Peripheral Blood CD8highCD57+ Lymphocyte Levels May Predict Outcome in Melanoma Patients Treated with Adjuvant Interferon-α

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Abstract. *Background: The objective of this study was to evaluate the significance of CD8highCD57+ lymphocytes for the survival of high risk melanoma patients treated with adjuvant interferon-α (IFN-α). Patients and Methods: The prognostic significance of peripheral blood CD8highCD57+ lymphocyte levels for survival was analysed retrospectively in 16 IFN-α-treated melanoma patients with resected regional lymph node metastases. The survival of the patients was analyzed using the Kaplan-Meier method. The difference between survival curves was determined using the log-rank test. Results: The median survival time of patients with >23% CD8highCD57+ lymphocytes prior to treatment with IFN-α was 14.2 months, whereas the median survival time of patients with < 23% CD8highCD57+ lymphocytes was not reached at the time of analysis (median follow-up 24.6 months). Conclusion: Larger prospective studies are justified to investigate the precise value of CD8highCD57+ lymphocytes in the selection of melanoma patients for adjuvant treatment with IFN-α.*

Interferon-α (IFN-α), given as an adjuvant treatment, shows consistent activity in high risk melanoma by prolonging the recurrence-free survival. However, the impact of IFN-α on overall survival remains controversial and new predictive factors are needed to select melanoma patients who could benefit from this treatment (1). There is evidence that the antitumour action of IFN-α in human patients is associated with the induction of CD8+ cell-mediated lysis of autologous tumour cells (2). Our previous observations have shown that the levels of peripheral blood CD8highCD57+ lymphocytes may predict the outcome of treatment with IFN-α of patients with metastatic renal cell carcinoma (3). Some evidence has suggested that the involvement of CD8+ lymphocytes in the immune response against melanoma is also associated with the expression of the CD57+ antigen (4). CD57 is a 110-kDa glycoprotein, first identified on CD16+ natural killer (NK) cells, but also present on a subset of T cells. CD8+ T lymphocytes can be distinguished from NK cells by the density of CD8 antigen, CD8highCD57+ T lymphocytes (CD3+, αβ-T-cell receptor-positive cells) express high levels of CD8, while CD8lowCD57+ NK cells (CD16+, CD56+) express low levels of surface CD8 (5).

In the present study the pre-treatment levels of peripheral blood CD8highCD57+ lymphocytes in melanoma patients receiving adjuvant IFN-α and their possible prognostic significance were investigated.

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Patients and Methods

Patients. This report is based on a retrospective analysis of melanoma patients who had received adjuvant immunotherapy with IFN-α at the Institute of Oncology, Vilnius University from December 1995 to October 2002. The patients with resected metastases in regional lymph nodes, were given IFN-α (interferon-α2b, Realdiron, SICOR Biotech UAB, Vilnius, Lithuania) administered intramuscularly at doses of 18 MU three times a week, starting within 2 months after lymph node dissection. Sixteen melanoma patients with resected metastases in their regional lymph nodes had blood samples drawn for flow cytometric analysis within 3 weeks before the administration of IFN-α. These patients had no prior treatment during the last 6 months before surgery and were selected for survival analysis.

FACS analysis of peripheral blood lymphocyte subsets. The CD57-fluorescein isothiocyanate/ CD8-phycoerythrin combination of fluorochrome conjugated monoclonal antibodies (Becton Dickinson, USA) was used. The samples of peripheral blood were

Table I. *Patient characteristics.*

Patient no.	Gender	Age	Lymph node dissection[a]	Disease interval (months)[b]	Duration of treatment (months)	Time to relapse (months)	Overall survival (months)
1	F	32	1/5	0	2.2	3.2	6.1
2	F	49	2/5	0	4.8[d]	5.5	7.2
3	M	41	1/1	12	2.8	3.7	22.3
4	F	37	1/2	13	1.9	2.3	14.2
5	F	59	1/1	0	16.0[d]	17.3	>33.4
6	F	49	4/10	4	55.8	>84.9	>84.9
7	M	46	1/9	0	10.7	12.4	>28.3
8	M	49	1/5	0	23.3[d]	24.3	26.9
9	M	38	1/5	0	60.5[d]	76.1	>85.0
10	F	73	2/4	18	3.2	6.3	10.5
11	F	67	3/3	39	1.0	5.3	16.1
12	F	58	3/5	19	1.0	6.1	8.4
13	M	59	2/6	0	9.6[c]	41.4	>41.4
14	F	74	1/6	0	18.3[e]	43.3	>45.8
15	M	53	1/5	10	1.0	10.1	>89.7
16	F	69	3/5	34	2.4[d]	3.4	12.2

[a]Results are given as the number of metastatic lymph nodes / total lymph nodes resected; [b]disease interval is given as the time period between the removal of the primary tumour and of the metastatic lymph nodes; [c]doses of IFN-α were reduced to 12 MU after 3 months of treatment; [d]doses of IFN-α were reduced to 9 MU after 2 months (patient no 2), after 3 months (patient no 5), after 15 months (patient no 8), after 49 months (patient no 9) and after 1 month (patient no 16) of treatment; [e]doses of IFN-α were reduced to 3 MU after 5 months of treatment.

analyzed on a FACSort® (Becton Dickinson) flow cytometer with a laser tuned at 488 nm. Forward and side scatter were used to gate the lymphocytes. The data were acquired and analyzed with LYSYS II software (Becton Dickinson). List mode files were collected for 10,000 cells from each sample. The CD8highCD57+ lymphocytes were defined as described in our previous publication (3). The absolute counts of CD8+ and CD8highCD57+ lymphocytes were calculated using the absolute number of white blood cells counted with a haemocytometer. The percentage of CD8highCD57+ lymphocytes in the CD8+ subset was determined from the flow cytometric data.

Follow-up and statistics. The patients periodically visited the Institute of Oncology and progression of disease was recorded during these visits. The disease-free survival was measured in months from the date of the radical lymphadenectomy until there was evidence of recurrence or metastasis. The survival status of the patients was determined with the help of the Lithuanian Cancer Registry. The overall survival was measured in months from the date of radical lymphadenectomy to the time of death or the last date the patient was known to be alive. The survival of patients was analyzed using the Kaplan-Meier method. The difference between survival curves was determined using the log-rank test.

Results

The characteristics of the patients selected for survival analysis are shown in Table I. There were 10 females and 6 males with a median age of 51 years (range 32 to 74 years). In 7 out of the 16 patients, the doses of IFN-α were reduced due to poor tolerance. The treatment with IFN-α was continued until disease progression for 8 patients (patient nos. 1-5, 7, 8, 16), and the treatment was stopped for reasons other than progression for the remaining 8 patients (patient nos. 6, 9-15). The median duration of treatment with IFN-α was 4.0 months (range 1.0-60.5 months). The median duration of follow-up was 24.6 months (range 6.1-89.7 months).

Since this was a retrospective study, data of repeated flow cytometric analysis was available only for 9 patients (patient nos. 1-9). For these patients, flow cytometry was repeated at 3 months (range 2.5-4 months) after treatment with IFN-α. The percentage of CD8highCD57+ lymphocytes in the CD8+ subset decreased in 4 patients and increased in 5 patients. It was found that all 4 patients with a decreasing percentage of CD8highCD57+ lymphocytes had pre-treatment values >23%, whereas all 5 patients with an increasing percentage of CD8highCD57+ lymphocytes had pre-treatment values <23% (Figure 1).

Subsequently, the analysis of the total group of 16 patients was performed to evaluate the survival of the patients based on these pre-treatment lymphocyte values. The median time to relapse for patients with >23% and with <23% CD8highCD57+ lymphocytes in the CD8+ subset was 5.3 months and 17.3 months, respectively. However, this difference did not reach statistical significance (data not shown, $p=0.097$). The overall survival of the IFN-α treated melanoma patients according to the pre-treatment level of CD8highCD57+ lymphocytes in the

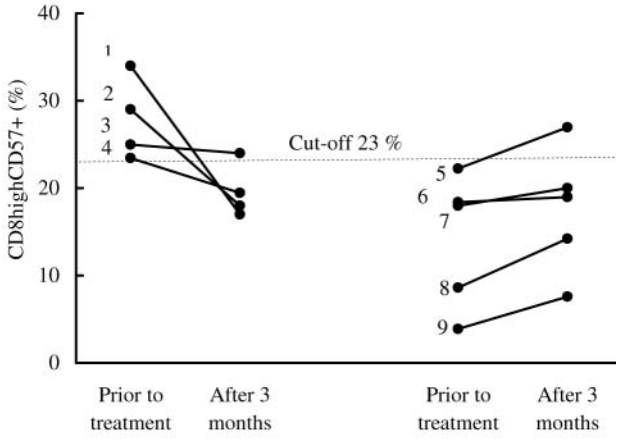

Figure 1. *Changes in the percentage of CD8highCD57+ lymphocytes in the CD8+ subset during the first 3 months of treatment of melanoma patients with IFN-α. Numbers (1-9) correspond to patient numbers in Table I. Cut-off 23% (dotted line) separates patients with decreasing CD8highCD57+ lymphocyte values from patients with increasing CD8highCD57+ lymphocyte values.*

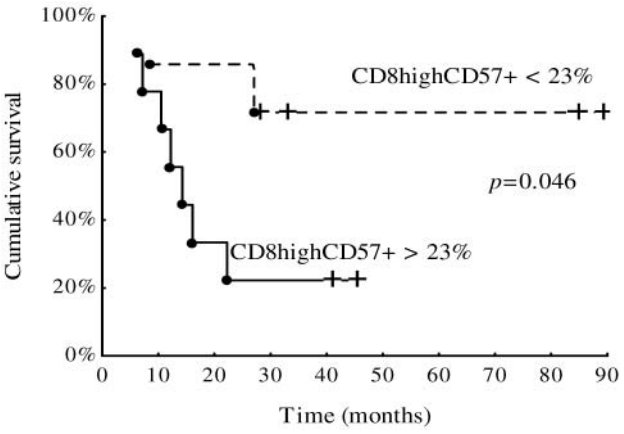

Figure 2. *Overall survival of IFN-α-treated melanoma patients with <23% (n=7) or >23% (n=9) CD8highCD57+ lymphocytes in the CD8 subset prior to treatment.*

CD8+ subset is shown in Figure 2. The median survival time of the patients with >23% CD8highCD57+ lymphocytes was 14.2 months, whereas the median survival time of the patients with <23% CD8highCD57+ lymphocytes was not reached at the time of analysis.

Discussion

In our small series of 16 melanoma patients treated with adjuvant IFN-α, remarkable differences in survival were observed depending on the pre-treatment levels of CD8highCD57+ lymphocytes in the peripheral blood. The patients with <23% CD8highCD57+ lymphocytes in the CD8+ subset prior to treatment survived considerably longer than patients with >23% CD8highCD57+ lymphocytes. The cut-off level of 23% was found by separating the patients with increasing values of CD8highCD57+ lymphocytes from those with decreasing values. This means that lower (<23%) pre-treatment values of these lymphocytes may tend to increase, whereas higher values may tend to decrease during the first few months of treatment with IFN-α. Thus, it can be assumed that not the pre-treatment value itself, but an early increase in the levels of CD8highCD57+ lymphocytes may play a role in the success of adjuvant immunotherapy with IFN-α of high risk melanoma. The relationship between the increase in CD8+ lymphocyte subsets and positive outcome of cancer immunotherapy has also been observed by other authors (6). Jorkov *et al.* (7) found that metastatic melanoma patients responding to treatment with IL-2, IFN-α and histamine dihydrochloride tended to have lower pre-treatment peripheral blood counts of CD3-positive T cells and NK (CD56+/CD3-) cells than patients with progressive disease. However, a peak value of T cells in the blood was observed earlier in patients with a tumour response than in patients with progressive tumours.

As the present study did not have a control group of patients untreated with IFN-α, the possibility that the observed changes in CD8highCD57+ lymphocytes were not caused by IFN-α cannot be excluded. However, a causal relationship between IFN-α treatment and the increase in percentage of CD8highCD57+ lymphocytes is plausible, since IFN-α can promote the survival of effector T cells (8). Thus, IFN-α may exert its antitumour action in the adjuvant treatment of melanoma through the expansion of the pool of cytotoxic CD8highCD57+ lymphocytes. It could be hypothesized, that melanoma tumour cells become adapted to a certain level of T lymphocyte cytotoxicity. This adaptation may be overcome by a rapid increase in cytotoxic T cell numbers. It is of interest to note in this context that a 1-month adjuvant treatment with high-dose IFN-α might be sufficient to reduce the risk of relapse and death in high risk melanoma (9). On the other hand, some evidence has indicated that IFN-α may also contribute to the elimination of over-activated T cells (10). Therefore, a causal relationship between the treatment with IFN-α and the decrease in CD8highCD57+ lymphocytes seen in the melanoma patients with higher pre-treatment values of these lymphocytes cannot be excluded.

The results of the present study in melanoma seem to contradict our previous results showing a negative role of CD8highCD57+ lymphocytes in advanced renal cell

carcinoma (3). This contradiction may be explained by the fact that the expression of CD57 antigen by CD8+ T lymphocytes has been associated not only with cytotoxic potential (11) but also with an immunosuppressive effect (12). It remains unclear, however, why the manifestation of cytotoxic or immune suppressive properties of CD8highCD57+ lymphocytes would depend on the type of tumour (melanoma or renal cell carcinoma) or the clinical setting (postoperative or advanced disease). Additional markers, such as perforin (13), IFN-γ and CD94/NKG2A (14) probably could help to distinguish between cytotoxic and inhibitory CD8highCD57+ lymphocytes. Studies along these lines are underway in the Institute of Oncology.

In conclusion, the possible relationship between a pre-treatment immunological parameter value (CD8highCD57+ lymphocytes in peripheral blood) and survival of melanoma patients after adjuvant IFN-α treatment is shown for the first time. Larger prospective controlled studies are justified to investigate the precise value of CD8highCD57+ lymphocytes in the selection of melanoma patients for adjuvant treatment with IFN-α.

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