Interleukin-2 based systemic and locoregional immunotherapy

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Op interleukine-2 gebaseerde systemische en locoregionale immunotherapie

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De mens heeft drie mogelijkheden om zijn handelen te bepalen:
ten eerste door na te denken; dat is de edelste,
ten tweede door na te apen; dat is de makkelijkste,
ten derde door de ervaring; dat is de bitterste.

Confucius

Aan Ling, Andrew en Kaitlyn
Aan mijn ouders

CONTENTS

The major impact of recent clinical research with interleukin-2 (IL2) has been the demonstration that a strictly immunological manipulation can mediate the regression of established cancer in humans through the activation of cytotoxic lymphocytes and the release of secondary cytokines.

Since 1985 a variety of clinical studies have been carried out in metastatic cancer patients with the use of interleukins, interferons, and lymphokine activated killer cells. These studies have either employed a single agent approach or combined modality treatment also including hormonal and chemotherapy.

Although the majority of human cancers are systemic diseases by nature, some tumor types are predilected to reside in one organ site or cavity. For example, metastatic colon cancer is often confined to the liver for prolonged periods of time. Ovarian cancer is usually restricted to the abdominal cavity, whereas mesothelioma mostly does not extend the pleural cavity until death.

It is for these reasons that the clinical investigations described in this thesis are based on a systemic approach on the one hand and on a locoregional approach on the other hand in selected tumor types. The study treatments comprised single agent IL2 and combinations of IL2 and interferon (IFN)-α with or without chemotherapy.

Regarding the systemic administration of IL2 based immunotherapy, we have chosen for a constant infusion schedule rather than intermittent bolus intravenous administration, based on available data in the literature of treatment equivalence and less toxicity accompanied with the continuous infusion method.

For the locoregional treatment of liver metastases we have used a continuous arterial infusion method. It appeared that intra-arterial as well as peripheral intravenous administration of IL2 resulted in endothelial damage leading to thrombophlebitis and thromboembolic complications. These untowards side effects have led us to use central venous catheters (CVCs) for the administration of IL2 in the treatment of systemic disease. In principle, this approach will reduce the risk of thromboembolic complications, but create a new problem, namely the occurrence of catheter related infections. Consequently, we have investigated the safety of tunneled CVCs.

Chapter 1

IMMUNOTHERAPY OF METASTATIC RENAL CELL CANCER

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INTRODUCTION

Renal cell cancer (RCC) is a disease afflicting approximately 27,000 individuals per year in the United States (1). It is the 10th most common cancer, constitutes 3% of all adult malignancies, affects men more than women, and occurs at a median age of 64 years. One third of the patients presents with distant metastases while 30-40% of the others will eventually develop these (2). The median survival of metastatic disease is 7 months and only 1-2% of patients survive 5 years or more (3). Surgery is the standard treatment for localized disease; however, its role in the presence of distant metastases is limited to palliation of symptoms or resection of solitary metastases in highly selected patients. The role of radiotherapy is limited to palliation of symptoms, while hormonal treatment and chemotherapy are ineffective (4), the latter possibly partly because of the high expression of the multidrug resistance (MDR1) gene in human RCC (5).

In search for better treatment immunotherapy has also been studied extensively. The basis for this approach originated from observations that metastases though infrequently (6), may regress following nephrectomy as a consequence of an immune response. Early attempts to induce an immune response involved application of crude tumor cell preparations, bacillus Calmette-Guérin, or Corynebacterium parvum. Such non-specific immunotherapy was proven ineffective (7). Efforts were subsequently directed towards activation of the patients' immune system by biologic response modifiers (BRMs) or the adoptive transfer of activated immune cells. The interest in immunotherapy revived with the potentials of genetic engineering, mass cell culturing, the improved techniques in protein and nucleic acid sequencing, and hybridoma technology (8) through which highly purified molecules such as interferons, interleukins, tumor-necrosis factor and monoclonal antibodies directed against tumor-associated antigens became available. In renal cell cancer the most significant treatment results are obtained with interferons and interleukin-2. This review focuses on the use of these cytokines.

IMMUNOMODULATION WITH INTERFERON

Interferons were initially identified as antiviral agents (9), but in addition these molecules are potent regulators of cell gene expression, cell structure and cell function, and they exhibit direct antiproliferative activity. Three major classes of

interferons have been characterized: Interferon-alpha (IFN-α) produced by leukocytes, interferon-beta (IFN-β) produced by fibroblasts, lymphoblastoid cells, macrophages and epithelial cells, and interferon-gamma (IFN-γ) produced by activated lymphocytes. Interferons have antitumor properties, either mediated through a direct cytotoxic effect on tumor cells, augmentation of the immunogenicity of tumors by upregulation of major histocompatibility complex (MHC) and tumor-associated antigens, and/or activation of macrophages, T-lymphocytes, and natural killer (NK) cells.

Initially, human leukocyte derived IFN-α was used (10-12), yielding response rates not exceeding 15% in patients with renal cell cancer. The development of recombinant DNA techniques made it possible to develop highly purified preparations with considerable biologic activity. Studies using IFNα-2a have shown response rates of approximately 15% (a total of 420 patients; 95% confidence interval (CI) of 12-19%) (13-21). With IFNα-2b similar response rates have been reported (239 patients; 95% CI of 12-22%) (17, 22-25). The experience with IFN-β in renal cell cancer is very limited (response rate of 7% in only 28 patients) (26,27). IFN-γ yielded a 10% response rate (255 patients;95% CI of 6-14%) (23,28-36). In most series, patients with a good performance status, low tumor burden or mainly lung metastases were more likely to respond to IFN. The time to response varied from 1-3 months, the median duration of response was 6 months. Survival data vary from 7-18^{+} months and are likely to be influenced by patient selection (15,37).

In a meta-analysis of 1600 RCC patients, treatment with IFN resulted in a response rate of 15% including 2% complete responses (38). Approximately 3% of responding patients survived for prolonged periods without additional therapy. Selection bias and small sample size may account for much of the variability in reported response rates.

Although the optimal dose and schedule of IFN-α has yet to be determined, in 2 small randomized trials the highest therapeutic index was achieved with a total daily dose of 5-10 MU (12,13,39). The route of administration (s.c. or i.v.) does not seem to be a major factor in determining response.

Side effects of interferon therapy are proportional to dose, more pronounced in elderly patients and fully reversible (40). Acute effects involve an influenza-like syndrome, characterized by fever, chills, tachycardia, malaise, myalgia, headache, dizziness, but dissipate after 1 week of continued therapy. Chronic effects involve

primarily fatigue, weakness, and anorexia, which have been dose-limiting in the majority of trials. Other common and usually mild side effects include decreased ability to concentrate, nausea, emesis, diarrhea, elevation of liver enzymes, proteinuria, alopecia, anemia, leukopenia, and thrombocytopenia (40). Moderate to severe changes in behavior are possibly due to direct effects of IFN on the brain (41,42). Toxicity can be reduced by using induction treatment with relatively high dose over a short period of time followed by maintenance therapy with a lower dose (15). Patients can be treated symptomatically with acetaminophen and benzodiazepines. The development of neutralizing anti-interferon antibodies in patients during therapy with IFN has been described. The observed frequency showed a wide range, varying from 5 to 44% (13,22,43,44). The clinical relevance of these antibodies remains controversial and further investigations are necessary to determine the effect of their formation on antitumor response.

It has been suggested that treatment results with IFN-α may be improved by the addition of cytotoxic drugs, in particular vinblastine, which is considered to have some activity in RCC (46). However, while response rates in relatively small non randomized studies range from 0-45%, a randomized study (47) could not show any benefit of adding vinblastine to IFN-α.

In conclusion, single agent therapy with interferon in metastatic renal cell cancer manifests modest clinical antitumor activity. It is not clear whether any one IFN preparation or dosage scheme is superior, nor is there definitive evidence for the superior efficacy of interferon combinations or interferon in conjunction with chemotherapy.

THE INTERLEUKINS

The interleukins are mediators secreted by a variety of cell types that can activate and regulate growth and/or differentiation of immune cells. Through recombinant DNA technology the list of known, well-characterized, multifunctional interleukins has extended steadily. To date, 15 interleukins have been identified and cloned (Table 1). The availability of purified, recombinant interleukins has enabled extensive *in vitro* and *in vivo* studies, to characterize their biologic effects and mechanisms of action. This has considerably enhanced our understanding of how these multifunctional cytokines regulate immune-, hematopoietic- and inflammatory processes, through binding to unique cell surface receptors. In most instances the action of one particular cytokine can cause a cascade of events in

Table 1. The Interleukines

Interleukin(s)	Source	Biologic effects	Ref.
IL1	Monocytes/macrophages; dendritic cells; B, T, and NK cells; keratinocytes; fibroblasts; endothelial cells; epithelial cells; glial cells; neuronal cells	Costimulates B-cell proliferation; induces maturation of pre-B-cells; induces IL2 production, increases IL2 receptor number and binding on T-cells, augments T-cell-derived lymphokine-activated killer (T-LAK) activity; activates macrophages; induces acute phase responses	48-50
IL2	T-cells	Costimulates T-cells; activates cytotoxic responses and chemotaxis of T-cells; co-factor for growth and differentiation of B-cells; generates NK-derived LAK cells	51-54
IL3	T-cells; thymic epithelium; myelomonocytic cells; keratinocytes; neuronal cells	Stimulates early progenitor cell growth; synergizes with Steel factor for early multipotential progenitor cell expansion; B-cell differentiation; augments LAK-activity in the presence of IL2	55-59
IL4	T-cells; mast cells; macrophages; bone marrow stroma	B-cell growth factor; induces MHC class II antigens on monocytes and B-cells; activation of IL2 stimulated LAK cells; synergizes with IL12 in NK cell proliferation	57, 60-63
IL5	T-cells; mast cells	Induces IL2 receptor on T-cells, B-cells and NK-cells; B-cell growth factor II; increases IgA and IgM secretion; co-factor for induction of CTL differentiation; induces proliferation and differentiation of eosinophils	57, 61, 64, 65
IL6	T-cells; monocytes; fibroblasts; endothelial cells; tumor cells	B-cell differentiation factor; co-stimulator of T-cells; induces IL2 production; megakaryocyte differentiation factor; induction of acute phase proteins; activates NK-cells	66-70
IL7	Bone marrow stromal cells	Supports the growth of B-cell precursors; induces cytokine secretion and tumoricidal activity by monocytes; maintains viability and responsiveness to IL2 of NK precusors	63, 71-73
IL8	Monocytes	Chemoattractant for neutrophils	74
IL9	T-helper cells	Stimulates proliferation of mitogen activated T-cells; potentiates IL4 induced IgE synthesis by B-cells; supports erythroid colony formation	75-77
IL10	T-cells; B-cells; macrophages	Cytokine synthesis inhibitory factor for T-helper cells; costimulates with IL2 and IL4 the proliferation of activated T-cells; augments CTL activity	78-80
IL11	Bone marrow stroma	Stimulates T-cell-dependent development of Ig-producing B-cells; stimulates hematopoiesis	81-83
IL12	B-cells; monocytes	Induces the production of IFN gamma by human T-helper type 1 cells; synergizes with IL2 in induction and proliferation of CTL; induces NK-cell lytic activity and proliferation; enhances IL3 dependent hematopoiesis in the presence of IL11 and Steel factor	84-87
IL13	T-cells	Induces proliferation and differentiation of B-cells; induces IgE synthesis; regulates inflammatory cytokine production and nitric oxide production by monocytes	88-90
IL14	T-cells	Induces B-cell proliferation	91
IL15	T-cells	Stimulates T-cell proliferation; induces LAK-cells; interacts with the beta chain of the IL2 receptor	92, 93

which complex interactions between a number of distinct secondarily secreted cytokines and effector cells occur. The interleukins, their sources and biologic effects are depicted in Table 1.

Some of the interleukins may have a direct or indirect inhibitory effect on tumor growth (e.g. by activation of T-lymphocytes or macrophages) and thus may be useful for cancer therapy. Immunotherapy of RCC is predominated by interleukin-2 (IL2) so we focus on this cytokine in the framework of this review.

Immunomodulation with IL2

IL2, first described in 1976, is a glycoprotein lymphokine mainly produced by T lymphocytes of the helper subset (51-53). IL2 induces proliferation of antigen stimulated T-cells and, at high concentration, non-specific lymphokine-activated killer (LAK) cells. When appropriately activated, the expansion of T-cells in the presence of IL2 can be maintained in culture systems for many weeks or months. IL2 is also the predominant cytokine activating NK cells to fully function and IL2-activated NK cells lyse fresh syngeneic NK-resistant murine and human tumor cells (94,95).

IL2 was also used to grow lymphocytes directly from the peripheral blood or spleen. Proliferating lymphocytes could be identified by their ability to lyse fresh tumor cells but not fresh normal cells, the socalled LAK phenomenon. These LAK cells were initially distinguished from NK cells since they could be derived from sites where NK activity was not usually found such as thoracic duct fluid, whereas LAK could also kill fresh tumors which were resistant to NK lysis (96-98). It is now clear that the majority of cells with LAK activity are large granular lymphocytes (LGLs) that do not bear B-cell and T-cell receptors or have functional rearrangements of the T-cell receptor genes. Such cells are capable of mediating NK activity, LAK activity, antibody-dependent cellular cytotoxicity, and are capable of secreting a variety of cytokines (99-101).

IL2 mediates its effects via specific cell surface receptors. Constitutive expression of the IL2 receptor (IL2R) beta chain is observed on macrophages. Antibodies blocking IL2 interaction with this chain prevent IL2 induced cytolytic maturation of macrophages but not the lytic activity induced by interferon-γ. Stimulation of macrophages with IL2 causes them to release large quantities of TGFβ (102,103) an important physiological, antagonistic regulator of tumor necrosis factor (TNF) synthesis and action. Multiple other cell types have been reported to express the beta chain of the IL2 receptor. Much of the effect of IL2 on

other cell types is mediated by the production of other cytokines, subsequent to IL2/IL2R interaction.

IL2 treatment induces alteration in circulating neutrophil Fc-receptor expression as well as reduction in chemotactic activity (104,105), which leads to a marked increase of the risk of catheter-related infections in IL2 treated patients, which requires prophylactic use of antibiotics and/or tunneled catheters (106,107). In addition, probably partly due to the induction of IL5, IL2 administration causes an increase in circulating eosinophils (108), which are capable of cytolysis of NK susceptible targets.

High dose bolus intravenous IL2

The initial experience with IL2 in humans was obtained with a regimen of intravenous bolus therapy given every 8 hours, which was developed by Rosenberg et al. (109). Limited phase I studies demonstrated that the maximum tolerated dose (MTD) of this schedule of IL2 was 600,000-720,000 IU/kg, given for 14-20 doses (110). This regimen of high-dose bolus IL2 has yielded an overall response rate in metastatic RCC of 20%, comprising 7% CRs and 13% PRs (110). A summary of the literature on the treatment of RCC with high-dose bolus IL2 alone is shown in Table 2. The overall response rate in these studies was 15% (110-114).

Table 2. High-dose bolus intravenous IL2 alone in metastatic RCC

IL-2 dose IU/kg x 10^{-3} (schedule)	Number of patients	Response Rate No. (%)	References
600 (q 8 hrs)	16	0 (0)	110
600 (q 8 hrs)	37	3 (8)	111
600 (q 8 hrs)	71	12 (17)	112
720 (q 8 hrs)	149	30 (20)	113
600-720 (q 8 hrs)	255	16 (14)	114
Total	528	81 (15)	

High-dose bolus IL2 is associated with considerable toxicity and frequently requires intensive care management. The most striking side effects are a capillary

leak syndrome and decreased peripheral vascular resistance, leading to interstitial and peripheral edema, hypotension and oliguria. In addition, IL2 can cause flu-like symptoms as described in the chapter on IFNs. Hence, dose reductions and treatment interruptions are necessary in 40-50% of patients. Based on growing experience, it has been suggested to reserve high-dose bolus IL2 for patients in good condition, with normal cardiac, pulmonary, renal and hepatic function. Although concern has been expressed that dose reduction may decrease the efficacy of IL2, there is no evidence to suggest that the response rates are indeed reduced. Yang et al. (115) performed a randomized trial comparing a high-dose bolus IL2 regimen (720,000 IU/kg i.v. every 8 hours), with a low-dose bolus schedule (72,000 IU/kg i.v. every 8 hours). The high-dose arm was associated with significantly more toxicity. The respective response rates were 15% for the low-dose regimen and 20% for the high-dose regimen. There was no difference in survival.

Overall, IL2 induces objective responses in 15-20% of patients with metastatic renal cell cancer. Although these response rates are not different from the results obtained with IFNs, approximately 1/3 of responders achieve a complete response, and moreover responses to IL2 appear to last longer. Some of the patients in the early studies are now disease-free for more than 7 years, and thus it is conceivable that they have been cured.

High-dose continuous intravenous IL2 infusion (c.i.v.)

In an effort to reduce the toxicity related to high-dose bolus IL2, continuous intravenous (c.i.v.) infusion has been studied. Some of these studies have suggested that c.i.v. IL2 produces greater immunostimulatory effects such as greater rebound lymphocytosis, LAK-cell yield, and *in vivo* immunostimulation (116, 117).

West et al. reported that the antitumor effects of c.i.v. IL2 were similar to those obtained with high-dose bolus IL2 (118). Toxicity appeared to be somewhat less with c.i.v. than with high-dose bolus IL2. With c.i.v. IL2 response rates of approximately 15-20% are achieved. The results of the most important trials are summarized in Table 3.

The overall response rates and types of side effects are similar for high-dose bolus IL2 and high-dose c.i.v. IL2. The only randomized trial comparing bolus IL2 to c.i.v. IL2 was conducted by Weiss et al. (124). Forty-eight patients received IL2 by c.i.v. at a dose of 18-22.5 MIU/m^2/day, and 46 patients received 600,000 IU/kg

($\simeq$ 24 MIU/m^2) of IL2 every 8 hours by i.v. bolus, i.e. a 3-fold difference in total dose per course in favor of bolus administration. Infections were more frequent

Table 3. High-dose c.i.v. IL2

IL2 dose/day (IU/m^2 x 10^{-6})	Median LAK cells given (x 10^{-10})	Number of patients	Response Rate No.(%)	References
18	1	51	14 (27)	119
18	9	47	4 (9)	120
18-24	15	25	4 (16)	121
18	7	21	2 (10)	122
2-6	18	42	14 (33)	123
18-23	14	48	7 (15)	124
18	9	46	7 (15)	125
	Total	280	52 (19)	

in patients on c.i.v. IL2, while thrombocytopenia was more frequent in patients on bolus IL2. Response rates (15 and 20% respectively) were not significantly different. Another conclusion from these studies was that the cumulative MTD of IL2 is less for c.i.v. administration than for bolus administration.

Subcutaneous IL-2 administration

After subcutaneous (s.c.) administration, many biological response modifiers yield sustained blood levels for up to 24 hours. Therefore, some investigators explored the s.c. route of administration in order to simplify IL2 therapy and to reduce side effects (Table 4). This treatment can be given on an outpatient basis. Buter et al. (126) administered IL2 s.c. once daily for 5 days a week for 6 weeks. The resulting 20% response rate could not be confirmed by others using comparable doses and regimens (127,128). Several studies have combined IL2 s.c. with IFNα. The reported response rates varied from 12%-25% (127,129,130). Clearly, the toxicity of s.c. IL2 is modest in comparison with the intravenous route and mostly consist of local inflammation at the injection site and flu-like symptoms.

In summary, low-dose s.c. IL2 regimens have modest antitumor activity and may be an option with greater safety indicated for patients with concomitant disease such as cardiovascular abnormalities. However antitumor effects are difficult to interpret, because most reported studies are small phase I-II trials.

Table 4. Subcutaneous IL2

IL2 dose $IU/m^2 \times 10^{-6}$ (schedule)	IFN-α dose $U/m^2 \times 10^{-6}$ s.c. (schedule)	Number of patients	Overall response (%)	Reference
9-18 (5d/w, 4-6w)	-	46	9 (20)	126
6-30 (5 d)	-	14	0 (0)	128
1.8-9 (q12h 5d/w x 6)	-	14	0 (0)	127
12 (4d)15, 4w)	9 (2d/w)	42	5 (12)	129
1.5-7.5 (5d/w)	2.5-12.5 (3d/w)	16	4 (25)	130
5-20 (3d/w x 6)	6 (1-3d/w x 6)	51	14 (27)	127
5-20 (3d/w x 6)	6-9 (1-3 d/w x 8) plus 5FU 750 mg/m^2 bolus i.v., 1x/5-8	35	17 (49)	127
		218	49 (22)	

COMBINATION THERAPY

The rationale for using combinations of different cytokines is the observation that some of these agents such as IL2 and IFN-α have shown synergistic antitumor activity against a variety of tumors in preclinical studies (131-132). In many weakly or non-immunogenic tumors, the combination of IL2 and IFN-α has shown substantial antitumor activity in settings where either agent alone is ineffective. Despite the enhancing effect of interferons on class I MHC expression, the ability of IFN to enhance the activity of IL2 appears more dependent on NK cells, since the synergy between the two cytokines is decreased in NK-deficient beige mice and in mice depleted of NK cells (132). Synergy between IL2 and interferon in murine models is not restricted to antitumor activity, but pertains to toxicity as well (135,136).

Many clinical trials have been performed to evaluate combinations of IL2 and IFN-α. Rosenberg et al. used a high-dose regimen of bolus intravenous IL2 given

every 8 hours for 5 days, week 1 and 3, at doses ranging from 3-13.5 MIU/m^2, combined with IFN-α 3 MU/m^2 intravenously. At the highest dose levels of this study response rates of 38% for RCC were observed (137). However this high-dose schedule of the 2 cytokines appeared to result in increased cardiac-, neuropsychiatric-, and hepatic toxicity (143,155). Therefore, subsequent trials attempted to decrease toxicity by administering lower dosages. Numerous doses and schedules of IL2 and IFN-α have been tested. Intermediate to high dose schedules yielded response rates of approximately 16% (112,138-142,144-148,155). A summary of these trials is given in Table 5.

Table 5. Combinations of IL2 and IFN-α

	# patients	# studies	overall response (%)	references
High dose i.v. IL2	221	5	35 (16)	112, 138-140,155
Intermediate i.v. dose IL2	183	7	32 (17)	141,142,144-148
Low dose s.c. IL2	378	7	89 (23)	129, 149-154

Low dose: < 20 MIU/day
Intermediate dose: 20-32 MIU/day
High dose: 32-150 MIU/day

Several small studies have focused on outpatient low-dose regimens (129,149-154). Although these regimens produce less acute toxicity and are therefore easier to administer than high-dose IL2 regimens, most patients developed chronic fatigue and a decrease in performance status requiring modification or cessation of therapy.

The Surgery Branch of the National Cancer Institute - USA has reported the long-term follow-up evaluation of their initial phase I/II study (137) and concluded that the combined use of IL2 and IFN-α was not indicated based on a lack of survival benefit and increased toxicity (155). These findings are in agreement with those of a randomized study comparing IL2 + IFN-α versus IL2 alone (112). In this trial IL2 alone resulted in a response rate of 17%, while the combination yielded a response rate of 11%. The median survival in the 2 treatment arms was 15,5 months and 16 months, respectively.

Presently, an interesting area of research involves the application of chemo-immunotherapy. Several investigators have added 5-fluorouracil (5-FU) to the

combination of IL2 and IFNα and reported response rates varying from 24-49% (127,156,157). Whether the addition of 5-FU to IL2/IFN-α represents a benefit will have to be addressed in randomized studies.

The combination of IL2 and IFN-γ has also been used in the treatment of RCC. Although IFN-γ was not able to increase IL2 receptor expression, it did increase serum β-2-microglobulin levels (159), IL2 receptors on T-cells, HLA-DR expression on NK cells, and Fc receptors on macrophages (160). Increases of LAK and NK activity correlated with absence of disease progression (160). IFN-γ appeared not to worsen IL2 toxicity, but early clincal studies have shown poor treatment results, so that the combination of IL2 and IFN-γ has not been elaborated (159,163).

Studies on the combination of IL2 with IL4, intended to synergize the activation and proliferation of MHC-restricted cytotoxic T-lymphocytes, failed to demonstrate enhancement of immune stimulation or antitumor effect (164-167).

In tumor bearing animals interesting though unexplained synergistic antitumor effects have been demonstrated with the combination of TNFα and IL2 with a clear dose and schedule dependency (168-170). Optimal results have been obtained when TNFα was used at its MTD prior to IL2 administration (169). Clinical trials have shown that administration of TNF-α prior to IL2 did not appear to increase the toxicity of IL2, while administration of IL2 prior to or concurrent with TNF-α appeared to worsen the side effects typical of high-dose IL2 (171-174). Although responses were seen, there was no evidence to suggest improved efficacy in comparison to IL2 alone.

In summary combination cytokine therapy failed to improve the clinical efficacy of IL2 alone despite promising preclinical data.

ADOPTIVE CELLULAR IMMUNOTHERAPY

Adoptive cellular immunotherapy (ACIT) of cancer is a form of passive immunization in which immune cells with antitumor properties are transferred to the tumor-bearing host. These cells may mediate their cytolytic effects either via direct contact with and killing of their targets, or indirectly by producing substances with tumoricidal or immunomodulatory properties. In ACIT autologous immune cells that have been activated and expanded ex vivo are reinfused in the tumor-bearing host, often in combination with IL2 or other therapeutic agents. The effector cells employed for ACIT can be divided in specific (MHC-restricted) or non-specific (MHC-unrestricted) immune cells. Lymphokine-activated killer (LAK) cells are non-specific, while another population of cytotoxic lymphocytes, tumor-

infiltrating lymphocytes (TIL) recognize tumor-associated antigens (TAA) in a specific MHC-restricted manner.

ACIT with LAK

The cultivation of peripheral blood lymphocytes (PBL) in media containing IL2 (usually at concentrations of 6000 IU/ml) results in the generation of LAK cells. LAK is best considered as a functional state of immune cells which may be generated from both NK cell and T lymphocyte populations (175).

After it was demonstrated that large numbers of in vitro activated cells could safely be infused in humans, various clinical studies of IL2 and LAK with or without IFNα have been performed. These trials used a variety of regimens. The response rates ranged from 9-34% (mean 22%) (120,123-125,177-181). These studies are summarized in Table 6.

Table 6. Phase II studies of IL2 and LAK

IL2 dose IU x 10^{-6} (schedule)	Median LAK cells given (x 10^{-10})/course	Number of patients	Response rate No. (%)	References
0.6/kg (q 8w)	10	49	15 (32)	177
18/m^2/d	9	47	4 (9)	120
18/m^2/d	7	46	7 (15)	125
18/m^2/d	6.5	102	17 (18)	178
18-23/m^2/d	14	48	7 (15)	124
16/m^2/d	5.6	23	6 (26)	179
2-6/m^2/d	18	42	14 (33)	123
8-16/m^2/d + IFN-α 12 MU/m^2 3x/w	11	40	8 (20)	180
18/m^2/d + IFN-α 5 MU/m^2/d	9.6	68	23 (34)	181
	Total	465	101 (22)	

In general, the addition of LAK cell infusion to high-dose IL2 therapy has not been shown to be superior over single agent high-dose IL2. Three randomized trials have been carried out to determine whether the addition of LAK offers a therapeutic benefit, but none showed a higher response rate or longer survival (111,177,182). The reasons for this may be various. LAK cells were shown not to localize selectively in tumors (183,187). In addition, the therapeutic effect of LAK is probably related to the cytotoxic effects of tumor specific T-lymfocytes at the site of the tumor (184-188). Immunophenotypic analysis of cellular infiltrates of regressing melanoma lesions revealed the presence of $CD3^+$ but not $CD3^-$ $CD16^+$ infiltrating cells, suggesting that T-lymphocytes rather than NK cells were responsible for the antitumor effect (185,186). Taken together, these findings indicate that direct (IL2 mediated) or indirect (through release of secondary cytokines) activation of tumor-specific T-cells form the basis of adoptive cellular immunotherapy of cancer.

ACIT with TIL

Another population of cytolytic lymphocytes, that can be used as effector cells in combination with IL2, are tumor-infiltrating lymphocytes (TIL). These immune cells possess potent antitumor activity and are derived from lymphocytes which have infiltrated tumor lesions. In contrast to LAK, murine and human TIL demonstrate cytolytic specificity only against the tumor from which they are derived or against closely related tumors (189,190). In murine systems TILs are 50-100 times more potent than LAK in their cytolytic capacity (189). Murine TILs are predominantly $CD3^+$ $CD4^-$ $CD8^+$, while human TILs are phenotypically more heterogenous, which may reflect the difficulties encountered in obtaining true TIL from human tumors, which by definition also contain PBC. This heterogeneity may be the reason that in vitro cytotoxicity of TIL is not a good predictor for their ability to cause tumor regression in vivo (191). An exception to this rule are TILs derived from melanomas, which manifest MHC-restricted interactions with tumor targets in at least 50% samples. TILs with specific lytic activity for autologous tumor but not for normal autologous tissues or for allogeneic tumor can be derived from approximately one-third of all patients with melanoma (191,192). For this reason, clinical trials of TILs in humans have concentrated on melanoma and response rates of 20-30% have been reported (125,193-195). In RCC the experience with TIL is very limited and antitumor responses are rare (193,195-197). The same holds true for small studies with TILs and IL2 in combination with IL4 or IFNα (198-200).

FUTURE PERSPECTIVES

From the above reviewed literature it becomes clear that no standard immunotherapy of RCC can be recommended, since the cytokines and effector cells mentioned do not offer clearcut benefit for larger groups of patients. However, it is for the first time in the long history of immunotherapy that treatment strategies can be rationally designed on the basis of our increased knowledge of the function of the immune system and its interaction with tumor target cells, whereas at the same time these strategies can be materialized with the help of recently developed methods of molecular engineering.

What is clearly needed in the clinical arena is the development of efficient effector cells which are tumor selective. Although T-lymphocytes are equipped with T-cell receptors which can recognize and bind TAA, this is clinically futile since tumor cells often do not express their TAA adequately in association with MHC molecules, which makes recognition by the T-cell receptor impossible. The CD3 antigen is an activation molecule physically associated with the T-cell receptor. Binding of the CD3 antigen to TAA leads to activation of the T-cell, followed by lymphokine production and cytolytic activity (211). This approach of targeting immune cells to tumor cells has been elaborated in preclinical and clinical studies.

ACIT with T-lymphocytes retargeted with bispecific monoclonal antibodies

Bispecific monoclonal antibodies (bs-MAb) are hybrid antibodies constructed from two parent MAbs: one specific for the immune effector cell and the other specific for a TAA on the target cell. Bs-MAb-mediated cross-linking of the effector T cell to the tumor target cell results in activation of the lymphocyte leading to lysis of the tumor cell (201-205,211). Bs-MAbs are usually constructed by somatic hybridization of 2 mouse hybridomas. Several bs-MAbs have been developed with specificity against RCC, ovarian cancer and breast cancer (211-116).

One of the first clinical trials testing this concept was in ovarian cancer with a bs-MAb against the overexpressed folate-binding protein on the tumor cells and against the activation antigen CD3 (206-208). A clinical phase I-II study was carried out in ovarian cancer patients with recurrent disease confined to the peritoneal cavity. Patients were treated with daily intraperitoneal infusions of autologous in vitro expanded T-lymphocytes retargeted with the bs-MAb. Infusions were given with low-dose IL2. The overall intraperitoneal response rate was 27% (209). The development of neutralizing human anti-mouse antibody (HAMA) was observed in all patients after approximately 2-3 weeks from the start of treatment (210).

The use of mouse monoclonal antibodies has several disadvantages such as a short serum half-life, limited potential to trigger human effector cell functions and HAMA response (217-219). These problems have led to the construction of "humanized" antibodies, which have been shown to be less immunogenic than their murine counter parts (220-223).

However, despite this improvement in antibody technology, the use of bs-MAbs can still be hampered by the inaccessibility of solid tumor to antibody penetration. In addition, bs-MAb redirected T-cells retain the antibody for limited periods of 48-69 hours due to dissociation from the T-cell surface. It has also been demonstrated that bs-MAb redirected T-cells lose their signal transducing and lytic capacity following target cell recognition (209,224). To circumvent the limitations associated with bs-MAb, a new approach has been adopted in which T-cells are "gene-grafted" with a permanent antibody dictated specificity.

Gene modified chimeric T-cell receptor

By the construction of a chimeric immunoglobulin T-cell receptor complex (Ig-TCR), tumor selectivity of T lymphocytes may be obtained. To be effective such T lymphocytes would require stable expression of the engineered Ig-TCR receptor at the lymphocyte surface and its functional association with the CD3 signal-transducing element. This has been achieved by the introduction and expression of chimeric Ig/TCR genes, in which the variable (V) gene segments of the TCRα and TCRβ chains are replaced by the variable gene segments of the heavy and light chain of an Ig with known specificity (225-230). Retroviral gene transfer is employed to transduce the chimeric receptor into activated T lymphocytes. The transduced T lymphocytes stably express the receptor for >4 months of in vitro culture (230). Stimulation of the chimeric receptor results in T cell activation, cytokine production and lysis of target cells (228-230).

In contrast to BsMAbs redirected lymphocytes, lymphocytes transduced with chimeric Ig-TCR genes show recycling of the cytolytic process (230). An essential feature of Ig-TCR targeted lymphocytes is their ability to recognize tumor-associated antigens in a MHC-unrestricted manner.

Cytokine gene modified tumor cells

Cytokines provide costimulatory signals important for T lymphocyte activation. Because most cytokines have a short serum half-life, local delivery may be attractive and in addition potentially less toxic. One locoregional approach is to introduce genes encoding for cytokines into tumor cells sothat the cytokine is

continuously secreted by the tumor cell, resulting in effective cytokine concentrations in the vicinity of tumor cells and tumor antigens, but not elsewhere in the body. Many cytokine genes have been introduced into tumor cells with varying effects on both tumorigenicity and immunogenicity. This new tumor vaccine approach has been best developed in murine models (231-235). Pilot studies of cytokine transfer by vaccination with engineered tumor cells in patients have started (236,237). However, adequate TAA expression and recognition remains a problem (238).

Cytokine gene modified effector cells

Although it has been relatively easy to express cytokine genes in tumors cells, this is not the case in lymphocytes, where it is difficult to achieve constant high levels of cytokine production, which is probably due to regulatory mechanisms (239). Moreover, large numbers of T cells are required for adoptive therapy, and consequently, a high transduction efficiency is needed to enable the growth of adequate numbers of gene modified cells. Recently, gene therapy of RCC has focused on TNF gene transfer into TIL (240).

There are other possibilities for the introduction of cytokine genes into TIL. For example, TIL transduced with the gene for the IL2 receptor may increase their sensitivity to IL2. The introduction of IFN-α or IFN-γ genes into TIL could cause direct antiproliferative effects and upregulation of MHC antigens, increasing the immunogenicity of the tumor. However, it should be remembered that the existence of true TIL in RCC is still controversial.

Tumor vaccines

Active specific immunotherapy with vaccines constructed of TAA is conceptually an attractive approach to treatment and possibly to prevention of cancer. The rationale is that such vaccines may be able to stimulate the immune system more vigorously and activate silent precursor lymphocytes with tumor specificity. However, in most types of human cancer, there is little evidence that primary or metastatic tumors induce immunity in patients. Occasionally, melanoma patients were found to have demonstrable tumor specific antibodies and cytotoxic T lymphocytes (241-243). The problem with human tumors appears to be poor immunogenicity of relevant antigens which permits tumor cells to escape the immune attack and active inhibition of the host immune response by the tumor cells (244). Thus far, a number of antigens have been identified as targets for recognition by cytotoxic lymphocytes, mostly in melanoma, but also in other

tumors types (245-251). Vaccination studies with immunogenic peptides in humans have recently started (252,253).

It can be concluded that the past 15 years have witnessed revolutionary developments in tumorimmunology and experimental immunotherapy. Firstly, our understanding of the communication network between immune cells has significantly increased. Secondly, the availability of recombinant DNA techniques has enabled the isolation and production of a whole series of interleukins which can be studied in preclinical and clinical models. Finally, new and refined methods of tumor specific targeted immunotherapy are on the brink of clinical application.

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Chapter 2

INTRAPLEURAL ADMINISTRATION OF INTERLEUKIN-2 IN PLEURAL MESOTHELIOMA; A PHASE I-II STUDY

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SUMMARY

Twenty-three patients with pleural mesothelioma stage I-IIA were entered in a study of continuous daily intrapleural infusion of interleukin-2 (IL2) for 14 days, repeated every 4 weeks. IL2 was administered according to a groupwise dose escalation schedule (Group A: 3×10^4, B: 3×10^5, C: 3×10^6, D: 6×10^6, E: 18×10^6, and F: 36×10^6 IU/day). Each group consisted of at least 3 patients.

Intrapleural administration of IL2 was associated with acceptable toxicity. All patients were treated on an outpatient basis except for the patients at dose levels E and F. Dose limiting toxicity was observed at level F: 36×10^6 IU daily and consisted of catheter infection, fever, and flu-like symptoms.

Intrapleural IL2 levels were high (> 20,000 IU/ml) at levels E and F, while serum levels in most patients were not or barely detectable (<3 - 30 IU/ml). Intrapleural IL2 levels were up to 6000 fold higher than systemic levels. Intrapleural tumour necrosis factor-alpha (TNFα) levels varied greatly and did not correlate with IL2 dosage. Intrapleural mononuclear cells (MNC) displayed IL2-induced lymphokine-activated killer (LAK) activity in all patients.

Two patients were not evaluable for response due to catheter-related problems which precluded the delivery of IL2. Partial response (PR) occurred in 4 of 21 evaluable patients (19%; 95% confidence interval 5 - 42%) with a median time to progression of 12 months (range 5 - 37). Stable disease (SD) occurred in 7 patients with a median time to progression of 5 months (range 2 - 7). There were no complete responses (CRs). The median overall survival was 15.6 months (range 3.0 - 43). No relationship between the dose of IL2 and response rate was observed.

We conclude that IL2 given intrapleurally is accompanied with acceptable toxicity and has antitumour activity against mesothelioma. In view of the refractory nature of the disease IL2 may be a treatment option for mesothelioma. A formal phase II study is warranted. Based on the observed toxicity, the lack of dose response relationship, and the immuno-modulatory effects seen at relative low dose IL2, the recommended dose for a phase II study is 3×10^6 IU/day using the present treatment schedule.

INTRODUCTION

Untreated patients with pleural mesothelioma have a median survival of 9 months and may survive just as long as treated patients (Hillerdal, 1983; Law, et al. 1984). Current treatment methods do not appear to improve survival (Alberts, et al. 1988). Therefore, new treatment modalities should be investigated.

In intraperitoneal tumour models it has been demonstrated that intracavitary administration of interleukin-2 (IL2) can induce very high numbers of lymphokine activated killer cells (LAK) in the peritoneal exudate (Eggermont, et al. 1988 & 1989). Consequently, intrapleural administration of IL2 for the treatment of pleural mesothelioma appears to be a reasonable therapeutic approach, particularly since mesothelioma tends to be confined to the pleural cavity for the most part of the course of the disease. Yasumoto et al (Yasumoto, et al. 1987) reported the complete clearance of malignant cells after intrapleural instillations of IL2 in patients with pleurisy due to lung cancer. Astoul et al. (Astoul, 1993) reported objective responses in mesothelioma patients treated with continuous intrapleural IL2 instillation.

Based on these observations, we performed a phase I-II study with intrapleural IL2 in patients with pleural mesothelioma stage I-IIA, classified according to the Butchart staging system (Butchart, et al. 1976).

PATIENTS AND METHODS

Patients

Staging and diagnosis of mesothelioma was based on computed tomographic (CT) scan of the chest, thoracoscopic findings, and histologic examination of biopsy samples.

All biopsies were reviewed by our institution's pathologist. The staining techniques used included haematoxylin and eosin, special stains for reticulin and mucins (such as mucicarmine, periodic acid-Schiff after diastase, and the alcian blue stain with and without prior digestion with hyaluronidase), and the immuno-histochemistry stain for CEA, keratin, CAM-5.2, and an epithelial membrane antigen MOC-31.

According to Butcharts' staging system (Butchart, et al. 1976) stage I is defined as tumour confined within the capsule of the parietal pleura, i.e. involving only ipsilateral pleura, lung , diaphragm, and external surface of the pericardium within the pleural reflection. Stage IIA is defined as mesothelioma invading chest wall or

mediastinal tissues with or without lymph node involvement ipsilaterally inside the chest.

Eligibility criteria required histologically confirmed pleural mesothelioma stage I-IIA, sufficient pleural effusion to insert an intrapleural catheter, no signs of loculation on the CT scan, no prior chemo-, radio-, or immunotherapy, age < 76 years, Karnofsky performance status ≥ 80, no cardiovascular disease, a white blood cell count ≥ 4000/ml, a platelet count ≥ 100,000/ml, haematocrit ≥ 30%, serum bilirubin and creatinine levels within the institution's normal range, no active infection, no use of corticosteroids, and obtained informed consent.

Treatment

One to two weeks prior to the first administration of IL2 a port-a-cath system was surgically inserted under general anaesthesia. The correct intrapleural position of the catheter was examined radiographically and a Technetium 99m colloid scan was made to evaluate the distribution of pleural fluid throughout the pleural cavity.

Recombinant human interleukin-2 (IL2) (Chiron B.V., Amsterdam, The Netherlands) was administered as a continuous intrapleural infusion at a dose according to a groupwise dose escalation schedule (Group A: 3×10^4, B: 3×10^5, C: 3×10^6, D: 6×10^6, E: 18×10^6, and F: 36×10^6 IU/day) for 14 days, repeated every 4 weeks. After 2 cycles, response to treatment was evaluated. Each group consisted of at least 3 patients. Patients with stable disease or response could receive up to a maximum of 6 cycles. No intrapatient dose escalation was performed.

All patients were seen weekly at the outpatient clinic. Masks and sterile gloves were used for all dressing changes, and dressings were changed only by trained nursing personnel.

Response and toxicity

Response was evaluated after every 2 treatment cycles using CT-scan of the chest. Tumour response and toxicity were assessed according to the criteria of the World Health Organization (WHO) (WHO handbook, 1979). In case of measurable disease, complete response (CR) was defined as the disappearance of all known disease for at least 4 weeks; partial response (PR) as a decrease > 50% in tumour size for at least 4 weeks; stable disease (SD) as a decrease of < 50% in tumour size. Progressive disease (PD) was defined as an increase > 25% in the diameter of any lesion or the appearance of a new lesion.

In case of unmeasurable but evaluable disease a CR was defined as the complete disappearance of all known disease for at least 4 weeks; a PR as an estimated decrease in tumour size of ≥ 50% for at least 4 weeks; SD as an estimated decrease of less than 50%, and lesions with estimated increase of less than 25%. PD was defined as the appearance of any new lesion not previously identified or estimated increase of 25% or more in existent lesions.

Pleural effusion was not considered an adequate parameter of response or progression by itself. It would not detract from a PR or SD. However, if remaining present, it would reduce a CR to a PR.

Time to progression and survival were calculated from the start of treatment to the date of progressive disease or death, respectively, according to the Kaplan and Meier method (Kaplan, 1958).

Toxicity was recorded and analysed using the WHO grading system. For toxicities not included in the WHO guidelines, a grading system was used ranging from mild (grade 1) to life-threatening (grade 4).

Immunomonitoring

All samples were taken at the same time of the day (preferably in the morning) since a circadian rhythm has been described for a number of functions. Mononuclear cells (MNC), serum samples and pleural fluid (if present) were collected weekly during each course and cryopreserved until tested.

Immunophenotyping

MNC were isolated from heparinized pleural and peripheral blood samples by density centrifugation (Ficoll-Isopaque). MNC were washed, resuspended at a concentration of 1 x 10^6/ml in phosphate buffered saline (PBS) + 1% bovine serum albumin (BSA) and stained with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated monoclonal antibodies (mAbs).

The following mAbs were used: anti-Leu4/FITC (CD3); anti-Leu3a/FITC (CD4); anti-Leu2a/PE (CD8); anti-Leu 11c/PE (CD16); anti-Leu12/FITC (CD19) and anti-Leu19/PE (CD56). All mAbs were purchased from Becton and Dickinson Immunocytometry Systems (BDIS, San Jose, CA, USA).

After incubation with the mAb for 30 minutes at 0^0C, MNC were fixed in PBS containing 1% paraformaldehyde, stored at 4^0C and flow cytometry was performed within 24 h using a FACScan (BDIS).

Cytotoxicity assays

Cytotoxic activity of MNC was determined in a standard 3-h ^{51}Cr release assay. Briefly, lymphocytes were seeded in triplicate in 96-well, round-bottomed microtiter plates. Target cells labelled with ^{51}Cr were added and at the end of the incubation period (37^0C and 5% CO_2), supernatants were collected, and ^{51}Cr release was measured. Target cells were the NK-sensitive K562 chronic myelogenous leukemia cell line and the NK-resistant, LAK sensitive Daudi Burkitt lymphoma cell line.

Determination of cytokine levels

In the patients where pleural fluid could easily be obtained, this was done prior to and during treatment with IL2. At the same time blood samples were taken in order to compare intrapleural IL2 and tumour necrosis factor-alpha (TNFα) levels with simultaneous blood IL2 and TNFα levels.

IL2 was measured with a double antibody radio-immunoassay using a polyclonal antiserum (IRE-Medgenix, Fleurus, Belgium). The detection limit is about 0.5 U/ml (3.0 IU/ml). The interassay coefficient of variation at a level of 10^4 U/ml is 6.8%. One unit in this assay corresponds with 6 IU.

TNFα was measured with a coated tube immuno-radiometric assay (IRE-Medgenix, Fleurus, Belgium). The detection limit is 5 ng/l and the interassay coefficient of variation at a level of 131 ng/l is 7.2%.

RESULTS

Toxicity profile and tumour response

Twenty-three male patients with epithelial type malignant pleural mesothelioma stage I or IIA, without prior treatment, were eligible for the study. Most of them were shipyard workers with a history of asbestos exposure. Their median age was 57 (range 47-71) and their median Karnofsky performance status 100 (range 90-100).

Twenty-one patients were evaluable for toxicity and tumour response (Tables I and II). Two patients were inevaluable for toxicity and response due to catheter-related problems which prevented the delivery of any IL2. At least one treatment cycle of 14 days was received by 5 patients at 3×10^4 IU/day, 2 patients at 3×10^5 IU/day, 3 patients at 3×10^6 IU/day, 3 patients at 6×10^6 IU/day, 3 patients at 18×10^6 IU/day, and 1 patient at 36×10^6 IU/day. Five patients were started at the highest dose level but only one patient was able to receive one cycle. The other 4

patients were unable to complete one treatment cycle because of catheter-related infections which required catheter removal before the completion of the first cycle. After 5 patient entries at this dose level the study was stopped. Hence, this dose was not tolerated by 4 of the 5 patients (Table I).

In general, IL2-mediated toxicity was mild to moderate, except at the highest dose level. At this level, in addition to the catheter-related infections, fever and flu-like symptoms were dose limiting. The systemic side effects such as fever and skin toxicity corresponded with serum IL2 levels, which reached 30 IU/ml in group F. All patients received their treatment in an outpatient setting except for the last 5 patients who were treated at level F: 36 x 10^6 IU/day. No serious systemic adverse effects such as hypotension, cardiovascular disturbances, pulmonary oedema, liver and renal dysfunction were observed, with the exception of one patient in group F, in whom grade 3 nephrotoxicity was noted. Mild leucocytosis (10,000-12,000 cells/ml) and eosinophilia (2000-3500 cells/ml) in the peripheral blood were seen in most patients at all dose levels.

Treatment-related complications were infection of the port-a-cath systems in 7 patients, 4 of whom were treated at the highest dose level in group F.

Clinical signs of empyema were noted in 5 of the 7 patients with a port-a cath infection. These 5 required removal of the port-a-cath system in combination with drainage by thoracotomy. As can be seen in Table I a variety of micro-organisms were cultured from pleural samples and removed port-a-cath systems of these patients which suggests a secondary bacterial infection in necrotic tissue.

Patients received from less than one up to 6 complete treatment cycles. There were no complete responses. Partial response occurred in 4 of 21 evaluable patients (19%; 95% confidence interval 5 - 42%) with a median time to progression of 12 months (range 5 - 37). Stable disease occurred in 7 patients, the median time to progression was 5 months (range 2 - 7). Six patients had progressive disease. The median overall survival was 15.6 months (range 3.0 - 43). Responses occurred at different dose levels, therefore no dose-response relationship can be established (Table II). Figure 1 shows a representative example of a tumour response.

Three of the 4 PRs with a longlasting response underwent thoracotomy. In patients 10 and 8, an infected port-a-cath with concomitant empyema was noted after 1 and 4 cycles, respectively (Table II).

Table I. Toxicity in relation to intrapleural IL2 in 21 evaluable patients (WHO grade ≥ 2)

Dose level (IU)	$3x10^4$	$3x10^5$	$3x10^6$	$6x10^6$	$18x10^6$	$36x10^6$
Group	A	B	C	D	E	F
Patients	5	2	3	3	3	5
# cycles	14	8	11	10	7	4
Fever > 38°C	-	1*	1	1	2	5
Flu like	-	1	1	-	-	4
Myalgia	-	1	-	1	-	2
Arthralgia	-	1	-	1	-	-
Diarrhea	1	-	-	-	-	1
Non-productive cough	1	1	2	1	1	1
Dyspnea	1	-	1	2	2	-
Arrhythmia	-	-	-	1	-	-
Anorexia	-	-	-	1	1	1
Skin	-	-	-	-	-	3
Creatinine elevation	-	-	-	-	-	1
Leucocytosis (>10,000 cells/ml)	1	1	1	3	3	3
Eosinophilia (>2,000 cells/ml	-	1	2	2	1	1
Infection	-	1	1	-	1	4
Bacterial culture	-	1 s.aureus	1 s.aureus	-	1 streptococcus	4 s.epidermidis (3) eubacterium (1)
Thoracotomy due to empyema	-	1	2	-	1	1

* number of patients per dose level experiencing toxicity

Table II. Clinical data and response to therapy in 21 evaluable patients with pleural mesothelioma treated with intrapleural IL2

Pat. nr.	IL2 (IU/d)	Age	# Cycles	Response	Progression Free Survival (months)	Overall Survival (months)
1	$3x10^4$	51	4	PR	5	15
2	$3x10^4$	47	1	SD	4	8
3	$3x10^4$	50	0	NE°	-	7.5
4	$3x10^4$	63	5	SD	5	9
5	$3x10^4$	71	2	PD	1	5
6	$3x10^4$	57	2	PD	1	19
7	$3x10^5$	49	0	NE°	-	10
8	$3x10^5$	54	6	PR	17	23
9	$3x10^5$	52	2	PD	1	10
10	$3x10^6$	59	1	PR	12	31
11	$3x10^6$	62	4	SD	3	6
12	$3x10^6$	51	6	PR	37	43
13	$3x10^6$	63	6	SD	5,5	28
14	$3x10^6$	64	2	PD	1	16
15	$3x10^6$	62	2	PD	1	17
16	$18x10^6$	69	2	SD	7	10
17	$18x10^6$	62	3	SD	2	18
18	$18x10^6$	59	2	PD	2	3
19	$36x10^6$	48	2x½	SD	6	10
20	$36x10^6$	49	½	SD	-*	9
21	$36x10^6$	55	1	SD	5	26
22	$36x10^6$	54	½	SD	-*	16
23	$36x10^6$	59	3/4	SD	-*	26+

* Pts 20 - 22 - 23 received additional cisplatin and etoposide, thus appropriate PFS cannot be determined.

° Pts 3 and 7 did not receive IL2 intrapleurally due to catheter related problems and were thus inevaluable.

In both these patients massive tumour necrosis was found. Bacterial cultures revealed S.aureus in both cases. In patient 8, whose near-complete response lasted 17 months, a fenestration of the thoracic wall had to be performed in order to prevent recurrent empyema. In a second stage, the pleural cavity, which was macroscopically still free of tumour, was closed by a pedicled omentoplasty. The patient did very well for 17 months, until mesothelioma developed in the contralateral pleural cavity. In patient 12, a fistula developed after 5 cycles and required ribresection. Thoracotomy showed a completely necrotized pleural

mesothelioma. Bacterial cultures were negative. One of the multiple pleural biopsies contained mesothelioma cells. Two patients with SD, patients 19 and 16, showed clinical signs of empyema after 1 and 2 cycles, respectively. A thoracotomy was performed and extensive necrosis was found in the remaining tumour. Bacterial culture revealed streptococcus in one and was negative in the other patient. In these 3 patients (12, 16 and 19) CT-scans showed no tumour regression whereas histologic examination revealed extensive tumour necrosis. These findings underscore the difficulties in the clinical evaluation of response in mesothelioma.

Immunomonitoring

After intrapleural administration of IL2 a mild increase of $CD3^-56^+$, $CD3^+56^+$, and $CD3^-16^+$ lymphocytes was observed in pleural effusion as well as in peripheral blood. All other T-lymphocyte subsets remained at normal levels.

At dose levels A through D cytotoxicity assays showed a significant induction of LAK activity by pleural effusion derived MNC but not by peripheral blood MNC. At dose levels E and F, LAK activity was induced in MNC from both sites. Of note, no differences in LAK activity were seen between responders and nonresponders.

Determination of cytokine levels

Intrapleural as well as serum IL2 levels were determined. Intrapleural levels were very high and correlated with the administered dose of IL2. Intrapleural levels varied from 6 - 110 IU/ml in group A to as high as 66,000 - 192,000 IU/ml in group F. Serum IL2 levels became measurable only in groups E and F, and varied in the 8 patients treated at those levels from < 3 IU/ml to 30 IU/ml. Intrapleural IL2 levels were up to 6000 times higher than serum levels.

Intrapleural TNFα levels varied from 50 - 125 pg/ml in group A to 235 - 405 pg/ml in group F. However, no clear relationship between TNFα and IL2 levels was observed as TNFα levels in groups B-E varied from 292 - 1141 pg/ml.

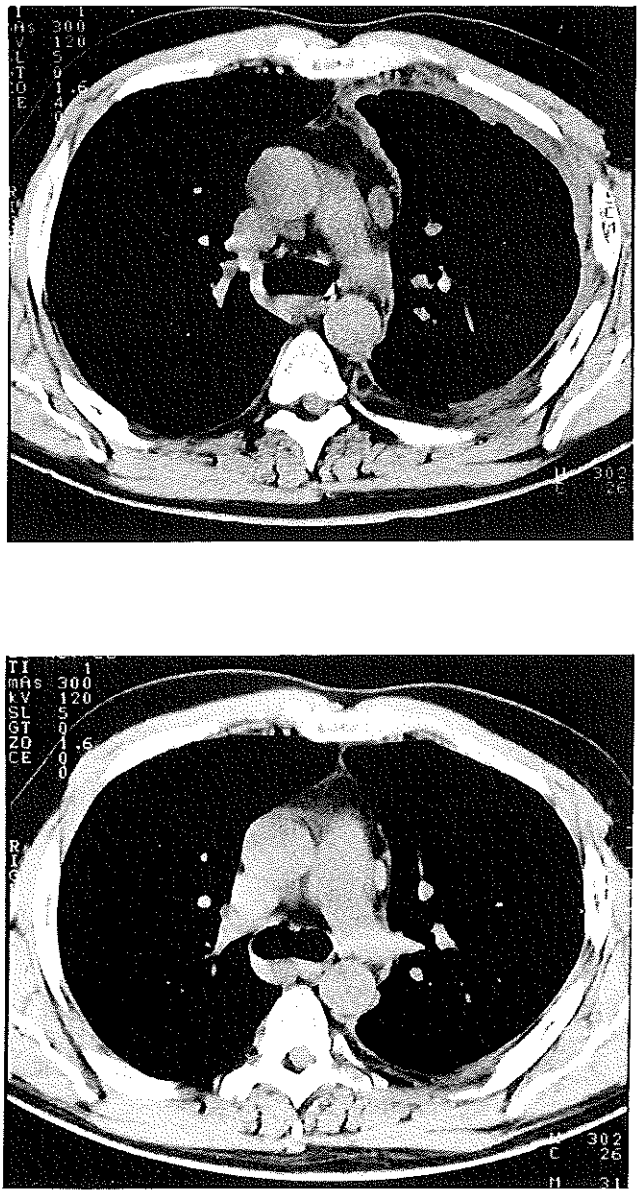

Figure 1.
Partial response of pleural mesothelioma (evaluable disease) and a mediastinal lymph node (measurable disease) metastasis in the left hemithorax. Top: pretreatment CT-scan. Bottom: after 2 cycles of IL2.

DISCUSSION

In this phase I-II study on the toxicity and efficacy of intrapleural administration of IL2 in patients with pleural mesothelioma we observed antitumour activity with acceptable toxicity. The response rate of 19% in 21 patients is of interest as mesothelioma is known to be refractory to treatment.

The basis of this study was the observation of a dose-response relationship in experimental tumour models (Rosenberg, et al. 1987 & 1989; Ettinghausen 1986; Eggermont, et al. 1988). Systemic high dose IL2 therapy is associated with severe toxicity, which may prohibit the possibility to apply doses with optimal antitumour effects (Herberman, 1989). Intrapleural administration of IL2 is therefore a logical approach as it can be expected that very high local levels of IL2 can be delivered without severe systemic adverse effects. We have demonstrated that high intrapleural levels IL2 are associated with mild to moderate toxicity, except in those patients treated at the highest dose level F with 36 x 10^6 IU/day. All patients in groups A to E were treated on an outpatient basis.

Intrapleural administration of various biological response modifiers may have significant antitumour effects against intrapleural malignant disease. Uchida et al. (Uchida, et al. 1984) reported that intrapleural instillation with the biologic response modifier OK-432 significantly increased autologous tumour killing by tumour associated large granular lymphocytes. The intrapleural instillation of natural beta-interferon was reported to be effective against malignant pleural effusions by Rosso et al. (Rosso, et al. 1988). Recently, Markowitz et al. (Markowitz, et al. 1992), reported on the efficacy of intracavitary administration of IFN-α. Boutin et al. (Boutin, et al. 1991) reported 4 CRs and 2 PRs after weekly intrapleural administration of gamma-interferon in 22 patients with mesothelioma, also underscoring the therapeutic potential of locoregional cytokine therapy.

Antitumour effects after intrapleural administration of TNFα have been reported by Karck et al (Karck, et al. 1990). Intrapleural administration up to 200 microgram/m^2 weekly in 7 patients with malignant pleural effusion led to complete disappearance of tumour in 3 patients without side effects. The same group reported that in patients with ovarian cancer and recurrent ascites intraperitoneal administration of TNFα resulted in the disappearance of ascites in 7 of 9 patients.

Yasumoto et al. (Yasumoto, et al. 1987), demonstrated that low intrapleural doses of IL2 were sufficient to induce lymphokine-activated killer activity in the pleural exudate and to reduce malignant pleural effusions. Manning et al.

(Manning, et al. 1989) have shown that these activated lymphocytes were able to kill NK cell-resistant human mesothelioma cells.

Astoul et al (Astoul, et al. 1993) reported a relatively high response rate (7/15 patients) in mesothelioma patients who were treated with intrapleural IL2 in a dose escalation study. The preponderance of responses was observed in stage I disease.

We made similar observations in our study. Significant LAK-activity was displayed by MNC collected from the pleural effusions after intrapleural administration of IL2. No LAK-activity was displayed by the peripheral MNC, except at the highest 2 dose levels in groups E and F. However, LAK activity and response did not correspond with IL2 dose level, intrapleural IL2 level or intrapleural TNFα level.

Immunophenotyping of intrapleural and peripheral MNC showed some changes after intrapleural administration of IL2. In this study, the dose of IL2 did not correlate with response, and none of the parameters investigated by immunophenotyping, cytotoxicity assays and determination of cytokine levels corresponded with the outcome of treatment.

Dosages of IL2 in this study were tolerable up to and including level E: 18 x 10^6 IU/day. However, at level F: 36 x 10^6 IU/day, catheter-related infection was the most pronounced and dose-limiting side effect. Others have reported neutrophil dysfunction during IL2 administration including decreased chemotaxis and Fc receptor expression (Klempner, et al. 1990; Jablons, et al. 1990; Murphy, et al. 1988). High dose IL2 increased the risk of bacteremia significantly, which led to a reduction of catheter indwelling time (Richards, et al. 1991). These facts may explain the high incidence of catheter-related infections in our patients, particularly in group F, and after repeated administration of IL2 at the lower doses.

It remains uncertain if and to what extent these infections have played a role in the induction of an antitumour response, as 3 of the 4 responders developed an empyema, but 2 of the 5 patients with an empyema had no response. In addition, IL2 by itself induced high intrapleural levels of TNFα in the majority of the patients treated.

We conclude that intrapleural administration of cytokines should be explored further as a new mode of treatment for pleural mesothelioma. IL2 given intrapleurally appears to have antitumour activity against mesothelioma. We are aware of the difficulty recommending a daily dose of IL2 because of the small numbers of patients in each group and the frequent occurence of empyema, but given the facts that a) no correlation was found by us and by others between IL2

dose and antitumour response, b) low intrapleural IL2 doses are sufficient to induce LAK-activity by MNC which can kill NK cell-resistant human mesothelioma cells, and c) mild toxicity was seen below 6 x 10^6 IU of IL2, we suggest a daily dose of 3 x 10^6 IU of IL2 for a phase II study.

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Chapter 3

PROLONGED CONTINUOUS HEPATIC ARTERY INFUSION WITH INTERLEUKIN-2 IN UNRESECTABLE LIVER METASTASES OF COLORECTAL CANCER: A PHASE IA-B STUDY

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SUMMARY

Background: A clinical study to investigate the effects of prolonged continuous infusion of interleukin-2 (IL-2) via the hepatic artery in patients with unresectable liver metastases of colorectal cancer was performed.

Patients and methods: Of fourteen selected patients 9 received interleukin-2. Five patients had to be excluded due to anomalous arterial anatomy. IL-2 was administered by continuous arterial infusion in 2 cycles of 12 days at 3 week intervals. Intrapatient dose escalation was applied. A minimum of 3 evaluable courses were applied. Doses ranged from 1.5 - 12 x 10^6 IU/m^2/day.

Results: In the patients receiving the lowest 2 dose levels of IL-2 very little toxicity was seen. At the dose of 6 x 10^6 IU/m^2/day however considerable hepatic toxicity but relatively little systemic toxicity was observed. The maximal tolerated dose (MTD) of IL-2 was 6-12 x 10^6 IU/m2/day. Thrombosis of the hepatic artery was observed in 2 of 5 patients treated at the lower IL-2 dose levels and in 3 of 4 patients at the highest IL-2 dose levels. These thromboembolic complications are possibly due to endothelial damage by the prolonged infusion of high doses of IL-2. The rate of this complication was so high that the study was stopped after 9 patients. Eight patients had progressive disease, one patient had stable disease for a period of 6 months.

Conclusions: This study demonstrates that small caliber arteries like the gastroduodenal artery are not suitable for prolonged infusion of IL-2. We do not recommend this approach for the regional treatment of liver metastases with IL-2.

INTRODUCTION

The liver is a major site of metastatic spread of primary colorectal cancer; in as many as 30% of the patients it is also the sole site of initial tumor recurrence [1]. In the majority (75%) of these patients the metastases are not resectable and most patients can be considered candidates for locoregional therapies as the effect of systemic chemotherapy results in relatively low response rates with minimal effect on survival [2]. Unfortunately, although improved response rates with hepatic arterial infusion chemotherapy have been reported, convincing evidence of improved survival with these techniques is also lacking [3, 4, 5]. Therefore the search for new drugs and new treatment modalities is warranted. Many clinical studies on the effects of the systemic administration of the cytokine interleukin-2 (IL-2) have been performed, but relatively few studies on regional administration of IL-2 have been published. The most favorable results of IL-2 based immunotherapy have been achieved in patients with renal cell cancer, melanoma and Non-Hodgkin's Lymphoma. Results obtained in other malignancies have been disappointing, but incidental long lasting remissions in patients with colorectal cancer have been observed [6]. Although no clear dose-response relationship has been observed in humans, this has been clearly demonstrated in experimental tumor models [7, 8, 9].

High dose IL-2 therapy is associated with severe systemic toxicity, characterized by the malfunction of vital organs [6]. This does prohibit the systemic application of doses with optimal antitumor effects [10, 11]. Since hepatic metastases derive their blood supply almost totally from the hepatic artery [12, 13], higher local IL-2 levels can be achieved by intraarterial administration. High local levels of IL-2 may lead to increased numbers of activated liver associated large granular lymphocytes (LGL) and increased numbers of LGL in the liver as has been shown in rats by Bouwens and coworkers [14]. Moreover high local IL-2 levels may lead to optimal activation of tumor infiltrating lymphocytes (TIL), which are known to be abundantly present in colorectal hepatic metastases [15]. The regional advantage of achieving high levels of cytokines with minimal systemic toxicity has been observed in other regional settings such as after intracavitary administration [16].

Therefore we performed a phase IA-B study in patients with unresectable liver metastases of colorectal cancer in order to determine the maximal dose of IL-2 that can be administered via the hepatic artery for a prolonged period of time.

PATIENTS AND METHODS

Eligibility criteria

Patients were required to have histologically confirmed unresectable measurable hepatic metastases of colorectal cancer not exceeding 50% of liver volume. Patients were required to have no signs of extrahepatic disease on CT scans of the brain, thorax and abdomen. Other inclusion criteria were: age < 70 years, no prior chemotherapy or radiotherapy during the 4 weeks prior to the administration of IL-2, no prior immunotherapy, a Karnofsky performance status ≥ 80, no prior malignancies or concurrent second primary malignancies, no significant cardiovascular, renal, pulmonary or central nervous system disease, no active infections, a white blood cell count > 4,000/ml, platelets > 100,000/ml, hematocrit > 30%, partial thromboplastin time, prothrombin time, creatinine and bilirubin serum levels within the institution's normal range. Patients with a positive test for anti-human immune deficiency viral (anti-HIV) antibodies or hepatitis-B-surface antigen (HBsAg), and requirements for corticosteroids administration were excluded. Informed consent was obtained from all patients prior to study entry.

Study design

A preoperative hepatic arteriogram that included celiac and mesenteric angiography was done before catheter placement to define the arterial blood supply of the liver. Patients were excluded if there was evidence of anomalous hepatic arterial anatomy. At laparotomy the catheter was placed into the gastroduodenal artery and positioned at the junction with the common hepatic artery. Ligation of the distal gastroduodenal artery, right gastric artery, and any other branches supplying the stomach and duodenum arising from the common hepatic distal to the gastroduodenal take-off was performed. A cholecystectomy was performed. A Port-a-Cath® (PAC) was implanted in a subcutaneous pocket overlying the lower part of the sternum or the right chest wall. After canulation the patency of the catheter was checked. The catheter was flushed twice daily with 5000 IU heparin until IL-2 administration.

The administration of recombinant human IL-2 (Proleukin® Chiron BV, Amsterdam, NL) was instituted 2 weeks after the PAC placement. Therapy consisted of the continuous hepatic artery infusion (HAI) of IL-2 in 2 cycles of 12 days at 3 week intervals according to a dose-escalation scheme. This intrapatient dose escalation scheme was chosen since IL-2 has a relative short half life (t 1/2 α = 7 minutes and t 1/2 β = 30-60 minutes) so that by the time the second cycle was given most

if not all the side effects from the first cycle might have already disappeared. This procedure would require fewer patients than a more conventional phase I study design.

Dose escalation scheme and dose adjustment

According to the intrapatient dose escalation scheme the dose administered during the second cycle was twice the dose of the first cycle as is illustrated in Table 1. Three eligible patients were to be entered per scheme. In the first cycle, the patients received IL-2 for 12 days, followed by a rest period of 3 weeks. If toxicity was acceptable in the first cycle and the patients had recovered, he/she was then to receive a second cycle, identical to the first but at a higher dose. If no dose limiting toxicity occured in 2/3 patients then the next 3 patients were to be entered in the next scheme. If 2/3 patients experienced dose limiting toxicity during the first cycle of a scheme, the next 3 patients were to be treated with the dosage used in the second cycle of the previous scheme. This dose was then to be considered the maximal tolerated dose (MTD). If in 2/3 patients unacceptable toxicity occured during the second cycle of a scheme, then the next 3 patients were to be treated with the dosage used in the first cycle of this scheme, with no dose escalation for the second cycle. This dose was then to be considered the MTD. Patients with stable disease (SD) or better after 2 cycles should receive 1-2 additional cycles after a 4 weeks rest period. The dose of this cycle should be the highest dose previously given to the patient, not exceeding the MTD.

Table 1. Dose escalation scheme

		IL-2 dosage (10^6 IU/m^2/day x 12)
Scheme I	cycle 1 cycle 2	1.5 3
Scheme II	cycle 1 cycle 2	6 12
Scheme III	cycle 1 cycle 2	18 24
Scheme IV	cycle 1 cycle 2	30 36

Follow-up studies and toxicity- and response assessment

During IL-2 infusions, daily: vital signs, weight (q 12 hrs), hematology (including differential and platelets), electrocardiogram (ECG) (if indicated), chest-X-ray (if indicated), survey for infection (if indicated), once every two days the following parameters were analysed, total serum bilirubin, serum creatinine, LDH, alkaline phosphatase, ASAT, ALAT, gamma-GT.

Weekly during rest periods: weight, hematology (including differential and platelets), total serum bilirubin, serum creatinine, LDH, alkaline phosphatase, ASAT, ALAT, gamma-GT, CEA.

Within 4 weeks after treatment completion: relevant scans for tumor assessment, CEA.

Toxicity was recorded using the WHO grading system [17]. For toxicities not included in the WHO guidelines, a grading system was used ranging from mild (grade I) to life-threatening (grade IV). Tumor response was classified using standard WHO criteria [17]. Duration of response and survival were calculated from the day of treatment initiation.

RESULTS

Fourteen patients entered the study, but five had to be excluded after arteriography because of an anomalous arterial anatomy. The characteristics of the 9 remaining eligible patients are shown in table 2. All patients had liver metastases in both lobes. No complications occurred in the 2 week period between the PAC placement and the start of IL-2 treatment.

Table 2. Patients' characteristics

Sex	males females	6 3
Age	median range	51 years 38-64
Karnofsky	median range	100 90-100
Prior therapy	surgery systemic therapy radiotherapy	all 1* 1

* 5FU intravenously + FUDR intra-arterially

The distribution of patients per dose level and the reasons for treatment discontinuation are shown in table 3.

Table 3. Patients per dose level and reasons of treatment discontinuation

IL-2 dose	patient no.	treatment duration (days)	discontinuation reasons
1.5 x 10^6 IU/m^2/day x 12	1	6	thrombosis + PAC dislocation
	2	12	
	3	12	
	4	12	
	5	12	
3 x 10^6 IU/m^2/day x 12	2	12	
	3	2	thrombosis
	4	12	
	5	12	
6 x 10^6 IU/m^2/day x 12	6	12	
	7	12	
	8	12	
	9	7	thrombosis
12 x 10^6 IU/m^2/day x 12	6	12	
	7	0	thrombosis + dislocation PAC
	8	6	dislocation PAC + upper GI bleeding

Patient no. 1 received 6 days of IL-2 after which a dehiscence of the laparotomy wound was noted. An angiogram revealed thrombosis of the hepatic artery and dislocation of the catheter tip into the peritoneal cavity. Patient no. 3 received the first cycle of IL-2 without any problems. On the third day of the second cycle the catheter appeared obstructed and blood could not be withdrawn from the PAC. An angiogram revealed thrombosis of the hepatic artery. In an effort to restore the patency of the catheter a laparotomy was performed. Due to massive adhesion around the hepatic artery no further attempt could be made to isolate the artery. Patient no. 6 received 2 cycles without experiencing severe toxicities. However, at

the end of the second cycle the PAC appeared occluded due to thrombosis of the hepatic artery as seen on angiography. In patient no. 7 thrombosis of the hepatic artery and dislocation of the catheter tip was noted on angiogram after the first cycle. Patient no. 8 experienced an upper gastro-intestinal (GI)-bleeding during the second cycle. At gastroduodenoscopy the source of the bleeding appeared to be in the duodenum. Angiography revealed that the catheter tip was dislocated outside the gastroduodenal artery, with an aneurysm of the gastroduodenal artery adjacent to the duodenal wall, resulting in the upper GI-bleeding. Embolization of the hepatic artery was succesfully performed. Patient no. 9 had problems with the delivery of IL-2 on the ninth day of the first cycle which was found to be related to thrombosis of the hepatic artery, dislocation of the catheter and intimal dissection of the gastroduodenal artery, as revealed by angiography. No attempt was made to restore the catheter because of coinciding progressive disease (PD). IL-2 related toxicity is summarized in Table 4. At the dose levels 1.5 and 3.0 x 10^6 IU/day only few and moderate side effects were noted. None of the patients developed fever, flu-like symptoms, gastrointestinal symptoms, pulmonary, renal or hepatic toxicity. Apart from a mild rise in serum levels of alkaline phosphatase and gamma-GT no signs of hepatic toxicity were noted. Hematologic toxicity only consisted of a mild leukocytosis and eosinophilia. At the dose levels of 6 x 10^6 IU/day severe but reversible renal and hepatic toxicity was observed. Four patients started at this dose level. Two of them (patients no. 7 and 9) developed a thrombosis of the hepatic artery during the first cycle. Patient 9 also developed grade III hepatic toxicity, another reason to stop the administration. Bilirubin levels returned to normal rapidly. Patient no. 7 developed grade III hepatic and renal toxicity at the end of the first cycle. During the treatment free period a thrombosis of the hepatic artery was noted with dislocation of the PAC. Patient no. 6 also received the dose of 12 x 10^6 IU/day with only moderate side effects. She experienced high fevers, fatigue and some mild nausea and vomiting, and at the end of the second cycle she developed a thrombosis of the hepatic artery. As mentioned previously, patient no. 8 developed an upper GI-bleeding during the second cycle. None of the patients developed severe cardiovascular, pulmonary, renal, or CNS toxicity. Dose limiting toxicity in 2 patients was hepatic toxicity probably related to IL-2 induced cholestasis. Apart from the rise in serum bilirubin levels, a 2 to 4 fold increase in alkaline phosphatase, SGOT, SGPT and gamma-GT levels was noted. One patient had stable disease for a period of 6 months, the other 8 patients progressed.

Table 4. Toxicity in relation to Dose Level

	IL-2 dose levels/m^2/day			
TOXICITY	1.5 x 10^6 IU 5 pts	3 x 10^6 IU 4 pts	6 x 10^6 IU 4 pts	12 x 10^6 IU 2 pts
Mild toxicity				
Flu-like symptoms	-	-	4	1
Fever > 39°C	-	-	3	1
Chills	-	-	3	1
Nausea/vomiting	-	-	3	1
Moderate toxicity				
Skin rash	-	-	3	1
Arthritis/-algia	-	-	1	-
Fatigue	1	1	2	1
Leukocytes				
10-15,000/ml	4	3	-	-
15-20,000/ml	-	-	4	1
Eosinophilia > 10%	2	2	3	1
Hepatic enzymes ≥ 50%	2	2	4	1
Severe Toxicity				
Creatinin > 3.0 mg/dL	-	-	1	-
Bilirubinemia ≥ 4 mg/dL	-	-	2	-

DISCUSSION

The results of this study indicate that the dose limiting toxicity of prolonged administration of IL-2 via the hepatic artery is hepatic toxicity and that relatively little systemic toxicity is observed at the maximum tolerated dose (MTD) of between 6 and 12 x 10^6 IU/m2/day. This MTD corresponds with the results reported by Mavligit and coworkers [18] using a 5-day continuous infusion of IL-2 via the hepatic artery (ie. 9 x 10^6 IU/m^2/day) and is about 50% of the MTD observed with 5-day continuous intravenous infusion of IL-2 [19].

The period of 12 days continuous infusion of IL-2 in our study is however significantly longer than the 5-day treatment cycles in the other studies. It may well be that this prolonged infusion period was related to the very high rate of thrombotic catheter related complications observed in this study. Using an identical surgical

technique in a previous chemotherapy hepatic artery infusion protocol also not applying routine heparinization during drug infusion, we observed a complication rate of less than 7 % as opposed to a 67% thrombosis rate of the hepatic or gastroduodenal artery in the present study.

Interleukin-2 causes substantial endothelial damage in the form of endothelial swelling and the appearance of pores between endothelial cells [20]. It also activates the coagulation and fibrinolytic systems in vivo, and these changes resemble the perturbations observed after tumor necrosis factor-α (TNFα) administration [21]. Moreover through the induction of TNFα and IL-1 focal thrombosis may be induced in a Schwartzman type reaction [20, 22, 23], believed to be a possible cause for transient focal neurological deficits that may complicate IL-2 therapy [24]. The presence of a corpus alienum in the hepatic artery may cause turbulence in the blood stream facilitating the aggregation of blood platelets. The addition of heparin during IL-2 administration was avoided since heparin is diluted in solvents which caused precipitation of Proleukin®. Moreover, Proleukin® should not be mixed with other drugs. Of note, thrombosis of the hepatic artery was also described by others during hepatic arterial infusion of floxuridine (FUDR) in patients with liver metastases from colorectal cancer [5, 25]. Apparently a small caliber artery such as the gastroduodenal artery is not suited for prolonged IL-2 infusions. Tumor responses were not observed in this study. This disappointing result is in accordance with the scarce literature on this approach. Mavligit and coworkers reported on 14 patients with 5-day continuous IL-2 infusions via the hepatic artery and 14 patients with infusions via the splenic artery. They observed one partial remission in each arm of the study [18]. Klasa [26] reported only 2 minor responses in 20 patients treated by 5-day splenic artery infusions with IL-2. Especially in Japan studies have been performed on the locoregional application of LAK cells. Okuno and coworkers [27] infused only LAK cells via the hepatic artery in a patient with unresectable hepatoma without achieving a CT-documented response. However the patient had a transient significant decrease in alpha-foetoprotein levels and in ascites after therapy. Recently, Matsuhashi et al. [28] reported PRs in 2 out of 5 patients with hepatocellular carcinoma, treated by both LAK cell and IL-2 infusions via the hepatic artery. No responses were observed in ten patients with hepatocellular carcinoma after the injections of LAK cells via the hepatic artery in combination with the systemic adminstration of IL-2 [29]. Direct injections of IL-2 into the tumor in 5 patients with hepatocellular carcinoma resulted in partial remissions in 2 patients in one report [30], but no

responses were observed in another study where both LAK cells and IL-2 were injected into the tumors of patients suffering from hepatocellular carcinoma [31].
Although these clinical studies demonstrate that incidental antitumor responses can be observed after the locoregional administration of IL-2 with or without LAK cells the overall results are disappointing.
With respect to hepatic artery infusion of IL-2 we must also take into account that a significant percentage of patients (in our study 5 out of 14) are not suited for hepatic artery infusion therapy because of anatomical variations.
We cannot recommend this approach in treating hepatic metastatic disease.

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Chapter 4

INTERLEUKIN-2 AND INTERFERON ALPHA-2A DO NOT IMPROVE ANTITUMOUR ACTIVITY OF 5-FLUOROURACIL IN ADVANCED COLORECTAL CANCER

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SUMMARY

Treatment using a combination of 5-Fluorouracil (5-FU), interferon-alpha (IFNα-2a), and interleukin-2 (IL-2) has been shown to mediate disease regression in selected patients with advanced colorectal cancer. This phase II study was designed to evaluate the anti-tumour activity and toxicity of the combination of IL-2, IFNα-2a and 5-FU in patients with advanced colorectal cancer. Forty-four patients with metastatic colorectal cancer were treated, predominantly at an outpatient basis, with subcutaneous IFNα-2a and IL-2 three times per week followed by once a week bolus intravenous 5-FU injections. There were 6 (14%) partial responses among the 43 evaluable patients (95% confidence interval [CI] = 5%-28%). Twenty-four patients had stable disease (56%) and 13 patients (30%) showed progressive disease. The median time to progressive disease in 43 patients was 19 weeks (range 2-72 weeks), and in responders 34 weeks (range 24-30 weeks). The median overall survival was 47 weeks (range 2-85 weeks) and in responders 60 weeks (range 35-71 weeks). Treatment related toxic effects included fatigue, nausea and vomiting. Granulocytopenia was the main reason for the dose reductions or treatment interruptions in 32 out of 44 patients. One patient died of toxicity due to renal failure. Serial assessments of immunophenotyping and cytolytic activities of peripheral blood lymphocytes did not show changes in the numbers of circulating natural killer (NK) cells or in the levels of NK and lymphokine-activated killer (LAK) cytolytic activities. This regimen of IL-2 and IFNα-2a with 5-FU has only modest antitumour activity in advanced colorectal cancer.

INTRODUCTION

The treatment of advanced and metastatic colorectal cancer remains unsatisfactory despite the availability of many cytotoxic agents. Since 1957 5-fluorouracil (5-FU) has been the mainstay of therapy for disseminated colorectal cancer (Heidelberg, 1957).

Biochemical modulation of the effect of 5-FU with methotrexate or with leucovorin has marginally improved survival (Moertel, 1994). Another approach appeared to be the combination of 5-FU with interferon-alpha (IFNα-2a), (Wadler *et al.*, 1989; Pazdur *et al.*, 1990; Kemeny *et al.*, 1990). A potential way to further improve the reported results was suggested by Onodera et al. (Onodera *et al.*, 1990). They studied the effects of 5-FU + leucovorin on the interleukin-2 (IL-2) related lymphocyte immune response. Rather than being immunosuppressive, the use of 5-FU + leucovorin appeared to augment natural killer (NK)- and lymphokine activated killer (LAK) activity. Promising clinical results were recently reported by Yang et al. (Yang *et al.*, 1993) applying a combination of 5-FU, leucovorin and IL-2, and by Atzpodien et al. (Atzpodien *et al.*, 1994) using a combination of 5-FU, IL-2 and IFNα-2a in metastatic colorectal cancer. These studies provided the basis for the design of the here reported study with a schedule of IL-2, IFNα-2a and 5-FU in patients with advanced colorectal cancer. We used the combination of IFNα-2a and IL-2 upfront based on preclinical and clinical data suggesting synergistic antitumour activity of this schedule (Cameron *et al.*, 1988; Rosenberg *et al.*, 1989). IFNα can upregulate major histocompatibility antigens class I (MHC-I) expression on tumour cells (Weber and Rosenberg, 1988) which are usually downregulated when the tumour becomes more invasive (Feldman and Eisenbach, 1991; Smith *et al.*, 1988). It augments LAK-activity (Chikhala *et al.*, 1990), and it has direct antiproliferative and cytotoxic properties against tumour cells (Gresser, 1989). These properties may alter the malignant phenotype of tumour cells so that they become more susceptible to the cytolytic activity of immune cells.

MATERIALS AND METHODS

Patient eligibility

Patients were required to have histologically confirmed metastatic or locally advanced measurable adenocarcinoma of the colon or rectum, not previously treated with systemic therapy. Patients were required to be $\leq$ 75 years of age, to have a neutrophil count of $\geq$ 1.5 x 10^9/l and a platelet count of $\geq$ 100 x 10^9/l, serum bilirubin $\leq$ 1.25 x upper limit of normal unless due to metastatic liver

disease, serum creatinine ≤ 1.25 x upper limit of normal, life expectancy ≥ 3 months, normal cardiopulmonary function as assessed by non-invasive clinical examination and a Karnofsky score ≥ 70. Patients with evidence of symptomatic CNS metastases, positive for anti-HIV antibodies or HBsAg, or requiring glucocorticoid administration were excluded. Written informed consent was obtained from all patients prior to entry into this study.

Pretreatment evaluation

Pre-study screening included clinical assessment, haematology tests including white blood cell count and differential, platelet count and haemoglobin, biochemistry including bilirubin, alkaline phosphatase, ALAT, ASAT, electrolytes, creatinine, special laboratory tests including prothrombin, partial thromboplastin time, thyroxine, thyrotropin, thyroglobulin, anti-thyroid microsomal antibodies, HIV-antibody and HBs-antigens, chest X-ray, ECG and computer tomography (CT) of the chest and abdomen. Serum samples of anti-IL-2 and anti-IFNα-2a antibodies were taken before treatment and were repeated before each cycle. Antibody analysis was performed by enzyme immunoassay (EIA) in screening for binding antibodies and by a biological assay for the detection of neutralizing antibodies.

Treatment

The treatment regimen is shown in Table 1. The first 6-weeks cycle consisted of IFNα-2a (Roferon®-A, Hoffmann La Roche Ltd., Basel, CH) 9 MIU subcutaneously (sc) 3 times a week (tiw) for 6 weeks except for day 1 in week 2; IL-2 (Proleukin®, Chiron BV, Amsterdam, NL) 9 MIU sc tiw, weeks 2 to 5, preceded by loading doses of 9 MIU sc 3 times a day on days 1 and 2 in week 2; and 5-FU at a dose of 750 mg/m^2/day as a continuous intravenous (iv) infusion on days 15 to 20 followed by iv bolus injections of 750 mg/m^2 on day 29 and 36. Thereafter, a maximum of five 4-weekly cycles were administered, consisting of IFNα-2a 9 MIU sc tiw for 4 weeks; IL-2 9 MIU sc tiw for 3 weeks; and 5-FU 750 mg/m^2 iv bolus weekly for 4 weeks.

Table 1 Immunotherapy in advanced colorectal cancer: Treatment scheme

Drug	Dose	Schedule
IFNα-2b	9 million U sc	Cycle 1: 3 times per wk, wk 1 and 3 through 6 2 times per wk, wk 2 Cycles 2-6: 3 times per wk, wk 1 through 4
IL2	9 million IU sc	Cycle 1: thrice daily, d 1+2 and once daily, d 3 in wk 2; 3 times per wk, wk 3 through 5 Cycles 2-6: 3 times per wk, wk 1 through 3
5-FU	750 mg/m^2/day	Cycle 1: CIV d 1-5, wk 3 IV bolus once weekly, wk 5+6 Cycles 2-6: IV bolus once weekly, wk 1 through 4

Evaluation of toxicity, dose modifications and concomitant medication

Toxicity was graded according to the WHO criteria (WHO Handbook, 1979) and assessed weekly.

No dose modifications were required in case of grade I toxicity. In case of grade II toxicity, the dose of 5-FU had to be reduced to 500 mg/m^2; IFNα-2a to 4.5 MIU and IL-2 to 4.5 MIU. If recovery occurred within 1 week, the 3 drugs were given at full dose. If the toxicity recurred, the decreased dose was re-introduced. In case of grade III toxicity, all 3 drugs were discontinued. If recovery to grade 0 occurred within 28 days of treatment discontinuation, 5-FU was resumed at 500 mg/m^2, IFNα-2a at 4.5 MIU and IL-2 at 4.5 MIU. In case no full recovery occurred within 28 days or the occurrence of any grade IV toxicity, the patient went off study.

Patients could be given paracetamol 500 mg 6 times daily to reduce flu-like symptoms; codeine phosphate 30-60 mg 4 times daily for diarrhoea; sucralfate mouthwash for stomatitis and metoclopramide or a 5HT3 antagonist for nausea and/or vomiting. Patients were substituted with levothyroxine in case of hypothyroidism. Other concomitant antitumour therapies or systemic steroids were not allowed.

Definition of response and statistical analysis

Tumour assessment was performed according to WHO criteria (WHO Handbook, 1979). Evaluation of response was performed after the 2nd, 4th and 6th cycle. Further therapy was withheld in case of progressive disease (PD) at any time. In case of no response in the first 10 patients treated for at least 10 weeks, the trial was to be terminated. Otherwise the sample size was to be large enough to confirm or exclude a 40% response rate by 95% confidence intervals using Pearson-Clopper range limits.

Overall survival and time to disease progression were calculated from the start of treatment until the date of death or progression. The Kaplan-Meier method was used to calculate the probability of survival or time to progression.

Immunological monitoring

Absolute numbers of lymphocyte subsets and cytolytic activities of peripheral blood mononuclear cells (PBMC) were assessed immediately prior to and at the end of the 1st, 2nd and 3rd week of the first cycle, immediately prior to the 2nd, 3rd and 4th cycle (i.e., weeks 6, 10 and 14) and at the end of the 4th cycle (i.e., week 18). The PBMC were isolated by Ficoll-Isopaque density centrifugation of 30 ml heparinized venous blood samples. An aliquot was processed immediately for immunophenotyping and the remainder was cryopreserved in liquid N_2 to allow the cytotoxicity assays on all samples from a single patient to be tested on the same occasion to exclude the effects of interassay variability. The lymphocyte subsets defined by CD3 and CD56, CD4 and CD8, CD16 and CD19 monoclonal antibodies were assessed by multicolor immuno-fluorescence and flow cytometry as described elsewhere (Gratama *et al.*, 1996). Cytolytic activities were determined by a standard 3-hour ^{51}Cr-release assay as described previously (Gratama *et al.*, 1993). The K562 erythromyeloid leukemia cell line and the Daudi Burkitt's lymphoma cell line were used as sources of target cells for the assessment of NK and LAK activities, respectively.

RESULTS

Patients

Fifty-one patients were entered in this study between January 1991 and September 1992. Six patients were considered ineligible because they did not fulfil the inclusion criteria. One patient withdrew consent, and another patient was not evaluable for response because no post-treatment tumour assessment was available. Thus, 43 patients were evaluable for response and 44 for toxicity.
The patient characteristics are shown in Table 2.

Table 2 Patients' characteristics

Sex	
males	25
females	19
Age	
median	59
range	31-71
Karnofsky	
90-100	28
80	12
70	4
Sites of disease	
liver	35
lung + pleura	11
lymphnodes	6
peritoneum	4
skin	4
other	13

Evaluation of toxicity

A total of 159 treatment cycles were given with a median of 4 per patient. One patient died due to treatment related renal failure. There were no other cases of drug-related renal toxicity neither were there other grade IV toxicities. Table 3 summarizes the percentage of patients experiencing WHO grade II-IV toxicities. The most frequently occurring grade III adverse events were fatigue, nausea and

vomiting. Thirty-two patients required one or more temporary dose reductions or treatment interruptions, which was mostly due to granulocytopenia.
The mean total dose per cycle of the trial medication in relation to the planned dose is shown on Table 4. It appears that the percent dosage actually given decreases with the number of cycles.

Table 3 Side effects observed in 44 patients (159 courses, analyzed according to the highest toxicity-grade per patient)

Adverse events*	No. of patients	WHO Grade (%)		
		II	III	IV
Fever	34	63	7	-
Fatigue	34	46	12	-
Nausea-vomiting	32	49	23	-
Stomatitis	19	28	7	-
Diarrhoea	24	28	2	-
Cutaneous	14	14	-	-
Local inflammation at injection site	10	21	-	-
Hypotension	9	9	-	-
Granulopenia	16	23	10	-
Renal	1	-	-	2

Table 4 Mean total dose per cycle

Cycle	IL2 (MIU) received (%planned)	IFNα (MIU) received (%planned)	5-FU (mg) received (%planned)
1	134 (93%)	128 (84%)	9151 (96%)
2-6	55 (68%)	73 (68%)	4002 (73%)

Immunological monitoring
Lymphocyte subset enumerations and assays of cytolytic functions were performed in 24 of the 43 evaluable patients (Figure 1). Prior to therapy, the median values of the absolute numbers of NK lymphocytes ($CD56^+,3^-$; panel A) and cytotoxic/suppressor T lymphocytes ($CD8^+$; panel D) were at the upper limit of

the normal range and the absolute number of lymphocytes ($CD3^+$; panel B) were within the normal range, whilst that of the helper/inducer T lymphocytes ($CD4^+$; panel C) was slightly below the normal range. These lymphocyte subset counts remained essentially unchanged throughout the period of treatment and shortly thereafter. Prior to therapy, the median NK activity of peripheral blood lymphocytes was increased (panel E), whilst LAK activity was absent in most donors (panel F). The median values of both activities increased slightly during the first therapeutic cycle to persist at those levels thereafter, i.e., increased relative to the normal range for NK activity and within the normal range for LAK activity.

Antibody formation against IL-2 and IFNα-2a

Serial serum samples of 29 patients were available. Thirteen (45%) developed antibodies against IFNα-2a, 6 (21%) against IL2. Three (10%) had antibodies against both IL2 and IFNα. Two of these 3 patients achieved a PR despite the presence of neutralizing antibodies. Two patients (7%) had anti-IFNα-2a antibodies at baseline; none had anti-IL-2 antibodies at baseline. The development of antibodies did not appear to be related to specific side effects or severity of side effects.

Response to treatment

Six of the 43 patients evaluable for response achieved a partial response. Thus, the overall response rate was 14% (95% confidence interval 5-28%). Twenty-four patients (56%) had stable disease and 13 (30%) showed progressive disease. The median time to progressive disease in 43 patients was 19 weeks (range 2-72), and in responding patients 34 weeks (range 24-36). The median overall survival was 47 weeks (range 2-85), and in responding patients 60 weeks (range 35-71).

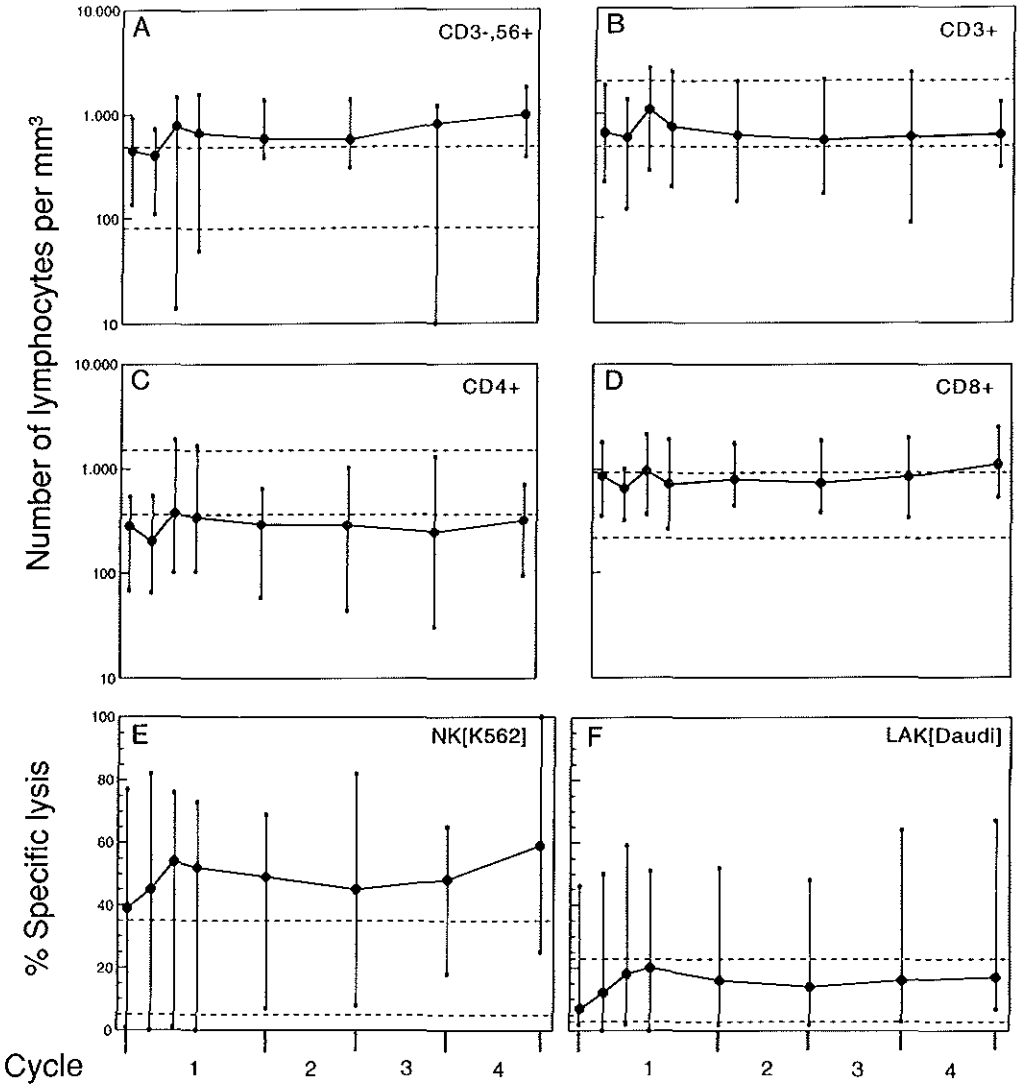

Figure 1 Median absolute numbers and ranges of $CD3^-,56^+$ NK lymphocytes (panel A), $CD3^+$ T lymphocytes (panel B), $CD4^+$ helper/inducer lymphocytes (panel C) and $CD8^+$ suppressor/inducer lymphocytes (panel D), and NK (panel E) and LAK (panel F) cytolytic activities of peripheral blood mononuclear cells in 24 patients. Logarithmic scales have been used for the vertical axes in order to compress the figure. Closed circles and vertical bars represent median values and confidence limits as defined by the 5th and 95th percentiles, respectively. The shaded areas represent the normal range as defined by the 5th and 95th percentiles of 72 (panels A to D) and 29 (panels E and F) apparently healthy control persons. Cytolytic activities were expressed as the weighed mean of specific lysis of 4 effector to target (E:T) ratios (i.e., ranging between 50 and 6.3), calculated for E:T ratio = 17.7 (Gratama *et al.*, 1993).

DISCUSSION

Preclinical studies have previously shown a synergistic interaction between 5-FU and IFNα (Miyoshi *et al.*, 1983; Elias and Crisman, 1988), which formed the basis to investigate this combination in patients with advanced cancer. Initial clinical studies (Wadler *et al.*, 1989; Pazdur *et al.*, 1990; Kemeny *et al.*, 1990; William *et al.*, 1993) had suggested higher response rates than usually achieved with 5-FU alone.

Other preclinical data suggested synergy between IL-2 and IFNα (Cameron *et al.*, 1988), which appeared to be confirmed in clinical studies in melanoma and renal cancer (Rosenberg *et al.*, 1989; Marincola *et al.*, 1995). The logical next step was to study the combination of the 3 drugs. However, we observed a meager 14% partial response rate with a median response duration of 34 weeks. Although 78% of the patients completed at least 2 full courses, 32 of them (63%) required treatment interruptions or dose reductions. The most common reason for this was granulocytopenia. The majority of these dose modifications occurred during the first 2 courses. So, one could argue that the low response rate might be attributable to the moderate dose intensity achieved. Another possible reason could be the fact that 5-FU in this study was administered after IL-2 and IFNα-2a, thereby not taking full advantage of the possible eradication of T-suppressor cells with chemotherapy before immunotherapy (Berendt and North, 1980). It was also found that 5-FU cytotoxicity was enhanced by concomitant or subsequent exposition to IFNα, while the reverse sequence, IFNα followed by 5-FU, abrogated the cytotoxic effect of 5-FU suggesting that pretreatment with IFNα could protect tumour cells (Wadler *et al.*, 1988). Prolonged administration of IFNα (i.e., three times a week) can induce a persistent block of tumour cells in G_0-G_1, thus reducing the S-phase fraction and therefore diminish the anticancer activity of 5-FU (Cascinu *et al.*, 1993). To date, we have deliberately chosen a regimen using a loading dose of IL-2 and IFNα-2a preceding 5-FU, in order to enable upregulation of MHC-I molecules on tumour cells (Weber and Rosenberg, 1988), to augment LAK-activity (Chikhala *et al.*, 1990), and to exploit the antiproliferative and cytotoxic properties of IFNα (Gresser, 1989). However, we did not observe any changes in the tested immune parameters throughout the study. Occasionally, the lack of anti-tumour response has been associated with the development of neutralizing antibodies to IL-2 and IFNα used. However, in this study 2 of the 3 patients who developed neutralizing antibodies against IL-2 and IFNα-2a, nevertheless achieved a partial response.

As previously stated, at the time this study was designed, interferon-alpha seemed to be an effective biomodulating agent for increasing 5-FU activity in the treatment of advanced colorectal cancer. However, recently published randomized studies were not able to confirm this.

Hill et al. (Hill *et al.*, 1995a) randomized 155 patients to receive either protracted continuous intravenous infusions (civ) of 5-FU at a dose of 300 mg/m^2/day for 10 weeks in combination with IFNα-2b 5 MIU sc tiw, or the civ 5-FU only. In the 5-FU/IFNα-2b-group there were significantly more episodes of mucositis (p=0.008), leucocytopenia (p=0.001), granulocytopenia (p=0.004), and alopecia (p=0.0002). The overall response rate in the 5-FU/IFNα-2b-group was 22% and in the 5-FU-group it was 33% (p=0.12). With a follow-up time of 861 days, the median survival in the 5-FU/IFNα-2b-group was 161 days, and in the 5-FU-group it was 193 days. The differences did not reach statistical significance. Premature withdrawals due to toxicity in both groups of patients were equal and cannot explain the lack of IFNα-2b benefit.

The same group (Hill *et al.*, 1995b) performed another randomized controlled phase III study in advanced colorectal cancer patients using a different dose and scheduling of 5-FU and IFNα-2b. At the start of treatment, 106 patients received a contiuous infusion of 5-FU at a dose of 750 mg/m^2/d for 5 consecutive days. Fifty-two patients were randomized to receive IFNα-2b at a dose of 10 MU sc tiw 2 to 4 hours after initiating 5-FU. During the second week, these patients continued on IFN-α-2b and had the first dose of bolus IV 5-FU 750 mg/m^2/d at the beginning of week 2. Fifty-four patients were randomized to receive 5-FU alone, and this was given at the beginning of week 2. Treatment was continued until progression of disease or unacceptable toxicity for up to 12 months. In the 5-FU/IFNα-2b-group there was significantly more leucopenia (p = 0.013), lymphopenia (p = 0.01), depression (p = 0.014), and withdrawal due to adverse events (p = 0.003). There were 4 toxic deaths, all of which occurred in patients who received IFNα-2b. The overall response rate was 19% (all PRs) in the group that received 5-FU + IFNα-2b, and 30% in the 5-FU-alone group (3 CRs and 13 PRs) (p = 0.21). Neither progression-free survival nor overall median survival showed any significant differences in the two groups.

Likewise, in a randomized phase III study performed by the Corfu-A Study Group (Corfu-A-Study Group, 1995), the biochemical modulation of 5-FU by either IFNα-2a or leucovorin was studied. In 247 patients 5-FU was given at a dose of 370 mg/m^2/day i.v. bolus for 5 days in combination with leucovorin (LV) 200 mg/m^2/day i.v. for 5 days, repeated every 4 weeks. The other group consisted of 245 patients,

who received 5-FU 750 mg/m^2/day civ for 5 days, followed after a 9-day interval by a weekly bolus i.v. injection at the same dose in combination with IFNα-2a 9 MIU s.c. tiw throughout the treatment period. In the 5-FU/LV-group there were more gastrointestinal toxicities while the 5-FU/IFNα-2a-group the regimen was more myelosuppressive (p=0.0001). The overall response rate in the 5-FU/LV-group was 18% and in the 5-FU/IFNα-2a-group it was 21% (p=0.57). After a follow period of 20 months, the median survival time for the 5-FU/LV-group was 11.3 months versus 11 months for the 5-FU-IFNα-2a-group (p=0.98). These results suggested that biochemical modulation of 5-FU by either leucovorin or IFNα-2a yield comparable response and survival data. The addition of IFNα-2a to high dose 5-FU plus leucovorin was studied by Köhne et al (Köhne *et al.*, 1995) in a 3-arm randomized study. Chemotherapy-naive patients were randomized to receive 5-FU 2600 mg/m^2 i.v. as a 24-hour infusion, combined with either leucovorin 500 mg/m^2 as a 2-hour infusion (arm A), or IFNα-2b 3 MIU sc tiw (arm B), or the combination of leucovorin plus IFNα-2b as in arms A and B (arm C). Treatment was repeated weekly for 6 weeks followed by a 2-week rest period until tumour progression. Because of the occurrence of 2 toxic deaths (septicaemia due to mucositis and diarrhoea) among the first 17 patients treated in arm C, the 5-FU dose was reduced to 2000 mg/m^2 for all patients in arm C. Despite this dose reduction another patient died of severe diarrhoea. An interim analysis was then performed after the first 93 of 149 randomized patients. Among patients treated in arm A, and in arm C, objective tumour responses occurred in 39% (95% confidence interval; 21-56%) and in 38% (95% confidence interval; 20-56%, respectively). This interim analysis showed that the rates of objective responses observed in treatment arm A and C were equivalent. Due to the increased toxicity observed in arm C this treatment arm was closed. No report on the response rate in treatment arm B was given because randomization between arm A and arm B was continuing. The authors concluded that the addition of IFNα-2b to 5-FU plus leucovorin did not increase efficacy and was associated with life threatening toxicity.

Heys et al (Heys *et al.*, 1995) performed a randomized controlled phase III study comparing the efficacy of 5-FU plus leucovorin (5-FU/LV) with 5-FU plus leucovorin plus IL-2 (5-FU/LV/IL2) in patients with unresectable or metastatic colorectal cancer. In the 5-FU/LV group, 68 patients received 5-FU 600 mg/m^2 day bolus i.v. once a week for 6 weeks in combination with leucovorin 25 mg/m^2/day bolus i.v. to be repeated after 2 weeks rest. In the 5-FU/LV/IL2 group, 65 patients received IL-2 18 MIU/m^2/day civ from day 1-5, followed by 5-FU 600 mg/m^2/day bolus i.v. in combination with leucovorin 25 mg/m^2/day bolus i.v. on days 7, 14 and

21. This treatment regimen was repeated on day 28. The objective response rates were not significantly different in both arms, 16% for 5-FU/LV and 17% for 5-FU/LV/IL2. With a follow-up duration of 30 months, there was no difference in the median survival, being 11.7 months and 11.4 months (p=0.11), respectively. Finally, in a small phase II study in 18 patients Ridolfi *et al.* (Ridolfi *et al.*, 1994) only achieved a 5% response rate using 5-FU, leucovorin, IL-2 and interferon-α, in advanced, pretreated colorectal cancer.

Despite different scheduling of IFNα and IL-2 in combination with different doses of 5-FU +/- leucovorin, all of these studies show that the addition of IFNα and IL-2 failed to improve clinical benefit over 5-FU alone. Apparently observations from laboratory studies cannot be translated clinically.

We conclude that our schedule of IL-2 and IFNα-2a combined with 5-FU has only modest antitumour activity, which does not appear to be better than what can be expected of 5-FU alone. This is confirmed by randomized studies which failed to confirm the ability of IFNα and/or IL-2 to augment the efficacy of 5-FU. In our opinion, further clinical investigation of IFNα and IL-2 in combination with 5-FU in advanced colorectal cancer is not justified.

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Chapter 5

FINAL REPORT OF A PHASE II STUDY OF INTERLEUKIN-2 AND INTERFERON-α IN PATIENTS WITH METASTATIC MELANOMA

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SUMMARY

Fifty-seven patients with metastatic melanoma were treated with interleukin-2 (IL2) 7.8 MIU/m^2/d as a continuous infusion for 4 days combined with interferon-α (IFNα) 6 MIU/m^2/d, subcutaneously day 1+4. The cycle was repeated every 2 weeks for a maximum number of 13 cycles. Of the 51 evaluable patients one (2%) achieved a complete and 7 (14%) a partial response, total response rate 16% (CI 7-29%). Median time to progression and median survival were 2.5 and 11.3 months, respectively. This regimen of IL2 and IFNα appeared to be only moderately active.

INTRODUCTION

Immunotherapy with recombinant interleukin-2 (IL2) has been reported to yield a 5-27% response rate in metastatic melanoma (Rosenberg et al., 1989a; Parkinson et al., 1990; Whitehead et al., 1991; Rosenberg et al., 1993; Sparano et al., 1993). Interferon-alpha (IFNα) alone in this group of patients has shown response rates of 12-22% (Robinson et al., 1986; Kirkwood, 1991).

Based on the synergistic activity of IL2 and IFNα in preclinical experiments (Brunda et al., 1987; Cameron et al., 1988; Iigo et al., 1988) and on the encouraging results of early clinical trials with this combination (Budd et al., 1989; Lee et al., 1989; Rosenberg et al., 1989b), we decided to perform a phase II study. Here, we report the final analysis after a median follow-up period of 10.5 months (range 1.1-47+ months).

MATERIAL AND METHODS

Patients

Fifty-seven patients with metastatic melanoma were entered in the study. 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 > 100,000/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. 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 I. The median time from initial diagnosis to immunotherapy was 24 months (range 1 to 142 months).

Treatment

Patients were treated with IL2 at a dose of 7.8 MIU/m^2/d by continuous

Table I. 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	22	(4%)
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%)

infusion on days 1-4 and with IFNα-2a 6 MIU/m^2/d 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.

Monitoring

Toxicity was recorded and analysed using the WHO grading system (WHO handbook, 1979). Side effects not described in the WHO guidelines were graded from mild (grade 1) to life-threatening (grade 4).

Response was evaluated according to the WHO guidelines (WHO handbook, 1979). 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.

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.

The overall response rate was 16% (95% confidence interval:7-29%), including 1 CR (2%) and 7 PRs (14%). Twenty patients (39%) had stable disease. In 23 (45%) patients progressive disease was documented. Three of the responders were male and 5 were female. Responses were seen in skin lesions (36%), lymph nodes (27%), lung (18%) and liver (18%). Of note bone metastases did not respond. All responses occurred in the first 3 months of treatment.

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). 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 patients was 11.3 months [Figure 1], and of the responding patients 20.2 months.

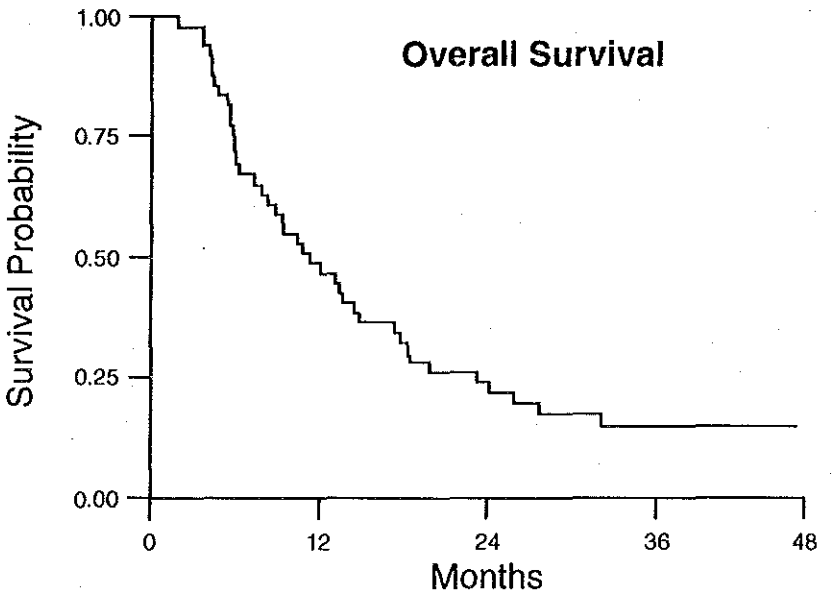

Figure 1. Survival curve (median survival 11.3 months).

Toxicity

An overview of the observed toxicity is presented in Table II. Frequently occurring side effects were fever, skin rash, nausea, vomiting, diarrhea and malaise. Two-third 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.

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

The most frequent manifestation of haematologic 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.

Table II. 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

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. These results are disappointing and not better than can be expected of conventional chemotherapy or immunotherapy with IL2 alone.

Response rates of 21-44% have been reported in some studies using the combination of both cytokines (Lee et al., 1989; Rosenberg et al., 1989b; Budd et al., 1992). However, low response rates of 10% or less were observed by others (Oldham et al., 1992; Dillman et al., 1993; Sparano et al., 1993;). The median response duration in these trials varied between 2 and 11 months, and the median survival was approximately 10 months (Lee et al., 1989; Rosenberg et al., 1989b; Oldham et al., 1992; Dillman et al., 1993; Sparano et al., 1993). We achieved comparable 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. Our 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 (Mule et al., 1984). However, trials using high-dose IL2 (18 MIU/m^2/day) by continuous infusion in patients with metastatic melanoma reported inferior response rates (Oldham et al., 1992; Dillman et al., 1993). An NCI Surgery Branch Study, administering high-dose bolus IL2 (>30 MIU/m^2/day) and IFNα found the highest response rates (Rosenberg et al., 1989b). On the other hand, the Extramural IL2 Working Group, using identical dose, schedule and patient selection criteria did not observe any evidence of enhanced response with the IL2/IFNα combination (Sparano et al., 1993). Summarizing, a dose-response effect for IL2 in the treatment of metastatic melanoma is not clear.

The side effects, we observed, were of comparable incidence and severity as reported earlier (Lee et al., 1989; Rosenberg et al., 1989b; Budd et al., 1992; Oldham et al., 1992; Sparano et al., 1993). Toxicity was managable and patients tolerated the therapeutic regimen relatively well. However, cumulative fatigue made it impossible to give patients more than 13 cycles of therapy.

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.

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Chapter 6

TUNNELED CENTRAL VENOUS CATHETERS YIELD A LOW INCIDENCE OF SEPTICAEMIA IN INTERLEUKIN-2 TREATED PATIENTS

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SUMMARY

We conducted a retrospective study on the incidence of catheter related complications and catheter indwelling time (CIT) during treatment with continuous interleukin-2 (IL2) infusion in patients with metastatic renal cell cancer (RCC), who were equipped with tunneled central venous catheters (CVC).

Seventy-two patients were treated with IL2 based immunotherapy. Two induction treatment cycles of 35 days each were used. Treatment consisted of IL2 as a continuous intravenous infusion (c.i.v.) with lymphokine activated killer cells (LAK) and interferon-alpha (*α*IFN) intramuscularly (i.m.). A tunneled CVC was inserted at the start of treatment and was kept in place for the duration of the therapy or until the occurrence of complications.

Thirty (42%) of 72 CVCs functioned uneventfully for a median CIT of 64 days. In another 12 clinically uncomplicated cases (16%) catheter tips were positive in routine culture after a median CIT of 33 days. In 18 patients (25%), CVC related infections were noted, including 8 (11%) local tunnel infections and 10 (14%) septic episodes. These complications occurred at a median CIT of 28 and 20 days, respectively. In 15 (83%) of these 18 catheter infections, S.aureus was isolated, whereas in the remaining 3 (17%) S.epidermidis was found.

Subclavian vein thrombosis was noted in 12 (17%) CVCs at a median CIT of 31 days; 5 (36%) of these were diagnosed in the first 14 patients. This prompted us to administer prophylactic heparin 15,000 IU c.i.v. daily during IL2 treatment. Thereafter the incidence of thrombosis dropped to 7 (12%) in the subsequent 58 inserted CVCs ($p = 0.03$).

In conclusion, in contrast to previous reports on the high incidence of CVC related septicaemia and thrombosis, we observed a relatively low incidence of these complications, which we ascribe to the use of tunneled catheters and prophylactic heparin.

INTRODUCTION

Interleukin-2 (IL2) is approved for the treatment of metastatic renal cancer. The drug has various side effects, which are well characterized (1-3). Because of the protracted administration of IL2 and the drug-induced tendency to clotting of peripheral veins at the site of infusion, indwelling central venous catheters are required. The frequency of CVC related bacterial infections has been reported to vary between 10-38% in studies using high dose IL2-regimens (4-6). In all of these studies non-tunneled CVCs were used. High dose IL2 appeared to double the relative risk of bacteraemia as compared to low dose IL2 regimens, leading to a related reduction of catheter indwelling time (CIT) by 40% (4). Skin colonization with S. aureus and IL2 induced desquamation of the skin increased the relative risk of S. aureus bacteraemia up to 14.5 fold (5). In addition, neutrophil dysfunction during IL2 treatment may also contribute to the risk of bacteraemia (7-9). In our IL2 study protocols, we have used tunneled CVCs to obtain continuous vascular access for a prolonged period of time. We selected a tunneled CVC procedure because of our previous experience showing a long CIT in immunocompromised patients (10). The study reported here presents a retrospective analysis of the usefulness of these CVCs as related to catheter infection, catheter related sepsis, catheter thrombosis, and CIT in IL2 treated patients.

PATIENTS AND METHODS

Eligibility

Patients with metastatic renal cell carcinoma (RCC) were treated in the framework of a phase II study of IL2 based adoptive cellular immunotherapy. Inclusion criteria included: measurable or evaluable metastatic RCC, age < 70 years, Karnofsky performance status ≥ 80, no evidence of brain metastases and normal organ functions. Patients with relevant clinical metabolic or endocrine disorders, systemic infections, positive HIV or HBsAg serology and patients requiring systemic corticosteroids were excluded. All patients gave informed consent according to institutional rules.

Treatment

IL2 was given as a continuous intravenous infusion (c.i.v.) at a dose of 18 MIU/m^2/day on days 1-5. Lymphapheresis was performed on days 7-9 and lymphokine activated killer (LAK) cells were reinfused in 60 minutes on days 12-15

together with IL2 18 MIU/m^2/day c.i.v. and interferon-alpha (*α*IFN) 5 MU/m^2/day intramuscularly (i.m.) on days 12-16. This cycle was repeated on day 36. After these two induction cycles, tumor evaluation was performed. Patients with objective response or stable disease continued to receive 4 monthly maintenance cycles with IL2 18 MIU/m^2/day c.i.v. and *α*IFN 5 MU/m^2/day i.m. on days 1-5. After 17 patients *α*IFN was also administered on days 1-5 of each induction cycle at a dose of 5 MU/m^2/day i.m.

Ex vivo activation of lymphocytes with IL2

We have previously reported the details of this procedure (11). Briefly, all lymphapheresis procedures were performed using a Travenol CS-3000 Blood Cell Separator (Travenol, Deerfield, IL). Buffy coats 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 RPMI-1640 78%, AIM-V 20% and autologous human plasma 2%. L-glutamine-2mM, streptomycin 50 μg/ml, and gentamycin 40 μg/ml were added to the medium. After incubation for 5 days in a 5% carbondioxide (CO_2), humidified, 37°C incubator, cells were harvested on a Fenwall Cell Harvester. The harvested cells were washed with saline 0.9% and resuspended in human serum albumin 5% supplemented with 6000 IU IL2/ml to a volume of 500 ml.

Surveillance for bacterial contamination of harvested cells involved culturing of samples from the culture bags in TCS medium (Gibco Ltd., Buckingham, U.K.):

a) immediately after lymphopheresis,

b) 24 hours prior to cell harvest, and

c) 1 hour before reinfusing LAK cells into the patient.

Catheters

Catheters used were double lumen Hemed CVACs 5200 (11 Fr) (Gish Biomedical Inc., Santa Ana, California, USA) and double lumen Groshong CVCs (9.5 Fr) (Cath-tech, Salt Lake City, Utah, USA). Both catheters were equipped with a Dacron cuff. All catheters were inserted under sterile conditions in the operating room by closed method under local anaesthesia by staff surgeons. The subclavian vein was the preferred site of insertion and all catheters were tunneled, placing the Dacron Cuff at the end of a 15-20 cm subcutaneous tunnel (2 cm from skin entry). After the procedure, a chest X-ray was performed to confirm the proper position of

the CVC and to rule out the existence of a pneumothorax. Mask and sterile gloves were used for all dressing changes, and dressings were changed daily by trained nursing personnel. The skin around the catheter was cleansed first with chlorohexidine 0.5% in alcohol 70%. Fixomull (Beiersdorf, Hamburg, FRG) was placed over sterile gauze on all dressings to cover the insertion site. In between treatment courses dressings were changed once a week and catheter lumina were flushed with 2 ml of a 0.9% saline solution containing 150 U/ml of heparin. If an infection was suspected or at the end of treatment, catheters were removed in a sterile fashion after cleansing the skin surrounding the entry site. The distal 2 cm of the catheter was submitted for culture using a semiquantative culture method as described by Maki et al. (12). If an infection was suspected, peripheral blood samples were drawn via peripheral venous puncture and cultured in TCS medium (Gibco Ltd., Buckingham, UK). Skin cultures were taken using a premoistened Stuart Culturette (Transwab, MW&E Co. Ltd., Potley, Corsham, Wiltshire, UK). All culture samples were incubated both aerobically and anaerobically at 37°C for 72 hours.

Definitions

Catheter related septicaemia was defined as the growth of the same micro-organisms from the catheter tip and the peripheral blood without evidence of other sources of infection. A tunnel infection was defined as tenderness and redness of the subcutaneous tunnel with or without evacuation of pus. A positive culture was defined as growth of 15 or more bacterial colonies per plate.

All catheters showing evidence of a tunnel infection were promptly removed, no attempts were made to save the catheter by antibiotic treatment. Catheter related thrombosis was defined as venous occlusion on angiogram.

Statistical methods

To compare variables between two groups the Fisher exact test or the chi-square test was used where appropriate. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Seventy-two patients were analyzed for the usefulness of their initial CVC. Median age of the study population was 54 years. Male : female ratio was 2 : 1. Median performance status was Karnofsky 100 (range 80-100). Sixty percent of the patients had ≥ 2 metastatic organ sites.

In 30 patients (42%) the CVCs functioned uneventfully for a median CIT of 64 days. In other words, these patients received 2 induction treatment cycles using one CVC. Twelve CVCs (16%), which were removed routinely because of treatment cessation (median CIT: 33 days), turned out to have positive tips in routine culture. There were 18 (25%) infectious episodes: 8 (11%) tunnel infections and 10 (14%) septicaemias.

Table 1. CVC and non-CVC related infections

	CVC-related	CVC-unrelated
S.aureus tunnel infections	7 (10%)*	
S. aureus sepsis	8 (11%)	2 (infected LAK culture) (3%)
S. epidermidis tunnel infection	1 (1%)	
S. epidermidis sepsis	2 (3%)	
E.coli sepsis		1 (urinary tract infection) (1%)
Routine CVC-tip culture: - S. aureus - S. epidermidis	 4 (5%) 8 (11%)	

* Percentages related to all 72 patients

All catheter related infections were caused by staphylococci. S. aureus was the isolated micro-organism in 15 (83%) of 18 catheter infections (see table). In 2 additional patients S. aureus septicaemia was due to contaminated LAK cells, the culture results of which became positive on the third day after the start of LAK cell reinfusion. S. epidermidis was isolated in 3 (17%) of 18 catheter infections.
In 12 cases of routine removal of clinically uncomplicated CVCs after discontinuation of treatment, routine culture was found to be positive with 4 cultures showing S. aureus (5%) and 8 cultures showing S. epidermidis (11%).
Thrombosis of the subclavian vein occurred in 12 (17%) of the 72 CVCs; 5 (36%) of these occurred in the first 14 inserted CVCs. Due to this high incidence all subsequent patients received prophylactic heparin at a dose of 15,000 IU c.i.v. per

24h during IL2 treatment. With this regimen only 7 (12%) thrombotic events were observed in the subsequent 58 CVCs ($p = 0.03$).

DISCUSSION

Prolonged central venous access is frequently necessary in cancer patients treated with continuous IL2 therapy because of the thrombophlebitis inducing potential of IL2. In addition, the i.v. administration of IL2 is often associated with hemodynamic changes that require volume replacement and i.v. medication, which are usually given via a second lumen of the CVC.

In view of our previous experience with CVCs in immunocompromised patients (10), we have chosen the tunneling procedure for CVC placement in our IL2 treated patients, since this insertion technique was found to be safe and rapid and resulted in a long CIT. In an attempt to further reduce the hazard of CVC related sepsis, we used a CVC equipped with a Dacron cuff. Both the Dacron cuff and the tunnel are intended to act as a barrier against invading micro-organisms. Of all CVCs inserted initially, 42% (30/72) functioned uneventfully for a median CIT of 64 days. In addition, 16% (12/72) were removed routinely at the end of treatment and had thus served their purpose, although routine bacterial culture of the catheter tip turned out to be positive. Overall, these results indicate that in 58% of patients only one operation for catheter insertion was sufficient, avoiding the risk of pneumothorax related to multiple blind CVC insertions.

The frequency of staphylococcal bacteraemias has been reported to range from 10-38% in patients receiving IL2 (4-6,9). IL2 related side effects such as transient impairment of neutrophil function (7-9), dose dependent incidence of staphylococcal bacteraemia (4), colonization with S. aureus and skin desquamation (5), are well documented risk factors for the development of catheter infection and sepsis.

Richards et al (4) reported a septicaemia incidence of 18% after a mean CIT of 20 days, associated with low dose (9 MIU/m^2/d) IL2 treatment. In case of high-dose bolus IL2 (1.8 MIU/kg/d $\sim$ 72 MIU/m^2/d) the incidence of septicaemia rose to 38% and the mean CIT decreased to 12 days. An uncuffed non-tunneled double lumen CVC was used in their patients. Our patients received intermediate doses (18 MIU/m^2/d c.i.v.) of IL2. This dose as a continuous infusion has been shown to be equitoxic to bolus administration of 72 MIU/m^2/d of IL2 (13). Hence, the relatively low incidence of septicaemia of 14% taken together with a long overall median CIT of 43 days in our series compare favourably. In a prospective randomized study comparing prophylactic oxacillin i.v. with placebo in high dose bolus IL2 (1.8

MIU/kg/d) treated patients, Bock et al (6) demonstrated that in the oxacillin arm no catheter related septicaemia was seen while in the placebo arm 10% of the patients experienced septicaemia (p=0.05). Moreover, catheter colonization was reduced significantly in the oxacillin arm (10%) versus placebo arm (44%, p=0.0001). To date, in their study CIT was deliberately kept short at ± 4 days and therefore their results cannot easily be compared with ours. Moreover, Vlasveld et al (14) found 34 (63%) catheter related infections out of a total of 54 CVCs which were inserted in cancer patients treated in a phase I-II study with low dose IL2 (0.18-9 MIU/m^2/d) c.i.v. via an implantable Port-a-Cath®. In order to prevent infection, subsequent patients received prophylaxis with oxacillin (4 x 1 g i.v. for 24 hours) starting one hour before CVC insertions followed by oral pefloxacin (1 x 400 mg daily), given during the entire period of IL2 treatment. These investigators could not demonstrate a reduction in the risk of infection by prophylactic antibiotics. In contrast, we have found a 14% rate of septicaemia after a median CIT of 20 days, despite the fact that we used a much more intensive regimen of cytokines without antibiotic prophylaxis, since we wanted to avoid the development of bacterial resistance and superinfection related to long-term antibiotic treatment.
The relatively high rate of catheter related thrombosis observed in the first 14 patients (36%) might well be attributable to the production of secondary cytokines such as tumor necrosis factor-alpha (TNFα) during IL2 administration (15). Baars, et al (16) reported that IL2 activates the coagulation and fibrinolytic systems in vivo, which changes resemble the perturbations observed after TNFα administration. By the institution of prophylactic heparin during IL2 administration the frequency of CVC related thrombosis decreased significantly to 12% in the subsequent 58 patients (p = 0.03).
In conclusion, 58% of our patients required only one CVC for the duration of their treatment. The infection rate related to the technique of CVC insertion that we used, is relatively low in view of the high doses of IL2 used and the avoidance of prophylactic antibiotics. This compares favourably to previously reported experience. Therefore, we recommend the use of this technique in long-term IL2 treatment schedules. Low dose heparine should be given prophylactically to avoid thrombotic complications.

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CONCLUSIES EN PERSPECTIEVEN

De laatste 20 jaar is er een opleving in de belangstelling voor immunotherapie, mede door de recombinant DNA-technieken, de hybridoma technologie en de opkomst van de genetische modificatietechnieken. Dit heeft geresulteerd in het ter beschikking komen van voldoende hoeveelheden gezuiverde moleculen voor onderzoek en therapie bij de mens door middel van cytokinen, hematopoietische groeifactoren en monoklonale antilichamen gericht tegen tumor-geassocieerde antigenen. Ook de nieuwe mogelijkheden om autologe lymfocyten *ex vivo* te bewerken tot geactiveerde immuuncellen heeft een stimulans gegeven aan de adoptieve immunotherapie. Dit proefschrift beschrijft diverse toedieningsvormen van immunotherapie met interleukine-2 (IL2) en combinaties van IL2 en IFN-α met of zonder chemotherapie bij de behandeling van solide tumoren.

Hoofdstuk 1 geeft een uitgebreid overzicht van de reeds toegepaste vormen van adoptieve immunotherapie met cytokinen en geactiveerde lymfocyten bij de behandeling van gemetastaseerde nierkanker. De resultaten en beperkingen van deze vorm van immunotherapie worden bediscussieerd. Tenslotte worden potentieel nieuwe en meer verfijnde methoden van tumorspecifieke immunotherapie aangegeven.

Hoofdstuk 2 beschrijft een fase I-II dosis-escalatie studie waarbij IL2 interpleuraal werd toegediend bij mesothelioom. IL2 werd op 14 opeenvolgende dagen toegediend. Dit schema werd elke 4 weken herhaald. Partiële responsen (PR) werden bij 4 van de 21 evalueerbare patiënten waargenomen (19%). IL2 spiegels in het pleuravocht bleken 6000 maal hoger dan serumspiegels. Tumor necrose factor alfa (TNF-α)-spiegels in het pleuravocht, waren zeer variabel en niet gecorreleerd aan IL2 doseringen. Mononucleaire cellen in het pleuravocht vertoonden bij alle patiënten lymfokine geactiveerde killer (LAK) activiteit. Dosis limiterende bijwerkingen werden gezien bij een dosis van 36 MIU IL2 per dag en bestonden uit catheter infecties, koorts en griepachtige verschijnselen. Er werd geen relatie gevonden tussen dosis en respons. Op grond van de waargenomen bijwerkingen, de afwezigheid van een dosis-respons relatie en het reeds bij lage doseringen optredende immunomodulerend effect, werd de aanbevolen IL2 dosis voor een fase II studie vastgesteld op 3 MIU per dag.

In **Hoofdstuk 3** worden de resultaten beschreven van een fase I-II dosis-escalatie studie, waarbij continue IL2 infusie via de arteria hepatica werd gegeven bij patiënten met een gemetastaseerd coloncarcinoom met niet-resectabele levermetastasen. Bij ongeveer 30% van deze patiënten blijkt de lever de enige lokalisatie te zijn. De huidige behandelingen met systemische of intra-arteriële chemotherapie hebben niet geresulteerd in een betere overleving. Derhalve ligt het voor de hand om locoregionale immunotherapie van de lever te bestuderen. In onze studie werd via een laparotomie een catheter ingebracht in de arteria gastroduodenalis en aangesloten op een Port-a-Cath systeem. Twee weken later werd gestart met de intra-arteriële toediening van IL2 gedurende 12 dagen, elke 3 weken. Een intrapatiënt dosis escalatie schema werd toegepast met IL2 doseringen variërend van 1.5-12 MIU/m^2 per dag. Bij doses van 6-12 MIU/m^2 per dag werd aanzienlijke hepatotoxiciteit waargenomen. Trombose van de arteria hepatica werd waargenomen bij 2 van de 5 patiënten bij de lage dosis IL2 en bij 3 van de 4 patiënten met de hoogste dosis IL2. Door deze complicaties werd de studie vroegtijdig gestopt. Geconcludeerd werd dat, als gevolg van de IL2-geïnduceerde endotheelschade, kleine arteriën zoals de arteria gastroduodenalis, niet geschikt zijn voor langdurige IL2 infusie.

Hoofdstuk 4 beschrijft het antitumor effect en de bijwerkingen bij 44 patiënten met een gemetastaseerd colon carcinoom. De patiënten werden behandeld met IL2, IFN-α en 5-FU. Tot op heden is 5-fluorouracil (5-FU) het enige actieve cytostaticum bij deze ziekte. Synergisme tussen 5-FU en IFN-α enerzijds en tussen IL2 en IFN-α anderzijds werd aangetoond in preklinische studies en aanvankelijk bevestigd in klinische studies bij gemetastaseerd coloncarcinoom, melanoom en niercelkanker. Derhalve werd de combinatie van deze 3 middelen hier bestudeerd. De behandeling werd voornamelijk poliklinisch gegeven waarbij 3 maal per week subcutaan IL2 en IFN-α, en eenmaal per week 5FU intraveneus werden toegediend. Er werden 6 (14%) partiële responsen gezien bij 43 evalueerbare patiënten. De mediane overleving was 47 weken (2-85 weken). De bijwerkingen bestonden uit moeheid, misselijkheid en braken. Granulopenie was de belangrijkste reden om dosis-uitstel en -reductie toe te passen. Seriële bepalingen van fenotypen en cytolytische activiteit van perifere lymfocyten voor en tijdens de therapie toonden geen veranderingen in aantal "natural-killer" cellen en de mate van LAK-activiteit. Concluderend heeft de combinatie van IL2 en IFN-α met 5-FU slechts een matige antitumor activiteit.

Hoofdstuk 5 beschrijft de behandelingsresultaten met IL2 en IFNα bij 57 gemetastaseerde melanoom patiënten. De behandeling met IL2 alleen resulteert in een responspercentage variërend van 5-27% terwijl met IFN-α een reponspercentage wordt bereikt van 12-22%. Op grond van synergistische activiteit tussen IL2 en IFN-α, zoals aangetoond in dierexperimenten, en de veelbelovende resultaten in vroege klinische studies, hebben we een fase II studie gedaan met de combinatie van deze twee middelen. Het behandelschema bestond uit 7,8 MIU IL2/m^2 per dag toegediend gedurende 4 dagen als continue intraveneuze infusie in combinatie met 6 MU IFN-α/m^2 per dag subcutaan op dag 1 en 4. De kuur werd om de 2 weken herhaald tot een maximum van 13 kuren. Van de 51 evalueerbare patiënten bereikte 1 (2%) patiënt een complete respons en 7 (14%) een partiële respons. De mediane responsduur was 8 maanden en de mediane overleving bedroeg 11 maanden. Cumulatieve chronische moeheid trad op na 3 maanden therapie waardoor slechts 2 patiënten meer dan 13 kuren hebben gekregen. Geconcludeerd kan worden dat deze combinatietherapie slechts een matige antitumor activiteit heeft.

Hoofdstuk 6 beschrijft de resultaten van een retrospectieve studie naar de frequentie van catheter complicaties en catheterisatieduur tijdens de behandeling met IL2 bij patiënten met gemetastaseerde nierkanker. Hierbij werd IL2 per continu infuus via een getunnelde centraal veneuze catheter (CVC) toegediend. Om de kans op infectie zo klein mogelijk te houden, werden de CVC's getunneld. Na het invoeren van deze methode en het geven van heparine profylaxe gedurende de behandeling werd een relatief lage frequentie van infectie en catheter gerelateerde trombose waargenomen.

Perspectieven

Op grond van de tot op heden gerapporteerde behandelingsresultaten, is het duidelijk dat adoptieve immunotherapie met IL2 niet als standaard therapie beschouwd kan worden bij de behandeling van solide tumoren. Een nieuwe ontwikkeling betreft het maken van tumorspecifieke effector cellen. Alhoewel T-lymfocyten voorzien zijn van T-cel receptoren (TCR) die tumor geassocieerde antigenen (TAA) kunnen herkennen en binden, heeft dit vooralsnog geen klinische betekenis omdat tumorcellen vaak hun TAA niet adequaat presenteren in de groeve van een MHC-molecule, waardoor herkenning door de TCR onmogelijk is.

Het CD3 antigen is een activatiemolecule geassocieerd met de TCR. Binding van een TAA aan het CD3 molecuul leidt tot activatie van de T-cel, gevolgd door lymfokineproduktie. T-lymfocyten kunnen op hun doel worden gericht en geactiveerd door gebruik te maken van bispecifieke monoklonale antilichamen (bs-Mab). Dit zijn hybride antilichamen verkregen door fusie van twee afzonderlijke Mabs; het ene deel specifiek voor het CD3 antigen en het andere deel specifiek voor het TAA van de tumorcel. Het bs-MAb bewerkstelligt kruiskoppeling tussen de T-cel en de tumorcel. Hierdoor vindt activatie van de T-cel plaats, hetgeen aanleiding geeft tot cytolyse van de tumorcel. Diverse bs-MAbs zijn ontwikkeld met specificiteit voor niercelkanker, eierstokkanker en borstkanker.

Een andere methode om T-lymfocyten specifiek te richten op een tumorcel is door genetische manipulatie van de TCR tot een zogenaamd chimeer immunoglobuline T-cel receptor complex (Ig-TCR). Om effectief te zijn behoort een dergelijke T-lymfocyt het Ig-TCR deel op een stabiele wijze in associatie met het CD3-antigen tot expressie te brengen. Dit wordt bereikt door de introductie en expressie van Ig-TCR genen, waarbij de variabele gen-segmenten van de TCRα en TCRβ ketens vervangen worden door de variabele gen-segmenten van de zware en lichte ketens van een Ig met bekende specificiteit. Een belangrijk kenmerk van dergelijke Ig-TCR lymfocyten is het vermogen om TAAs te herkennen onafhankelijk van het MHC molecule.

Een andere benadering om een specifieke T-cel respons op te wekken bestaat uit immunisatie met tumorcellen waarin cytokinegenen zijn gebracht. Dit resulteert in een effectieve concentratie van cytokines in de directe nabijheid van de tumorcel en niet elders in het lichaam. Fase I studies met vaccinatie met cytokine-gen getransduceerde tumorcellen bij melanoompatienten zijn reeds in gang. Bij deze benadering blijft adequate TAA expressie door tumorcellen en hun herkenning een probleem. Hoewel het relatief eenvoudig is om cytokine-genen in tumorcellen tot expressie te brengen, is dit klaarblijkelijk niet het geval bij lymfocyten. Het is moeilijk om hoge cytokine produktiespiegels te verkrijgen, mogelijk door regulatie mechanismen van de lymfocyt.

Actieve specifieke immunotherapie met vaccins verkregen uit TAA is conceptueel een aantrekkelijke benadering bij de behandeling en mogelijk ook preventie van kanker. De gedachte achter dit concept is dat dergelijke vaccins in staat zijn om

het immuunsysteem beter te stimuleren en rustende tumorspecifieke T-lymfocyten te activeren. Tot dusver zijn een aantal antigenen geïdentificeerd die herkend worden door cytotoxische lymfocyten, voornamelijk bij melanomen, maar ook bij andere tumorsoorten. Vaccinatiestudies met immunogene peptiden bij de mens zijn recentelijk van start gegaan.

CONCLUSIONS AND PERSPECTIVES

CONCLUSIONS AND PERSPECTIVES

During the past two decades, renewed interest in immunotherapy was stimulated by the results from genetic engineering, improved techniques on protein and nucleic acid sequencing and hybridoma technology. Through these techniques, highly purified molecules including cytokines, hematopoietic growth factors and monoclonal antibodies directed against tumor-associated antigens became available for clinical studies. The use of recombinant cytokines to generate, *ex vivo*, autologous lymphocytes with antitumor activity has stimulated the further development of adoptive immunotherapy in humans. This thesis describes different approaches of immunotherapy with interleukin-2 (IL2), combinations of IL2 and interferon-α (IFN-α) with or without the addition of chemotherapy in the treatment of solid tumors.

Chapter 1 gives a comprehensive overview on the applied forms of adoptive immunotherapy with cytokines and activated lymphocytes in metastatic renal cell cancer (RCC). Furthermore, their results and limitations are discussed. Finally, in future perspectives, new and refined methods of tumor specific targeted immunotherapy are indicated.

In **Chapter 2** a phase I-II dose-escalation study of locoregionally applied IL2 in pleural mesothelioma is reported. IL2 was administered for 14 days, repeated every 4 weeks, according to a group-wise dose escalation schedule. Partial response (PR) occurred in 4 of 21 evaluable patients (19%). Intrapleural IL2 levels were up to 6000-fold higher than systemic levels. Intrapleural tumor necrosis factor-alpha (TNF-α) levels varied greatly and did not correlate with the IL2 dosage. Lymphokine-activated killer (LAK) activity was displayed by intrapleural mononuclear cells in all patients. Dose-limiting toxicity was observed at 36 MIU IL2 daily, and consisted of catheter infection, fever and flu-like symptoms. No relationship between IL2 dose and response was observed. Based on the observed toxicity, the lack of a dose-response relationship and the immunomodulatory effects seen at relatively low-dose IL2, the recommended IL2 dose for a phase II study is 3 MIU daily using this treatment schedule.

Chapter 3 describes the results of a phase I A-B dose-escalation study investigating the dose limiting toxicities and antitumor effects of prolonged continuous hepatic artery infusion with IL2 in unresectable liver metastases of

patients with colorectal cancer. In as many as 30% of colorectal cancer patients liver metastases encompass the sole site of initial tumor recurrence. So far, neither systemic nor local administration of chemotherapy improved survival in this disease. These patients can be considered as candidates for locoregional immunotherapy. A catheter connected to a Port-a-Cath system was inserted into the gastroduodenal artery at laparotomy and 2 weeks later continuous hepatic artery infusion (HAI) of IL2 in 2 cycles of 12 days at 3 weeks intervals was started. Intrapatient dose escalation was applied. Doses ranged from 1.5-12 IL2 MIU/m^2 daily. Considerable hepatic toxicity was observed at an IL2 dose of 6 MIU/m^2 daily. Thrombosis of the hepatic artery was observed in 2 of 5 patients at the lower IL2 dose levels and in 3 of 4 patients at the highest IL2 dose levels. Because of these complications the study was stopped prematurely. In conclusion this study demonstrates that due to IL2 induced endothelial damage, small caliber arteries like the gastroduodenal artery are not suitable for prolonged IL2 infusion.

Chapter 4 describes the antitumor activity and toxicity of 44 patients with metastatic colorectal cancer. The patients were treated with the combination of IL2, IFN-α, and 5-fluorouracil (5-FU). To date, 5-FU remains the most active agent against this disease. Synergistic interaction between 5-FU with IFN-α at one hand, and between IL2 with IFN-α on the other hand, were demonstrated in preclinical studies and appeared to be confirmed in early clinical studies in colorectal cancer, metastatic melanoma and RCC. Consequently, the combination of these 3 drugs were studied in this disease. The treatment was given predominantly on an outpatient basis with three times per week subcutaneous IFN-α and IL2 followed by once a week 5-FU as a bolus intravenous injection. There were 6 (14%) PRs among 43 evaluable patients. The median overall survival was 47 weeks (range 2-85 weeks). Toxic effects included fatigue, nausea and vomiting. Granulocytopenia was the main reason for dose reductions or treatment interruptions. Serial assessments before and during therapy of immunophenotyping and cytolytic activities of peripheral blood lymphocytes did not show changes in the numbers of circulating natural killer cells or in the levels of LAK activities. It can be concluded that the schedule of IL2 and IFN-α combined with 5-FU has only modest antitumor activity.

Chapter 5 describes the results of 57 patients with metastatic melanoma treated with IL2 and IFN-α. Immunotherapy with IL2 has been reported to yield a 5-27%

response rate in metastatic disease while IFN-α alone has shown response rates of 12-22%. Based on synergistic activity of IL2 and IFN-α in preclinical experiments and promising results of early clinical trials, we performed a phase II study with this combination. The treatment schedule consisted of IL2 given at a dose of 7.8 MIU/m^2/day for 4 days as a continuous intravenous infusion in combination with IFN-α 6 MIU/m^2/day subcutaneously on days 1 + 4. The cycle was repeated every 2 weeks for a maximum of 13 cycles. Of the 51 evaluable patients, 1 (2%) achieved a complete response (CR) and 7 (14%) a PR. The median response duration was 8 months and the median survival was 11 months. Chronic cumulative fatigue occurred after 3 months of treatment so that only 2 patients received more than 13 cycles. In conclusion, this combination therapy has only moderate antitumor activity.

Chapter 6 describes the results of a retrospective study on the incidence of catheter complications and catheter indwelling time (CIT) during continuous IL2 infusion in metastatic RCC patients. To reduce the chance of catheter related infection a tunneled central venous catheter (CVC) was used. With the institution of tunneled CVCs and prophylactic heparin, we observed a relatively low incidence of catheter sepsis and thrombosis.

Perspectives

Based on the treatment results of immunotherapy studies as reported in the literature, it becomes clear that adoptive immunotherapy with IL2 can not be recommended as standard treatment for solid tumors. A new approach comprising the development of tumor specific effector cells is needed. Although T-lymphocytes are equipped with T-cell receptors (TCR) which can recognize and bind tumor associated antigens (TAA), this is clinically futile since tumor cells often do not express their TAA adequately in association with MHC molecules, which makes recognition by the TCR impossible.

The CD3 antigen is an activation molecule physically associated with the TCR. Binding of the CD3 to TAA leads to activation of the T-cell, followed by lymphokine production. T-lymphocytes can be targeted to tumor cells and activated by the use of bispecific monoclonal antibodies (bs-MAb) which are hybrid antibodies constructed from two parent MAbs: one specific for the CD3 antigen and the other specific for a TAA on the tumor cell. BsMAb mediated cross-linking of the T-cell to the tumor cell resulting in activation of the T-cell leading to cytolysis of the tumor

cell. Several bs-MAbs have been developed with specificity against RCC, ovarian cancer and breast cancer.

By the construction of a chimeric immunoglobulin T-cell receptor complex (Ig-TCR), tumor selectivity of T lymphocytes may be obtained. To be effective such T lymphocytes would require stable expression of the engineered Ig-TCR at the lymphocyte surface and its functional association with the CD3 signal-transducing element. This has been achieved by the introduction and expression of chimeric Ig/TCR genes, in which variable gene segments of the TCRα and TCRβ chains are replaced by the variable gene segments of the heavy and light chain of an Ig with known specificity.
An essential feature of Ig-TCR targeted lymphocytes is their ability to recognize TAA in a MHC-unrestricted manner.

Another approach to induce a specific T-cell response is by immunization of the host with tumor cells transduced with genes encoding for cytokines. These cytokines are continuously secreted by the tumor cell resulting in effective concentrations in the vicinity of tumor cells but not elswhere in the body. Pilot studies of cytokine transfer by vaccination with engineered tumor cells in melanoma patients have started. However, with this approach adequate TAA expression by tumor cells and their recognition remains a problem.
Although it has been relatively easy to express cytokine genes in tumor cells, this is not the case in lymphocytes. It is difficult to achieve constant high levels of cytokine production, which is probably due to regulatory mechanisms.
Active specific immunotherapy with vaccines constructed of TAA is conceptually an attractive approach to treatment and possibly to prevention of cancer. The rationale is that such vaccines may be able to stimulate the immune system more vigorously and activate silent precursor lymphocytes with tumor specificity. Thus far, a number of antigens have been identified as targets for recognition by cytotoxic lymphocytes, mostly in melanoma, but also in other tumors types. Vaccination studies with immunogenic peptides in humans have recently started.

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