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Combined interferon and vinblastine treatment of advanced melanoma: evaluation of the treatment results and the effects of the treatment on immunological functions

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Summary. Thirteen patients with metastatic malignant melanoma received interferon α-2a (Roferon-A) and vinblastine. The interferon dosage was increased from 3×10^6 IU to 9×10^6 IU daily in 10 weeks and thereafter 9×10^6 IU was administered three times weekly intrasmuscularly. Vinblastine (0.075–0.15 mg/kg) was given every third week intravenously. One of the ten evaluable patients had partial remission (PR) (11%) for 10 months. The diseases was stabilized (NC) in three patients (30%) for 3, 6 and 9 months. Progression (PD) occurred in six patients. The treatment time varied from 5 weeks to 44 weeks. The median surival time from the beginning of this combination treatment was 5 months. The most common side-effects were fever, fatigue, loss of taste, weight loss and neutropenia.

The mitogen response to phytohemagglutinin and purified protein derivative of tuberculin decreased in all patients. The response to concanavalin A decreased less and began to increase again in the patients with PR and NC. The natural killer cell activity in PD patients decreased more than in the patients with PR and NC. The ratio of T4/T8-positive cells was restored in PR + NC patients but rose in PD patients indicating a difference in the immunomodulatory effect of the combination or of the advanced disease itself on T-cell function in PD patients.

This combination of daily interferon and vinblastine did not prove to be effective in melanoma. The depression of immunological functions, which was more marked in patients with PD, might indicate that vinblastine in this combination counteracts the immunostimulatory effect of interferon.

Introduction

Malignant melanoma is a rare disease with a rapidly rising incidence. The response of advanced malignant melanoma to radiotherapy and current chemotherapy has been poor. Dimethyltriazenoimidazolecarboxamide (DTIC) is the most effective single agent. Only slightly increased response rates (generally 20–40%) have been achieved with combination chemotherapy [6, 19, 20]. According to the literature, the addition of immunotherapy to cytostatic drugs has not yielded better results [13, 17, 19]. So far there exists no standard chemotherapy for metastatic disease.

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Interferons affect tumor growth both by immunomodulation and, also directly, as cytostatic drugs. Cytostatic agents, on the other hand, act synergistically with interferons and affect the immune functions. In patients, the natural killer cell activity has been shown to decrease shortly after an interferon injection [4]; however, it returns close to the pretreatment level in long-term measurements. Interferons increase natural killer cell activity in vitro [4, 5, 10].

Interferons alone have shown 10%–20% response rates in advanced malignant melanoma [1], and in order to increase the response rate, known potent cytostatic drugs have been combined with them [7–9].

In this study both the effectiveness of the combination of recombinant interferon α and vinblastine, and the changes in immunocompetence during the treatment were studied as a function of the responses.

Materials and methods

Patients. Thirteen patients entered this trial, ten of them are evaluable. The treatment of three nonevaluable patients was stopped before 1 month because of surgery for severe pain in the bone metastasis, breathing difficulties because of pulmonary metastases, and irradiation to brain metastases. All patients had histologically verified malignant melanoma, metastases were clinically, and usually also cytologically, verified (metastatic sites are presented in Table 1). The ages of the patients at the time of diagnosis varied from 28 years to 66 years and the disease-free interval varied from 4 months to 72 months. Patients with leptomeningeal or cerebral metastases, severe heart disease, heavily altered liver and renal function tests, or severe infections were excluded from the study.

Staging and follow-up. The follow-up registration included all symptoms and signs of disease, performance status (Karnofsky index), neurological examination, body weight and height, measurements of visible and palpable tumor lesions, chest X-ray, electrocardiography, liver and abdominal ultrasound scans. An abdominal or mediastinal computed tomography scan was performed if necessary for tumor follow-up. Usual laboratory tests were made weekly at the beginning of the treatment and thereafter every third week.

All patients were hospitalized first for 2 weeks for dosage increase and evaluation of acute side-effects. Response evaluation and immunological tests were made every

fourth week during the first 3 months and thereafter every third week.

Treatment schedule. Roferon-A was given with dosage increase from 3×10^6 to 9×10^6 IU intramuscularly daily for 10 weeks and after that 9×10^6 IU was administered three times per week. Vinblastine was given intravenously every 3 weeks increasing the dosage from 0.075 mg/kg to 0.15 mg/kg.

Criteria of response. The response was evaluated according to the criteria of the International Union Against Cancer (UICC) [12]. The patients whose therapy lasted less than 4 weeks were considered nonevaluable. Duration of treatment and survival were measured from the start of therapy.

Immunological tests. For the enumeration of lymphocyte subpopulations, the OKT3, OKT4, OKT8, OKIaI, B1 and My4 sera were used according to the instructions of the producer (Ortho Pharmaceutical Corporatin, Immunobiology Division, Raritan, NJ and Coulter Immunology, Hialeah, Fla, USA). The cells were counted using a Leitz SM-Lux microscope.

The natural killer cell activity was measured using K562 cells as targets in a ^{51}Cr-release assay as described by West et al. [21]. All effector:target ratios were used in the final analyses.

For the mitogen responses to phytohemagglutinin, concanavalin A and of purified protein derivative tuberculin (PPD), a whole-blood method described by Eskola et al. [2] was used as described in earlier reports [18]. The tests were carried out in triplicate. The results are expressed as net counts per minute (cpm), obtained by deducting the average value of three unstimulated control cultures from that of the stimulated cultures.

Results

Response and tolerance

Only one partial remission, which lasted for 10 months, was achieved (Table 1). In three patients the disease was stabilized for 3, 9 and 6 months. In the remaining six patients progression occurred. The median survival time from the beginning of the treatment was only 5 months and that from the primary treatment of the melanoma was 3.5 years. The treatment time varied from 5 weeks to 44 weeks.

Table 2. Toxicity of the combined treatment of interferon and vinblastine

Side-effect[a]	Number of patients (total evaluable ten)
Constitutional symptoms fever, fatigue, weight loss)	9
Leucopenia	8
Thrombopenia	3
Anemia	4
Neurological symptoms	1
Urticaria	1
Alopecia (mild)	1

[a] Elevation of liver function tests was observed, but the patients had intra-abdominal (visceral) metastases at the same time

The majority of patients with NC and PR had lymph node and lung metastases (Table 1) and progression occurred in patients with intra-abdominal metastases. There was no correlation between the disease-free interval and the response.

The most common side-effects were fatigue and fever, the other were thrombopenia and leucopenia (Table 2). The dose three times per week was much better tolerated than a daily dose of interferon.

Immunological tests

The mitogen responses to phytohemagglutinin and especially to PPD declined in both PD and PR + NC patients. However, the response to concanavalin A was slightly restored in the direction of the pretherapeutic value in the patients with NC and PR, but remained low in PD patients (Fig. 1, Table 3).

The reduction of NK cell activity was also more pronounced in every effector:target ratio in PD patients (Fig. 2 Table 4).

The subpopulations of T-lymphocytes and the helper:suppressor cell ratios (OKT4/OKT8) are shown in Tables 5 and 6. The total amount of T-cells remained at the same level in the patients with NC and PR and decreased in PD patients. At the same time the helper:suppressor cell ratio rose in PD patients but remained unchanged in the

Table 1. Patient characteristics and response to combined treatment of recombinant interferon α-2a and vinblastine

Patient number	Age at the time of diagnosis (years)	Disease-free interval[a] (months)	Clark level, metastatic site(s)[b]	Response[c] (duration in months)
1	55	4 (15)	Clark x, c, ia, pl	PD
2	35	18 (21)	Unknown primary, c, l, ln	PD
3	46	26 (33)	Clark III, l, s, ia	PD
4	37	72	Clark III, c, ia	PD
5	60	6	Clark IV, l, ln	PR (10)
6	40	9 (33)	Clark IV, l, h	NC (3)
7	28	37 (48)	Clark IV, c, ln	PD (9)
8	39	57 (59)	Clark IV, c, b, ia	PD
9	66	16	Choroid primary, l, h	NC (6)
10	60	49 (54)	Clark III, c, l, h, b	PD

[a] In parentheses the time interval in months from diagnosis to the beginning of the treatment
[b] Metastatic sites: c, cutaneous; ia, intra-abdominal; b, bone; h, liver; l, lung; ln, lymph nodes; pl, pleura; s, spleen
[c] PR, partial remission; NC, no change; PD, progressive disease

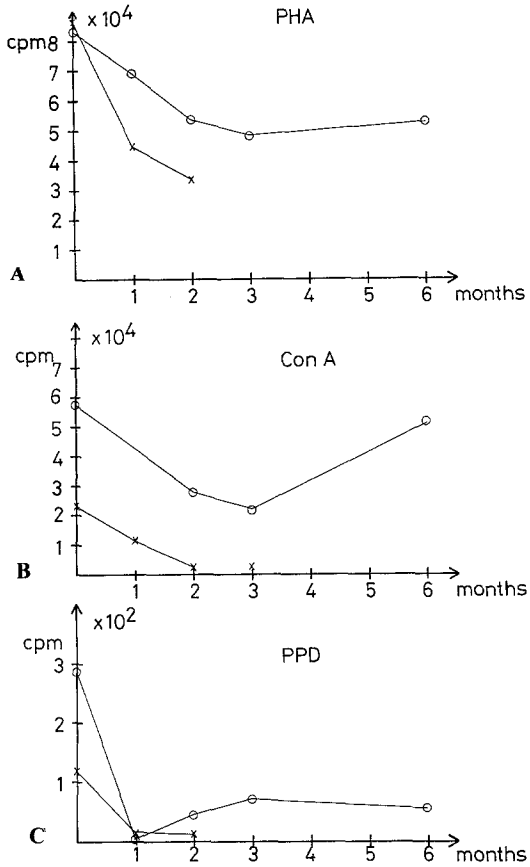

Fig. 1. Responses of peripheral blood lymphocytes to phytohemagglutinin (**A**), concanavalin A (**B**), or tuberculin purified protein derivative (**C**). x———x, Patients with progressive disease (PD) (six patients); O———O, patients with partial remission (PR) and no change (NC) (four patients)

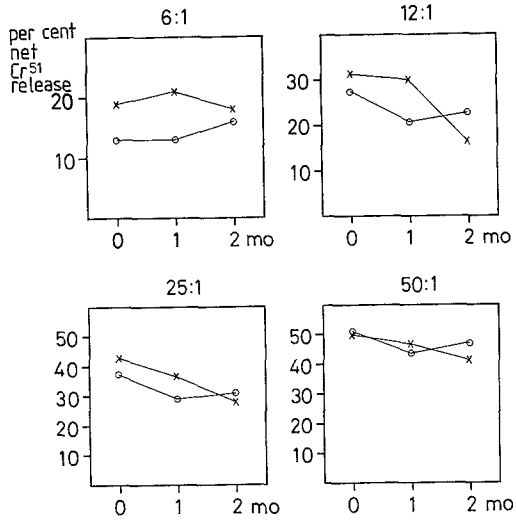

Fig. 2. Changes in natural killer activity at different effector: target ratios. x———x, Patients with PD (six patients); O———O, patients with PR and NC (four patients)

patients with NC and PR. The amount of macrophages increased respectively; other markers, e.g. My4, and B1, were restored at the same level (Table 6).

Discussion

The treatment response of ten patients with advanced melanoma in this phase I–II study was so low that the combination of interferon α (9×10^6 IU 3–7 times weekly) and vinblastine (0.1 mg/kg every third week) (only one partial remission was achieved) can not be recommmended for further evaluation, although the number of patients was small and most of them had a very advanced disease.

Table 3. Proliferative responses of peripheral blood lymphocytes to mitogen stimulation in the six patients with progressive disease (PD) and four patients with partial remission + no change (PR + NC)

Sample[a]	Phytohemagglutinin		Concanavalin A		PPD[c]	
	PD	NC + PR	PD	NC + PR	PD	NC + PR
I	87 400 ± 33 800[b]	82 700 ± 20 000	23 500 ± 14 500	58 900 ± 26 400	1240 ± 900	2900 ± 2930
II	45 000 ± 27 400	69 900 ± 2600	13 800 ± 22 200	18 400 ± 1800	170 ± 120	50 ± 60
III	34 600 ± 22 800	54 300 ± 7700	3100 ± 3500	29 600 ± 11 300	140 ± 210	480 ± 570
IV	N.T.[d]	49 800 ± 6600	N.T.	23 000 ± 9900	N.T.	720 ± 870
V	N.T.	53 700 ± 12 800	N.T.	51 200 ± 26 800	N.T.	630 ± 760

[a] I, baseline; II, 1 month after initiation of treatment; III, 2 months; IV, 3 months; V, 6 months
[b] The results are expressed as net cpm (mean ± SEM)
[c] PPD, purified protein derivative of tuberculin
[d] N.T., not tested

Table 4. Natural killer activity measured using different effector:target ratios; the results are expressed as the percentage specific lysis

Sample[a]	PD patients[b] effector:target ratio				NC + PR patients[b] effector:target ratio			
	6:1	12:1	25:1	50:1	6:1	12:1	25:1	50:1
I	19 ± 9	34 ± 13	43 ± 15	50 ± 20	13 ± 5	27 ± 8	38 ± 8	51 ± 7
II	21 ± 19	30 ± 22	37 ± 23	47 ± 23	13 ± 6	21 ± 9	29 ± 11	44 ± 14
III	18 ± 11	16 ± 12	28 ± 17	42 ± 22	16 ± 5	23 ± 7	31 ± 9	48 ± 11
IV	N.T.	N.T.	N.T.	N.T.	13 ± 7	27 ± 5	44 ± 7	53 ± 12
V	N.T.	N.T.	N.T.	N.T.	14 ± 8	21 ± 13	30 ± 16	34 ± 28

[a] I, baseline; II, 1 month after initiation of treatment; III, 2 months; IV, 3 months; V, 6 months
[b] PD, patients with progressive disease (six patients), NC + PR, patients with no change or partial remission (four patients)
N.T., not tested

Table 5. Percentage (mean ± SEM) of T-cell (T) subpopulations in patients during the combined treatment of recombinant interferon α-2a and vinblastine

Sample[a]	OKT3		OKT4		OKT8		Ratio OKT4/OKT8	
	PD	NC+PR	PD	NC+PR	PD	NC+PR	PD	NC+PR
I	48±17	51±16	31±13	25± 9	16±6	28±10	1.9	0.9
II	55±12	66± 4	36± 8	34± 6	15±9	31±11	2.4	1.1
III	33±11	56± 9	24±10	28± 9	8±3	22± 7	3.0	1.3
IV	N.T.	49±10	N.T.	25± 8	N.T.	22± 8	N.T.	1.1
V	N.T.	51± 7	N.T.	26±11	N.T.	23±12	N.T.	1.1

[a] See Table 4 for abbreviations

Table 6. Percentages of macrophages and monocytes as determined by My4 and Ia monoclonal sera, and B cells

Sample[a]	My4		Ia		B1 (B cells)	
	PD	NC + PR	PD	NC + PR	PD	NC + PR
I	20± 6	20±7	14±13	12±4	6±3	9±7
II	22± 7	8±6	19± 9	8±1	8±5	6±3
III	23±11	16±4	20± 2	9±5	4±2	7±5
IV	N.T.	24±9	N.T.	7±4	N.T.	4±1
V	N.T.	10±6	N.T.	13±4	N.T.	10±3

[a] See Table 4 for abbreviations

However, the treatment times of the patient with PR and two patients with NC were quite long.

The effect of this combination was similar to the effect of interferon alone. Two PD patients developed brain metastases. The side-effects were same as those described for interferon alone and there were no irreversible side-effects [7].

The response to mitogen stimulation declined in both patient groups, as shown in other studies on patients with advanced cancer. However, in these results responding patients slightly differ in the concanavalin A response. Our results are the opposite of those in the study by Hirsch and Johnson [5], in which α-2 recombinant interferon did not change the lymphoproliferative responses in multiple sclerosis patients.

The analysis of the NK cell activity in clinical studies in the literature differs from one study to another and thus it is difficult to compare them [16]. The statistical significance of immunological functions could not be tested, because of the small number of patients, but it was obvious that the reduction of NK cell activity was more pronounced in patients with PD. Also the amount of suppressor T-cells declined in these PD patients, as shown by the increase of the helper:suppressor cell ratio. The function of these cell subpopulations in tumor cell killing is not clearly understood [3]. The most pronounced change between the two treatment groups was the increase of macrophages in PD patients, which might be connected to greater tumor invasion. This is in agreement with the hypothesis that macrophages might help in tumor invasion, thus inducing the progression [14] or the change could merely be due to progression of the disease. The other hypothesis is that interferon could not decrease macrophage production since this cell type produces interleukin. Thus, according to these measurements, the amount of this cell population might be important for tumor progression and might be used as a prognostic marker, however, more patients should be analyzed with immunological tests.

The side-effects and the tolerance for the treatment were acceptable. Because of the low response rate, more effective combinations such as those with interleukins and LAK cells [15] or dimethyltriazeno-imidazolecarboxamide [11] should be sought for treatment of advanced melanoma. This study measured the long-term effects of the combination of interferon and vinblastine in the natural killer activity and in the other immunological functions of lymphocytes and showed that combination treatment changes them. This should be taken into account when planning new combination treatments.

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