Adoptive Immunotherapy with Interleukin-2 and Interferon-alpha in Metastatic Renal Cell Cancer and Melanoma

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Printed by: ICG Printing b.v.
Dordrecht
The Netherlands

ISBN: 90-9009936-0

Adoptive Immunotherapy with Interleukin-2 and Interferon-alpha in Metastatic Renal Cell Cancer and Melanoma

Adoptieve immunotherapie met interleukine-2 en interferon-alpha in gemetastaseerd niercarcinoom en melanoom

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam,
op gezag van de rector magnificus
Prof.dr. P.W.C. Akkermans M.A.
en volgens besluit van het college voor promoties.
De openbare verdediging zal plaatsvinden
op woensdag 27 november 1996 om 15.45 uur

door

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Geboren te Vlaardingen

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Ter nagedachtenis aan mijn moeder
Voor mijn vader

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Chapter I

Introduction and scope of the thesis

Introduction

Approximately one half of all newly diagnosed cancer patients will die of metastatic disease despite the application of the best available treatment consisting of surgery, radiation therapy and chemotherapy. Attempts to develop new approaches for the treatment of metastatic cancer by stimulating immune host defences against the tumor have received substantial attention. Initially most efforts to develop immunotherapies have involved nonspecific stimulation of the immune system with unspecific immunostimulants such as Bacille Calmette Guerin or Corynebacterium parvum. However, clinical trials have been disappointing and this immunotherapeutic approach has been abandoned. An alternative approach is that of adoptive immunotherapy, which is defined as the transfer of immunologic reagents or immune cells with antitumor reactivity to the tumor bearing host (1).

Interleukin-2 (IL2), formerly called "T cell growth factor", is a naturally occurring glycoprotein, first identified in 1976 (2,3). It is synthesized and released by activated T lymphocytes of the helper subset (2,4). IL2 acts as a pleiotropic mediator within the immune system, having a variety of effects via specific cell surface receptors. In vitro activities of IL2 include long-term proliferation of activated T cell clones, enhancement of lymphocyte mitogenesis, induction of T cell reactivity, augmentation of natural killer (NK) cell activity and induction of lymphokine-activated killer (LAK) activity (5-11). LAK cells are effector cells capable of lysing autologous and allogeneic tumor cell lines and fresh tumor. DNA technology has made it possible to clone the gene for IL2 and insert it into Escherichia coli to produce large amounts of recombinant interleukin-2 (12). When IL2 is injected in vivo it leads to the production of secondary cytokines, including IL1, TNFα, IFNγ, IL6, GM-CSF and M-CSF (13-15). These cytotoxic and antiproliferative cytokines may partly be responsible for the antitumor activity of IL2 (6,7,16-18). In animal studies IL2 mediates the regression of pulmonary and liver metastases from a variety of tumors (19).

These observations have led to clinical investigations with IL2 alone or in combination with LAK. It was shown that renal cell cancer and melanoma were the most sensitive tumors. The first results were published by Rosenberg et al (20-22). The first used schedule consisted of a high-dose intravenous bolus regimen,

administering IL2 (25 MIU/m^2) every 8 hours for 5 days. In the initial trials impressive responses were seen in up to 35% of patients (20-22). The side effects of high-dose intermittent doses of IL2 were substantial, including fever, chills, skin rash, anorexia, nausea, vomiting, diarrhea, hypotension, oliguria, weight gain, lung edema and cardiac rhythm disturbances (20-22).

In contrast to this high-dose bolus regimen, West et al introduced a schedule of continuous intravenous administration of IL2 18 MIU/m^2/day for 5 days and early studies suggested that IL2 by continuous infusion could also yield good antitumor activity with decreased toxicity (52,53). In the past years a great number of studies, administering high-dose IL2 alone or combined with LAK cells in a wide variety of schedules, have been published (25-41). In patients with metastatic renal cell cancer or melanoma response rates of 10-25% have been reported.

Interferon-alpha is produced by leukocytes upon stimulation by a virus and was originally detected by its ability to mediate in vitro antiviral activity (41). IFNα has a direct antiproliferative effect on tumor cells in vitro (42-44). It upregulates the expression of major histocompatibility complex (MHC) antigens class I and II, β-microglobulin as well as tumor associated antigens (42,43,45,46). The stimulated expression of tumor antigens may make neoplastic cells more susceptible to cytotoxicity by T cells of the host. IFNα has the ability to modulate the host-immune system by augmenting the activity of mature NK cells and by promoting their differentiation from precursors (43). Stimulation of NK cells may result in lysis of tumor cells. The development of cytotoxic T lymphocytes, monocytes and macrophages is enhanced by IFNα (43,44,46).

The antitumor activity of IFNα in vivo has been demonstrated in various mouse models (47). In patients IFNα has the highest antitumor activity in hematological malignancies, followed by metastatic renal cell cancer and melanoma with response rates of approximately 15-20% (48-53).

Several strategies have been proposed for improving the antitumor activity of immunotherapy with cytokines. There has been considerable interest in the combination of IL2 and IFNα for cancer treatment. Several animal experiments have shown that the combination of IL2 and IFNα enhances effector-cell mechanisms and produces superior antitumor activity when compared with the maximum-tolerated dose of the single agents in the same tumor models (54-57). Based on the

synergistic effects of IL2 and IFNα in murine models, clinical studies were conducted. A phase I trial of high-dose IL2 and IFNα by bolus injections performed by the NCI Surgery Branch produced response rates of 31% and 33% in patients with renal cell cancer and melanoma, respectively (58). Also other early clinical studies yielded encouraging response rates of up to 40% (59-61).

Scope of the thesis

The above mentioned observations of synergy between IL2 and IFNα in laboratory experiments and the encouraging initial treatment results of clinical phase I studies form the rationale for further investigations of immunotherapy based on various combination schedules of IL2 and IFNα. This thesis describes the results of phase II studies of IL2 and IFNα in patients with metastatic renal cell cancer and melanoma. The primary objective was to determine the response rate and the median duration of response. A second objective was to assess the toxicities associated with these combination schedules.

Chapter 2 describes our initial experience with the combined treatment of IL2 and IFNα in patients with metastatic melanoma, whereas in Chapter 3 the final analysis of this large European phase II study is reported. In this study patients received intermediate high doses of IL2 (7.8 MIU/m^2/day) and IFNα (6 MU/m^2/day) every other week. The treatment schedule was derived from a regimen developed by Sondel et al (62). They found that IL2 administration each week for 4 days resulted in a progressive increase of the rebound lymphocyte count at the completion of each cycle and that these lymphocytes demonstrated a 100-fold increase in cytotoxic potential.

In a dose escalating study Rosenberg et al found a dose-response relationship for the combination of IL2 and IFNα with response rates as high as 40% (58) In an attempt to confirm these results we carried out a trial in melanoma patients, administering high dose IL2 (11.7 MIU/m^2/day) and IFNα (3 MU/m^2/day) by intravenous bolus three times a day. The results of this study are presented in Chapter 4.

Initial studies suggested that the combination of IL2 and lymphokine-activated killer cells (LAK) was superior to IL2 alone. Consequently, we designed a study of

IL2, IFNα, and LAK in patients with metastatic renal cell cancer. The final analysis of this study is presented in Chapter 5.

Chapter 6 describes the high incidence and the severe nature of cardiotoxicity, observed in patients with metastatic melanoma treated with high-dose bolus IL2 and IFNα as reported in Chapter 4.

Several investigators have attempted to identify prognostic factors that predict a response to IL2-based immunotherapy. Some authors have suggested that the occurrence of hypothyroidism is positively related to clinical response (63). In Chapter 7 the incidence of thyroid dysfunction related to various treatment schedules is reported and the significance of thyroid dysfunction as a prognostic factor is analyzed.

In chapter 8 the present role of adoptive immunotherapy with IL2 and IFNα is discussed with special attention to new innovative treatment modalities and future perspectives that become available.

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Chapter II

Clinical experience with the combined use of recombinant interleukin-2 (IL2) and interferon-alpha-2a (IFNα) in metastatic melanoma

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Published in
Br J Haematol 1991;79(suppl):84-86

Summary

A multicenter study of IL2 and IFNα has been performed in 58 patients with metastatic melanoma. The scheme consisted of IL2 3.0 BRMP MU/m^2/day as a continuous infusion for 4 days combined with subcutaneous administration of IFNα 6 MU/m^2/day, day 1 + 4. The cycle was repeated every 2 weeks for a maximum duration of 26 weeks. 54 patients were evaluable for response. One (2%) achieved a complete and 10 (19%) a partial response. 19 (35%) patients were stable and 24 (44%) showed progressive disease. Common side effects included fever, chills, fatigue, skin rash, anorexia, nausea and diarrhea. Hypothyroidism was noted in 10% of the patients. These results show that this regimen of IL2 and IFNα is active but, in contrast to what could be expected, not superior to IL2 alone possibly due to suboptimal dosing.

In an ongoing study in Rotterdam and Nijmegen, a more intense schedule was chosen, consisting of three daily i.v. doses of IL2 4.5 BRMP MU/m^2 and IFNα 3.0 MU/m^2 for 5 days. This regimen was repeated at intervals of 3 weeks for a total of three cycles. Presently, nine patients have been entered. One patient achieved a complete response, four a partial response (overall 56%), three had stable disease and one progressed. Toxicity was severe and treatment was prematurely stopped in five patients: myocarcial infarction (one patient), atrial fibrillation (one patient), negative T waves and myocardial hypokinesia (one patient) and psychosis (two patients). This regimen can only be justified if the therapeutic results are superb, which has yet to be awaited.

Introduction

Interleukin-2 (IL2) and interferon-alpha (IFNα) are biologic substances involved in the regulation of the immune system. The administration of IL2 or IFNα can mediate tumor regression in patients with metastatic melanoma (1-7). The mechanisms by which IL2 and IFNα mediate antitumor effects are different. IL2 has the ability to expand and activate subpopulations of cytotoxic T cells, NK- and K cells (8-10). Administration of IL2 can also stimulate the antitumor reactivity of adoptively transferred IL2 activated lymphocytes (LAK) (1). IFNα exerts a variety of other effects such as direct antitumor cytotoxicity, increased expression of major histocompatibility class II (MHC) antigens on tumor cells, and the potential to stimulate the differentiation of malignant cells (11). There is considerable interest in the synergistic effects of IL2 and IFNα against tumor cells. Preclinical studies in animal tumor models showed that the combined administration of IL2 and IFNα mediated greater therapeutic effects against established subcutaneous, hepatic and pulmonary metastases than either agent alone (1,12-14). In a study with escalating dose levels of both IL2 and IFNα Rosenberg et al (15) have observed response rates as high as 44% in a group of patients with renal cell cancer and melanoma.

Based upon these observations, we have initiated two studies with different doses and schedules of IL2 and IFNα combination therapy in metastatic melanoma. The results of these studies are reported here.

Patients and methods

Patients

From December 1988 to October 1989 58 patients with metastatic melanoma were entered in the first phase II trial. Eligibility criteria included: age 18 - 70 years, Karnofsky performance status 80-100, no metastases in the central nervous system, no significant cardiovascular history, serum bilirubin and creatinine within normal range, normal bone marrow function (HCT > 30%, WBC > 4000/ml,

platelets > 100000/ml), normal coagulation parameters, no previous treatment with IL2 or IFNα, washout period of at least 4 weeks for cytotoxic chemotherapy and negative tests for HIV antibody and hepatitis-B antigen. Pre-treatment characteristics are shown in Table 1. Sites of metastatic disease were skin (34%), lymph nodes (66%), liver (29%), lung (31%) and bone (17%). In the second ongoing trial nine patients (six males and three females) with a median age of 47 years (range 28-60) have presently been entered. Patient characteristics and disease sites are comparable to those in the first trial.

TABLE 1 PATIENT CHARACTERISTICS (FIRST STUDY)

Age, median (range)	54	(21-72)
Sex		
female	30	(52%)
male	28	(48%)
Karnofsky performance status		
80	15	(26%)
90	22	(38%)
100	21	(36%)
Prior chemotherapy	20	(34%)
Prior radiotherapy	5	(9%)

Treatment

In the first study IL2 was given by continuous infusion of 3.0 BRMP (Biological Response Modifiers Program) MU/m^2/day for 4 days combined with a subcutaneous injection of IFNα 6 MU/m^2/day on day 1 + 4 followed by a rest period of 10 days.

The cycle was repeated at day 15. Evaluation of response was done after each four cycles. Patients received 13 cycles of treatment unless progressive disease was documented. During treatment other anticancer therapy was prohibited. Paracetamol or indomethacine were used to control fever. Vomiting and diarrhea were treated symptomatically. Fluid output was carefully monitored and corrected. In the second study treatment consisted of three daily i.v. bolus injections of IL2 4.5 BRMP MU/m^2 and IFNα 3.0 MU/m^2 for 5 days followed by a 16 day rest period. The intended treatment plan consisted of three cycles after which evaluation of response was done.

Patient monitoring

Response was evaluated according to WHO guidelines (16). A complete response was defined as the disappearance of all known disease (measurable and non-measurable) for at least 4 weeks. A partial response was defined as a reduction in the sum of the products of the largest perpendicular diameters of each lesion by at least 50% for at least 4 weeks. Stable disease denoted less than 50% tumor reduction and less than 25% tumor progression. Progressive disease was defined as the appearance of a new lesion or an increase in size of more than 25% of any lesion. Toxicity was recorded and analysed using the WHO grading system (16), if possible, or otherwise a grading system from 1 to 3, in which grade 1 = mild, 2 = moderate and 3 = severe toxicity.

Results

Response

a. first study

54/58 patients were evaluable for response. Two patients were entered, but never treated. One suffered a ketoacidotic coma and one refused treatment. One patient (2%), who entered the study just before its completion, achieved a complete response, for a duration of 4$^+$ weeks. There were 10 (19%) partial responses with a mean duration of 19.5$^+$ weeks (range 13-37$^+$ weeks). The overall response rate

was 21%. 19 patients had stable disease (35%). In 24 patients progressive disease was documented (44%). Responses were seen in lymph nodes (29%), skin (25%), adrenal gland (20%), lung (17%), liver (12%).

b. second study

Nine patients received a median of 24 doses (53%) (range 8-41) of the planned total of 45 IL2 and IFNα doses. Four patients completed fully all three treatment cycles. Treatment was discontinued in one patient after two cycles and in four patients after one cycle because of unacceptable toxicity. In four of the five patients who received more than one treatment course, IL2 and IFNα doses had to be reduced to 50%. Of the nine evaluable patients, one achieved a complete and four a partial response (overall 56%), three had stable disease and one progressed.

Toxicity

a. first study

All 58 patients were evaluable for toxicity. The side effects are listed in Table 2. Fever, chills, anorexia, nausea and diarrhea occurred in most patients. Other frequently reported adverse effects were fatigue, muscle pain and headache. Erythema was seen in 55% of patients, desquamation in 48% and pruritus in 26%. Hypotension was noted in 67% of the patients; in seven patients grade 3 and in two grade 4. Weight gain and fluid retention were generally modest and occurred in 43% of cases. Oliguria was seen in only 10% of patients. Ventricular arrythmia developed in three and atrial fibrillation in two patients. The most frequent manifestation of hematological toxicity was anemia (64%). Moderate and reversible increases in serum creatinine and bilirubin were seen in 15% of patients. No toxic death occurred and all toxicities resolved after cessation of IL2 and IFNα. Hypothyroidism was observed in 10% of patients. Chronic cumulative fatigue occurred after about 3 months of treatment. Consequently no patient did receive more than 13 cycles.

TABLE 2 ADVERSE EVENTS (FIRST STUDY)

adverse event	number of patients (%)	WHO grading			
		1	2	3	4
fever	58 (100)	1	30	27	0
skin rash/erythema	32 (55)	13	18	1	0
nausea/vomiting	55 (95)	7	37	11	0
diarrhea	42 (72)	14	21	7	0
malaise	18 (31)	1	13	4	0
weight gain	25 (43)	14	10	1	0
hypotension	39 (67)	12	18	7	2
tachycardia	21 (36)	5	13	3	0
dyspnea	8 (14)	2	5	1	0
creatinine	9 (16)	6	3	0	0
alkaline phosphatase	52 (90)	31	19	2	0
anemia	37 (64)	26	11	0	0

b. second study

This intensifed treatment schedule was accompanied by considerable toxicity. All nine patients suffered from malaise, fever, chills, nausea, vomiting and diarrhea. At the end of even the first cycle all patients complained of extreme fatigue. Nausea and vomiting were easily controlled by antiemetics, but diarrhea was sometimes intractable. Weight gain exceeding 5% of the starting body weight was observed in 5/9 patients. Transient hyperbilirubinemia and increased creatinine were seen in seven and nine patients, respectively. In every patient increases in transaminases (SGOT and SGPT) were observed. In four patients side effects led to a dose reduction of 50% in subsequent cycles, intractable diarrhea of several litres per day in one patient, hypotension grade 3 and anuria in two patients and hyperbilirubinemia $>$ 100 μmol/l in one patient. Treatment had to be terminated in five patients due to myocardial infarction (one patient), atrial fibrillation (one

patient), negative T waves and myocardial hypokinesia (one patient) and psychosis (two patients).

Discussion

Monotherapy with IL2 has resulted in 25% mostly partial responses in patients with metastatic malignant melanoma (5). Response rates of 3-36% have been observed in studies utilizing the combination of IL2 and LAK (3,5,6,17). Patients treated with IL2 and IFNα in three different phase I-II studies showed a 21-44% response rate (4,7,15). Higher doses of IL2 and IFNα appeared to correspond with a higher response rate (15).

In the first multicenter phase II study, reported here, antitumor efficacy was demonstrated, but the overall response rate of 21%, including only 2% complete response, was disappointingly low. This may have been due to suboptimal dose and schedule.

In an attempt to confirm the results obtained in the NCI escalating dose study (15), we have chosen dose level 3 of that particular study; the one but highest dose, which had yielded a 44% overall response rate, despite the fact that the toxicity precluded the administration of more than 60% of protocol dose; i.e. 30 doses of IL2 and IFNα. We introduced two modifications, a rest period of 2 weeks instead of one and a total of three cycles instead of two. Our preliminary results showed an encouraging response rate of 56%. The side effects, however, were impressive. In five patients grade 3-4 toxicities necessitated the discontinuation of further treatment and in four patients dose reductions had to be made. This treatment schedule can only be justified if the therapeutic results are superb, which has yet to be awaited.

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Final report of a phase II study of interleukin-2 and interferon-alpha in patients with metastatic melanoma

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Published (shortened version) in
Br J Cancer 1995;71:1319-1321

Summary

A multicentre study of IL2 and IFNα was performed in 57 patients with metastatic melanoma. The treatment scheme consisted of IL2 7,8 MIU/m^2/day as a continuous infusion for 4 days combined with subcutaneous administration of IFNα 6 MIU/m^2/day, day 1+4. The cycle was repeated every 2 weeks for a maximum number of 13 cycles. Further treatment beyond half a year was allowed. Evaluation was done after every 4 cycles.

Fifty-one patients were evaluable for response and toxicity. One (2%) achieved a complete and 7 (14%) a partial response, total response rate 16% (CI 7-29%). Twenty patients (39%) were stable and 23 (45%) showed progressive disease. Time to progression was 2.5 months for all patients and 8.2 months for the responding patients. Median survival of all eligible patients was 11.3 months and of the responding patients 20.2 months. Common side effects included fever, chills, fatigue, skin rash, anorexia, nausea and diarrhea.

These results show that this regimen of IL2 and IFNα is only moderately active and not superior to IL2 alone.

Introduction

In metastatic melanoma no life-prolonging therapies exist. The best chemotherapy available comprises dacarbazine (DTIC) or cisplatin, which yields response rates between 5% and 30%. Response duration is usually very brief and there is no survival benefit (1-3).

Immunotherapy with recombinant interleukin-2 (IL2) has been reported to yield a 5-27% response rate in metastatic melanoma (4-11). Early studies of IL2 with lymphokine-activated killer cells (LAK) reported high response rates of 30-50% (12,13), but other reports showed lower response rates of 3-23% (5,10,14-18). Several studies of IL2 combined with a chemotherapeutic agent such as DTIC or cyclophosphamide have been carried out and have yielded response rates of about 25% (19-23). Interferon-alpha (IFNα) has shown response rates of 12-22% (24,25).

Based on the synergistic activity of IL2 and IFNα in preclinical experiments (26-28) and on the encouraging results of early clinical trials with this combination (29-31), we decided to perform a phase II study. Here, we report the final analysis of the treatment results after a median follow-up period of 10.5 months (range 1.1-47+ months).

Material and methods

Patients

Fifty-seven patients were entered in the study. The protocol required histologic or cytologic documentation of metastatic melanoma and measurable, progressive metastatic disease. Eligibility criteria included: age 18-70 years, Karnofsky performance status 60-100, no metastases in the central nervous system, no significant cardiovascular history, normal pulmonary function, serum bilirubin and creatinine within normal range, normal bone marrow function (HCT > 30%, WBC > 4000/ml, platelets > 100000/ml), normal coagulation parameters, normal serum calcium and negative tests for HIV antibody and hepatitis-B antigen.

Previous treatment with IL2 or IFNα was not allowed. Prior radiotherapy or chemotherapy had to be completed at least 4 weeks before entry into the study (6 weeks for nitrosoureas or mitomycin). Any significant disease such as infection or peptic ulcer had to be controlled. Corticosteroids were prohibited.

The protocol was reviewed and approved by the institutional review board and the ethical committee of each participating centre.

Six patients were ineligible; 3 had unmeasurable disease, 2 had brain metastases, 1 was pretreated with interferon-2β. Fifty-one patients were evaluable for response and toxicity. The patient characteristics are shown in Table 1. The median time from initial diagnosis to immunotherapy was 24 months (range 1 to 142 months).

TABLE 1 PATIENT CHARACTERISTICS

Number of patients	51	
Age		
median	49	
range	21-72	
Sex		
male	29	(57%)
female	22	(43%)
Performance status (Karnofsky)		
median	90	
range	70-100	
Prior therapy		
none	25	(49%)
chemotherapy	19	(37%)
radiotherapy	5	(10%)
hormone therapy	2	(4%)

TABLE 1 CONTINUED

Distribution of metastatic sites		
lung	20	(39%)
lymph nodes	29	(57%)
skin	16	(31%)
liver	17	(33%)
bone	10	(20%)
Number of metastatic sites		
1	15	(29%)
2	14	(27%)
3	10	(20%)
4	9	(18%)
5	2	(4%)
6	1	(2%)

Treatment

Patients were treated with IL2 at a dose of 7,8 MIU/m^2/day by continuous infusion on days 1-4 and with IFNα-2a 6 MIU/m^2/day by subcutaneous injection on day 1 and 4 of each treatment cycle. IL2 (Teceleukin) and IFNα (Roferon-A) were supplied by Hoffmann-La Roche Ltd, Basle, Switzerland. Cycles were repeated every 2 weeks.

Evaluation of response was performed after 4 cycles and every 2 months thereafter. Patients with response and no change received 9 additional treatment cycles. Further continuation of treatment beyond half a year was allowed. During treatment other anticancer therapy was not allowed. Paracetamol or indomethacine were used to control fever. Vomiting and diarrhea were treated symptomatically. Fluid output was carefully monitored and corrected with parenteral infusion if necessary.

Monitoring

Toxicity was recorded and analysed using the WHO grading system (32). In case of grade 1 and 2 toxicity treatment was to continue as scheduled. When grade 3 neurotoxicity or cardiotoxicity occurred, treatment was discontinued permanently and the patient was carefully followed until the adverse event resolved. With all other grade 3 toxicities (except fever, nausea/vomiting, diarrhea and hypotension) treatment was withheld until the toxicity improved to grade 1 or resolved. Resumption of treatment was allowed in the next cycle. Treatment was permanently discontinued in case of grade 4 toxicity.

Response was evaluated according to the WHO guidelines (32). A complete response (CR) was defined as the disappearance of all known disease for at least 4 weeks. A partial response (PR) was defined as a reduction in the sum of the products of the largest perpendicular diameters of the tumor lesions by at least 50% for more than 4 weeks. Stable disease (SD) denoted less than 50% tumor reduction and less than 25% tumor progression. Progressive disease (PD) was defined as the appearance of a new lesion or an increase in size of more than 25% in any lesion. Duration of response and stable disease were calculated from the start of treatment to the day of disease progression. Time to progression and survival were calculated from the start of protocol treatment until the day progressive disease was first noted.

Results

Response

Of the 51 eligible patients, 24 (47%) received 2-4 treatment cycles, 12 (24%) 5-8 cycles, 13 (26%) 9-13 cycles, one patient 15 and one patient 16 cycles. Four patients were taken off study early; one due to intercurrent illness and 3 due to grade 4 toxicity.

Table 2 shows the treatment results. The overall response rate was 16% (CI:7-29%), including 1 CR and 7 PRs. Twenty patients (39%) had stable disease. In 23 patients (45%) progressive disease was documented. Responses were seen in skin

lesions (36%), lymph nodes (27%), lung (18%) and liver (18%). Of note bone metastases did not respond. Three of the responders were male and 5 were female. All responses occurred in the first 3 months of treatment.

TABLE 2 RESPONSE TO TREATMENT

Number of patients	51	
Complete response	1	(2%)
Partial response	7	(14%)
Stable disease	20	(39%)
Progressive disease	23	(45%)

The median duration of response was 8.2 months (range 4.5-39+ months). For all 51 patients the median time to progression was 2.5 months (range 0.5-39+ months) [Figure 1]. Time to progression for responding patients was 8.2 months (range 4.5-39+ months), for the patients with stable disease 3.6 months (range 1.7-9.4 months) and for progressive disease patients 1.2 months (range 0,5-2.0 months). The median survival of all 51 patients was 11.3 months [Figure 2], and of the responding patients 20.2 months.

Toxicity

An overview of the observed toxicity in the 51 patients is presented in Table 3. Frequently occurring side effects were fever, skin rash, nausea, vomiting, diarrhea and malaise. Two-thirds of patients had tachycardia and hypotension, mostly of mild to moderate grade. Life-threatening hypotension requiring vasopressors occurred in 3 patients, who were taken off study (see above). One patient developed ventricular extrasystoles and another patient atrial fibrillation. In a minority of patients neurological abnormalities and mental disturbances were seen. Neurotoxicity included aphasia, peripheral neuropathy, somnolence, confusion and agitation.

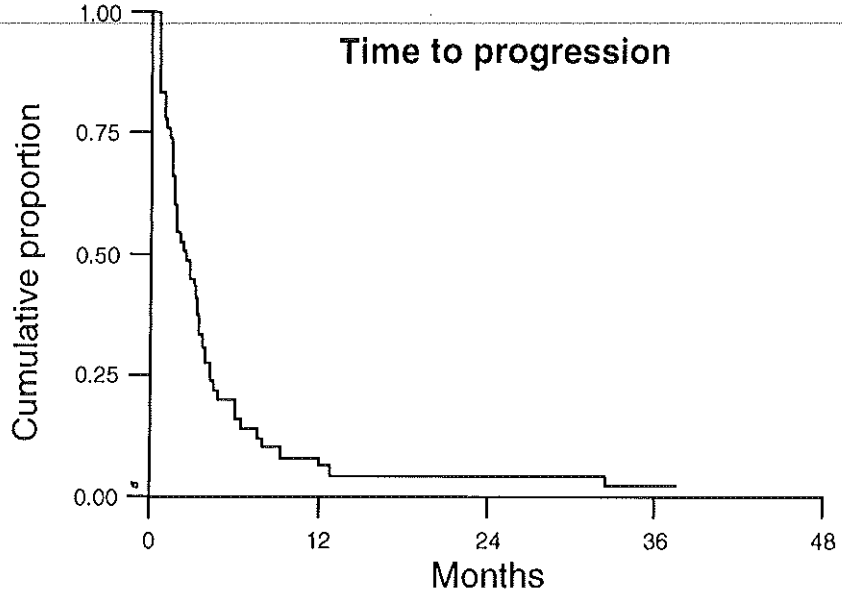

Figure 1. Time to progression (median 2.5 months)

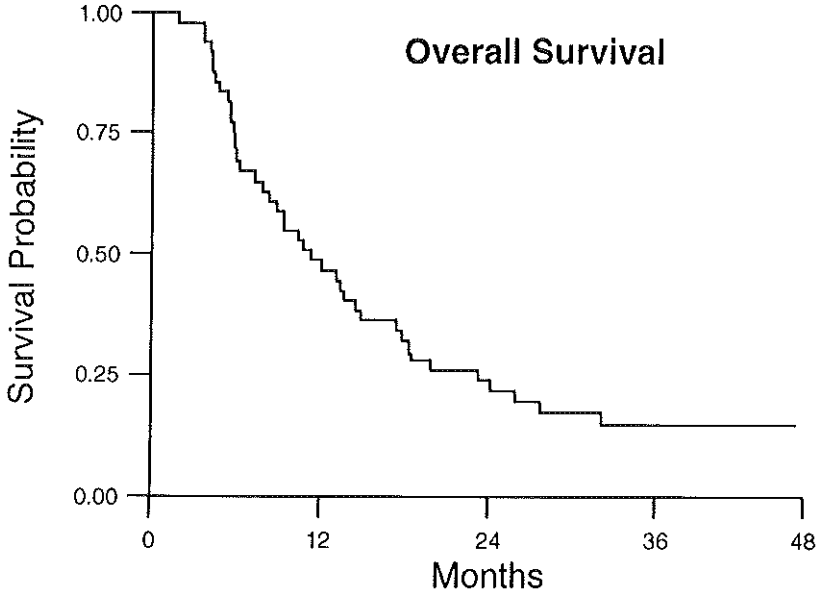

Figure 2. Survival curve (median survival 11.3 months)

Two patients required dose reductions because of adverse events and in 8 patients interruption of treatment was needed. No toxic death occurred and all toxicities resolved after cessation of IL2 and IFNα. Chronic cumulative fatigue occurred after about 3 months of treatment. Consequently only 2 patients did receive more than 13 cycles.

TABLE 3 ADVERSE EVENTS

Adverse events	Number of patients (%)		WHO grading 1	2	3	4
Fever	51	(100)	0	21	30	0
Skin rash/erythema	36	(71)	13	19	4	0
Nausea/vomiting	48	(94)	7	28	13	0
Diarrhea	38	(75)	10	20	8	0
Malaise	29	(57)	4	15	10	0
Weight gain	15	(30)	13	2	0	0
Hypotension	39	(76)	7	18	11	3
Tachycardia	36	(71)	13	20	3	0
Dyspnea	10	(20)	4	4	2	0
Mental disturbances	8	(16)	5	3	0	0
Creatinine	19	(37)	16	3	0	0
Alkaline phosphatase	30	(59)	12	15	3	0
Bilirubin	9	(18)	7	2	0	0
Anemia	36	(71)	17	14	5	0
Thrombocytopenia	9	(18)	7	2	0	0

The most frequent manifestation of hematologic toxicity was anemia (71%). Thrombocytopenia was seen in 18% of the patients. Moderate and reversible increases in serum creatinine and bilirubin occurred in a minority of patients.

A remarkable observation was the occurrence of thyroid function abnormalities in 18 patients (35%). An increase of serum thyroxine (T_4) levels with a concomittant decrease in thyrotropin (TSH) was seen in 10 patients (20%). Low T_4 levels

with high TSH were observed in 12 patients (24%). Treatment with levothyroxine for overt hypothyroidism was needed in 7 patients. Thyroid function abnormalities normalized after discontinuation of immunotherapy.

Discussion

In this study the combined use IL2 and IFNα in the treatment of metastatic melanoma resulted in a 16% response rate, including 2% complete responses. The responses occurred in patients with lymph node, lung and skin metastases. The median time to progression was approximately 3 months for the whole group of patients and 8 months for the responding patients. The median survival of all eligible patients was 11 months and of the responding patients 20 months. These results are disappointing and not better than can be expected of conventional chemotherapy.

The possible synergistic effects of IL2 and IFNα have led several investigators to study the effect of combined treatment with both cytokines. Response rates of 21-44% have been reported (29,30,33). However, low response rates of 10% or less were observed by others (11,18,34,35). In several studies the median response duration varied between 2 and 11 months, and the median survival was approximately 10 months (11,18,29,30,34). We achieved similar results.

We failed to confirm the ability of IFNα to augment the effect of IL2. This may have been due to suboptimal dose and schedule. Patients received moderate doses of IL2. In animal studies the efficacy of IL2 is dose dependent without reaching a plateau below the maximum tolerated dose (36). However, in trials using high-dose IL2 (18 MIU/m^2/day) given by continuous infusion in patients with metastatic melanoma inferior response rates were reported (18,34). An NCI Surgery Branch Study, administering high-dose bolus IL2 (>30 MIU/m^2/day) and IFNα found the highest response rates (29). On the other hand the Extramural IL2 Working Group (ILWG), using identical dose schedule and patient selection criteria did not observe any evidence of enhanced response with the IL2/IFNα combination (11). In an other ILWG study an inferior response rate and duration was demonstrated for patients with advanced renal cell carcinoma treated with high-dose IL2 plus IFNα compared with IL2 alone (37). In summary, a dose-response effect for

IL2 in the treatment of metastatic malignancy is not clear.

Another reason for the low results of the present study may be patient selection. In general the characteristics of our study population are comparable with other trials. However, a considerable number of patients had bone metastases and it has been suggested that bone metastasis respond poorly to immunotherapy (37-39).

The side effects, we observed, were of similar incidence and severity as in other studies with the combined use of IL2 and IFNα (11,29,30,33-35). Toxicity was manageable and patients tolerated the therapeutic regimen relatively well. However, cumulative fatigue made it impossible to give patients more than 13 cycles of therapy. Infectious complications are frequently encountered in IL2 based immunotherapy (40). We observed only one infection problem in our patient group. This may be related to the fact that we have not used central venous lines routinely. A remarkable observation was the considerable high incidence of thyroid function abnormalities. The thyroid dysfunction may be the result of an immunotherapy induced thyroiditis (41,42,43).

In conclusion, the combined therapy with IL2 and IFNα in the described regimen has only moderate activity in the treatment of patients with metastatic melanoma. Further clinical trials have to be designed to improve therapeutic results. Recently a decrescendo interleukin-2 dosing schedule has been developed with an encouraging response rate and reduced toxicity (44). New reports have shown promise for the use of combined or sequential chemo-and immunotherapy (45-47).

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Chapter IV

Dose-efficacy study of two schedules of high-dose bolus administration of interleukin-2 and interferon-alpha in patients with metastatic melanoma

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Will be published in
Br J Cancer 1996; in press

Summary

Background: Immunotherapy with interleukin-2 (IL2) and interferon-alpha (IFNα) is moderately active against metastatic melanoma.

Purpose: This study was undertaken in an attempt to increase the activity by high dose bolus administration of IL2 and IFNα.

Methods: In the first part of the study patients with metastatic melanoma were planned to receive IL2 11,7 MIU/m^2 i.v. plus IFNα 3 MIU/m^2 i.v. by bolus administration every 8 hours on days 1-5, to be repeated every 21 days for a total of 3 courses. Due to unacceptable toxicity only 18 patients were entered. In the second part, the treatment scheme was modified and the patients were treated with the same dosages of cytokines, however for 3 instead of 5 days per cycle.

Results: In the 5-day schedule 17 patients were evaluable for response and toxicity. Two (12%) complete responses and 5 (29%) partial responses were achieved for an overall response rate of 41% (95% confidence interval 18-67%). The median duration of response was 8.6 months. The study was prematurely discontinued because of severe cardiotoxicity in 7 patients (41%) and central nervous system toxicity in 5 patients (28%). Twenty-five patients were treated according to the 3-day schedule. Five partial responses were achieved (20%; 95% confidence interval 7-43%) with a median duration of response of 6.6 months. Toxicity was manageable.

Conclusion: The high dose 5-day schedule of IL2 and IFNα yielded a relatively high response rate at the expense of undesirable toxicity. The modified 3-day regimen was associated with a response rate of 20%, what was within the 10-25% range, expected of conventional IL2 and IFNα schedules.

Introduction

During the past decade two biological agents, interferon-alpha (IFNα) and interleukin-2 (IL2) have shown evidence of activity against metastatic melanoma. Interferon-alpha produced objective tumor regression in approximately 15% of the patients with advanced disease (1-3). Interleukin-2, with or without lymphokine-activated killer cells, yielded response rates from 10-25% (4-7). Experiments in murine models showed synergism between IL2 and IFNα (8-10). This observation has led to a number of clinical studies with the combination of these cytokines. The reported response rates varied between 0-44% (11-16). A phase I-II study with increasing dose levels of bolus IL2 and IFNα conducted at the US National Cancer Institute (NCI) produced an objective response in 33% of patients with metastatic melanoma. The highest response rate of 44% was reached with a schedule of IL2 11.7 MIU/m^2 and IFNα 3 MIU/m^2 3 times a day by intravenous bolus administration, 5 days per cycle (11). We performed a phase II study with this schedule in order to confirm these results.

Patients and methods

Patient population

The study was divided into two parts. Due to the occurrence of severe toxicity the 5-day regimen was replaced by a 3-day schedule. All patients had histologically confirmed melanoma and measurable, progressive metastatic disease. Eligibility criteria included: life expectancy of at least 12 weeks, Karnofsky performance status of at least 80, age between 18-70 years, no prior immunotherapy with IL2 and IFNα, no metastases in the central nervous system, no cardiovascular history, normal pulmonary function, serum creatinine $\leq$ 1.25 times the upper limit of normal, or creatinine clearance $\geq$ 50 ml/min, leucocyte count $\geq$ 4.0 x 10^9/l, platelet count $\geq$ 100 x 10^9/l, hemoglobin $\geq$ 9.5 g/100 ml, and normal liver function with the exception of liver function disturbances due to metastatic disease. Patients were also excluded in case of active infection, use of systemic corticoste-

roids or positive tests for HIV antibody or hepatitis-B antigen. Female patients of child-bearing age were required to have a negative pregnancy test. All patients gave informed consent.

Treatment

In part I of the study patients received recombinant IL2 at a dose of 11.7 MIU/m^2 (Teceleukin; Hoffman La Roche, Nutley, NJ) and recombinant IFNα at a dose of 3 MIU/m^2 (Roferon-A, Hoffman La Roche, Basle, CH), each administered as an intravenous injection over 15 minutes, every 8 hours on days 1-5. This 5-day cycle was repeated every 21 days up to a total of 3 cycles. The cytokines were given via a tunneled central venous double lumen catheter. With this schedule we were confronted with severe and life-threatening cardiotoxicity, albeit that the first 3 days of treatment were relatively well tolerated. Therefore, we decided to modify the treatment schedule, using the same daily dosages of IL2 and IFNα, but now for 3 instead of 5 days.

Patients were treated and monitored on the clinical ward with frequent assessments of vital signs, weight, and fluid balance. Hematologic and biochemical blood tests were performed on days 1,3, and 5 of each treatment cycle. A 12-lead ECG was obtained daily during therapy. Acetaminophen 500 mg orally every 4 hours was given to control fever. Nausea and vomiting were treated with alizapride 150 mg i.v. every 4 hours. In addition, pethidine for chills, loperamide for diarrhea, terfenadine for pruritis and pipamperon for mental confusion were given if indicated. Corticosteroid administration was not allowed, except in the event of life-threatening toxicity. Patients with weight loss secondary to vomiting or diarrhea were given supplementary i.v. fluids.

The World Health Organization (WHO) criteria were used to grade toxicity (17). Treatment was permanently discontinued if grade 3 neurotoxicity or cardiovascular toxicity occurred. In case of grade 3 hypotension, therapy was continued while giving symptomatic treatment with colloids. If volume expansion gave no improvement, dopamine was added (maximum dose of 5 μg/kg/min). Treatment was discontinued if the patient remained hypotensive and/or oliguric (urine production $<$ 80 ml/8 hours). In all other cases of grade 3 toxicity with the

exception of fever, nausea/vomiting and diarrhea, immunotherapy was discontinued until toxicity improved to grade 1 or resolved. Resumption of treatment at 50% of the previous dose was allowed in the next cycle if grade 3 toxicity decreased to grade 1 or less.

Response assessment

The first tumor assessment was performed at 8 weeks after the start of therapy. Response evaluations were repeated every 4 weeks. Tumor response categories were defined according to WHO criteria (17). A complete response (CR) was defined as the disappearance of all known disease for at least 4 weeks, a partial response (PR) as a reduction in the sum of the products of the 2 largest perpendicular diameters of all lesions by at least 50% for more than 4 weeks, without the appearance of any new lesion. Stable disease (SD) denoted less than 50% tumor reduction and less than 25% tumor progression. Progressive disease (PD) was defined as the appearance of a new lesion or an increase in size of more than 25% in any indicator lesion. The treatment results were analyzed on an intent-to-treat basis. The confidence interval of the observed response rate was computed using the exact binomial distribution. Duration of response, time to progression and survival were calculated from the start of treatment. The median survival was estimated using the Kaplan-Meier method.

Immunological monitoring

Absolute numbers of lymphocyte subsets and cytotoxic activities of peripheral blood mononuclear cells (PBMC) were assessed in each treatment cycle, immediately prior to the first administration of IL2 and IFNα and one week later. The PBMC were isolated by Ficoll-Isopaque density centrifugation of heparinized venous blood samples. An aliquot was processed immediately for immunophenotyping. The remainder was cryopreserved in liquid N_2 so that all samples from a given patient were tested for cytotoxicity at one occasion to exclude interassay variability. The lymphocyte subsets defined by double-staining with CD3 and CD56, CD4 and CD8, CD16 and CD19 monoclonal antibodies were assessed by multicolor immunofluorescence and flow cytometry as described elsewhere (18,19). Cytotoxic activities of lymphocytes were determined in standard 3-h ^{51}Cr-release

assays as described previously (18). The K562 erythromyeloid leukemia cell line and the Daudi Burkitt's lymphoma cell line were used as target cells for the assessment of natural killer (NK) and lymphokine-activated killer (LAK) activities, respectively.

Results

Patient characteristics

Forty-three patients were entered in the study, 18 in part I and 25 in part II. The patient characteristics are summarized in Table 1. All eligible patients were evaluable for response and toxicity. In the 5-day regimen, one patient was ineligible because of unmeasurable disease. Two patients had previously been treated with isolated limb perfusion with melphalan and one patient received palliative radiotherapy before study entry. In the 3-day regimen, 2 patients were pretreated with adjuvant BCG or Poly A-Poly U immunotherapy. The other patient characteristics were similar for both parts of the study.

TABLE 1 PATIENT CHARACTERISTICS

	Part I		Part II	
Evaluable patients	17		25	
Age				
median	48		41	
range	29-61		20-69	
Sex				
male	9	(53%)	16	(64%)
female	8	(47%)	9	(36%)
Performance status (Karnofsky)				
median	90		90	
range	80-100		80-100	

TABLE 1 CONTINUED

Prior therapy				
surgery	17	(100%)	25	(100%)
immunotherapy	0		2	(8%)
chemotherapy	2	(12%)	0	
radiotherapy	1	(6%)	0	
Distribution of metastatic sites				
lung	9	(53%)	10	(40%)
lymph nodes	7	(41%)	17	(68%)
subcutaneous	6	(35%)	12	(48%)
liver	6	(35%)	9	(36%)
other (adrenal, pancreas, bone)	8	(47%)	9	(36%)
Number of metastatic sites				
1	6	(35%)	7	(28%)
2	4	(24%)	7	(28%)
3	4	(24%)	7	(28%)
4	2	(12%)	4	(16%)
5	1	(6%)	0	

Treatment characteristics

Of the 17 evaluable patients in part I, 9 (53%) received 3 treatment cycles, 3 (18%) 2 cycles and 5 (29%) only 1 cycle. Eight patients were taken off study early; 6 due to grade 3-4 toxicity, one due to rapidly progressive disease and another patient refused further treatment. The actual cytokine doses given during the first, the second and the third cycle, expressed as percentage of the planned dose were 85%, 42% and 30%, respectively.

Of the 25 evaluable patients in part II, 14 (56%) received 3 cycles, 5 (20%) 2 cycles and 6 (24%) one cycle. The actual cytokine doses administered to these groups of patients were 95%, 71% and 54% of the planned dosages, respectively. Treatment was discontinued in one patient after only one single cytokine infusion due to the development of grade 3 toxicity. He was considered a treatment failure.

Treatment results

Table 2 shows the results of response and survival. With the 5-day schedule the overall response rate was 41% (95% confidence interval 18-67%), including 2 CRs. The overall survival was 10.2 months (range 2.6-37.5 months). The responding patients had a median survival of 27.4 months (range 2.7-37.5 months). With the 3-day schedule the overall response rate was 20% (95% confidence interval 7-43%). No complete responses occurred. The overall survival was 6.8 months (range 0.9-24+ months). The responding patients had a median survival of 14.2 months (range 6.6-21.5 months).

TABLE 2 RESPONSE TO TREATMENT

	Part I		Part II	
Evaluable patients	17		25	
Complete response (CR)	2	(12%)	0	
Partial response (PR)	5	(29%)	5	(20%)
Overall response rate	41%		20%	
Stable disease (SD)	3	(18%)	4	(16%)
Progressive disease (PD)	7	(41%)	16	(64%)
Median duration of response	8.6	months	6.6	months
(range)	(2.0-37.5)		(3.9-9.9)	
Median time to progression	3.2	months	2.0	months
(range)	(0.7-37.5)		(0.5-9.9)	
Median survival	10.2	months	6.8	months
(range)	(2.6-37.5)		(0.9-24+)	

Toxicity

Adverse effects are shown in Table 3. Fever, fatigue, nausea, vomiting and diarrhea were frequently observed. With the 5-day schedule a high incidence of

severe cardiac toxicity occurred. Seven patients (41%) experienced cardiac adverse events; cardiomyopathy in 4, acute cardiac arrest, myocardial infarction and negative T-waves on the ECG in one patient each. The details of these patients have been reported elsewhere (20). No cardiotoxicity was observed in the 3-day regimen.

Two-thirds of patients in the 5-day regimen suffered from neuropsychiatric disturbances such as agitation, disorientation, confusion and overt psychosis. With the 3-day regimen neuropsychiatric side effects were encountered in about one-third of patients, and were mild in most cases. Neurotoxicity completely resolved in all patients.

TABLE 3 ADVERSE EVENTS

	Part I		Part II	
Adverse events (%)	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Fever	12%	88%	52%	48%
Skin rash	76%	0%	64%	0%
Nausea/vomiting	76%	18%	40%	52%
Diarrhea	53%	35%	48%	44%
Malaise	24%	76%	40%	56%
Weight gain	35%	0%	16%	4%
Hypotension	41%	53%	60%	32%
Cardiac	6%	35%	0%	0%
Dyspnea	53%	24%	28%	8%
Neuropsychiatric	41%	29%	32%	4%
Oliguria	18%	18%	20%	12%
Creatinine	47%	0%	12%	0%
Alkaline phosphatase	76%	6%	56%	4%
Bilirubin	47%	12%	16%	12%
Transaminases	47%	53%	60%	24%
Anemia	53%	0%	36%	8%
Thrombocytopenia	41%	6%	48%	0%

Immunologic monitoring

Prior to therapy the absolute numbers of NK lymphocytes [$CD56^+$ 3^- and $CD16^+$], T lymphocytes [$CD3^+$], helper/inducer T lymphocytes [$CD4^+$], cytotoxic/suppressor T lymphocytes [$CD8^+$] and B lymphocytes [$CD19^+$] were within the normal range. During therapy, the $CD56^+$ 3^-, $CD3^+$ and $CD8^+$ lymphocyte counts gradually increased above normal, whilst the $CD4^+$ and $CD19^+$ remained within the normal range (Figure 1). The number of lymphocytes after each treatment cycle (rebound lymphocytosis) reached a higher value in the 5-day study (Figure 1, open symbols) compared to the 3-day study (closed symbols). NK and LAK cytotoxic activities of PBMC remained within the normal range in both parts of the study, with a large variation between patients (data not shown). There was no relationship between tumor response and immune parameters.

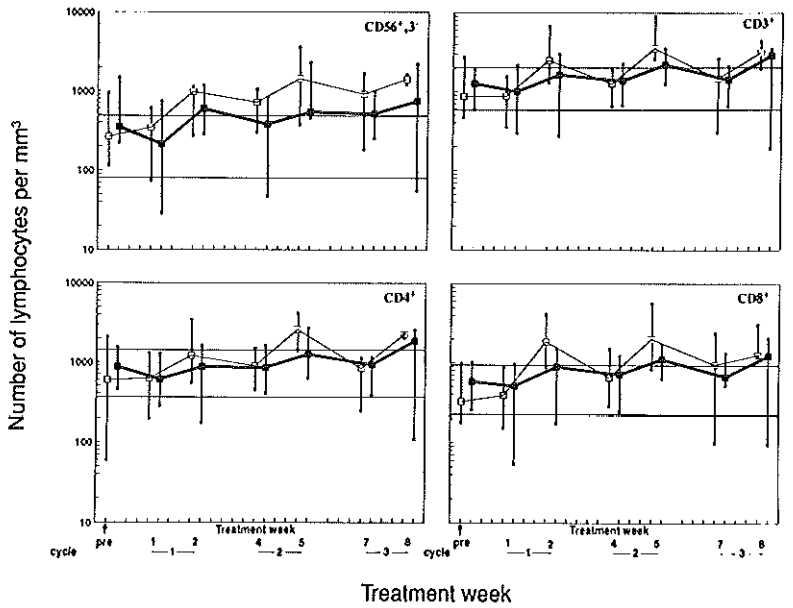

Figure 1. Median absolute numbers of $CD56^+$,3^- (upper left pannel), $CD3^+$ (upper right pannel), $CD4^+$ (lower left pannel) and $CD8^+$ (lower right pannel) lymphocytes in patients treated in the 5-day schedule (open symbols) and in the 3-day schedule (closed symbols). Logarithmic scales have been used for the vertical axes in order to compress the figure. Vertical bars represent confidence limits as defined by the 5th and 95th percentile of each group. The areas between the horizontal lines represent the normal range of the different lymphocyte subsets as defined by the 5th and 95th percentiles of healthy control persons.

Discussion

From an earlier carried out NCI dose escalating study of bolus IL2 and IFNα we selected the dosage schedule with the highest response rate in patients with metastatic melanoma (IL2: 11.7 MIU/m^2 and IFNα: 3 MIU/m^2, 3 times a day, during 5 days). This treatment schedule resulted in a 41% response rate (95% CI 18-67%), which is identical to the results reported by the NCI investigators (11).

However, we observed a high incidence of severe cardiotoxicity. Light- and electronmicroscopic examination revealed cardiomyopathy and myocarditis (20). Although severe myocardial toxicity was not so frequently encountered in the NCI study as we did, several cases of myocardial infarction and elevation of cardiac enzyme levels in 15% of treatment courses were reported (11, 21). Also other recent literature indicates that high-dose IL2 regimens bear a high risk of severe cardiotoxicity (22-24).

The second severe toxicity of this 5-day regimen consisted of severe neuropsychiatric disturbances. Evidence from earlier studies showed that the frequency and severity of these side effects are dose dependent (12,13,15,23,25-28). The NCI investigators observed severe neurotoxicity including coma in 54% of patients (21).

Thus, these promising results on response were accompanied by unacceptable toxicities. In an attempt to maintain the high antitumor activity and to reduce the side effects, we shortened in the second part of the study the treatment duration from 5 to 3 days. Indeed, the 3-day schedule was accompanied with acceptable toxicity, but the response rate dropped to 20%. A direct comparison between the two schedules is difficult, because it was not a randomized study and the confidence intervals showed considerable overlap. The response rate and survival duration of the 3-day schedule were in the same range as observed in most other reported studies of IL2 and IFNα in melanoma (12-14,16,21,27,29). In a recent progress report the NCI investigators observed a similar decrease in treatment results after modification of the treatment schedule (1 instead of 3 IFNα administrations per day), made necessary by the encountered toxicity (21). In general, the response rates and survival data of combined therapy with IL2 and IFNα in patients with metastatic melanoma appeared not to be superior to treatment with IL2 alone or IL2 in combination with lymphokine-activated killer cells (13,21,30-34).

With respect to the evaluated immune parameters we observed some difference in the degree of lymphocytosis between the 5-day and the 3-day regimen, but there was no association between lymphocyte counts, phenotypic changes or cytotoxic activity of lymphocytes and tumor response. This lack of relationship is also reported by others (35-37). It appears that the quantification of these general parameters gives insight into the degree of immune modulation achieved with a particular treatment rather than in the mechanism of its therapeutic effect.

In summary, the combination of IL2 and IFNα has not meaningfully improved the clinical results that may be obtained with IL2 alone. Because of the disappointing treatment results with cytokines alone in metastatic melanoma and because of the reported possibility of additive or synergistic effects between chemotherapy and cytokines (38-41), we participate at this moment in a phase III study of the EORTC Melanoma Cooperative Group, comparing the combination of IFNα, cisplatin and dacarbazine with or without IL2.

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High-dose regimen of interleukin-2 (IL2) and interferon-alpha (IFNα) in combination with lymphokine-activated killer cells (LAK) in patients with metastatic renal cell cancer

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Summary

Background: Immunotherapy with interleukin-2 (IL2) alone, IL2 and lymphokine-activated killer cells (LAK) or IL2 and interferon-alpha (IFNα) has resulted in significant antitumor effects in patients with metastatic renal cell cancer.

Purpose: This study was carried out to investigate if the combined use of all three components (IL2, IFNα and LAK) could improve the treatment results.

Methods: Seventy-two patients with metastatic renal cell cancer were treated. Seventeen patients were entered in a feasibility part of the study (protocol 1) and 55 in an efficacy part (protocol 2). Protocol 2 differed from protocol 1 by the addition of IFNα to the first 5 days of IL2 infusion. Each patient was planned to receive two induction cycles. IL2 18 MIU/m^2/day was administered continuous i.v. days 1-5 and IFNα 5 MIU/m^2/day (protocol 2) i.m. days 1-5, followed by 3 daily lymphaphereses on days 7-9. On day 12 treatment was resumed with IL2 and IFNα on days 12-15 and LAK reinfusions on days 12-14. Patients, whose disease stabilized or responded, received maintenance therapy with the same dose regimen of IL2 and IFNα during 4 days every 4 weeks up to a total of 4 cycles.

Results: In protocol 1, all 17 patients were evaluable. Three complete (CR) and one partial (PR) responses were achieved (response rate 24%). The median duration of response was 18.1 months. The median survival of the 17 patients was 13.9 months. The 3-year survival was 35%. Toxicity was manageable and no dose reductions were necessary.

Of the 51 evaluable patients in protocol 2, 6 achieved a CR and 13 a PR (response rate 37%). The median duration of response was 11.1 months. The median survival was 16.9 months. The 3-year survival was 35%. There were 3 treatment related deaths. Other severe toxicities included hypotension, cardiotoxicity, pulmonary edema, renal toxicity and infectious complications. In the 2 induction cycles only 54% and 42% of the planned doses of IL2 and IFNα could be administered.

Conclusion: Based on our experience with this treatment schedule of IL2, IFNα and LAK we conclude that the use of high-dose regimens with these cytokines is not warranted, unless we can accurately define the 25-35% of patients who will experience long-term survival as a result of this treatment.

Introduction

High-dose interleukin-2 (IL2) alone or combined with lymphokine-activated killer (LAK) cells leads to a response in 15-30% of patients with metastatic renal cell cancer (1-10). Interferon-alpha (IFNα) has modest activity against renal cell cancer with a response rate of approximately 15% (11-13). In preclinical experiments a synergistic antitumor effect of IL2 and IFNα has been demonstrated (14-16). In early clinical studies the combination of IL2 and IFNα has yielded response rates ranging from 22-40% (17-20).

The experience with multimodality treatment consisting of IL2, IFNα and LAK is limited. Only one study with this combination has recently been published (21). Here, we report a phase II study in which we evaluated the toxicity and antitumor efficacy of combination immunotherapy consisting of high-dose IL2 and IFNα with LAK in patients with metastatic renal cell cancer.

Patients and methods

Patient population

Seventy-two patients with progressive metastatic renal cell cancer were studied. Their primary tumor was removed by nephrectomy. Eligibility criteria included bidimensionally measurable disease, age $\leq$ 70 years, performance status Karnofsky index $\geq$ 80 (WHO 0-1), normal organ functions of heart, lung, kidney, bone marrow and normal serum bilirubin and coagulation tests. Prior immunotherapy or chemotherapy was not allowed. Patients with uncontrolled hypertension, a history of myocardial infarction or arrhythmias, central nervous system metastases, infections and use of steroid medication were excluded. To exclude significant cardiac dysfunction, every patient had to have normal ECG in rest and during exercise, cardiac multiple uptake gated acquisition (MUGA) scan and echocardiography.

Treatment

The treatment schedule is displayed in Figure 1. Seventeen patients were entered into a feasibility part of the study (protocol 1), using Proleukin IL2 (Eurocetus, Amsterdam, The Netherlands). Subsequently, after the treatment scheme proved to be safe, an efficacy study (protocol 2) was carried out, using Teceleukin IL2 (Hoffmann-LaRoche, Basle, Switzerland). In protocol two 55 patients were included. Protocol 2 differed from protocol 1 by the addition of IFNα (Roferon, Hoffmann-LaRoche, Basle, Switzerland) to the first 5 days of IL2 infusion in the 2 induction cycles. In both protocols two cycles of adoptive cellular therapy with IL2 and IFNα and LAK were given. Each cycle started with a 120 hour priming phase of IL2 18 MIU/m^2/day administered as a continuous infusion (civ) and IFNα 5 MIU/m^2/day on days 1-5 (protocol 2) as intramuscular (im) injections. After a rest period of 24 hours 3 daily runs of lymphapheresis were performed (days 7-9). The autologous lymphocytes obtained were incubated with IL2 for 5 days and reinfused on days 12-14. Infusion of IL2 civ (108 h) at the same doses as above was resumed at the start of LAK administration, together with daily im injections of IFNα 5 MIU/m^2/day (days 12-15). IFNα was administered 3 hours before infusion of LAK. IL2 was reconstituted without carrier protein in protocol 1 and with 0.5-0.7% human serum albumin which was a constituent of the Teceleukin vials in protocol 2 (22).

After a rest period of 3 weeks this cycle was repeated on day 36. After two induction cycles each patient was evaluated for response. Patients with stable disease or response were scheduled for maintenance treatment with 4 monthly cycles of IL2 18 MIU/m^2/day and IFNα 5 MIU/m^2/day on days 1-4.

Patients were treated and monitored on the clinical research unit during IL2 infusions. Vital signs, daily weights and urine volumes were obtained. Fluid balance was carefully monitored. Acetaminophen (Paracetamol), 500 mg every 4-6 hours, was used to control fever. Alizapride (Litican^R), loperamide (Imodium^R), and codeine were routinely given to suppress nausea, vomiting, and diarrhea. Prophylactic antibiotics were not used routinely. Because of possible nephrotoxicity, non-steroid anti-inflammatory drugs (NSAID's) such as indomethacine were avoided. Steroids were prohibited. Lymphaphereses and the

administration of lymphokines and LAK were performed via tunneled central venous catheters. Intravenous heparine 15,000 U/day was given during IL2 treatment episodes to reduce the risk of thrombo-embolic complications.

Toxicity was graded according to the World Health Organization (WHO) criteria (23). For toxicities not included in the WHO guidelines a grading system was used ranging from mild (grade 1) to life-threatening (grade 4). Initial treatment of hypotension and oliguria consisted of i.v. volume replacement. If volume expansion gave no improvement dopamine was added up to doses of 5 μg/kg/min to improve renal perfusion.

Two induction cycles of 35 days		
days 1 - 5	7 - 9	12 - 16
↑ ↑ ↑ ↑ ↑		↑ ↑ ↑ ↑ ↑
IL2		IL2
	↓ ↓ ↓	↑ ↑ ↑
	LY-PH	LAK
↑ ↑ ↑ ↑ ↑		↑ ↑ ↑ ↑
IFNα*		IFNα

Four maintenance cycles of 28 days
Days 1-4
↑ ↑ ↑ ↑
IL2
↑ ↑ ↑ ↑
IFNα

IL2: 18 MIU/m²/day CIV.
LY-PH: lymphapheresis.
IFNα: 5 MIU/m²/day IM.
* not administered in protocol 1.

Figure 1. Induction and maintenance treatment scheme of IL2 with LAK and IFNα

The administration of cytokines was interrupted if hypotension WHO grade 3/4, not responding to intravenous fluids or dopamine occurred, if oliguria (urine production < 15 ml/hour) developed or if the creatinine level rose above 400 μmol/l. Other reasons for interruption were metabolic acidosis, severe arrhythmia or myocardial ischemia, signs or symptoms of lung edema, agitation or persistent confusion not responding to piperidone and elevation of serum bilirubin > 85 μmol/l.

Treatment was discontinued until the side effects improved to grade 1 toxicity or resolved. Reductions in the dose of IL2 and IFNα by 50% were made in the subsequent cycles if the patient had experienced hypotension not responding to therapy within 8 hours, serum bilirubin > 85 μmol/l or creatinine > 525 μmol/l and WHO grade 3 central nervous system toxicity.

Treatment was permanently discontinued in case of documented myocardial ischemia, WHO grade 4 central nervous system toxicity, serum bilirubin or creatinine levels that failed to return to grade 1 toxicity levels or better.

Response assessment

Before the start of treatment all tumor lesions were assessed by routine CT scans of chest, abdomen and brain. The 2 largest perpendicular diameters of each indicator lesion were measured and multiplied. The sum of the products of these diameters was calculated. This procedure was repeated after 8 weeks and every 2 months thereafter. Response criteria were used according to the instructions of the WHO handbook (23). A complete response (CR) was defined as the disappearance of all known disease for at least 4 weeks, a partial response (PR) as a reduction in the sum of the products of the 2 largest perpendicular diameters of all lesions by at least 50% for more than 4 weeks, without the appearance of any new lesion. Stable disease (SD) denoted less than 50% tumor reduction and less than 25% tumor progression. Progressive disease (PD) was defined as the appearance of a new lesion or an increase in size of more than 25% in any indicator lesion.

The best response observed in a given patient was noted as the overall response. Response duration was calculated from the start of treatment, as were time to progression and survival, using the Kaplan-Meier method.

Activation of lymphocytes with IL2 *in vitro*

Buffy coats harvested by lymphapheresis (Fenwall CS-3000) were placed into culture using a semi-closed bag system: Travenol-Fenwall PL 732 bags, containing 1500 ml activation medium with 3 x 10^6 cells/6000 IU IL2/ml. Bags were loaded with cells and medium using a Travenol-Fenwall model SAV EX 2 Fluid Fill/Weight Unit. The activation medium consisted of 78 % RPMI-1640, 20% AIM-V and 2% autologous human plasma. 2 mM L-glutamine, 50 μg/ml streptomycin, and 40 μg/ml gentamycin were added to the medium.

Bags were incubated flat at 37°C, 5% CO_2, 95% humidity for 120 hours (5 days). Cells were harvested from the bags with a Fenwall Cell Harvester, washed using saline and resuspended in 5% human serum albumin supplemented with 6000 IU IL2/ml to a volume of approximately 500 ml.

Cultures for aerobic an anaerobic micro-organisms were obtained immediately after lymphapheresis, 24 hours prior to cell harvest and 1 hour before reinfusion of LAK cells into the patient. Samples of LAK were tested for viability and cytotoxicity.

Immunological studies

In vitro IL2 activated lymphocytes (LAK) were tested for lysis of K562 and Daudi target cells in a 4-hour standard ^{51}Cr release assay. Mononuclear cells isolated from patients on treatment were assayed for: a) cell surface markers detected by monoclonal antibodies in fluorescence assays and b) cytolysis against NK-sensitive (K562) and NK-resistant (Daudi) target cells. The sera from patients were collected at predetermined timepoints before and during treatment for the assay of IFNγ, TNF, IL2, soluble IL2 receptor, IL6, IL8 and CRP. We have reported a detailed description of these immunological analyses elsewhere (24).

Results

Patient characteristics

In total seventy-two patients were entered in both parts of the study; 17 in protocol 1 and 55 in protocol 2. Their median age was 54 years (range 30-69); 47 (65%) were male and 25 (35%) female. Patient characteristics per protocol are summarized in Table 1. Ten patients (14%) received palliative radiotherapy before study entry. The median time from initial diagnosis to start of treatment was 6 months. In 25% of the patients (18/72) the time that elapsed between the primary diagnosis of renal carcinoma and the development of metastases was more than 24 months.

TABLE 1 PATIENT CHARACTERISTICS

	Protocol 1	Protocol 2
Age, median (range)	58 (35-68)	54 (30-69)
Male:female	12 : 5	35 : 20
Karnofsky, median (range)	100 (90-100)	100 (80-100)
Prior radiotherapy	4 (24%)	6 (11%)
≥ 2 metastatic organ sites	8 (47%)	33 (60%)

Treatment characteristics

A total of 33 induction cycles were administered to the patients in protocol 1. One patient developed rapid progressive disease and received only one induction cycle. Therapy was continued with maintenance courses in 10 patients, who received a number of 37 additional cycles. The actual doses of cytokines administered in the 2 induction cycles, expressed as percentage of the planned dose were 100% and 94%, respectively. Dose reductions were not necessary.

In the second protocol, 18 patients (33%) received only one induction cycle: 3 (5%) died of toxicity, 11 (20%) developed severe adverse events, and 4 patients (7%) were taken off study due to rapid progressive disease. Five patients (9%) required dose reductions during the first course because of significant toxicities. Six patients (11%) did not receive any LAK infusions. Of the 37 patients (67%), who received a second induction cycle, toxicity necessitated treatment discontinuation in 4 (7%). Patients received an average of 54% (range 23-100%) and 42% (range 8-100%) of the planned dose of IL2 and IFNα in the 2 induction courses, respectively. An overview of the administered dose levels of cytokines during the induction cycles in protocol 2 is presented in Table 2. Twenty-five patients (45%) received maintenance therapy for a total of 71 cycles.

TABLE 2 PERCENTAGE OF PLANNED DOSE OF IL2 AND IFNα ACTUALLY GIVEN DURING INDUCTION TREATMENT IN PROTOCOL 2

	Patients (%)	
Dose	Cycle 1	Cycle 2
> 80%	29 (53%)	8 (15%)
60-80%	15 (27%)	7 (13%)
< 60%	11 (20%)	22 (40%)
0%	-	18 (33%)

Treatment results

Of the 68 evaluable patients in the whole study, 23 responded (response rate 34%; 95% confidence interval 23-46%). There were 9 complete (CR) and 14 partial (PR) responses. The treatment results per protocol are listed in Table 3. In protocol 1 all 17 patients were evaluable for response and toxicity. The response rate was 24% (95% confidence interval 7-50%), with 3 CRs and 1 PR. The median duration of response was 18.1 months (range 5.5-56.0+). The median survival was 13.9 months (range 1.9-56.0+).

In protocol 2, fifty-one patients were evaluable for tumor response. Three patients died due to toxicity and in the fourth patient post-treatment tumor evaluation could not be carried out. These patients were classified as treatment failures. Six CRs (12%) and 13 PRs (24%) were achieved for an overall response rate of 37% (95% CI 24-52%). The median duration of response was 11.1 months (range 2.9-31+ months). The median survival was 16.9 months (range 1.0-48+ months). In both protocols the 3-year survival was 35%.

TABLE 3 TREATMENT RESULTS

	Protocol 1		Protocol 2	
Evaluable patients	17		51	
Complete response	3	(18%)	6	(12%)
Partial response	1	(6%)	13	(25%)
Overall response	4	(24%)	19	(37%)
Median duration of response	18.1	months	11.1	months
Median time to progression	6.0	months	5.9	months
Median survival	13.9	months	16.9	months
3-Year survival	35%		35%	

Toxicity

An overview of the most important grade 3/4 toxicities is presented in Table 4. Toxicity was predominantly observed during the induction cycles. Frequently encountered side effects of any grade were fever, fatigue, malaise, nausea, vomiting, diarrhea and skin toxicity.

There were important differences in the frequency and intensity of observed toxicities between the two protocols. Treatment was relatively well tolerated in protocol 1 and toxicity did not require dose reductions or permanent treatment

discontinuation in any patient. Grade 3/4 hypotension occurred in 9 patients (53%) but only necessitated brief interruption of IL2 administration. Three patients developed supraventricular rhythm disturbances. Only a minority of patients experienced relatively mild neurological symptoms.

In contrast, toxicity was considerable in protocol 2. There were 3 treatment-related deaths. One patient, a 56-year old male, died of massive pulmonary embolism during the convalescence period 3 weeks after the first induction cycle was completed. A second patient, a woman of 68-years old, developed intractable hypotension and anuria with severe metabolic acidosis on day 4 of the first episode with IL2 and IFNα.

Despite cessation of immunotherapy and termination of its effects by i.v. corticosteroids and maximum efforts at the intensive care unit, she died of multiple organ failure. Blood, urine and stool microbial cultures were negative. In the third patient, a 61-year old woman, hypotension and oliguria occurred on day 2 of the second induction cycle, followed by signs of cardiac failure. Echocardiography showed hypokinesis of the left ventricle and low ejection fraction, indicating cardiomyopathy. Despite the infusion of vasopressors and artificial ventilation, a complete atrioventricular block developed, immediately followed by a dying heart rhythm.

Hypotension grade 3/4 was the most common dose-limiting adverse effect occurring in 42 patients (76%). In 11 patients (20%) treatment was discontinued because of cardiovascular and pulmonary complications: hypotension in 4, reversible cardiomyopathy in 3, rhythm disturbances in 2 and lung edema with respiratory insufficiency in another 2 patients. Transient renal failure grade 3 necessitated dose reductions in 12 patients (22%). Six patients (11%) required dose reduction because of grade 3 neurotoxicity. Sixteen patients (29%) developed infections, mostly catheter related.

Maintenance therapy was given without serious problems in the first protocol. In protocol 2 the toxicity was cumulative with regard to fatigue and renal function disturbances, leading to premature cessation of treatment in 9 of 25 patients.

TABLE 4 GRADE 3 AND 4 TOXICITY

Adverse event	prot. 1		prot. 2		prot. 1		prot. 2	
	Grade 3				Grade 4			
	Number of patients (%)							
Fever	11	(65)	49	(89)	0		0	
Fatigue	11	(65)	43	(78)	2	(12)	8	(15)
Anorexia	2	(12)	17	(31)	0		1	(2)
Skin	1	(6)	10	(18)	0		0	
Gastro intestinal								
Nausea/vomiting	8	(47)	19	(35)	0		1	(2)
Diarrhea	3	(18)	12	(22)	0		3	(5)
Hepatic								
Bilirubine	0		0		0		0	
Alk. phosphatase	2	(12)	8	(15)	0		3	(5)
Transaminases	1	(6)	12	(22)	0		4	(7)
Weight gain	0		6	(11)	0		1	(2)
Hypotension	8	(47)	33	(60)	1	(6)	9	(16)
Cardiac								
Cardiomyopathy	0		3	(5)	0		2	(4)
Arrhythmia	3	(18)	10	(18)	0		2	(4)
Pulmonary	3	(18)	13	(24)	0		2	(4)
Renal failure	0		12	(22)	0		0	
Neurologic	0		13	(24)	0		0	
Hematologic								
Anemia	0		5	(9)	0		0	
Thrombocytopenia	1	(6)	8	(15)	0		2	(4)
Infection	2	(12)	10	(18)	0		2	(4)

Immunologic parameters

We have recently published the results of the immunological monitoring of the patients in this study (24). In short, the most important findings were as follows. During IL2 infusion peripheral lymphopenia developed, followed by rebound lymphocytosis within 2 days after cessation of treatment and a return to normal during the subsequent 2 to 3 weeks. The eosinophil counts increased to supranormal levels and eosinophilia persisted during the entire treatment period. Serum concentrations of the secondary cytokines IFNγ and TNFα were increased. The peak levels of serum IL2, IFNγ and TNFα during IL2 infusion were 2-3 times higher in protocol 2 than in protocol 1, which was explained by the better bioavailibility of IL2 after reconstitution with carrier protein in protocol 2 (22). The cell phenotypes of the apheresis products were not significantly different between protocols 1 and 2. Differences between responders and non-responders treated according to the two protocols were not significant, except for the total number of lymphocytes obtained by apheresis, which was higher in responders than in non-responders, reaching statistical significance in multivariate analyses ($p = 0.02$).

Clinical prognostic factors

The effect of the following baseline clinical parameters on antitumor response and survival was investigated by multivariate analysis: (i) treatment protocol (1 vs 2): (ii) performance status (Karnofsky index 80-90 vs 100); (iii) time interval between diagnosis of the primary and start of treatment for metastasis ($\leq$ 24 vs $>$ 24 months); (iv) number of metastatic sites (1 vs $\geq$ 2); (v) absence vs presence of metastases in lymphnodes, lung, liver, bone, abdomen or soft tissues; (vi) weight loss. The only parameter of borderline statistical significance was lymphnode metastasis: 15 of 33 patients (45%) with predominant lymphnode metastases responded versus 8 of 35 patients (23%) with predominant visceral metastases without lymphnodes ($p = 0.049$). None of the above mentioned parameters had a significant effect on survival.

Discussion

This triple regimen of high-dose IL2 and LAK in combination with IFNα was developed in our institution in 1988. At that time treatment with IL2 combined with LAK drew considerable attention. Preclinical animal studies suggested that the addition of LAK cells to IL2 could markedly improve antitumor activity. Several clinical trials, using high-dose IL2 and LAK reported relatively high response rates of 30-35% in patients with metastatic renal cell cancer (2,25). A European multicenter study, in which we participated, yielded a response rate of 27% (3). Moreover, animal studies and early clinical studies of the combination of IL2 and IFNα appeared very promising with response rates of up to 40% (14-20).

Consequently, we wanted to investigate whether a triple combination of high-dose IL2 and LAK with IFNα could yield a high rate of responses of long duration in patients with metastatic renal cell cancer. To be able to confirm a predetermined response rate of approximately 40% we planned to perform an extended phase II study, comprising ≥ 40 patients. We had the intention to use the eventual good results of this phase II study as a basis for a subsequent phase III study, to examine the relative contribution of LAK. Initially, we were concerned about the use of IFNα in the "priming" phase (day 1-5), because IFNα can cause lymphopenia, which would jeopardize the lymphocyte harvest during lymphaphereses. Secondly, the addition of IFNα might aggravate IL2 related toxicities, particularly hypotension, which would also complicate the lymphapheresis procedure. However, the adverse effects observed in the feasibility part of the study proved to be manageable, whereas the treatment results with a response rate of 24% and a median survival of 18 months were reasonable.

Therefore, we felt encouraged to continue with an efficacy study. After having entered 41 evaluable patients, a preliminary analysis demonstrated a response rate of 39%, a median duration of response of 14 months, and notably a median survival of 28 months (26). Thus, protocol 2 appeared to be highly effective, although the adverse effects were much more outspoken than in protocol 1. We ascribe this increase in side effects not only to the addition of IFNα into the "priming" days 1-5, but particularly to the observed higher serum levels of IL2, TNFα, and IFNγ after the change from Proleukin to Teceleukin, which can be

explained by the better bioavailability of Teceleukin (24,27-29). At the time of the preliminary analysis, we concluded that this combination regimen was the best available therapy for metastatic renal cell cancer. Consequently, the protocol was kept open for the period of time which was needed for the design and the organization of a phase III comparative study of IL2 and IFNα with or without LAK. The phase II study, protocol 2 here reported, was closed after 55 patients were recruited. The final analysis showed an identical response rate of 37%, but a decrease in the duration of response from 14 to 11 months, and more importantly a decrease in the median survival time from 28 to 17 months. Attempts to find explanations for this unfavourable development of the treatment results suggested that the last 14 patients in the study have a less good performance status and more extensive disease.

The past few years have witnessed a flood of reports on immunotherapy studies in renal cell cancer. Trials of IL2 monotherapy in intermediate to high-dose yielded response rates from 13-20% (5,30-35). More recent studies of IL2 and LAK yielded response rates of 9-20% and could not confirm the earlier reported better treatment results compared with IL2 alone (7,25,36,37). In a recent randomized trial, combination therapy of IL2 and LAK was not superior to monotherapy with IL2 (38). More mature data on the efficacy of the combination of IL2 and IFNα also showed lower response rates of 8-12% (37,39,40). In a randomized study the efficacy of IFNα and high-dose IL2 versus IL2 alone was compared. The study was prematurely closed because the combination was ineffective (10). In a recent final report of the NCI Surgery Branch dose-escalating study the overall response rate of IL2 and IFNα decreased from 38% to 28% (17,41).

The only other report on a study of IL2, IFNα and LAK with similar dose-intensity, albeit a different schedule, has yielded a response rate of 24% and a median survival of 8 months (21). The investigators observed a toxicity profile of the same nature and severity as we did. Of note, we and others who studied intensive regimens of IL2 and IFNα could not administer more than 40-60% of these cytokines due to the severity of side-effects (10,21,41,42).

On the basis of our study and in view of other study data we conclude that these high-dose regimens of IL2 and IFNα with or without LAK are not warranted,

unless we are able to select reliably the 25-35% of patients who will have long-term survival as a result of this treatment.

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Chapter VI

Cardiotoxicity as dose-limiting factor in a schedule of high dose bolus therapy with interleukin-2 and interferon-alpha

An unexpectedly frequent complication

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Published in
Cancer 1994;74:2850-2856

Summary

Background: In a group of patients with metastatic melanoma treated with high dose immunotherapy, there was an unexpectedly high incidence of severe cardiac adverse effects.

Methods: Sixteen patients with metastatic melanoma were treated with high dose interleukin-2 (IL2) and interferon-alpha (IFNα). Each treatment cycle consisted of IL2 at a dose of 12 MIU/m^2 and IFNα at a dose of 3 MIU/m^2, given as intravenous bolus injections every 8 hours on days 1-5, every 3 weeks for a total of 3 cycles. Before treatment, careful cardiologic screening was performed, including electrocardiogram (ECG), stress test, cardiac multiple uptake-gated acquisition (MUGA) scan and echocardiography. During therapy, patients were monitored with daily ECG and creatine phosphokinase (CPK) measurements. Once cardiac damage was suspected, IL2 and IFNα were discontinued and echocardiography, stress test and MUGA-scan were repeated. If indicated, cardiac catheterization with endomyocardial biopsies was performed.

Results: Despite pretreatment cardiac screening, seven patients (44%) exhibited myocardial injury. Acute myocardial infarction occurred in one patient, cardiomyopathy developed in four, asymptomatic ECG changes appeared in one and 1 patient died of acute cardiac arrest. Echocardiography showed hypokinesis and decreased left ventricular ejection fraction. These abnormalities disappeared within 6 months. Cardiac catheterization in four affected patients revealed normal coronary arteries, but endomyocardial biopsies showed interstitial edema, vacuolation and degeneration of myocytes. Electron-microscopic examination showed fragmentation of myofibrils, swelling of mitochondria and loss of mitochondrial cristae.

Conclusions: This intensive treatment schedule of IL2 and IFNα is prohibited by severe and life-threatening cardiac toxicity.

Introduction

Interleukin-2 (IL2), either alone or in combination with other cytokines or activated lymphocytes, has shown antitumor activity against several forms of cancer, especially renal cell carcinoma and melanoma (1-5). The side effects of IL2 are considerable, including a flu-like syndrome with fever, rigors, lethargy, nausea, vomiting and diarrhea. In addition, cardiovascular, pulmonary, renal, hepatic and hematologic adverse effects have been observed (6,7). Hemodynamic and pulmonary disturbances are caused by a decrease in peripheral vascular resistance and a capillary leak syndrome, which can lead to an increased cardiac index, decreased left ventricular function, hypotension, oliguria, renal failure, peripheral and lung edema (8-11).

IL2 can also induce atrial and ventricular arrhythmias, angina pectoris and acute myocardial infarction (1,8,12-14). Autopsies and endomyocardial biopsies have shown myocarditis with myofibre degeneration and foci of myocyte necrosis. In most patients with myocarditis variable degrees of lymphocytic or eosinophilic infiltrations were demonstrated (15-18). It has been reported that treatment with other cytokines such as interferon-alpha (IFNα) and tumor necrosis factor (TNF) can also be associated with similar patterns of myocardial damage (19-23).

A recent National Cancer Institute Surgery Branch study suggested a steep dose response relationship for the combination of IL2 and IFNα in the treatment of metastatic melanoma (3). Consequently, we started a phase 2 study with this high dose intravenous bolus regimen of IL2 and IFNα in an attempt to confirm the high response rate of approximately 40%. However, we were confronted with a high incidence of severe and life-threatening cardiotoxicity that prohibited additional use of this regimen. This article reports the clinical and morphologic findings.

Patients and methods

Thirty patients with metastatic melanoma were required to estimate a response rate of 40%. Eligibility criteria included histologic proof of melanoma, bidimensionally measurable metastases, Karnofsky performance status index of 80 or greater,

age 18-70 years, a normal computed tomography scan of the brain, normal organ functions of heart, lung, kidney, and bone marrow; and normal results of serum bilirubin and coagulation tests.

Initially, cardiologic screening consisted of a 12-lead electrocardiogram (ECG). However, two of the first five patients treated in the protocol experienced severe cardiotoxicity. We decided, that every new patient had to undergo an extensive evaluation of cardiac function, which included a 12-lead ECG, stress test, cardiac multiple uptake-gated acquisition (MUGA) scan and echocardiography. Normal results of all these tests were required; otherwise, the patient was ineligible. In addition, patients with uncontrolled hypertension and a history of myocardial infarction or arrhythmias were excluded. Prior immunotherapy was not allowed. Prior chemotherapy was permitted. Written informed consent was obtained from each patient.

The treatment plan consisted of three cycles. Each cycle comprised IL2 (Hoffmann-La Roche Inc. Basle) at a dose of 12 MIU/m^2 and IFNα (Hoffmann-La Roche Inc. Basle) at a dose of 3 MIU/m^2, each given as an intravenous bolus injection every 8 hours on days 1-5, every 3 weeks.

Patients were treated and monitored on the clinical ward with frequent assessments of vital signs, weight and fluid balance. Hematologic and biochemical blood tests, including creatine phosphokinase (CPK) were performed on days 1, 3 and 5 of each treatment cycle. A 12-lead ECG was obtained daily during therapy. Acetaminophen was used to control fever. Vomiting and diarrhea were treated symptomatically with alizapride and loperamide. Corticosteroids were not permitted.

Toxicity was graded according to the World Health Organization (WHO) criteria (24). Initial treatment of hypotension and oliguria consisted of i.v. volume replacement. If volume expansion gave no improvement, dopamine was added at dosages as great as 5 μg/kg/min to improve renal perfusion. If higher dosages of dopamine were required, the patient was transferred to the intensive care unit and the administration of IL2 and IFNα was discontinued. Treatment was permanently discontinued if grade 3 cardiovascular toxicity or neurotoxicity occurred. In all other cases of grade 3 toxicity (except fever, nausea/vomiting, diarrhea) treatment was discontinued until the side effects improved to grade 1 or resolved. Resumpti-

on of treatment was allowed at 50% of the doses in the next cycle if grade 3 toxicity resumed to grade 1 or less. Patients with ECG changes or precordial pain were examined by the cardiologist. A full re-examination was performed, including stress test, echocardiography and ejection fraction measurement (MUGA-scan). If indicated, cardiac catheterization with endomyocardial biopsy was performed.

Results

Sixteen patients, 9 men and 7 women in excellent clinical condition (median Karnofsky index, 100), with a median age of 47 years (range, 29-61) were treated. Seven (44%) achieved a response (two complete and five partial). The study was closed prematurely becuase of prohibitive cardiac toxicity.

Severe and life-threatening cardiotoxicity occurred in seven patients, one with myocardial infarction, and four with cardiomyopathy; one patient died of cardiac arrest, and one had asymptomatic ECG changes. ECG abnormalities occurred between day 4 and 64 (mean day 23, median day 5) after the start of treatment. The intended treatment also was hampered by other serious adverse effects. Toxicities, that led to dose reduction of 50% in subsequent cycles were hypotension and anuria in two patients, intractable diarrhea in one and hyperbilirubinemia greater than 6,0 mg/dl (greater than 100 μmol/l) in one patient. Treatment was terminated prematurely in three patients because of neurotoxicity. One of these patients also developed a cardiomyopathy. Catheter related sepsis occurred in one patient, necessitating brief interruption of treatment.

Patients with cardiotoxicity received 82%, 54% and 53% of the planned total dose of IL2 and IFNα in the three respective treatment cycles. The entire patient group received 88%, 65% and 51% of the planned dose, respectively.

The details about patient characteristics, onset and type of cardiotoxicity, the results of cardiologic examinations and the final cardiac function status are reported here and summarized in Table 1.

TABLE 1 IL2 AND IFNα ASSOCIATED CARDIAC DYSFUNCTION

Pt.	Age/Sex	Interval start treatment to ECG changes (days)	Ischemia*	Hypo-kinesia RV/LV #	Myocard-itis@	Improvement cardiac function
1	45/m	5	3	+/+	NE	+
2	32/f	4	3	+/+	+	+
3	29/m	56	1	NE	NE	NE
4	50/f	21	2	+/+	+	+
5	40/m	5	1	+/-	+	+
6	43/f	5	2	-/+	+	+
7	43/m	64	acute cardiac arrest	NE	NE	NE

* Ischemia: 1 = negative T-waves without symptomes
2 = angina, ischemic changes on ECG
3 = acute myocardial infarction

@ Microscopic examination of endomyocardial biopsies showing degenerative changes of the myofibrils and/or cellular infiltration

\# Echocardiographic and/or cardiac catherization findings

NE: Not Evaluated

RV: Right Ventricle

LV: Left Ventricle

Patient 1 had signs of an acute myocardial infarction at day 5 of the first course. At that moment, treatment related toxicity consisted of fever, nausea, diarrhea and hypotension grade 2. The clinical picture started with chest pain, characteristic ECG changes consisting of ST-elevations in leads II and III and elevation of CPK-MB. One day later, he experienced severe pulmonary edema, hypotension and hypoxemia. He was transferred to the intensive care unit and treated with diuretics and vasopressors for a period of 4 days. Echocardiography demonstrated hypokinesia of the ventricular septum, the anterior wall and the apex of the left ventricle. He recovered uneventfully and was discharged 2 weeks later in good clinical condition without any physical restriction. Treatment with IL2 and IFNα was discontinued permanently. No cardiac catheterization was performed.

Subsequent ECG showed a negative T-wave in lead III and a small q-wave in lead aVF.

Patient 2 experienced chest pain and hypotension on day 4 of the first cycle. During the first 3 days only minor toxicities such as fever, chills, nausea and vomiting were observed. Typical ECG changes and elevation of CPK-MB suggested myocardial infarction. Echocardiographic examination on day 5 revealed hypokinesia of the ventricles. The left ventricle ejection fraction (LVEF) decreased from a pretreatment value of 67% to 23% (normal value $\geq$ 50%). Cardiac catheterization was performed on day 11. Normal coronary arteries were found. Endomyocardial biopsies showed degeneration of the myofibrils with interstitial edema and lymphocytic cell infiltration compatible with myocarditis. The biopsy procedure was complicated by cardiac perforation with symptoms of cardiac tamponade. Successful pericardiocentesis was performed and the patient recovered uneventfully. Immunotherapy was discontinued permanently. An echocardiogram 2 months later revealed normal cardiac dimensions with an ejection fraction of 55%. ECG was normalized at that time.

Patient 3 received three full cycles of therapy without any severe or life-threatening toxicity and without cardiac symptoms or complaints. The most important adverse effect present during immunotherapy was hyperbilirubinemia (102 μmol/l). However, on day 56 after the start of treatment, 8 days after the completion of IL2 and IFNα administration, negative T-waves were observed in the precordial leads of a routine ECG. His malignant disease was progressive at that time and the patient returned abroad without additional cardiologic evaluation.

Patient 4 underwent the first cycle uneventfully. The main adverse effects were fever, fatigue, nausea and vomiting. At the start of the second cycle she complained about chest pain during rest and dyspnea during exercise. Her ECG revealed negative T-waves in standard leads I, II, III, aVL, aVF and in the precordial leads V3-V6 (Figure 1, top). CPK-MB was not elevated. Echocardiographic examination revealed hypokinesia of the ventricles. The ejection fraction decreased from 60% to 45%.

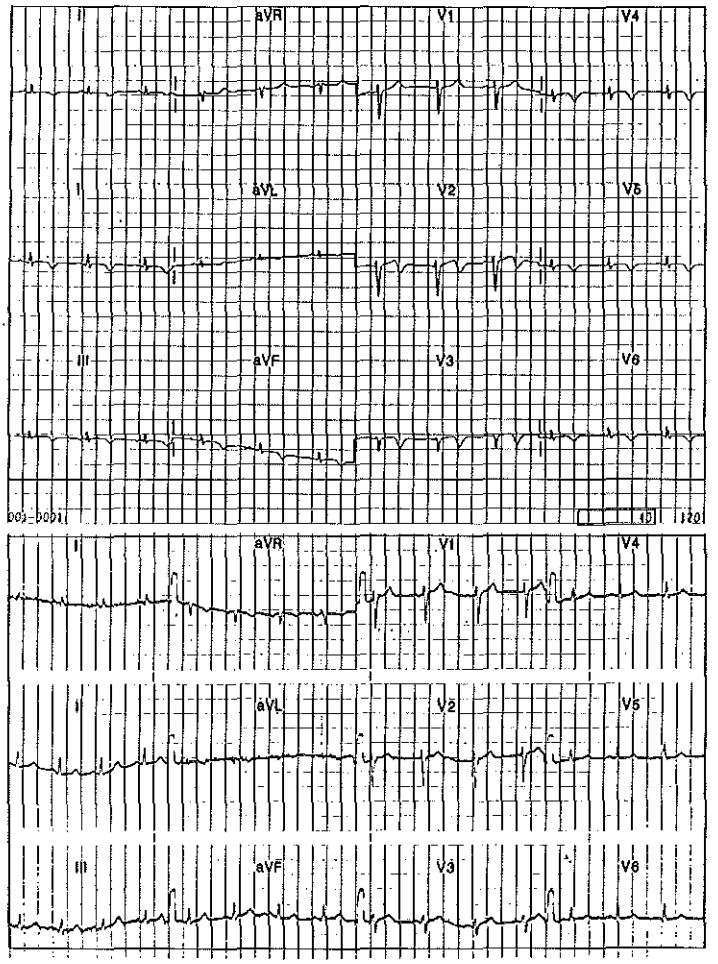

Figure 1 Electrocardiogram of patient 4 (Top). Negative T-waves in the standard and precordial leads (I, II, III, aVL, aVF, V2-V6), as detected 3 weeks after therapy was started (Bottom). A normalization of the ECG 4 months later.

The second cycle was withheld and cardiac catheterization was performed on day 28. Normal coronary arteries were found. Endomyocardial biopsy was taken from the left ventricle and histologic examination revealed interstitial edema, vacuolation of the myocytes and myofibrillar loss. No lymphocytic or eosinophilic

infiltrates were seen (Figure 2). Electron microscopic examination of these specimens, as shown in Figure 3, revealed degenerative changes of the myofibrils with myofibrillar loss, mitochondrial changes consisting of abnormal shape and size and loss of cristae. The symptoms disappeared during the course of several weeks.

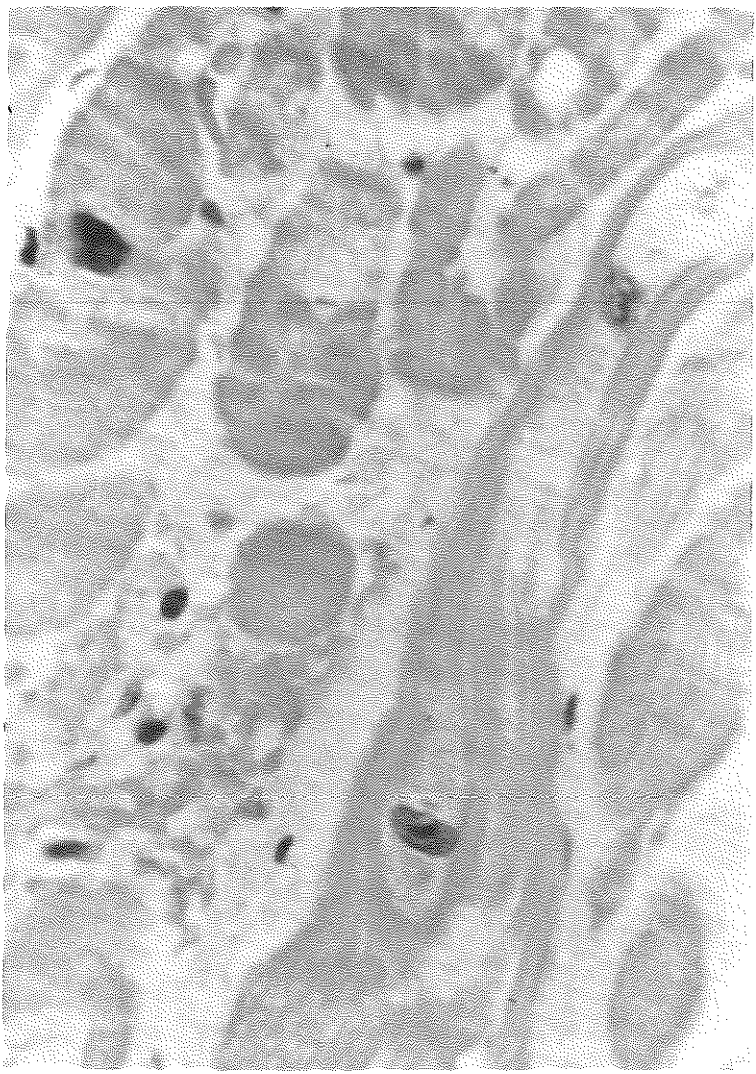

Figure 2 Endomyocardial biopsy from patient 4 obtained 4 weeks after therapy was started. Myofibrillar loss, focal vacuolation of the myocytes, and disruption of the normal architecture with interstitial edema. No lymphocytic or eosinophilic infiltration (hematoxylin and eosin; magnification x 400).

Echocardiographic hypokinesia improved gradually during a period of 2 months with normalization of the left and right ventricular dimensions and wall movements. ECG normalized after 4 months (Figure 1, bottom). IL2 and IFNα were not restarted.

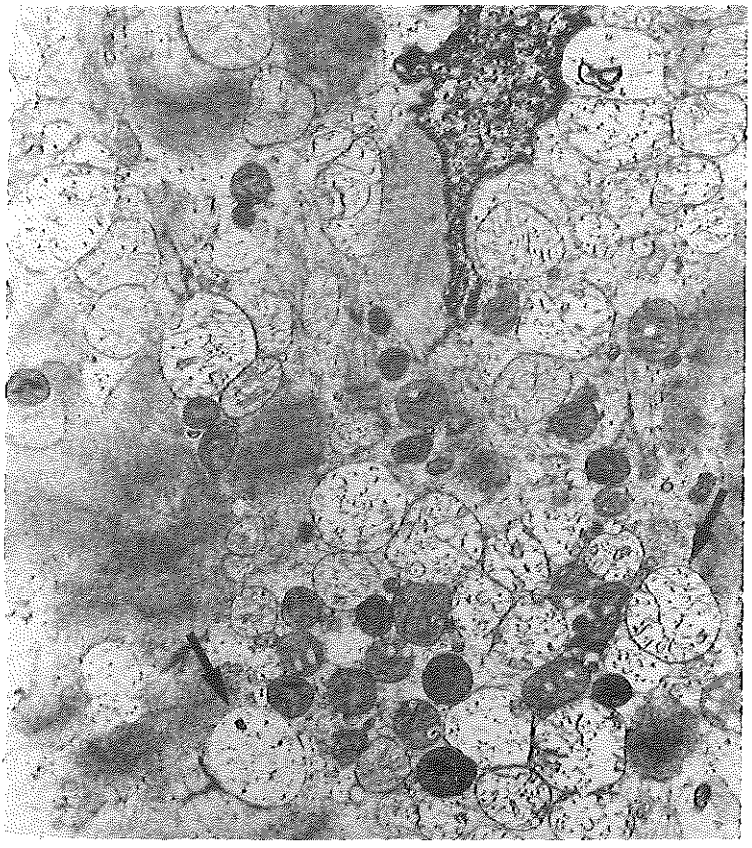

Figure 3 Electronmicroscopic picture of the same specimen as shown in Figure 2 showing degenerative changes with myofibrillar loss, abnormal shapes and sizes of the mitochondria (arrow) with loss of cristae (magnification x 7000).

Patient 5 had asymptomatic ECG changes detected at day 5 of the first course with negative T-waves in standard leads III and aVF. An additional significant side effect was confusion. Echocardiographic examination revealed focal akinesia at the inferior region of the heart. The LVEF decreased from 75% to 42%. Cardiac catheterization, performed at day 21, showed normal coronary arteries. Endomyocardial biopsies taken from the left and right ventricle demonstrated the same light and electron microscopic findings as in patient 4. Because of the focal localization of the myocardial abnormalities and the recovery of the LVEF we deemed it acceptable to re-treat the patient with IL2 and IFNα in reduced doses. However, on day 4 of the second cycle, there was worsening of the ECG changes with negative T-waves occurring in leads V3-V5 and aggravation of the preexisting changes in leads III and aVF. Treatment was stopped permanently.

Patient 6 received the first cycle with the usual adverse effects caused by IL2 but without severe hypotension. On day 5 she reported an oppressive sensation on the chest, accompanied with negative T-waves and ST-elevations in leads III, aVF, V3-V6. Echocardiographic examination revealed hypokinesia in the anterolateral and posterobasal region. CPK-MB was slightly elevated, indicating myocardial damage. The LVEF decreased from 78% to 44%. Cardiac catheterization on day 8 showed normal coronary arteries. Endomyocardial biopsies taken from the left and right ventricle revealed only interstitial edema. There was a normal architecture of the myofibrils. No lymphocytic or eosinophilic cell infiltrates were seen. Because of the absence of damage in the myofibrils, therapy with cytokines was resumed, despite the persistance of negative T-waves in III and aVF. The doses were reduced with 50%. The patient received the second and third cycle without worsening of her ECG. Two months after completion of therapy, there was normalization of the ECG and echocardiogram.

Patient 7 died at home 5 weeks after the completion of three cycles of IL2 and IFNα. Ten years earlier he was examined by a cardiologist because of palpitations. Cardiologic examination at that time revealed a supraventricular tachycardia and a prolapse of the mitral valve. A diagnosis of viral myocarditis was suggested, but not proven. He was treated with a β-blocking agent (atenolol) until the start of

immunotherapy. His pretreatment cardiologic screening was normal. Because of the absence of cardiac signs and symptoms, the patient was accepted for the protocol. During the course of the study there were no ECG abnormalities. Treatment was complicated by the occurrence of hypotension grade 3 and neurotoxicity grade 3 in the third cycle. The patient made a complete recovery. One month after the completion of the third cycle of therapy, he was seen by the cardiologist because of palpitations. A supraventricular tachycardia was demonstrated, but echocardiography and results of a stress test were normal. He was treated with digoxin and flecainide. One week later, he was found dead at home. No autopsy was performed.

Discussion

A wide variety of cardiac toxicities have been attributed to IL2 therapy, including arrhythmias, ischemia, myocarditis, and hypocontractility. Arrhythmias, mostly supraventricular, occur in as many as 10% of patients (1,4,7,9). In large series, ischemia was reported in 3-10% of patients and myocardial infarction in 1-4% (1,7,9). In addition, several incidental cases of myocardial infarction and cardiomyopathy have been described (8,13,15-18,25). Strikingly, in most of these patients there was no evidence of coronary artery disease at coronary angiography or autopsy (8,9,13). Cardiomyopathy related decrements in left ventricular ejection fraction and echocardiographic abnormalities appear to be reversible and disappear during the course of several months (9,10,25).

Histologic examinations of cardiac biopsies have basically shown the picture of degenerative changes and necrosis of myocytes, with or without eosinophilic or lymphocytic infiltration. The suggested mechanisms underlying these abnormalities include toxic and allergic reactions (15-18,20,22).

Interferons also have been reported to be a potential cause of cardiotoxicity with features similar to that associated with IL2 administration. The frequency of severe signs and symptoms is reported to be 1-3% (19-22).

We report an unexpectedly high incidence of severe and life-threatening cardiotoxicity in a patient group treated with high doses of i.v. bolus IL2 and IFNα. One patient experienced an acute myocardial infarction and four patients

experienced cardiomyopathy. One patient died of an acute cardiac arrest. His death may have been caused by IL2 and IFNα therapy, but a relation with flecainide cannot be excluded, because this drug can induce re-entrance arrhythmias and cardiac arrest (26,27). The occurrence of cardiac problems appeared unpredictable on the basis of age, performance status and cardiovascular functional status at the start of treatment. Ischemic heart disease before immunotherapy in our series was ruled out by extensive cardiologic examinations.

Most studies of the combination of IL2 and IFNα have not shown an increased frequency and severity of cardiac adverse events, compared with what would be expected of IL2 administration alone (28-36). In reports, describing the use of low-dose intravenous or subcutaneous IL2 and IFNα, cases of severe cardiotoxicity are rare (28,32,36). Most IL2 trials used continuous infusion in doses not exceeding 18 MIU/m^2/day, and patients with cardiotoxicity were only incidentally encountered (29-31,33-35). In one such a study using IL2 (7,5-15 MIU//m^2/day, continuous i.v.), IFNα, LAK and immunomodulatory doses of chemotherapy, 10% of patients experienced myocardial infarction or cardiomyopathy (37). In trials, using high doses of i.v. bolus IL2 (18-24 MIU/m^2/day) alone or in combination with IFNα i.v. a 9-14% incidence of myocardial infarction and myocarditis has been observed (38,39). In the National Cancer Institute Surgery Branch study with high-dose IL2 and IFNα in a similar schedule as ours, 25% of treatment courses were associated with an increase in creatine phosphokinase MB, indicating myocyte damage (3). Thus, severe cardiac toxicity seems to be related to the use of high-dose IL2, whereas synergism between cytokines in causing myocardial damage cannot be excluded. The use of i.v. ultra-high bolus administration appears to be an especially great risk factor.

The histologic changes that we observed were primarily degenerative. Of the four patients who underwent biopsies, only one had an infiltrate of lymphocytes. Other findings were interstitial edema, vacuolation and necrosis of myocytes and myofibrillar loss. Electronmicroscopic examination of the biopsies of one patient revealed degenerative changes of the myofibrils, mitochondrial changes and loss of mitochondrial cristae. These morphologic changes are similar to the type of cardiotoxicity that has been reported for anthracyclines (40,41).

We conclude that this intensive schedule of i.v. bolus IL2 36 MIU/m^2/day and IFNα 9 MIU/m^2/day for 5 days is responsible for an unacceptable incidence of severe and life-threatening cardiotoxicity. This finding is additional supported by our not observing signs or symptoms of ischemia or cardiomyopathy in a consecutive cohort of 25 identical patients treated with the same daily dosages of IL2 and IFNα for 3 instead of 5 days per cycle (60% of the initial dose-intensity).

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Chapter VII

Interleukin-2 induced thyroid dysfunction is correlated with treatment duration but not with tumor response

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Published in
J Clin Oncol 1993;11:921-924

Summary

Purpose: To analyse the putative relationship between immunotherapy associated dysthyroidism and the probability of a tumor response.

Patients and methods: A total of 89 consecutive patients with advanced cancer were treated with interleukin-2 (IL2)-based immunotherapy in a prospective study.

Results: Twenty patients developed thyroid dysfunction. Repeatedly positive tests for thyroid antibodies developed in 28% of the patients. Twenty-two patients achieved a response. There was no relationship between the formation of antibodies and the probability of response. There appeared to be a trend towards a relationship between thyroid dysfunction and response ($p=0.23$). A strong relationship was found between response on the one hand and cumulative dose of IL2 ($p=0.01$) and treatment duration with IL2 ($p=0.025$) on the other hand. The frequency of thyroid dysfunction was also significantly correlated with treatment duration ($p=0.001$). After adjustment for cumulative dose of IL2 and treatment duration, no relationship between thyroid dysfunction and response remained ($p=0.99$).

Conclusion: There is no relationship between thyroid dysfunction and the probability of tumor response. Thyroid dysfunction is merely a function of treatment duration and cumulative dose of IL2.

Introduction

In 1988, Atkins et al reported a high incidence of hypothyroidism in interleukin-2 (IL2)-treated patients (1). This phenomenon appeared to be related to a higher probability of tumor response. Several other investigations have been carried out to examine this potential relationship (2-6). We have performed a prospective study in 89 consecutive patients with metastatic solid tumors, who were treated with IL2-based immunotherapy. Here we report an analysis of the incidence of thyroid dysfunction in relationship to tumor response, the cumulative dose of IL2 received, and the duration of IL2 treatment.

Patients and methods

Eighty-nine patients with progressive measurable metastatic solid tumors were treated with IL2-based immunotherapy. Thirty-two were women, and 57 were men. The median age was 51 years, range 22-69. Thirty-eight patients had melanoma, 39 renal cell cancer, 8 non-small cell lung cancer, 1 breast cancer, 1 gastric cancer, 1 sarcoma, and 1 had germ cell cancer. Thus, 77 patients (87%) had melanoma or renal cell cancer, which are the tumor types most responsive to IL2. Treatment regimens comprised high dose IL2 continuous intravenous infusion (c.i.v.), high-dose IL2 c.i.v. with interferon-alpha (IFNα) subcutaneously (s.c.), high-dose IL2 bolus i.v. with IFNα bolus i.v., low-dose IL2 c.i.v. with IFNα s.c., high-dose IL2 c.i.v. with ex vivo IL2-activated lymphocytes (LAK), and high-dose IL2 c.i.v. with IFNα s.c. and LAK. Table 1 shows the distribution of patients according to tumor type and protocol regimens. Three types of IL2 preparations were used with different specific activities on a per weight basis. The activaties were converted into international units (I.U.) for the purpose of this analysis:
3 million Cetus units = 6.9 million Roche units = 6.9 Glaxo units = 18 million international units (MIU).

Thyroid studies

Serial determinations of serum concentrations of thyroxine (T4) and thyroid stimulating hormone (TSH) were performed every 4 weeks. In case of abnormal thyroid function these tests were performed every week. Serum T4 concentrations were measured with a radioimmunoassay (normal value 60-160 nmol/l) and serum TSH with a very sensitive immunoradiometric assay (normal value 0.10-4.0 mIU/l). Thyroid dysfunction was defined as follows: hypothyroidism, T4 < 60 nmol/l and TSH > 4.0 mIU/l; subclinical hypothyroidism, TSH > 4.0 and T4 60-160; hyperthyroidism, TSH < 4.0 and T4 > 160. Biphasic thyroid dysfunction was defined as hyperthyroidism followed by hypothyroidism. All patients who developed decreased T4 levels were treated with levothyroxine substitution therapy.

Antithyroglobulin and antithyroid microsomal antibodies were determined semi-quantitatively by an immunofluorescence method. The results were reported as positive, weakly positive or negative. This method was developed and performed by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service. Two micron frozen sections of thyroid tissue derived from patients operated for thyrotoxic goiter were incubated with patients serum (dilution 1:10) for 30 minutes. In a second 30 minutes incubation step, fluorescein isothiocyanate-labeled anti-human sheep immunoglobulin was added. Hereafter the samples were examined by fluorescence microscope.

Results

Twenty patients (22%) developed thyroid dysfunction. Seventeen of them (85%) showed an initial rise of T4 > 160 nmol/l (peak values 161-320) with a concomitant decrease of TSH < 0.10 mIU/l (hyperthyroidism) at 3-12 weeks (median, 7 weeks) after the start of IL2. In 9 of them, a biphasic pattern was seen with a subsequent drop of T4 below normal and an increase in TSH with peak levels of 12-48 mIU/l (hypothyroidism). In 2 patients, T4 normalized in the presence of elevated TSH (subclinical hypothyroidism). Both T4 and TSH normalized in 3 patients. The course of thyroid dysfunction could not be followed in 3 patients because of early death due to cancer. Hypothyroidism without an

initial phase of hyperthyroidism was seen in 3 patients. Hypothyroidism emerged between 6-15 weeks (median, 11 weeks) after the start of IL2. Goiter did not occur.

The 12 patients with T4 < 60 nmol/l and TSH > 4.0 mIU/l were treated with levothyroxine during further immunotherapy. After cessation of IL2, levothyroxine was stopped and thyroid function became normal in the 10 patients who could be followed. The remaining 2 patients died of progressive cancer.

Antithyroglobulin and antimicrosomal antibody determinations before and during immunotherapy were available in 18 of the 20 patients with thyroid dysfunction. Before treatment, 2 patients had a weakly positive antibody test, which persisted during IL2 therapy. Of the remaining 18 patients, 6 (33%) developed a weakly positive test at only one point in time and 5 (28%) became permanently seropositive. The tests became positive simultaneously with the development of high T4 values.

Thyroid antibodies were determined in 62 of the 69 patients who remained euthyroid. Before therapy, 2 patients were positive and during treatment antibodies became positive in 3 other cases.

In 4 patients an ^{123}I thyroid uptake scan was performed in the period of high T4 values. The observed uptake was only 0.5%, 1%, 2% and 2%, respectively.

Abnormal thyroid function was observed in 5 of the 6 treatment regimens. The incidence varied from 0-53% (Table 1). There was a highly significant difference ($p=0.005$) between the short-term and the low-dose IL2 regimens (schedules 3 and 4) on the one hand and the high-dose prolonged IL2 regimens on the other hand.

Twenty-two of the 89 patients achieved a response (25%); 9 complete and 13 partial. Response rates varied greatly between the different regimens, even within one and the same tumor type (Table 1). Seven of the 20 patients with thyroid dysfunction (35%) achieved a response, as compared to 15 of 69 euthyroid patients (22%). This trend towards a difference does not reach statistical significance ($p=0.23$ chi-square test). Thirty-one percent of the patients with positive antibodies, and 25% of the patients with negative antibodies achieved a response. This difference is not significant.

TABLE 1 RATES OF THYROID DYSFUNCTION AND RESPONSE IN DIFFERENT PROTOCOLS

Regimen	Malignancy	Pts	Thyroid Dysfunction	Resp
			N (%)	N (%)
1. IL2 c.i.v. (phase I)	Miscellaneous	8	3 (38)	2 (25)
2. IL2 c.i.v. + IFNα s.c.	Melanoma	17	9 (53)	2 (12)
3. IL2 bolus i.v. + IFNα bolus i.v.	Melanoma	18	1 (6)*	7 (39)
4. IL2 low dose c.i.v. + IFNα s.c.	NSCLC	8	0 (0)*	0 (0)
5. IL2 c.i.v. + LAK	Renal	6	2 (33)	1 (17)
6. IL2 c.i.v. + IFNα i.m. + LAK	Renal	32	5 (16)	10 (31)
Total		89	20	22

* p=0.005
NSCLC: non-small cell lung cancer

Data regarding the influence of the cumulative dose of IL2 on the development of thyroid dysfunction and its relationship with tumor response are presented in Table 2. After dichotomizing cumulative dose around the mid-range IL2 dose of 400 MIU/m^2, there are more cases of thyroid dysfunction in the higher dose group, but this difference does not reach significance (p=0.09 chi-square test). However, a significantly greater proportion of the responding patients ($^9/_{22}$=41%) received a cumulative dose > 400 MIU/m^2) than the non-responding patients ($^{10}/_{67}$=15%) (p=0.003).

TABLE 2 INFLUENCE OF CUMULATIVE DOSE OF IL2 (MIU/m^2) ON FREQUENCY OF THYROID DYSFUNCTION AND RESPONSE

Thyroid function	Cumulative dose		Response	Cumulative dose	
	≤ 400	> 400		≤ 400	> 400
Abnormal	13 (19%)	7 (36%)	yes	13 (19%)	9 (47%)
Normal	57	12	no	57	10
Total	70	19	Total	70	19
	p = 0.09			p = 0.01	

Table 3 shows the influence of duration of therapy calculated as total days of IL2 administration on the development of thyroid dysfunction and the relationship with response. Treatment duration is dichotomized around the mid-range value of 25 days. A treatment duration of 25 days or more resulted in a higher frequency of thyroid dysfunction (45% vs 10%) ($p<0.001$) and is correlated with a higher response rate ($p=0.025$). When treatment duration is calculated as weeks from the first until the last IL2 dose, including rest periods, the results are identical at a mid-range value of 12 weeks (data not shown).

TABLE 3 INFLUENCE OF IL2 TREATMENT DURATION (CALCULATED AS TREATMENT DAYS) ON FREQUENCY OF THYROID DYSFUNCTION AND RESPONSE

Thyroid function	Treatment duration		Response	Treatment duration	
	< 25 days	≥ 25 days		< 25 days	≥ 25 days
Abnormal	6 (10%)	14 (45%)	Yes	10 (17%)	12 (39%)
Normal	52	17	No	48	19
Total	58	31	Total	58	31
	p = 0.001			p = 0.025	

Because of the apparent influence of cumulative dose of IL2 and treatment duration on the development of thyroid dysfunction, and the fact that responding patients received higher cumulative doses of IL2 with longer treatment duration (Tables 2 and 3), it is possible that a putative relationship between thyroid dysfunction and tumor response is biased by cumulative dose and/or treatment duration. Therefore, we analysed the response and thyroid dysfunction relationship in the four dose and treatment-duration strata as shown in Table 4. With the use of the Mantel-Haenszel approach for combining 2x2 tables (7), it is clear that after adjustment for cumulative dose and treatment duration no statistically significant relationship can be demonstrated between thyroid dysfunction and response ($p=0.99$).

TABLE 4 RELATIONSHIP OF THYROID DYSFUNCTION AND TUMOR RESPONSE WITHIN 4 TREATMENT STRATA WITH IL2 (FORMED BY CUMULATIVE DOSE AND TREATMENT DURATION

Cumulative Dose (MIU/m^2)	Treatment Duration (days)	Response	Thyroid Dysfunction	Euthyroidism
0-400	1-24	yes	1	8
		no	4	43
0-400	25-52	yes	3	1
		no	5	5
401-800	1-24	yes	0	1
		no	1	0
401-800	25-52	yes	3	5
		no	3	6

$p = 0.99$

Discussion

We report on a prospective study of thyroid function in 89 consecutive patients treated with IL2-based immunotherapy. The observed frequency of thyroid dysfunction in this series is 22%. Originally, hypothyroidism was the only recognized form of IL2-induced thyroid dysfunction (1), but later reports have shown that hyperthyroidism preceeds hypothyroidism in the majority of cases (2-6,8). We have seen this biphasic pattern in 65% of the patients who developed thyroid dysfunction. This phenomenon can also be produced by interferons (9-11).

It has been suggested that IL2 can induce autoimmune thyroiditis (2,5). Our findings of biphasic dysfunction, the development of thyroid antibodies and the absence of ^{123}I uptake are consistent with that concept. In a recent report, Jacobs et al (8) draw attention to the possibility of a non-autoimmune toxic side effect of IL2-based immunotherapy as a second mechanism. One argument to support that hypothesis was the fact that thyroid function rapidly normalized in 75% of their affected patients after cessation of immunotherapy. To date, our findings are identical with a 100% recovery in the patients followed.

A total of 61% of our patients with thyroid dysfunction developed thyroid antibodies, demonstrated by a semi-quantitative immunofluorescence method, which is high in comparison with other reports (1-6,8). However, if we exclude the patients who were weakly seropositive at only one point in time, the incidence of antibody formation is 28%, which is in agreement with the findings in the literature.

The reported frequency of thyroid dysfunction in the literature varies from 15-91% (1-6,8). These differences may be the reflection of additional treatment components such as IFNα and LAK, but may also be a function of treatment duration and cumulative doses of drugs.

The main purpose of this study was to determine a possible relationship between thyroid dysfunction associated with immunotherapy and the probability of response, as well as to elucidate the eventual influence of cumulative dose of IL2 and treatment duration. Since our analysis comprises 6 treatment schedules, we chose to calculate treatment duration as the total number of days that a patient had actually received IL2, as well as the period from the start to the finish of IL2 therapy. A trend towards a higher response rate was observed in patients who developed thyroid dysfunction (35 vs 22%). Table 2 shows that 7 (36%) of 19 patients who received more than a cumulative dose of 400 MIU/m^2 of IL2 developed thyroid dysfunction and that 9 (47%) of these patients obtained a response. Similarly, Table 3 shows that 14 (45%) of 31 patients with a treatment duration of 25 days or more developed thyroid dysfunction and that 12 (39%) of these patients achieved a response. The relationship between thyroid dysfunction and cumulative dose of IL2 is of borderline significance ($p=0.09$). The relationship between thyroid dysfunction and treatment duration is highly significant ($p=0.001$). In addition, the probability of response is significantly correlated with cumulative dose of IL2 ($p=0.01$) and treatment duration ($p=0.025$).

When we finally analyzed the relationship between thyroid dysfunction and response to treatment after adjustment for cumulative dose and treatment duration, no prognostic influence of thyroid dysfunction on the probability of response remained (Table 4, $p=0.99$). Consequently, we conclude that there is no correlation between thyroid dysfunction and tumor response. Thyroid dysfunction is merely a function of treatment duration and cumulative dose of interleukin-2.

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Chapter VIII

The role of adoptive immunotherapy in solid cancers

Review, general discussion, perspectives and conclusions

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Neth J Med
Accepted

Introduction

Immunotherapy, unlike chemotherapy and endocrine therapy, which are now established treatment methods in cancer, is still in the developmental stage, although it has a long history. At the beginning of this century Paul Ehrlich was the first, who proposed that the cellular immune system has a role in the recognition and destruction of malignant cells (1). However, in the past, efforts to use immunotherapy as a strategy against cancer were by and large unsuccessful due to the inability to evoke an immune response against tumor-associated antigens and the lack of immunocompetence of the host immune system.

Thanks to recent biotechnological advances interest in this treatment modality has been renewed. The antitumor activity of biological response modifiers such as interleukins and interferons in vitro and in vivo was impressive enough to expect them to exhibit significant anti-cancer activity in men. Dramatic responses and rejection phenomena in animal models led to high hopes that cytokines might be a panacea for the treatment of cancer. The cloning of cytokine genes enabled the production of large amounts of these substances by recombinant technology and the beginning of clinical trials. In humans, only renal cell carcinoma and melanoma have some immunogenic properties and investigators have concentrated their treatment efforts on these tumor types. The most significant results are obtained with interleukin-2 (IL2) and interferon-alpha (IFNα). For this reason we will focus in this review on the use of these two cytokines in renal cell cancer and melanoma.

Rationale for the use of immunotherapy in cancer

Studies already published in the 1960s provided the first evidence that human cancers can evoke both humoral and cell mediated immune responses (2-5). Although cell mediated reactions are probably of greater significance, antibodies may have important implications for host resistance. They may be directly lytic for tumor cells or recruit immune cells carrying Fc receptors. In the cell mediated immune response two different cytolytic mechanisms can be identified. One involves the specific recognition of an antigen and is mediated by classical CD3/T-

cell receptor-bearing lymphocytes that are major histocompatibility complex (MHC)-restricted. The other involves non-specific recognition and cytolysis, largely mediated by so-called natural killer (NK) cells.

The targets for activation of the immune system are often tumor-associated antigens (TAA), shared by neoplastic and embryonic cells (6). Cancer is associated with an accumulation of mutations affecting oncogenes and growth regulating proteins. Recent data suggest that subtle changes, such as only one mutation in an oncogene can be recognized by T cells (7). In melanoma specific cytolytic T cell clones have been identified (8,9). Cytotoxic T lymphocytes, able to recognize melanoma associated antigens in a MHC-restricted manner, have recently been described (10).

Cell mediated immune responses can cause destruction of certain human tumors in vivo (5,11,12). Some cancer patients have undergone long-lasting complete response following the infusion of large numbers of in vitro expanded autologous tumor-infiltrating lymphocytes (13). In addition, clinical responses have been seen in patients given monoclonal antibodies to tumor-associated antigens (14,15). A part of the patients, who have been immunized with preparations containing tumor antigens have displayed immunity to those antigens and a few of these immunized patients have experienced tumor regression (16-19). Therefore, if a cellular immune response directed against tumor cells can be demonstrated, one can wonder why tumors escape immune surveillance. This may be explained by a tolerance state or anergy, which has been reported in various models (20). This failure to eradicate tumor growth may also be explained by the high rate of tumor growth which exceeds the capacity for tumor rejection by the immune system (21). Activation of the immune system by administration of cytokines such as IL2 and IFNα may help to boost antitumor immunity.

Interleukin-2

IL2 is a hormone that was first described in 1976 and has been named T cell growth factor (22,23). It is a glycoprotein lymphokine almost exclusively produced by T lymphocytes of the helper subset after activation by interleukin-1 derived from macrophages (23). IL2 acts as a pleiotropic mediator within the immune

system, having a variety of effects via specific cell surface receptors. Most prominent is the expansion and activation of T lymphocytes, lymphokine-activated killer (LAK) cells and natural killer (NK) cells (24-30). When IL2 is injected in vivo it leads to the production of secondary cytokines, including IL1, TNFα, IFNγ, IL6, GM-CSF and M-CSF (31-33). These cytotoxic and antiproliferative cytokines may partly be responsible for the antitumor activity of IL2 (25,26,34-36). In animal studies IL2 mediated the regression of pulmonary and liver metastases from a variety of tumors (37). In mice high dose IL2 eradicated disseminated murine leukemia (38). Irradiated tumor-bearing mice did not respond to high doses of IL2, demonstrating that IL2 alone is devoid of antitumor activity in the absence of IL2 responsive cells (39).

These animal data formed the basis of investigations on the role of IL2 in the treatment of cancer in humans. Studies have been carried out mainly in patients with renal cell cancer and melanoma. An overview of trials including at least 25 patients is given in Table 1. The first used schedule consisted of a high dose intravenous bolus regimen and was developed by Rosenberg et al., administering IL2 (25 MIU/m^2) every 8 hours for 5 days. In the initial trials impressive responses were seen in up to 35% of patients (40). In more recent updates and in studies from other groups response rates of 8-22% for renal cell cancer and 5-24% for melanoma were reported (41-49). The median survival ranged from 14 to 21 months for renal cell cancer and 10 to 12 months for melanoma, respectively. This schedule, however, is associated with considerable toxicity and frequently requires intensive care management. Dose reductions and treatment interruptions are necessary in 40-50% of patients. An intermittent high-dose bolus regimen three times weekly yielded a response rate of 12% (50). In a randomized study of two bolus IL2 schedules in renal cancer a low-dose regimen (10% of the high dose) reduced intensive care unit need and yet produced similar response rates (15% versus 20%) (51). IL2 conjugated to polyethylene glycol (PEG) exhibits a markedly prolonged circulating half-life with retained biological activity. It is sufficient to give PEG-IL2 once a week. A regimen of initial high-dose IL2 followed by chronic maintenance therapy with PEG-IL2 was well tolerated and resulted in comparable treatment results as with high-dose IL2 alone (52).

TABLE 1 (studies including ≥ 25 patients)

IL2 monotherapy

author	dose-scheme per cycle	nr. pts.	resp. rate (%)	resp. duration in months	surv. in months	ref.
A. Renal carcinoma						
* *bolus i.v.*						
Rosenberg	720,000 IU/kg, t.i.d., d 1-5	54	22	25	NE	41
Rosenberg[1]	720,000 IU/kg, t.i.d., d 1-5	48	21	29	14	45
Rosenberg	720,000 IU/kg, t.i.d., d 1-5	149	20	21	21	48
Bukowski	60 MIU/m^2/d, 3 d/week	41	12	4	11	50
McCabe[1]	600,000 IU/kg, t.i.d., d 1-5	37	8	NE	NE	44
Atkins[2]	24 MIU/m^2, t.i.d., d 1-5	71	17	20	16	46
Yang	720,000 IU/kg, t.i.d., d 1-5	65	20	8	NE	51
	72,000 IU/kg, t.i.d., d 1-5	60	15	8	NE	51
Fyfe	720,000 IU/kg, t.i.d., d 1-5	255	14	20	16	49
* *continuous i.v.*						
Negrier	18 MIU/m^2/d, d 1-5, 12-15	32	19	7	9	56
Negrier	18 MIU/m^2/d, d 1-5, 12-15	25	12	NE	NE	59
v.d. Maase	18 MIU/m^2/d, d 1-5, 12-15	51	16	12	9	57
Geertsen	18 MIU/m^2/d, d 1-5, 12-15	30	20	NE	NE	58
Gore	18 MIU/m^2/d, d 1-5, 8-12	133	14	11	11	61
Escudier	24 MIU/m^2/d, d 1-2	104	19	14	7	62
Whitehead	6.9-13.0 MIU/m^2/d, d 1-4	47	13	14	15	63
Murray Law[1]	9 MIU/m^2/d, d 1-5, 13-17	34	9	11	11	64
* *subcutaneous*						
Sleyfer	9-18 MIU/m^2/d, d 1-5	26	23	11	13	68
Buter	9-18 MIU/m^2/d, d 1-5	46	20	11	12	69
B. Melanoma						
* *melanoma*						
Rosenberg	720,000 IU/kg, t.i.d., d 1-5	42	24	8	NE	41
Rosenberg[1]	720,000 IU/kg, t.i.d., d 1-5	22	27	7	12	52
Rosenberg	720,000 IU/kg, t.i.d., d 1-5	134	17	14	12	48
Parkinson	600,000 IU/kg, t.i.d., d 1-5	46	22	11	NE	42
Whitehead	36-60 MIU/m^2/d, 3 d/week	42	10	11	NE	43
McCabe[1]	600,000 IU/kg, t.i.d., d 1-5	45	16	NE	NE	44
Sparano[2]	6,0 MIU/m^2, t.i.d., d 1-5	44	5	12	10	47
* *continuous i.v.*						
Dorval	16-24 MIU/m^2/d, d 1-3/5	37	22	NE	NE	60
Legha	12 MIU/m^2/d, d 1-4	33	22	6	10	65

t.i.d = three times per day
NE = not evaluated
MiU = 10^5 IU
[1] = randomized trial IL2 versus IL2/LAK
[2] = randomized trial IL2 versus IL2/IFNα

Early studies suggested that IL2 by continuous instead of bolus infusion could yield good antitumor activity with decreased toxicity (53,54). However, a randomized study comparing continuous infusion and bolus injections at equivalent doses found similar toxicity (55). Several trials using continuous i.v. IL2 in dosages of 7-24 MIU/m^2/day have shown response rates of 9-20% in renal carcinoma and 17-22% in melanoma (56-65).

In order to simplify IL2 therapy and to reduce toxicity, several investigators attempted to utilize the subcutaneous administration route. This treatment can generally be given on a outpatient basis. The side effects are usually mild to moderate (66-69). Antitumor effects are difficult to interpret, because most studies are small phase I-II trials. The only study including a sufficient number of patients reported a response rate of 20% in renal cancer (69). The use of small amounts of natural IL2 by inhalation has been suggested as a non-toxic local treatment of pulmonary metastases (70).

Overall, IL2 induces objective responses in 15-20% of patients with metastatic renal carcinoma and melanoma without significant differences according to route, schedule and dosage. Patients with a prolonged disease-free interval, good performance status and a limited number of sites of disease are more likely to have a response (71,72). IL2 may offer a survival benefit to responding patients, especially those with a complete remission are reported to have a long progression-free and overall survival (48). In one study IL2 treated patients showed a longer survival than patients who received chemotherapy (72).

The administration of IL2 has also been investigated in other solid tumors such as carcinoma of colon, breast, lung, ovary, bladder, pancreas, and head and neck. All studies included only small numbers of patients. In general, the reported response rates are low (< 10%), durations of response are short (< 6 months) and the effects on survival not demonstrable or not reported (40,41,73-75).

IL2-related toxicity

The toxicity of IL2 administration can involve virtually every organ system. A vascular leak syndrome (VLS) appears to be an important underlying mechanism. The increase in vascular permeability causes an egress of intravascular fluid into

the interstitium with edema, weight gain, pleural effusions and ascites. The similarities between the major IL2 associated toxicities, i.e. VLS and hypotension and the features of septic shock suggest that the underlying systemic activation of inflammatory mediator systems (complement, coagulation and fibrinolytic systems), culminating in endothelial damage is a common factor. The principal cytokines thought to be involved include TNFα and IL1, produced as part of the cytokine cascade (76-78). Corticosteroids reverse most adverse effects but also eliminate antitumor activity (79-81).

Cardiovascular toxicity consists of hypotension, tachycardia, decreased vascular resistence and decreased ejection fraction (82-86). In general, a weight gain of up to 10-20% can occur. With high-dose schedules hypotension is often dose limiting and the use of vasopressors is needed in up to 50-70% of patients (40,53,82-87). Other reported cardiac toxicities are ventricular and supraventricular arrhythmias, ischemia, myocardial infarction and cardiomyopathy (82-90).

Another manifestation of VLS is pulmonary edema, leading to abnormalities in gas exchange, hypoxemia, and increase in total lung water (83,84,86,91,92). Pleural effusions may also contribute to respiratory distress. Renal dysfunction is manifested by azotemia, oliguria, low sodium excretion and elevated renin activity (85,86,93-96). Prerenal hypoperfusion of the kidneys is mainly responsible for renal dysfunction, but a direct toxic effect of IL2 on the kidneys can not be excluded (97).

Gastrointestinal toxicities include anorexia, xerostomia, glossitis, stomatitis, nausea, vomiting and diarrhea (85,86,98,99). Elevations in liver function tests are almost always seen and reversible cholestasis is common (100,101). Possible manifestations of neuropsychiatric toxicity are agitation, forgetfulness, confusion, hallucinations, and occasionally seizures or coma (86,102-104).

Other toxicities frequently seen with IL2 therapy include chills, fever, flu-like symptoms and fatigue. Cutaneous manifestations are macular erythema, burning, pruritis and desquamation (98,105). Anemia, thrombocytopenia and thyroid function abnormalities occur relatively frequently (106-111). There is an increased incidence of bacterial infections (112,113).

In general IL2 related toxicities will begin to reverse within 24 hours of cessation of treatment with complete resolution usually within a few days to a

week. Most adverse effects are predominantly associated with the application of intermediate to high-dose regimens. Outpatient therapy with subcutaneous IL2 is associated with moderate toxicities such as fever, chills, fatigue, skin changes, diarrhea, nausea, vomiting, stomatitis and irritation at the injection site.

Interferon-alpha

In 1957 the interferons were first described as a group of proteins, produced by cells in response to virus infections (114). Now it is known that they play a key role in host responses by modulating immune cell function. IFNs are classified into IFNα, IFNβ and IFNγ. Interferon-alpha has antiproliferative and direct cytostatic effects on tumor cells (115-117). It upregulates the expression of MHC class I and II, β-microglobulin as well as tumor associated antigens (115,116,118,119). The stimulated expression of tumor antigens may make neoplastic cells more susceptible to cytotoxicity by immune T cells of the host.

IFNα also has the ability to stimulate the activation of mature NK cells and to promote their differentiation from precursors (116). Stimulation of NK cells may result in lysis of tumor cells. The development of cytotoxic T lymphocytes, monocytes and macrophages is supported by interferon-alpha (116,117,119).

After the demonstration of the antitumor activity of IFNα in various mouse models (120), the administration in patients was studied. In metastatic renal carcinoma response rates varied from 5 to 27% with an average of 15% (121-128). The median duration of response and survival were approximately 6 and 11 months, respectively. The adminstered dosages ranged between 3 and 36 MU/m^2/day. There does not appear to be a dose-response relationship for IFNα, albeit that a certain threshold of 3 MU/m^2/ day seems necessary to induce responses (127,128). Responses occur predominantly in patients with good performance status, prior nephrectomy and limited metastatic disease, particularly when confined to the lung. A study comparing patients treated with IFNα with chemotherapy suggested a survival benefit for the cytokine group (129).

The overall response rate of IFNα in metastatic melanoma is about 16% (range 6-27%) with approximately one-third complete remissions (130-135). The median duration of remission is 4-6 months, however complete responses of several years

have been reported. Survival is mostly very short (6 months). The best therapeutic results in melanoma have been achieved with uninterrupted schedules, regardless of route. There is no clear advantage of lower or higher dosages of IFNα in the range between 10 and 50 MU/m^2/day (135). Patients with a lower performance status or visceral metastases have a reduced response rate.

Several well-described side effects are ubiquitous after first exposure to IFNα. A flu-like syndrome with fever, chills, headache, malaise, myalgias, arthralgias, and fatigue occurs in the majority of patients and diminishes over time with continued administration (135,136). Long-term toxicity consists of anorexia, weight loss and fatigue. The latter symptom is often dose-limiting. Neuropsychiatric adverse effects are somnolence, lethargy, overall mental and motor slowing and confusion (135,136). Frank depression and psychosis are rare events and occur mostly with the high dose regimens. The most important hematological toxicity is leukopenia with a decrease in both granulocyte and lymphocyte counts (135,136). These changes are caused by a redistribution of circulating leucocytes, rather than by true myelosuppression. Anemia and thrombocytopenia occur less frequently.

Gastrointestinal toxicity consists of anorexia, aberrant taste, nausea, vomiting, and diarrhea. Diarrhea can be severe at high doses (136). Elevation of transaminase levels is observed in 30% of patients, although the incidence is higher at high doses (136). Effects on the cardiovascular system and renal function are less common with IFNα. Symptoms such as tachycardia, rythm disturbances, vasoconstriction and hypotension may be directly related to the febrile reaction. Nonetheless, caution must be used in patients with a history of ischemic heart disease. The most common renal toxicity is proteinuria, occurring in approximately 15% of patients (136).

IL2 and IFNα

Since the mechanisms of action of IL2 and IFNα are partly different, it can be expected that the combination of these cytokines may act additively or even synergistically. Experimental data from a variety of animal tumor models showed that the combined administration of IL2 and IFNα mediated greater therapeutic

effects against established subcutaneous, hepatic and pulmonary metastases than either agent delivered alone (137-139).

In renal cancer and melanoma many clinical trials have been carried out, using a great variety of doses and routes of administration (Table 2). The reported response rates showed a wide range, varying from 0 to 40% (140-156). The NCI Surgery Branch undertook a phase I dose escalating study administering bolus injections of IL2 and IFNα (140). In the highest dose regimens response rates of 38% for renal carcinoma and 43% for melanoma were observed. However, a high incidence of severe toxicities forced the investigators to modify the treatment schedule and to select a lower dose level (151). Especially cardiac and central nervous system toxicity were dose-limiting. The response rates decreased to 25% with the modified dose schedule. A Dutch study, trying to confirm the initial encouraging response rate of the NCI trial, observed a similar drop in treatment results from 41% to 20% after modification of the dose, which was necessitated by the associated severe toxicity of the high dose schedule (90,156).

Schedules administering IL2 continuous i.v. in intermediate to high doses yielded response rates of 7-33% for renal cell cancer and 10-29% for melanoma, respectively (141-145,149,152,155). The demonstrated median survival varied between 10 and 21 months. The differences in treatment results are difficult to interpret due to the wide variation in schedule and dosage. Keilholz et al., using a decrescendo schedule of initial high dose IL2, subsequently tapered to a lower maintenance dose reached a high response rate of 41% in melanoma (149). However, the study was small and no confirmation trials have been performed.

Two studies in patients with renal carcinoma have been carried out administering the most intensive regimen of immunotherapy reported sofar, consisting of the combination of high-dose IL2, IFNα and lymphokine-activated killer cells (LAK) (157,158). Response rates of 24% and 39%, respectively, were achieved. However, the associated toxicity was considerable with several toxic deaths, and the patients could tolerate only 40-50% of the planned dose of cytokines.

A limited number of randomized trials have been done comparing the efficacy of the combination of IFNα and high-dose IL2 versus IL2 alone (46,47). Combined treatment appeared not to achieve superior results. In renal cancer response rates of

17% for IL2 alone and 11% for the combination were reported (46). In melanoma responses were seen in 5% and 10% of patients, respectively (47).

TABLE 2 (studies including ≥ 25 patients)

IL2 + IFNα

author	dose-scheme per cycle	nr. pts.	resp. rate (%)	resp. duration in months	surv. in months	ref.
A. Renal carcinoma						
* *bolus i.v.*						
Rosenberg	IL2 2.6-11.7 MIU/m^2, t.i.d., d 1-5 IFN 3-6 MIU/m^2, t.i.d., d 1-5	35	31	10	NE	140
Bergmann	IL2 18 MIU/m^2/d, o.d.d., d 1-14 IFN 10 MIU/m^2/d, o.d.d., d 1-14	35	26	10	NE	146
Atkins[1]	IL2 14.4 MIU/m^2, t.i.d., d 1-5 IFN 3 MIU/m^2, t.i.d., d 1-5	28	11	NE	16	46
Marincola[2]	IL2 2.6-15.6 MIU/m^2, t.i.d., d 1-5 IFN 3-6 MIU/m^2, t.i.d., d 1-5	75	28	37	31	151
Negrier[3]	IL2 24 MIU/m^2, t.i.d., d 8-12, 19-23 IFN 20 MIU/m^2/d, d 1-5 IFN 5 MIU/m^2, t.i.d., d 8-12, 19-23	29	24	6	8	157
* *continuous i.v.*						
Ilson	IL2 7 MIU/m^2/d, d 1-4 IFN 5 MIU/m^2/d, d 1-4	34	12	NE	11	141
Figlin	IL2 4.5 MIU/m^2/d, d 1-4 IFN 6 MIU/m^2/d, d 1-4	30	30	12	12	142
Sznol	IL2 7-14 MIU/m^2/d, d 1-5, 11-16 IFN 12 MIU/m^2/d, 3 d/week	40	20	8	NE	143
Oldham	IL2 18 MIU/m^2/d, d 1-5 IFN 3 MIU/m^2/d, d 1,3,5	83	7	3	11	144
Lipton	IL2 2.3-9.2 MIU/m^2/d, d 1-4/5 IFN 3-12 MIU/m^2/d, 2-3 d/week	39	33	15	21	145
Stoter[3]	IL2 18 MIU/d, d 1-5, 12-16 IFN 5 MIU/d, d 1-5, 12-15	41	39	14	15	158
* *subcutaneous*						
Vogelzang	IL2 12 MIU/d, d 1-4 IFN 9 MIU/d, d 1 + 4	42	12	NE	15	147
Ravaud	IL2 18 MIU/m^2/d, d 1 + 2 IL2 3.6 MIU/m^2/d, 5 d/week, sq IFN 5 MIU/m^2/d, 3 d/week	38	18	7	8	150
Facendola	IL2 9 MIU/m^2/d, d 1 + 2 IL2 1.8 MIU/m^2, b.i.d., 5 d/week IFN 5 MIU/m^2/d, d 1,3,5	50	18	12	12	153
Atzpodien[4]	IL2 5-20 MIU/m^2/d, 3 d/week IFN 6-9 MIU/m^2/d, 1-3/week	152	25	NE	NE	154

author	dose-scheme per cycle	nr. pts.	resp. rate (%)	resp. duration in months	surv. in months	ref.
B. Melanoma						
* *bolus i.v.*						
Rosenberg	IL2 1-4.5 MIU/m^2, t.i.d., d 1-5 IFN 3-6 MIU/m^2, t.i.d., d 1-5	39	33	7	NE	140
Sparano[1]	IL2 4.5 MIU/m^2, t.i.d., d 1-5 IFN 3,0 MIU/m^2, t.i.d., d 1-5	41	10	12	10	47
Marincola[2]	IL2 2.6-15.6 MIU/m^2, t.i.d., d 1-5 IFN 3.0-6.0 MIU/m^2, t.i.d., d 1-5	82	24	10	20	151
Kruit	IL2 4.5 MIU/m^2, t.i.d., d 1-3/5 IFN 3,0 MIU/m^2, t.i.d., d 1-3/5	42	29	7	9	156
* *continuous i.v.*						
Oldham	IL2 18 MIU/m^2/d, d 1-5 IFN 3 MIU/m^2/d, d 1,3,5	66	10	2	10	144
Keilholz	IL2 18 MIU/m^2/d, d 8-12 IFN 10 MIU/m^2/d, d 1-5	27	18	6	11	149
Keilholz	IL2 18 MIU/m^2/6-24 h, d 8-12 IFN 10 MIU/m^2/d, d 1-5	27	41	11	NR	149
Kruit	IL2 7.8 MIU/m^2/d, d 1-4 IFN 6 MIU/m^2/d, d 1 + 4	51	16	8	11	152

b.i.d. = two times per day
o.d.d. = every other day
t.i.d. = three times per day
NE = not evaluated
NR = not reached
sq = sequential
[1] = randomized trial IL2 versus IL2/IFNα
[2] = one patient cohort received IFNα 6 MIU/m^2/day
[3] = also treatment with LAK
[4] = some patients also received 5-FU 750 mg/m^2/week

The subcutaneous administration of IL2 combined with IFNα as an outpatient treatment has also been explored, especially in renal cancer. The reported response rates ranged from 12-25% (147,150,153,154). The median survival with the subcutaneous route seemed to be somewhat shorter than with intravenous administration.

In other solid tumors experience with the combination of IL2 and IFNα is very limited. A few studies have been done in breast and head and neck cancer, but patient numbers are insufficient to draw conclusions (75,159).

In conclusion, the addition of IFNα to IL2 has not meaningfully improved the treatment results in comparison with monotherapy with IL2 alone. The combination

of high-dose bolus IL2 and IFNα may have a somewhat higher response rate. However, it is doubtful whether this can be translated into a survival benefit. The accompanied severe toxicity remains a serious problem.

IL2 and chemotherapy

Metastatic renal cell carcinoma and melanoma are poorly sensitive to chemotherapeutic agents and as yet no standard chemotherapy has been established (160, 161). The combination of chemo- and immunotherapy may be a new therapeutic approach. In an animal model the administration of cyclophosphamide prior to IL2 resulted in improved response of lung metastases (162). Similar results have been observed in other models with the use of chemo-immunotherapy (163,164). Based on these preclinical data several studies in humans have been carried out, especially in melanoma. A summary of the most important trials is given in Table 3. There was some concern that cytostatics may impair the stimulatory effects of IL2. However, no obvious impairment of IL2 induced immune mechanisms has been detected (165).

In renal cancer the combination of IL2 and cyclophosphamide showed disappointing results with only incidental partial responses (166-168). An outpatient three-drug combination regimen consisting of IL2 and IFNα subcutaneously with 5-fluorouracil yielded an objective response rate of 49% (169). Systemic toxicity was mild to moderate.

In melanoma dacarbazine (DTIC) has been the most commonly used chemotherapeutic agent in combination with IL2. Response rates of 16-26% with a median survival of 9-13 months has been observed (170-173). Cisplatin (CDDP) alone or combined with DTIC added to IL2 resulted in response rates of approximately 40% (174-176). Recently, more aggressive multi-agent combinations of chemo-immunotherapy have been developed. Intensive regimens consisting of IL2 and IFNα in combination with CDDP (177), or CDDP, DTIC and carmustine (178,179), or CDDP, DTIC and vinblastine (180) or DTIC and carboplatin (179,181) yielded relatively high response rates of 35-57%. The preliminary survival data show a median duration of about 10 months.

TABLE 3 (studies including ≥ 25 patients)

Chemo-immunotherapy in melanoma

author	scheme per cycle	number of patients	response rate (%)	response duration in months	survival (months)	ref.
Flaherty	IL2/DTIC	32	22	5	9	170
Dillman	IL2/LAK/DTIC	27	26	4	10	171
Stoter	IL2/DTIC	25	24	7	13	172
Dummer	IL2/DTIC	57	16	14	9	173
Demchak	IL2/CDDP	27	37	4	NE	174
Flaherty	IL2/CDDP/DTIC	32	41	8	10	175
Atkins	IL2/DTIC/CDDP/Tam	38	42	6	11	176
Khayat	IL2/IFNα/CDDP	39	54	6	11	177
Richards	IL2/IFNα/DTIC/CDDP/BCNU/Tam	42	57	8	12	178
Atzpodien	IL2/DTIC/IFNα/Carbo	40	35	8-19	NE	179
Atzpodien	IL2/IFNα/DTIC/BCNU/Tam	27	56	7-11	NE	179
Legha	IL2/IFNα/DTIC/CDDP/Vin	60	53	NE	NE	180

NE = not evaluated
DTIC = dacarbazine
CDDP = cisplatin
BCNU = carmustine
Carbo = carboplatin
Vin = vinblastin
Tam = tamoxifen

The role of IL2 in combination with 5-fluorouracil (5-FU) or with 5-FU and leucovorin (LV) has been studied in colorectal cancer. In small phase I and II studies response rates of approximately 30% have been reported (74). However, a randomized trial comparing IL2 by continuous infusion in combination with 5-FU and LV versus 5-FU and LV alone did not show differences in response rates (17% vs 16%) (182).

In conclusion, although these studies suggest that chemo-immunotherapy results in better response rates than either modality alone, especially in patients with melanoma, there is no clear proof that response duration is improved. The demonstration of eventual survival benefit requires phase III trials.

IL2 and effector cells

As previously mentioned IL2 induces the expansion and activation of non-specific lymphokine-activated killer cell activity (LAK). These LAK cells are predominantly NK cells. Preclinical animal studies showed that the combination of

LAK cells and IL2 markedly improved antitumor activity (162,183). In vitro studies have also shown that tumors of many different histological types are susceptible to lysis by human LAK cells (26).

As a rule, patients are given IL2 for 3-5 days to increase the number of circulating LAK cell precursors. Subsequently, these precursors are harvested by apheresis and incubated in vitro with IL2 to generate LAK cells. These effector cells are reinfused into the patient over a period of 3-4 days with concurrent IL2 administration.

The overall experience with IL2 and LAK cells showed response rates varying from 3-35% (Table 4) (40,41,44,45,55,56,64,159,184-189). Studies using high-dose bolus IL2 (25 MIU/m^2 every 8 hours) reported response rates of 13-35% in metastatic renal cancer and 12-22% in melanoma (40,41,44,45,55,184,185). Treatment schedules of continuous i.v. IL2 (9-18 MIU/m^2/day) resulted in response rates of 3-27% and 3-23%, respectively (55,56,64,159,186-189). Data about survival and response duration are limited. Median survival durations ranged between 9 and 20 months for renal cancer and 6 and 11 months for melanoma (45,55,56,64, 159,187,189). The contention is that combined treatment with IL2 and LAK does not increase the side effects as compared to IL2 alone.

It is important to determine in a randomized fashion whether the addition of LAK cells offers improved therapeutic benefit. Until now 3 randomized trials have been carried out (44,45,64). None showed a higher response rate or longer survival for the LAK arm, neither in renal cancer nor in melanoma. In one study there was a trend toward more complete responses in patients treated with the combined modality (45). In conclusion, in renal cell cancer as well as in melanoma, the combination of IL2 plus LAK has not yielded superior treatment results compared to IL2 alone. Also in other solid tumors there is no indication that the addition of LAK has improved the response rates (41,75,159).

An other population of cytolytic lymphocytes, that can be used as effector cells in combination with IL2, are tumor-infiltrating lymphocytes (TIL). These T lymphocytes are found within solid tumors. Unlike non-specific LAK cells, TILs are able to recognize tumor-associated antigens in a specific MHC-restricted manner (190,191). TILs are capable of mediating cytotoxic activity against autologous and allogeneic tumor targets in vitro (192,193). In the presence of IL2,

TILs can be activated and expanded to great numbers in cultures of several weeks. The adoptive transfer of TILs plus IL2 to tumor-bearing animals appeared to be 50-100 times more effective than LAK cells plus IL2 (190,191,194).

TABLE 4 (studies including ≥ 25 patients)

IL2 + LAK

author	scheme per cycle	nr. pts.	resp. rate (%)	resp. duration in months	surv. in months	ref.
A. Renal carcinoma						
* *bolus i.v.*						
Rosenberg	720,000 IU/kg, t.i.d., d 1-5, 11-15	36	33	NE	NE	40
Rosenberg	720,000 IU/kg, t.i.d., d 1-5, 11-15	72	35	11	NE	41
Rosenberg[1]	720,000 IU/kg, t.i.d., d 1-5, 11-15	49	31	13	20	45
Fisher	600,000 IU/kg, t.i.d., d 1-5, 12-16	32	16	12	NE	184
McCabe[1]	600,000 IU/kg, t.i.d., d 1-5, 11-15	30	13	NE	NE	44
Weiss	600,000 IU/kg, t.i.d., d 1-5, 11-15	46	20	NE	NE	55
* *continuous i.v.*						
Negrier	18 MIU/m^2/d, d 1-5, 12-15	51	27	6	13	56
Parkinson	600,000 IU/kg, t.i.d., d 1-3, 18 MIU/m^2/d, d 9-14	44	9	NE	NE	187
Thompson	14 MIU/m^2/d, d 1-5, 4.6-14 MIU/m^2/d, 12-16	42	33	5-18	NE	189
Weiss	18 MIU/m^2/d, d 1-5, 22.5 MIU/m^2/d, d 11-16	48	15	NE	NE	55
Dillman	18 MIU/m^2/d, d 1-5, 11-15	46	15	7	9	159
Murray Law[1]	9 MIU/m^2/d, d 1-5, 13-17	35	3	NE	13	64
B. Melanoma						
* *bolus i.v.*						
Rosenberg	720,000 IU/kg, t.i.d., d 1-5, 11-15	48	21	6	NE	40
Rosenberg[1]	720,000 IU/kg, t.i.d., d 1-5, 11-15	27	22	42	11	45
Dutcher	600,000 IU/kg, t.i.d., d 1-5, 11-15	32	19	5	NE	185
McCabe[1]	600,000 IU/kg, t.i.d., d 1-5, 11-15	49	12	NE	NE	44
* *continuous i.v.*						
Bar	600,000 IU/kg, t.i.d., d 1-3 18 MIU/m^2/d, d 9-15	50	14	21	NE	186
Dutcher	18 MIU/m^2/d, d 1-5 22.5 MIU/m^2/d, d 11-15	33	3	NE	NE	188
Dillman	18 MIU/m^2/d, d 1-5, 11-15	53	23	4	6	159

t.i.d. = three times per day
NE = not evaluated
MIU = 10^6 IU
[1] = randomized trial IL2 versus IL2/LAK

These murine studies led to clinical trials with TILs and IL2 demonstrating regression of metastatic disease in patients with renal cell cancer and melanoma. The best treatment results have been shown in metastatic melanoma with response rates of 19-34%, whereas in renal cell carcinoma responses have been rare (13,159,195-199). TILs with MHC-restricted specificity to the autologous tumor have predominantly been demonstrated in melanoma (198). In other tumors the existence of MHC-restricted TILs is equivocal. The rather disappointing clinical results may be explained by lack of tumor specific recognition by TILs or inhibition of their lytic capacities by immunosuppressive factors produced at the tumor site (200). Additionally, in spite of earlier suggestions, preferential trafficking of TILs to tumor deposits could not be confirmed (201,202). Further important limitations of TIL therapy are the labor-intensive laboratory procedures and the limited number of patients from whom tumor specific TILs can be isolated.

Tumor targeting

New strategies for the improvement of adoptive immunotherapy focus on targeting immune cells to tumor cells (203). One attempt to improve treatment efficacy is the development of gene modified TILs. TILs can be transduced with several cytokine genes, which dictates the delivery of increased amounts of cytokines such as IL2, IL4, TNFα, IFNγ and GM-CSF at the tumor site. In this way transfected TILs can enhance the local antitumor effects. Several studies have demonstrated that gene transduction can be successfully carried out in autologous TILs of patients without altering their properties (201,204,205). However, given the above mentioned restrictions associated with TILs, it is difficult to foresee a more fruitful clinical application of this therapeutic approach.

The concept that T cells can be activated and targeted to tumor cells by either bispecific monoclonal antibodies (BsMAbs) or by genetic modification of the T cell receptor is under intensive scrutiny. BsMAbs are hybrid antibodies constructed from two parent MAbs: one specific for the immune effector cell, e.g. the CD3/TCR complex and the other specific for a tumor-associated antigen on the target cell (206-209). BsMAb-mediated cross-linking of the effector T cell to the tumor target cell via specific cytotoxic trigger molecules results in activation of the

lymphocyte lytic machinery and destruction of the tumor target in vitro and in vivo (207,208,210-213). BsMAbs can also be used to target and activate monocytes, macrophages, NK cells and neutrophils (209,214,215). The advantage of the use of BsMAbs is that all cytotoxic T lymphocytes regardless of their TCR specificity can be redirected and activated to lyse tumor cells, provided that these cells express tumor-associated antigens.

In pilot studies in patients with ovarian cancer and glioma, proof of concept was demonstrated since antitumor responses were indeed observed (216,217). In these studies, BsMAb-coated T cells were given locoregionally. The systemic administration of BsMAbs has also been investigated in phase I studies in renal cell cancer, ovarian cancer and breast cancer (218-222). The observed toxicities comprise fever, chills, nausea, vomiting, dyspnea, hypotension and anaphylactic reactions at low doses, indicating that this route of administration may be not very suitable for clinical use (218,220). In addition, the use of BsMAbs has other limitations. BsMAb-targeted lymphocytes only retain their antibody-dictated specificity for a short period of time (48-96 h), due to dissociation of BsMAb from the cell surface (203,208,223). More-over, BsMAb redirected lymphocytes can lose their signal transducing and lytic capacity (223). It is unknown whether systemically administered BsMAb redirected immune cells can migrate from the blood circulation to the tumor site (203). Thus, BsMAbs may be most effective when applied locoregionally.

To circumvent the limitations associated with BsMAbs and to achieve long-lasting and systemic immune responses a novel approach has been developed, in which T lymphocytes are modified by molecular engineering with a permanent antibody-dictated specificity (224-228). This technique is based on the construction of a chimeric immunoglobulin-T cell receptor (Ig-TCR) with a laboratory chosen tumor-associated antibody gene. To become effective, these lymphocytes require the expression of the engineered Ig-TCR on their surface, in association with the CD3 signal transducing complex. This was achieved by the construction of chimeric Ig-TCR genes in which the variable segments of the TCR genes were replaced by the variable segments of an immunoglobulin gene with known specificity (224-228).

It has been demonstrated that chimeric Ig-TCR genes can be transduced into T lymphocytes, resulting in the functional expression of the chimeric receptors on their cell surface. Stimulation of the chimeric receptor results in T cell activation, cytokine production and lysis of target cells (227-231). In contrast to BsMAb redirected lymphocytes, who lose their lytic capacity, lymphocytes transduced with chimeric Ig-TCR genes show recycling of the cytolytic process (223,231). An important feature of Ig-TCR lymphocytes is that they recognize tumor-associated antigens in a MHC-unrestricted manner. Hence their antitumor activity is not adversely affected by tumor cells, which downregulate their MHC complex or do not adequately express antigens in the groove of the MHC complex (232). Studies in patients, using this promising treatment approach are in preparation.

Other methods of improving tumor targeting focus on ways to mimick tumor immunogenicity by vaccination. It has been demonstrated that cytokine genes can successfully be introduced into tumor cells. Experimental studies have shown that genetically modified tumor cells expressing cytokines such as IL2, IL4, IL6, IFNγ, or GM-CSF were capable of inducing immune responses (233-237). Tumor cells engineered with cytokine genes act as self-replicating minipumps secreting increased amounts of that particular cytokine. Vaccination experiments in tumor bearing animals showed localized secretion of cytokines which mediated tumor rejection (238-240). Neoplastic cells transduced with the IL2 gene were able to generate specific cytotoxic lymphocytes with memory against subsequent tumor challenges (233,234,240). Further experiments demonstrated that human tumor cells can also be engineered with cytokine genes (241-244). On the basis of these findings pilot studies of cytokine transfer by vaccination with irradiated allogeneic tumor cells in patients with malignant tumors have been started (245,246).

Immunization efforts are also focused on the development of antigen-based vaccines to activate silent precursor cytotoxic lymphocytes. Thus far, a number of antigens have been identified as targets for recognition by cytotoxic lymphocytes, mostly in melanoma (MAGE, Melan A/MART, tyrosinase, gp 100), but also in other tumors (HER2/neu, p21ras) (10,247-253). These antigen peptide epitopes are recognized by T cells in the context of MHC class I or II molecules. The first experience with vaccination with these immunogenic peptides in humans demonstrated antigen-specific delayed type hypersensitivity reactions and an increase of

antigen-specific cytotoxic lymphocyte reactivity (254,255). Besides pruritis and skin induration at the injection site no significant adverse effects were observed. It is too early to draw conclusions about possible antitumor effects, but it appears that this vaccination strategy may be most appropriate as adjuvant therapy after resection of the primary tumor or in patients with a low tumor load. Vaccination with whole proteins containing multiple antigenic epitopes may increase the chance of multidirectional T cell activation (256).

Conclusions

Adoptive immunotherapy with cytokines such as IL2 and IFNα with or without the use of MHC-unrestricted effector cells yields good results in a small proportion of patients, almost exclusively with metastatic renal cell cancer and melanoma. Tumor responses occur in 10-25% of patients with 5-10% complete responses. Especially the complete responders can have durable responses and survival benefit. Higher dose regimens do not improve the treatment results but do increase the toxicity. Chemo-immunotherapy, especially in melanoma patients, may be more promising than either modality when used alone, since several studies reported encouraging response rates. However, as yet, no randomized trials have been carried out.

For improvement of these treatment results a tumor-specific or tumor-targeted immunotherapy strategy is essential. The use of tumor-infiltrating lymphocytes as possible specific MHC-restricted effector cells has clearly demonstrated antitumor effects. However, except in melanoma, TILs appear to lack sufficient specificity to be of great importance in the treatment of other cancers. Whether engineered TILs, tranduced with cytokine genes, are endowed with a better specificity and give improved results has to be awaited.

The concept of redirecting lymphocytes towards tumor cells using bispecific monoclonal antibodies is an attractive treatment approach. The advantage is that all T lymphocytes may be activated irrespective of the specificity of their receptor. However, it is unlikely that BsMAb redirected immune cells can be used systemically because of the anaphylactoid reactions observed.

More interesting is the development of the chimeric Ig-TCR receptor with its MHC-unrestricted tumor selective recognition features. This treatment modality has not yet reached the clinic.

Finally vaccination studies with cytokine transduced tumor cells or with antigenic peptides/proteins has demonstrated the possibility of inducing tumor-specific cytotoxic lymphocytes. This opens perspectives for the composition and application of antigen-specific cancer therapy.

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Summary

The cytokines interleukin-2 (IL2) and interferon-alpha (IFNα) have several immunomodulatory and antitumor effects. Immunotherapy with these two cytokines, administered as single agents, has demonstrated activity against metastatic renal cell cancer and melanoma. Based on preclinical evidence and initial treatment results of phase I studies, has it been suggested that the combination of IL2 and IFNα has a better antitumor effect than either cytokine alone. This thesis describes the results of several phase II studies of IL2 and IFNα in patients with metastatic renal cell carcinoma and melanoma.

Chapter 1 gives a short overview of the most important immunomodulatory and antiproliferative effects of IL2 and IFNα. The results of clinical studies with monotherapy of IL2 or IFNα are summarized. The rationale for combining IL2 and IFNα in the treatment of cancer is described.

In **Chapter 2** our initial experience with the combined use of IL2 and IFNα in two different schedules in patients with metastatic melanoma is presented. The preliminary response rates of these two studies were 21% and 56%, respectively.

The final analysis of a multicenter phase II study in patients with metastatic melanoma is reported in **Chapter 3**. Patients were treated with IL2 7.8 MIU/m^2/day for 4 days by continuous infusion and IFNα 6 MU/m^2/day, day 1+4 subcutaneously. Fifty-one patients were evaluable for response and toxicity. Eight of them (16%) obtained an objective response, one complete (2%) and 7 partial (14%). The median duration of response and the median survival were 8 and 11 months respectively. Common side effects included fever, chills, fatigue, skin rash, nausea, vomiting and diarrhea. It was concluded that this regimen of IL2 and IFNα in an intermediate dose intensity was only moderately active and not superior to IL2 alone.

In **Chapter 4** the results of two schedules of high-dose bolus administration of IL2 and IFNα in metastatic melanoma are presented. Patients were planned to

receive IL2 11.7 MIU/m^2 and IFNα 3 MU/m^2 by intravenous bolus administration every 8 hours for a total of 3 cycles. In the first schedule treatment duration was 5 days per cycle. Of the 17 evaluable patients, 2 (12%) achieved a complete and 5 (29%) a partial response (overall response rate 41%). This part of the study was closed prematurely due to the occurrence of severe cardiotoxicity (41%) and central nervous system toxicity (28%). In the second schedule the same dosages of cytokines were administered, however for 3 instead of 5 days per cycle. In the second part of the study 5 of the 25 evaluable patients responded (20%, only partial responses). The associated adverse effects were manageable. It was concluded that this 5-day schedule of high-dose IL2 and IFNα, although it had a relatively high response rate, was accompanied by unacceptable toxicity. The modified 3-day schedule gave treatment results not better than with IL2 monotherapy.

The results of single center study with an immunotherapy regimen consisting of IL2, IFNα and lymphokine-activated killer (LAK) cells in patients with metastatic renal cell cancer are reported in **Chapter 5**. Seventy-two patients were included, 17 in a feasibility part (protocol 1) and 55 in an efficacy part (protocol 2). Treatment consisted of IL2 18 MIU/m^2/day, day 1-5 and 12-15 by continuous infusion and IFNα 5 MIU/m^2/day, day 12-15 intramuscularly. LAK infusions were given on day 12-14. In protocol 2 IFNα was also administered on day 1-5. Each patient was planned to receive two induction cycles. Patients, whose disease stabilized or responded, received maintenance therapy with the same dose regimen of IL2 and IFNα during 4 days every 4 weeks up to a total of 4 cycles. Of the patients in protocol 1, 3 achieved a complete and one a partial response (overall response rate 24%). The median duration of response and the median survival were 18 and 14 months, respectively. Toxicity was manageable and no dose reductions were necessary. In protocol 2 response rate was 37%, 6 complete and 13 partial remissions. The median response duration and the median survival were 11 and 17 months, respectively. Toxicity was considerable with three treatment related deaths. In protocol 2 only approximately 40-50% of the planned doses could be administered. It was concluded that the use of high-dose schedules of IL2 and

IFNα is not warranted, unless we can define more accurately the subgroup of patients who will experience long-term survival as a result of this treatment.

Chapter 6 gives a detailed description of several cases of severe cardiotoxicity observed in a subgroup of patients treated with high-dose bolus IL2 and IFNα as reported in chapter 4. Despite pretreatment cardiac screening almost half of the patients exhibited myocardial injury. Four patients developed cardiomyopathy, one a myocardial infarction and one negative T-waves on the electrocardiogram. Another patient died of acute cardiac arrest. Echocardiography showed hypokinesis and decreased ventricular ejection fraction. Endomyocardial biopsies revealed edema, vacuolisation and degeneration of myocytes. It was concluded that treatment with a combination of high-dose IL2 and IFNα can induce severe and life-threatening cardiac toxicity.

The incidence of thyroid dysfunction during IL2-based immunotherapy and the significance of abnormal thyroid function as a prognostic factor, that can predict a tumor response was analyzed in a group of 89 patients, receiving various treatment schedules, and the results are presented in **Chapter 7.** Twenty patients (22%) developed thyroid dysfunction. The relationship between thyroid function abnormalities and cumulative dose of IL2 was of borderline significance, whereas the relationship with treatment duration was highly significant. In contrast with data from the literature no relationship between thyroid dysfunction and probability of response could be observed after adjustment for cumulative dose of IL2 and treatment duration.

In **Chapter 8** the present role of adoptive immunotherapy with IL2 and IFNα is discussed. Therapy with the cytokines IL2 and IFNα with or without LAK cells yields good results in a small proportion of patients, almost exclusively with metastatic renal cell cancer and melanoma. Tumor responses occur in 10-25% of patients with 5-10% complete responses. Combination schedules or higher dose regimens do not improve the treatment results but do increase toxicity.

For improvement of these treatment results a more tumor-specific or tumor-targeted immunotherapy strategy is essential. Innovative new treatment modalities that are now already available and may develop to important new immunotherapies in the near future, are gene modified tumor-infiltrating lymphocytes, gene modified tumor vaccines, bispecific monoclonal antibodies and the chimeric T cell receptor.

Samenvatting

De cytokinen interleukine-2 (IL2) and interferon-alpha (IFNα) hebben diverse immuunmodulerende en antitumor effecten. Bij patiënten met kanker is van beide eiwitten aangetoond, dat zij ieder afzonderlijk werkzaam zijn tegen uitgezaaide nierkanker en melanoom. Op grond van laboratorium onderzoek en op basis van resultaten van fase I studies bij patiënten, is de veronderstelling naar voren gekomen, dat gecombineerde behandeling met beide cytokinen tezamen betere antitumor resultaten geeft dan behandeling met elk eiwit apart. Dit proefschrift beschrijft een aantal klinische studies over de toepassing van de combinatie IL2 en IFNα bij de therapie van patiënten met uitgezaaide nierkanker en melanoom.

Hoofdstuk 1 geeft een kort overzicht van de belangrijkste immuunmodulerende, groeiremmende en antitumor effecten van IL2 en IFNα, zoals aangetoond in diverse laboratorium- en proefdier studies. De resultaten van monotherapie met IL2 of IFNα, zoals deze tot nu toe bereikt zijn, worden hierin samengevat. De redenen om IL2 en IFNα bij de behandeling van kanker te combineren worden beschreven.

In **hoofdstuk 2** worden onze eerste ervaringen beschreven met de gecombineerde toediening van IL2 en IFNα in twee verschillende schema's aan patiënten met gemetastaseerd melanoom. De voorlopige resultaten lieten respons percentages zien van respectievelijk 21% en 56%.

Hoofdstuk 3 bevat de eind analyse van een multicentrische fase II studie, uitgevoerd in patiënten met gemetastaseerd melanoom. Patiënten werden behandeld volgens het volgende schema; IL2 7.8 MIU/m^2/dag continu intraveneus gedurende 4 dagen samen met IFNα 6 MU/m^2/dag, per subcutane toediening op dag 1 en 4. Een-en-vijftig patiënten waren evalueerbaar voor respons en toxiciteit. Responsen werden gezien in 8 patiënten (16%), 1 compleet (2%) en 7 partieel (14%). De mediane respons duur en de mediane overleving waren respectievelijk 8 en 11 maanden. De meest voorkomende bijwerkingen waren koorts, koude rillingen, vermoeidheid, roodheid van de huid, misselijkheid, braken en diarree. De eind

conclusie van deze studie was, dat dit schema van toediening van IL2 en IFNα, in een intermediaire dosis intensiteit, slechts een beperkte antitumor activiteit had en niet beter was dan monotherapie met IL2 alleen.

De resultaten van twee schema's met hoge doses IL2 and IFNα, gegeven als intraveneuze bolus injecties aan patiënten met gemetastaseerd melanoom, zijn samengevat in **hoofdstuk 4**. De patiënten kregen gedurende iedere kuur IL2 en IFNα toegediend door middel van intraveneuze injecties in een dosering van respectievelijk 11.7 MIU/m^2 en 3 MU/m^2, elke 8 uur. In het totaal werden 3 kuren gegeven. In het eerste schema bedroeg de behandelingsduur 5 dagen per kuur. Van de 17 evalueerbare patiënten, bereikten er 2 (12%) een complete en 5 (29%) een partiële respons (totale respons percentage 41%). Echter, dit gedeelte van de studie werd voortijdig gesloten als gevolg van het optreden van ernstige cardiotoxiciteit (41%) en neurotoxiciteit (28%). Vervolgens werd in het tweede deel van de studie de duur van een kuur ingekort van 5 naar 3 dagen. Van de 25 evalueerbare patiënten in dit schema kregen er 5 een respons, (20%, allen partieel). De met dit schema samenhangende toxiciteit was hanteerbaar. Concluderend, gaf het 5-daagse schema met hoge dosis IL2 and IFNα een relatief hoog respons percentage, maar veroorzaakte het een onacceptabele toxiciteit. Met een gemodificeerd 3-daags schema vielen de bijwerkingen mee, maar waren de behandelingsresultaten niet beter dan met IL2 monotherapie.

In **hoofdstuk 5** worden de resultaten gerapporteerd van een intensief immunotherapie regime bestaande uit IL2, IFNα en lymfokine-geactiveerde killer (LAK) cellen. Deze behandeling werd gegeven aan patiënten met uitgezaaide nierkanker. Twee-en-zeventig patiënten werden in de studie opgenomen, waarvan 17 in het eerste deel van de studie om de haalbaarheid van het behandelingsschema te onderzoeken (protocol 1) en 55 in het tweede deel ter beoordeling van het antitumor effect (protocol 2). De behandeling bestond uit IL2 18 MIU/m^2/dag, dag 1-5 en 12-15 door middel van continue infusie en IFNα 5 MIU/m^2/dag, dag 12-15 als intramusculaire injectie. LAK infusies werden gegeven op dag 12-14. In protocol 2 werden ook IFNα injecties gegeven op dag 1-5. In principe kregen de patiënten twee van deze inductiekuren, waarna bij een gunstig resultaat de

behandeling met maximaal 4 onderhoudskuren kon worden gecontinueerd. Van de patiënten in protocol 1 kregen er 3 een complete en 1 een partiële respons (respons percentage 24%). De mediane respons duur en de mediane overleving waren respectievelijk 18 en 14 maanden. De bijwerkingen waren alleszins aanvaardbaar en dosis reducties waren niet nodig. Het respons percentage in protocol 2 bedroeg 37%, met 6 complete en 13 partiële responsen. De mediane duur van de respons en de mediane overleving waren respectievelijk 11 en 17 maanden. De toxiciteit in protocol 2 was echter aanzienlijk, drie patiënten overleden, mede als gevolg van de behandeling. Slechts ongeveer 40-50% van de geplande doseringen aan IL2 en IFNα kon worden gegeven. De conclusie van deze studie was, dat de toepassing van gecombineerde intensieve schema's met IL2 en IFNα voorlopig niet gerechtvaardigd is totdat we beter in staat zijn de subgroep van patiënten te identificeren, die een langdurige overleving bereiken met deze therapie.

Hoofdstuk 6 geeft een gedetailleerde beschrijving van een aantal gevallen van ernstige cardiotoxiciteit, dat zich voordeed in een groep patiënten, behandeld met hoge doses IL2 en IFNα in het schema zoals beschreven in hoofdstuk 4. Ondanks uitvoerige cardiale screening voorafgaande aan start van de therapie ontwikkelde de helft van de patiënten hartschade. Vier patiënten kregen een cardiomyopathie, één een myocard infarct en een ander ontwikkelde negatieve T-toppen op het elektrocardiogram. Een zevende patiënt overleed aan een acute hartstilstand. Echocardiografisch onderzoek liet verminderde wandbewegingen van de ventrikels en een verlaagde ejectiefractie zien. Endocard biopten toonden oedeem, vacuolisatie en degeneratie van myocyten. Concluderend kan behandeling met hoge doses IL2 en IFNα leiden tot ernstige en levensbedreigende cardiotoxiciteit.

Hoofdstuk 7 beschrijft een onderzoek naar het voorkomen van schildklierfunctiestoornissen gedurende behandeling met immunotherapie met IL2 in een groep van 89 patiënten. Tevens werd gekeken naar de mogelijk voorspellende waarde van het ontstaan van een gestoorde schildklierfunctie voor het optreden van een antitumor respons. Twintig patiënten (22%) ontwikkelden schildklierdysfunctie. De relatie tussen het ontstaan van schildklierfunctie afwijkingen en de cumulatieve dosis IL2, aan een patiënt toegediend, bleek net niet

significant te zijn. De relatie met de duur van de behandeling was zeer significant. De conclusies waren, dat schildklierdysfunctie een frequente complicatie van IL2 behandeling was en, dat in tegenstelling tot eerdere literatuur gegevens er geen relatie kon worden vastgesteld tussen het optreden van een afwijkende schildklier functie en de waarschijnlijkheid van een respons na correctie voor cumulatieve dosis IL2 en behandelingsduur.

In **hoofdstuk 8** wordt de huidige rol van adoptieve immunotherapie met IL2 en IFNα in de behandeling van solide tumoren besproken en bediscussieerd. Behandeling met IL2 en IFNα met of zonder LAK cellen geeft gunstige resultaten bij een beperkt aantal patiënten, voornamelijk patiënten met uitgezaaide nierkanker en melanoom. Tumor responsen worden waargenomen bij 10-25% van de patiënten, waarvan 5-10% compleet. Combinatie schema's of hoge doses regimes verbeteren de resultaten niet maar leiden wel tot meer bijwerkingen.

Om tot verbetering van de behandelingsresultaten van immunotherapie te komen is een meer tumor-specifieke en tumor gerichte strategie essentieel. Voorbeelden van nieuwe veelbelovende technieken zijn genetisch gemodificeerde tumor-infiltrerende lymfocyten, genetisch gemodificeerde tumor vaccins, bispecifieke monoclonale antilichamen en de chimere T cel receptor. De eerste ervaringen hiermee zijn bijzonder interessant en met toch zekere verwachtingen wordt uitgekeken naar meer definitieve resultaten van lopende studies.

Dankwoord

Hoewel de omslag van dit boekje zou kunnen doen vermoeden, dat promoveren berust op de activiteit van één persoon, moet een proefschrift en dat geldt zeker voor deze dissertatie, beschouwd worden als het "eindprodukt" van de inspanningen en het enthousiasme van velen. Iedereen, die haar of zijn bijdrage leverde bij het tot stand komen van dit werkstuk wil ik van harte bedanken.

Meer in het bijzonder wil ik een aantal mensen noemen.

Allereerst Prof. Dr. G. Stoter. Beste Gerrit, jij bent degene geweest, die mij, na aanvankelijke aarzelingen mijnerzijds, hebt overgehaald om te promoveren. Ik bewonder je vermogen om hoofd-en bijzaken van elkaar te onderscheiden en de essentie van iets naar boven te brengen. Ik dank je voor de nauwgezette wijze waarop je me begeleid hebt bij het vervaardigen van de diverse manuscripten.

Mijn collega S.H. Goey wil ik bedanken voor onze samenwerking bij de behandeling en de begeleiding van de immunotherapie patienten. Beste Hoo, dit proefschrift was niet tot stand gekomen zonder jouw bijdragen daaraan.

De mensen van het immunologie laboratorium, met name Dr. R.L.H. Bolhuis, Dr. J.W. Gratama en Dr. C.H.J. Lamers dank ik voor hun onmisbare hulp bij de meer basaal immunologische aspecten van dit proefschrift.

Dr. Ir. P.I.M. Schmitz ben ik erkentelijk voor zijn belangrijke hulp bij de statistische analyse van de onderzoekgegevens.

Dr. A.A.M. Eggermont bedank ik voor zijn nuttige adviezen en kritiek bij het maken van de verschillende artikelen.

De inzet van de researchverpleegkundigen D. Batchelor en B. Visser was van grote betekenis bij het verzamelen en samenvatten van de patientengegevens.

Tevens wil ik mijn erkentelijkheid uitspreken voor de medewerkers van de audiovisuele dienst van de DDHK voor het maken van de foto's en figuren. Ook moeten de dames van de medische bibliotheek genoemd worden, die nauwgezet zorgdroegen voor de noodzakelijke literatuurvoorziening.

Heel speciaal wil ik Thérèse van Eijk en Petra Bos bedanken. Zij boden de broodnodige secretariële ondersteuning.

Als laatste wil ik mijn vader bedanken. Hij leverde misschien wel de meest belangrijke bijdrage. Hij was mij tot grote steun en creëerde een sfeer waarin ik ongestoord heb kunnen werken.

Curriculum Vitae

Willem Harm Jan Kruit werd op 8 februari 1957 geboren te Vlaardingen. In 1975 behaalde hij het Gymnasium-β diploma en begon in datzelfde jaar met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam. Het doctoraal examen werd in juni 1980 behaald en het arts examen werd in december 1981 afgelegd. Ter vervulling van de militaire dienstplicht was hij in de periode van april 1982 tot juni 1983 werkzaam als arts-assistent in het Militair Hospitaal Dr Mathijsen te Utrecht, eerst op de afdeling interne geneeskunde (hoofd: Dr. M. van Zoeren) en later op de afdeling cardiologie (hoofd: Dr. B.K. Bootsma). Vervolgens werkte hij, in afwachting van een opleidingsplaats interne geneeskunde, van augustus 1983 tot januari 1985 als arts-assistent in algemene dienst in het Bonifatius Hospitaal te Leeuwarden. In maart 1985 werd begonnen met de opleiding tot internist op de afdeling interne geneeskunde van het Bergweg Ziekenhuis in Rotterdam (opleider: Dr. G.J.H. den Ottolander). Sinds september 1989 is hij, aanvankelijk als arts-assistent daarna als internist, werkzaam op de afdeling interne oncologie van de Dr. Daniel den Hoed Kliniek in Rotterdam (hoofd: Prof. Dr. G. Stoter). In deze periode is dit proefschrift tot stand gekomen. In maart 1990 is hij geregistreerd als internist en in mei 1993 volgde de registratie voor het aandachtsgebied oncologie.

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