor site, lesions were located in head and neck region in 16 cases, on the limbs in 70 and on the trunk in 75 cases. In 92 patients tumors were assessed as SSM type, in 3 patients as LMM and in 66 patients as NM type. The level of invasion was Clark III in 23 cases (but thickness more than 1.5 mm), Clark IV in 101 cases, and Clark V in 12 cases.

Patient distribution by various potential prognostic factors is presented in Table 1. Our

analysis showed that both protocol groups, i.e. HHLI and HCON, were similar as to their sex and age distribution. Also, there was no major difference in site and type of primary tumor, and neither in its level of invasion.

Statistical analysis

The statistical analysis was done using the Kaplan-Meier product-limit method^{11, 12} which is a non-parametrical method to estimate the probability of an event occurring during a given time-interval. Statistical significance of graphed survival curves was tested using logrank program which performs a chi-square like analysis.^{13, 14, 15}

Table 1. Comparison of HLI and CON group according to sex and age distribution, type and localization of primary tumor and level of invasion.

Data		H L I No.	Group Percentage	C O N No.	Group Percentage
Sex:	M	70	44 %	71	44 %
	F	90	56 %	90	56 %
Age:	<53	100	63 %	81	50 %
	>53	60	37 %	80	50 %
Type:	NM	127	79 %	66	41 %
	SSM	31	20 %	92	57 %
	LM	2	1 %	3	2 %
Local.:	Trunk	79	49 %	75	47 %
	HNeck	15	9 %	16	10 %
	Limbs	68	41 %	70	43 %
Clark	III	39	24 %	38	23 %
	IV	109	68 %	101	62 %
	V	12	8 %	12	7 %
TOTAL		160	100 %	161	100 %

Results

Survival analysis

Survival curves of patients in HHLI and HCON group are presented in Figure 2. Survival of patients treated with human leukocyte interferon (HHLI.dbf) is significantly higher than in the control (HCON.dbf) group ($p < 0.005$).

Survival curves of patients according to sex distribution in control group (HCON) are presented in Figure 3. Control female patients had significantly higher survival (HCONF.dbf curve) when compared with male controls (HCONM.dbf curve), which is consistent with

previous observations about influence of sex on the prognosis. The difference between both groups is significant ($p < 0.001$). Similar is the situation in HHLI group (Figure 4), though the difference between female (HHLIF.dbf curve) and male (HHLIM.dbf curve) patients is not significant ($p = 0.07$). Primary tumor site influenced the survival of patients in the control group. The difference between group of patients with tumors on limbs (HCONL.dbf curve) and patients with tumors in trunk region (HCONT.dbf curve) is significant ($p < 0.05$) in favour of limbs site, which is presented in Figure 5. In HLI group the similar difference was not significant (HHLIL.dbf curve versus

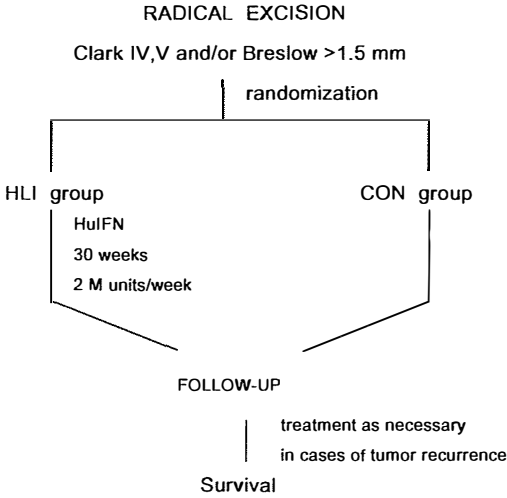


Figure 1. Protocol summary.

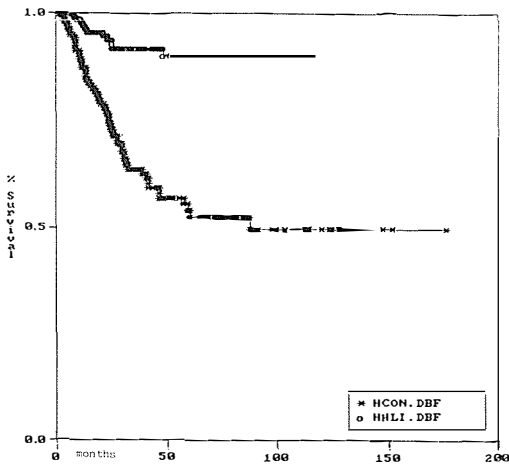


Figure 2. Survival analysis of melanoma patients in both control groups. (HCON.dbf – patients in control group, HHLI.dbf – patients in treated group; the difference is significant, $p < 0.001$).

HHLIT.dbf curve, $p = 0.5$). The type of primary tumor did not significantly influence the survival in both protocol groups (Figure 6), and also the impact of age of patients could not be established, as illustrated in Figure 7.

The difference between treated and control patients is significant also by sex stratification (Figure 8). Females in the treated group had

better survival than female controls; likewise, male patients treated with interferon survived longer than male patients in the control group (HHLIF.dbf curve vs. HCONF.dbf curve, and HHLIM.dbf curve vs. HCONM.dbf curve; $p < 0.005$).

Interferon treatment was well tolerated by majority of patients and no patient declined it because of toxic side effects. In all patients the

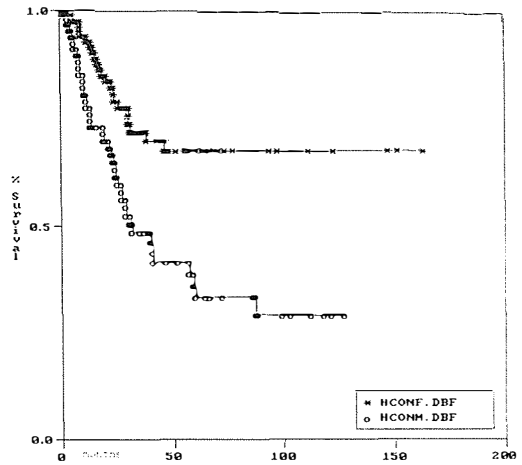


Figure 3. Survival comparison between male and female patients in control group. (HCONM.dbf – males, HCONF.dbf – females; the difference is significant, $p < 0.001$).

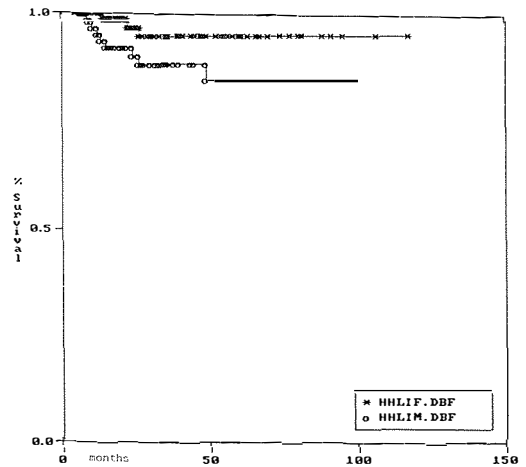


Figure 4. Survival comparison between male and female patients in treated group. (HHLIM.dbf – males, HHLIF.dbf – females; the difference is not significant, $p = 0.07$).

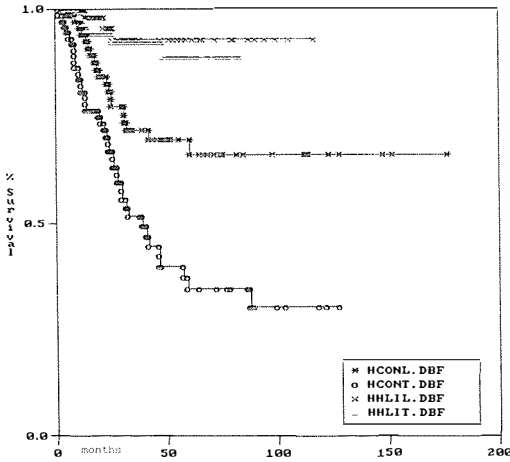


Figure 5. Survival comparison in both protocol groups according to the primary tumor site. (HCONL.dbf – control patients, primary tumor on limbs, HCONT.dbf – control patients, primary tumor in trunk, HHLIL.dbf – treated patients, primary tumor on limbs, HHLIT.dbf – treated patients, primary tumor in trunk; HCONL.dbf vs. HCONT.dbf significant, $p < 0.05$; HHLIL.dbf vs HHLIT.dbf not significant, $p = 0.5$).

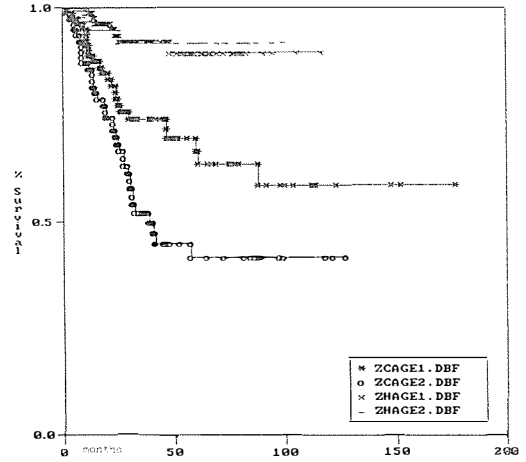


Figure 7. Survival comparison in both protocol groups according to the age of melanoma patients. (ZCAGE1.dbf – control patients, age <53 years, ZCAGE2.dbf – control patients, age >53 years, ZHAGE1.dbf – treated patients, age <53 years, ZHAGE2.dbf – treated patients, age >53 years).

application of interferon was followed by mild up to moderate fever (less than 39°C) which was transient. The patients experienced also

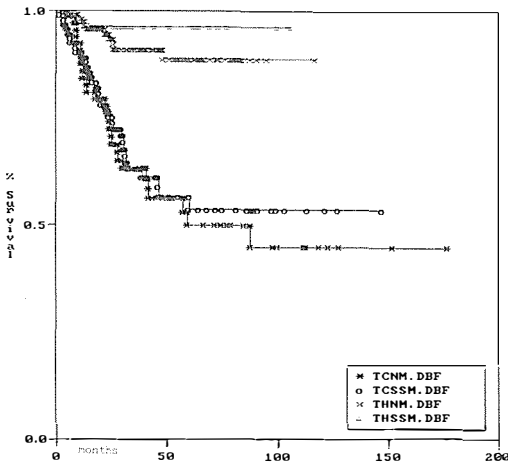


Figure 6. Survival comparison in both protocol groups according to the primary tumor type. (TCNM.dbf – control patients, nodular melanoma, TCSSM.dbf – control patients, superficial spreading melanoma, THNM.dbf – treated patients, nodular melanoma, THSSM.dbf – treated patients, superficial spreading melanoma).

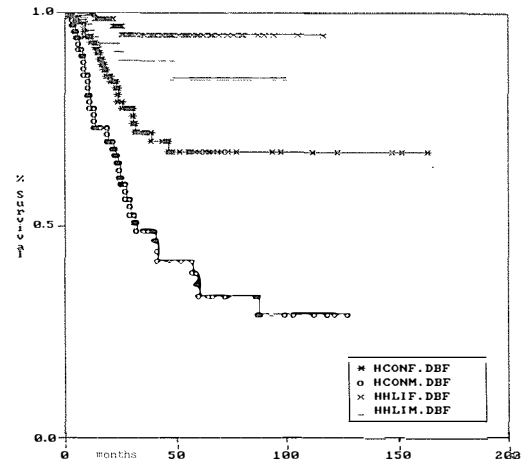


Figure 8. Comparison of survival between both protocol groups by sex stratification. (HHLIF.dbf – treated females, HHLIM.dbf – treated males, HCONF.dbf – female controls, HCONM.dbf – male controls; HHLIF.dbf vs. HCONF.dbf and HHLIM.dbf vs. HCONM.dbf significant, $p < 0.005$).

flu-like syndrom, which was anticipated. The application of interferon in performed dosage exerted no effect on blood counts and chemistry. In one case, as reported previously,¹⁶

moderate allergic reaction manifested with urticaria followed the second course of the treatment. Since the fever and flu-like syndrome were transient, after pilot study it was decided that the regimen should be applied on out-patients basis.

Discussion

The increasing incidence of melanoma and its tendency to affect younger adults, as well as relative ineffectiveness of treatment in advanced stages, point out the need for an effective adjuvant therapy.

In our study interferon treatment was found to have influenced the survival of patients, since the difference between both protocol groups (HLI and CON) was significant. In addition, the difference between male as well as female patients of both groups was also significant. According to these results, it can be postulated that the interferon treatment prolonged the survival of patients in treated group.

Also, the treatment was not associated with significant toxic side effects. The reason for allergic reaction in one case is most probably in crude extract of human leukocyte interferon containing various potential allergens.

Possible mechanisms of interferon action have not been fully explained yet. Theoretically, interferons could exert their antitumor effects in three ways: (1) via the host immune system; (2) by altering some non-immune host/tumor cell interactions; or (3) by direct effects on the tumor cells. It is known that tumors sensitive to interferon alpha tend to be slow growing and moderately well differentiated. Moreover, in contrast to other types of cancer therapy, responses to alpha interferons are typically slow, with haematological and bone marrow improvement often taking several months in leukemia cases. Data from laboratory animals suggest that interferon act best when tumor load is low, such as was the situation in our study.^{4,9} Interferons are also an important part of lymphokine cascade. It is reasonable to conclude, that interferon could act as biological

response modifier through many yet unknown mechanisms including lymphokine cascade.

According to our results, the interferon adjuvant treatment can be advised in cases with prognostically unfavourable melanoma, i.e. primary melanoma tumors with level of invasion Clark IV, V and/or thickness more than 1.5 mm.

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