ANTICANCER RESEARCH *25*: 3149-3158 (2005)

Phase II Randomized Study of Interleukin-2 with or without 13-cis Retinoic Acid as Maintenance Therapy in Patients with Advanced Cancer Responsive to Chemotherapy*

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Abstract. *Aim: In a previous phase 1B study, we determined the optimal biological dose of interleukin-2 (IL-2) and 13-cis retinoic acid (RA), given as maintenance therapy to patients with a variety of solid tumors, responding to chemotherapy, with a high risk of relapse. This therapy produced a statistically significant increase of the $CD4^+/CD8^+$ ratio, natural killer (NK) and lymphocyte cell counts and a decrease of vascular endothelial growth factor (VEGF). The aim of this phase II randomized study was to verify the role of RA in this drug combination. Patients and Methods: One hundred and twelve patients, with locally advanced or metastatic tumors responding to chemotherapy, were randomized to receive IL-2, $1.8x10^6$ I.U. for 5 days/week for 2 consecutive cycles of 3 weeks, with a 1-week interval (arm A), or the same regimen plus oral RA, 0.5 mg/Kg (arm B). VEGF, the $CD4^+/CD8^+$ ratio, NK and tumor markers were assessed every 2 months and response every 4 months. Results: The baseline characteristics were well balanced between the two treatment arms for age, performance status, type of disease, amount of previous chemotherapy and baseline values of NK, $CD4^+/CD8^+$ and VEGF. Toxicity was minor in both arms. After a median follow-up of 42 months, all immunological parameters improved in both arms with respect to the baseline values; this improvement was statistically more significant in arm B. There was no statistically significant difference in progression-free and in overall survival between the two arms. Conclusion: These data show that low-dose IL-2 and oral RA is more effective than IL-2 alone in improving all known prognostically significant parameters in a variety of solid tumors, including an increase of lymphocytes and a decrease of VEGF.*

In recent years, substantial progress has been made in the treatment of advanced human malignancies. Nonetheless, after chemotherapy, a majority of patients experience lethal relapse due to chemotherapy-resistant minimal residual disease (MRD). MRD manifests itself by the presence of tumor cells, both in tissues and hematopoietic autografts from patients with early-stage malignancies, or from patients in clinical complete remission after chemotherapy (1). The remaining cells, that often have long generation times or may even be in the G0-phase, may leave their state of dormancy to form a new tumor mass. This may be explained by cell kinetics alone, by an escape from immunological or other control mechanisms, or by the occurrence of an event that promotes an "*angiogenic switch*" (2). In addition, patients with advanced tumors have an impaired immune system (3). The onset of such immune disorder may occur early during the course of disease and is worsened by chemotherapy (4). Indeed, one of the most important independent negative prognostic factors in cancer patients is lymphocytopenia (5) that may be caused by the chemotherapy-related decrease in the production of IL-2 which, in turn, impairs cell-mediated immune functions, creating an imbalance in the T cell subset for a prolonged period of time (3). IL-2, defined as the T cell growth factor, has pleiotropic activities on cell-mediated and humoral immunity. IL-2 improves T cell proliferation, increases the generation of

*Presented in part at the 7th International Conference of Anticancer Research, 25-30 October 2004, Corfu, Greece.

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Key Words: Maintenance therapy, IL-2, 13-cis retinoic acid, solid tumors.

cytotoxic T lymphocytes and induces activation of T and B cells. Moreover, IL-2 increases the tumoricidal activity of NK cells (6). The interaction of IL-2 with its specific membrane receptor (7) is amplified through a paracrine route, involving IFN-γ (6). Furthermore, angiogenic activity is inhibited by IL-2 through the induction of IFN-γ, which in turn induces p-10, an inducible protein with potent anti-angiogenic activity (8). The same anti-angiogenic activity is shared by retinoids that have several synergistic effects with IL-2. In fact, retinoids co-operate with IL-2 in augmenting IFN-γ and IL-2 production by human peripheral monocytes. IL-2 in cell cultures with 13-cis retinoic acid (RA) produces a synergistic increase in IFN-γ production (4- to 90-fold), while anti-IL-2 antibodies abrogate this effect (9). In addition, retinoids increase both the number of IL-2 receptors and the percentage of peripheral blood lymphoid cells expressing surface markers for T-helper cells (9). Finally, retinoids inhibit the proliferation of various cell lines, inducing differentiation and apoptosis.

Bulky solid tumors, refractory to chemotherapy, have responded to high-dose intravenous IL-2 immunotherapy (10); however, at the price of considerable toxicity. In the attempt to find a maintenance therapy, and in order to decrease the toxicity profile described for intravenous high-dose IL-2, we conducted a phase IB study, associating subcutaneously administered IL-2 with oral RA. In this study, the Optimal Biological Dose (11) of IL-2 was found to be as low as $1.8x10^6$ I.U., in association with 0.5 mg/Kg of RA, administered 5 days/week, for 2 cycles of 3 weeks, followed by a 1-week interval each month, for up to 3 years. Such a dosing and administration schedule was easily feasible, well tolerated and improved total lymphocyte and NK counts, the $CD4^+/CD8^+$ ratio and decreased VEGF in patients with tumor response or stabilization after standard chemotherapy. In a further phase II study conducted in a large cohort of patients, we observed that 7.5% of patients entering immunotherapy as partial responders were converted to complete responders after a median time of 4.5 months (12). IL-2 with RA may, therefore, be the optimal cytokine association able to restore the cell-mediated immune function, destroy residual cancer cells with sublethal damage caused by chemotherapy (13) and decrease the possibility of an angiogenic switch. The objective of this larger phase II randomized study was, therefore, to determine the role of RA added to low-dose IL-2 in the improvement of immunological parameters such as lymphocyte and NK count, the $CD4^+/CD8^+$ ratio and in the reduction of VEGF and to confirm results obtained in previous studies. Secondary objectives were the evaluation of progression-free survival (PFS) and overall survival.

Patients and Methods

Patient selection. Patients were eligible for inclusion in the study if they were aged between18 and 81 years and had histologically confirmed stage III or IV (locally advanced or metastatic) malignant disease of the breast, lung, gastrointestinal tract, pancreas, liver, genitourinary tract, ovary or uterus. Patients exhibiting a complete response to chemotherapy, but with a high risk of relapse, or a partial response or disease stabilization with measurable disease, had to have a life expectancy of 3 months or greater. Adequate baseline bone marrow function [absolute neutrophil count (ANC) ≥1,500/μL, platelet count ≥100,000/μL], adequate baseline hepatic function [serum bilirubin ≤2.0 mg/dL, transaminase (AST, ALT) ≤5 x the upper limit of institutional normal] and adequate renal function (creatinine <1.5 mg/dL) were required. Patients with uncontrolled or severe cardiovascular disease were not eligible. Patients with brain metastases were included in the study, but those with malignancies other than curatively-treated skin and cervical cancer or previously treated with IL-2 and RA were excluded. All patients had to sign a consent form approved by the Ethical Committee of the Civilian Hospital of Avezzano, Italy, in adherence with provisions set forth in the Helsinki agreement.

Randomization and treatment schedule. A dynamic randomization procedure was used to assign patients to arm A receiving IL-2 alone or to arm B receiving IL-2 plus RA, with stratification based on type of response to chemotherapy and type of cancer. Patients in arm A were treated with self-administered subcutaneous IL-2, at a dose of $1.8x10^6$ I.U. daily, at bedtime, 5 days/week for 3 weeks each month. In arm B, where randomization for lung cancer was 2 to 1 due to our previous experience with lung cancer treated with RA (14, 15), patients received the same dose of IL-2 plus oral RA at a dose of 0.5 mg/Kg body weight, administered with meals for 5 days/week. This weekend-sparing regimen has been used previously by our group for various tumor types (14, 15), showing efficacy with good tolerability and a manageable toxicity profile. The sites of injection were rotated daily, using primarily the lower abdomen and upper and lower extremities. After a 1-week interval, patients started a new 3-week course of therapy. Two months were considered as 1 cycle of therapy. After completion of 1 year of treatment, responding patients continued to receive the same therapy as maintenance for 2 weeks each month. During the following years, therapy was continued for 5 days each month until progression. Patients exhibiting evidence of disease progression were removed from the study, treated with a salvage chemotherapy and were included in the analysis according to an intent-to-treat principle.

Study evaluations. Before random assignment, all patients underwent a complete workup to document the extent of the disease, including clinical examination, complete blood cell count, plasma urea, electrolytes, tumor-specific markers, triglycerides, liver function tests, thyroid function tests, electrocardiogram, the $CD4^+/CD8^+$ ratio, NK count and computed tomographic (CT) or magnetic resonance imaging (MRI) scans of affected regions. X-rays of abnormal areas of bone scan uptake were performed and CT scanning was used to evaluate hepatic lesions. Before each subsequent course of treatment, all patients had a further blood cell count and measurement of plasma urea, electrolytes, serum

creatinine, AST, ALT, alkaline phosphatase, bilirubin, the $CD4^+/CD8^+$ ratio and NK count. In addition, a blood cell count was repeated weekly with follow-up visits performed monthly during treatment. Tumor measurements and response assessment were performed every 2 courses of therapy (4 months), or sooner if the patient appeared to have disease progression. Patients showing disease progression were removed from the study, treated with a salvage chemotherapy regimen, specific for the type of tumor, and were followed for overall survival.

Response and toxicity evaluation. Tumor measurements were taken by physical examination and measurable disease was documented prior to the initiation of therapy. Complete response (CR) maintenance was defined as the absence of any clinical or radiological evidence of disease, by normalization of markers for at least 1 month. Partial response (PR) maintenance was defined as no growth of new lesions and a decrease by 50% or more of the sum of the diameters of measurable lesions. For bone metastases this included partial reclassification of osteolytic lesions and no concomitant occurrence of new lesions, while for liver metastases a 50% decrease in size of measurable tumors on CT scan. Stable disease (SD) maintenance was defined as a decrease of less than 50% or an increase in tumor size of less than 25% compared to the original measurements. No deterioration in symptoms or performance status, other than that secondary to drug toxicity, was admitted. Progressive disease (PD) was defined as an increase in tumor dimensions of 25% or greater compared to the original measurements of the sum of the 2 largest diameters of any measurable lesion. Analysis of the data was performed on September 30, 2004.

Vascular endothelial growth factor (VEGF) analysis by ELISA. The levels of VEGF in serum samples, kept at –20˚C until assayed, were determined by the Human VEGF Colorimetric ELISA Kit (Pierce Endogen, Rockford, IL, USA), a sandwich ELISA that utilizes antibodies raised against human $VEGF_{165}$. The assay was carried out in accordance with the manufacturer's instructions: the microplate provided was coated with antibodies that captured VEGF in standards and in samples added to the plate, after which unbound proteins were removed by washing. The biotinylated detecting antibody that binds to a second site on the VEGF protein was then added, followed by washing to remove excess detecting antibody. Streptavidin-horseradish peroxidase (SA-HRP) was then added. The enzyme-substrate reaction with TMB (3,3',5,5'-tetramethylbenzidine) generated a colorimetric signal that was read on a Titertek Multiskan plus spectrophotometer. The absorbance at 450 nm minus the absorbance at 550 nm was proportional to the amount of human VEGF in the standards or samples. VEGF concentrations (pg/ml) were determined from the standard curve generated.

Statistical methods. Considering a type I error of $\alpha=0.05$ and a confidence interval of 90%, 56 patients per arm were required in order to detect a 20% response rate differential. The Student's *t*-test was used for statistical analysis of immunological parameters. Relapse was defined as the recurrence, following a period of response, of a former lesion, its enlargement or the formation of new lesions, including central nervous system disease. The date of relapse was defined as the time when recurrent disease was diagnosed. Progression-free survival (PFS) was defined as the length of time from the date of the first course of immunotherapy to any relapse, the appearance of a second primary cancer or death, whichever occurred first. PFS and overall survival were estimated by means of the Kaplan and Meier product-limit method (16). Overall survival was measured from study entry to death, or September 30, 2004 for censored patients. All comparisons between patient characteristics, response rates and toxicity profiles were performed by Pearson's χ^2 contingency table analysis.

Table I. *Characteristics of patients.*

Characteristics	Arm A		Arm B	
	No.	%	No.	%
No. of patients	56	100	56	100
Age, years				
median	53.5		51	
range	27-80		21-81	
Sex				
males	23	41	35	63
females	33	59	21	38
Performance status (ECOG)				
0-1	55	98	55	98
2	1	2	1	2
3	0	0	0	0
Site of primary disease				
colo-rectal	5	9	5	9
stomach	6	11	3	5
breast	9	16	7	12
pancreas	2	4	2	4
ovary	9	16	5	9
lung	8	14	18	32
kidney	2	4	4	7
head and neck	4	7	6	11
lymphoma	3	5	1	2
uterus	2	4	1	2
other	6	11	4	7
Stage of disease				
III B	24	43	22	39
IV	32	57	34	61
Metastatic sites				
liver	7	13	3	5
lung	6	11	15	27
abdomen	14	25	8	14
bone	6	11	9	16
nodes	10	18	19	34
soft tissues	4	7	4	7
brain	3	5	0	0
adrenals	1	2	0	0
locally advanced	24	43	22	39

Arm A: 6 patients had 2 metastatic sites and 1 patient had 3 metastatic sites.
Arm B: 15 patients had 2 metastatic sites and 1 patient had 3 metastatic sites.

Table II. *Baseline immunological parameters.*

Parameter	IL-2 arm A	IL-2+RA arm B	*p* value
Lymphocyte (ml)	1581±112	1522±94	0.69
NK (ml)	275.7±27.64	285.9±28.03	0.79
CD4+/CD8+ ratio	1.718±0.12	1.654±0.18	0.09
VEGF (pg/ml)	290±39.1	384.4±39.5	0.09

Results

Patient characteristics. Patients were entered into the study from April 2000 to December 2001. The mean patient age was 53.1 years in arm A (range 27-80) and 51 years in arm B (range 21-81). Patients in arms A and B had previously received a total of 1,038 courses of standard chemotherapy (median 9.2 courses. $p=1$) and 1 and 2 courses of high-dose chemotherapy with peripheral progenitor cell support, respectively (Table I).

Seventeen patients in arm A and 19 patients in arm B had received radiation therapy. Forty-two patients in arm A had been radically operated, while 14 had had palliative surgery or a biopsy. In arm B, 46 patients had been operated radically, while 10 patients had undergone palliative surgery or a biopsy. In arm A, response to chemotherapy before entering the study was CR 64%, PR 25% and SD 11%, while in arm B a CR was observed in 57%, PR in 25 % and SD in 18% of patients. The patient characteristics are listed in Table I. No statistically significant differences with respect to baseline immunological characteristics of all parameters were observed in either arm (Table II).

Response. The 112 patients accrued into the study received a total of 836 courses of IL-2/RA therapy. All patients were evaluated on an intent-to-treat basis for toxicity, PFS and overall survival. Two patients in each arm, progressing after the first month of therapy, could not be evaluated due to unavailability of their laboratory data. Compliance with treatment was, in general, good. The baseline values of all parameters were well balanced in each arm for lymphocyte ($p=0.69$), the $CD4^+/CD8^+$ ratio ($p=0.09$), NK number ($p=0.79$) and VEGF ($p=0.09$) (Table II). During the course of therapy all immunological parameters improved, progressively in patients that continued to respond. A statistically significant increase in lymphocyte number was observed in both arms after the first course of immunotherapy ($p<0.0001$) (Figure 1). However, after the sixth course of immunotherapy, the improvement was higher

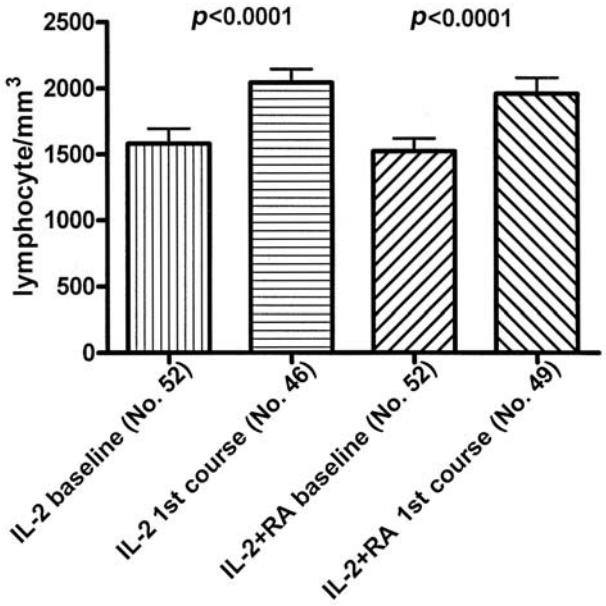

Figure 1. *Lymphocyte baseline and after the first course of immunotherapy (mean±SD). The improvement was statistically significant in both arms.*

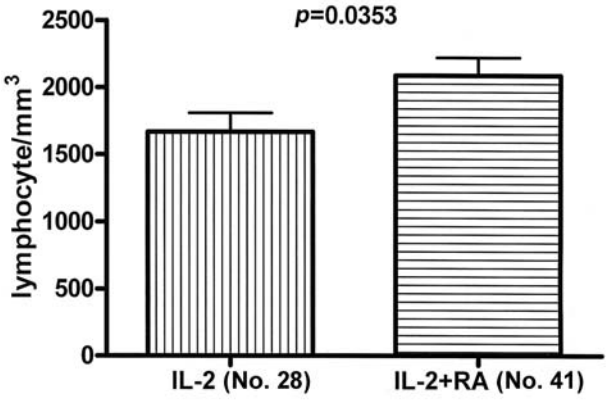

Figure 2. *Lymphocyte number after the sixth course of immunotherapy was higher in arm B (mean±SD).*

in arm B ($p=0.0353$) (Figure 2). The same pattern was observed for the NK count: a statistically significant increase in the number of NK cells with respect to baseline values was observed in both arms after the first course of immunotherapy (Figure 3). After the sixth course of therapy, the increase of NK cells was higher in arm B (Figure 4). The $CD4^+/CD8^+$ ratio improved in both arms after the first course of immunotherapy, without statistical significance (Figure 5). The improvement of the $CD4^+/CD8^+$ ratio from baseline mean values of 1.71 and 1.65 in arms A and B, respectively, reached statistical significance in arm B only

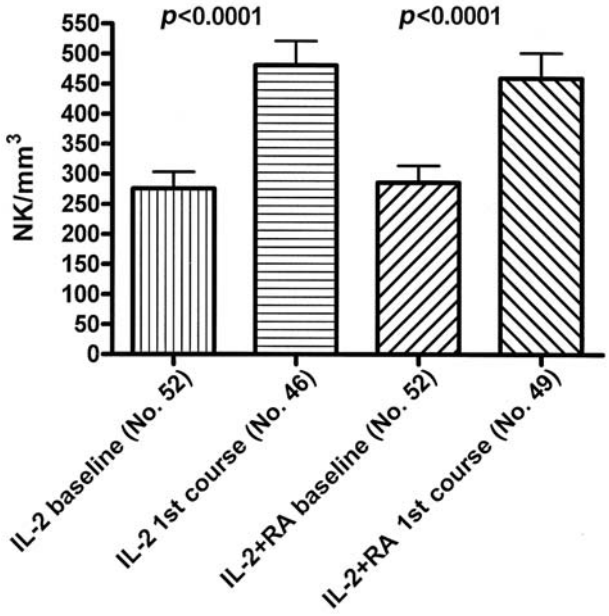

Figure 3. *NK baseline and after the first course of immunotherapy (mean±SD). The improvement was statistically significant in both arms.*

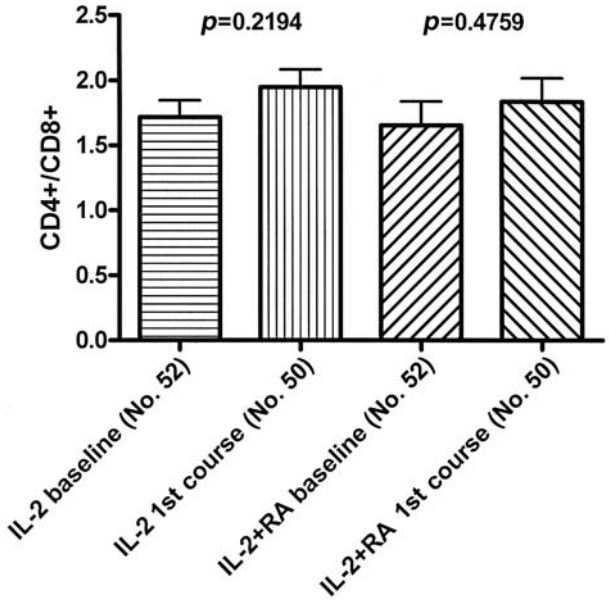

Figure 5. *The CD4+/CD8+ ratio baseline and after the first course of immunotherapy in both arms (mean±SD).*

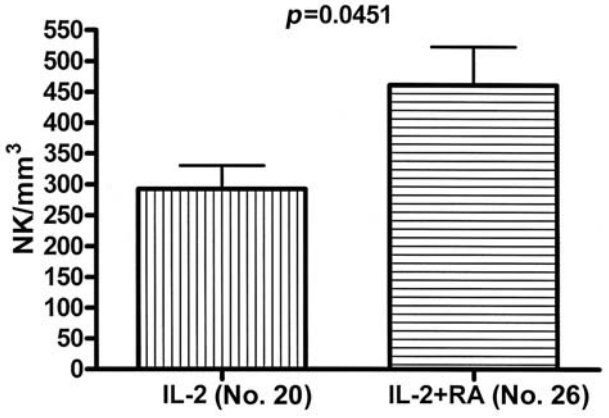

Figure 4. *NK after the sixth course of immunotherapy (mean±SD). The improvement was higher in arm B.*

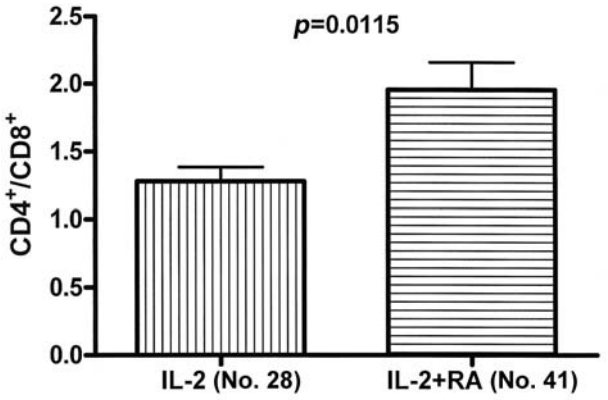

Figure 6. *The CD4+/CD8+ ratio after the sixth course of immunotherapy was higher in arm B (mean±SD).*

after the sixth course of therapy ($p=0.048$) (Figure 6). A reduction in VEGF levels was observed as a result of the therapy. The decrease of VEGF between baseline values and values of the first course was not statistically significant in either arm (arm A $p=0.912$, arm B $p=0.834$). After the sixth course of therapy, the value of VEGF was lower in arm B (Figure 7).

After a median follow-up of 42 months for surviving patients (minimum 30 months), 17 patients in arm A (30.4%) and 22 patients in arm B (39.3%) were progression-free (arm A median PFS 12.3 months, arm B PFS 28.4 months, $p=0.185$ by log-rank test) (Figure 8). Fifty-one percent of patients were alive in both arms and median overall survival had not been reached in either arm (log-rank test, $p=0.8092$) (Figure 9). Two patients in arm A, entering the study with a PR, were converted to a CR after a median time of 6 months and are still alive after a median time of 50 months. In arm B, 2 patients with a PR and 7 patients with SD were converted to a CR after a median time of 6 months and 5 of them are alive after a median

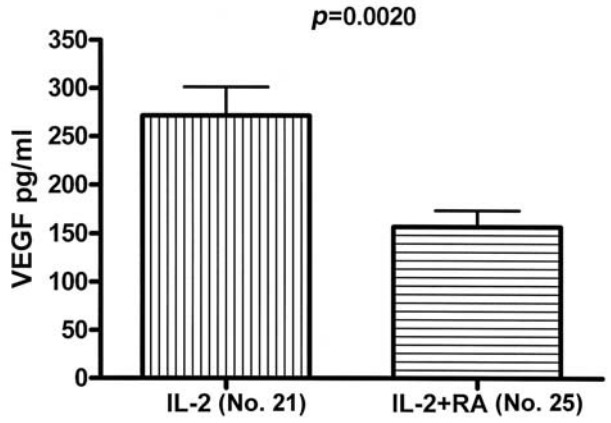

Figure 7. *VEGF after the sixth course of immunotherapy was lower in arm B (mean±SD).*

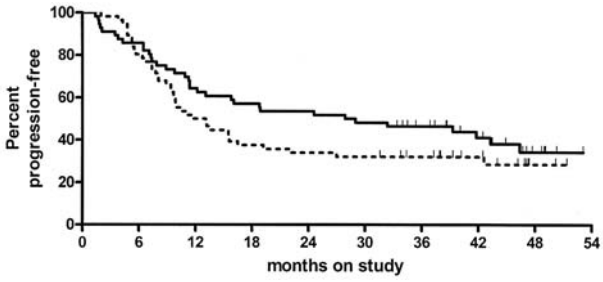

Figure 8. *Progression-free survival (PFS).*
—/— *IL2-RA: median PFS, 28.45 months.*
---/--- *IL-2: median PFS, 12.35 months. log-rank test, p=0.185. (Hazard ratio 0.7356, 95% CI of ratio 0.46 to 1.163)*

time of 46 months (Table III). As of September 30, 2004, 364 courses of immunotherapy had been delivered to arm A and 472 to arm B ($p=0.0432$). This difference was due to earlier failures of patients in arm A.

Toxicity. Standard World Health Organization (WHO) criteria for assessing toxicity were used. No treatment-related death was observed. The toxicity profile was mild, with none of the 112 patients entered into the study having grade 3 or 4 toxicity. Cutaneous WHO grade 2 toxicity was observed in 7 patients in arm A and 8 patients in arm B. Fever was observed in 6 patients in both arms. Mild hypothyroidism occurred in 2 patients in each arm, while grade 1 triglyceride increase was observed in 10 patients in arm B (Table IV). Such toxicity did not interfere with the cardiac function of patients with a previous history of coronary artery disease, who were closely monitored. We think that such low toxicity may be due to the interruption of therapy on weekends and for 1 week every month.

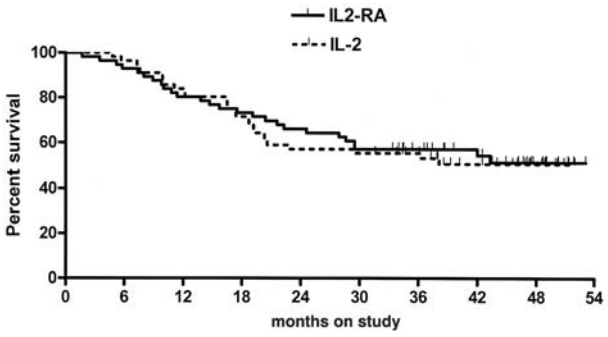

Figure 9. *Overall survival of all patients. The median survival has not been reached in either arm, after a median follow-up of 35.5 months. Log-rank test, p=0.809. (Hazard ratio 0.9360, 95% CI of ratio 0.5452 to 1.606)*

Discussion

We conducted this intent-to-treat study to determine whether RA added to IL-2 could be safely delivered as maintenance immunotherapy after chemotherapy and to determine whether this drug combination could improve the immunological parameters known to be prognostically significant. Patients with different types of tumors, who had achieved a benefit from chemotherapy (partial and complete remission or disease stability), were enrolled in the study consecutively, over a 20-month period, and assigned randomly to one of the two treatment arms. The study rationale was that long-term administration of therapy that did not interfere with the quality of every-day life would result in continued augmentation of the immunological response and decrease of VEGF, that should produce further regression of the disease and, therefore, improve survival.

Since 1984, recombinant IL-2, used in high-dose bolus in the treatment of patients with metastatic renal cell carcinoma and melanoma, despite an overall response rate (RR) of approximately 20% and some long-term remissions (17), has resulted in significant toxicity, often requiring patient admission to the intensive care unit (10). Intravenous continuous infusion at intermediate doses improved tolerance, but necessitated close monitoring of hospitalized patients (18). Subcutaneous administration of IL-2 at lower doses on an outpatient basis (19-21) has given response rates identical to those observed with intravenous infusion. Evidence supporting the efficacy of high-dose intravenous IL-2, rather than low-dose IL-2, is limited. Furthermore, one of the biases of previous trials has been the limited duration of therapy, varying from a few days to several months, due to the toxicity of high and intermediate doses of IL-2 (22).

The long-term administration of IL-2 might be necessary to eradicate MRD: MRD cells may remain dormant in tissues for long periods of time, as has been observed in estrogen

Table III. *Patients with partial response or stable disease, converted to complete response after therapy.*

	Initials Age	Disease	Site of metastasis	Previous chemotherapy courses	Response to chemo-therapy	Cycles of IL-2/ RA	Response to immuno-therapy	Time to progression (months)	Overall survival (months)
IL-2 + RA	M.S./61	cholangio-carcinoma	liver bones	8	PR	20	CR	39.3	49.4+
	F.G./77	NSCLC	controlateral lung	8	SD	12	CR	16.1	28.6
	G.N./51	H&N	nodes	6	SD	20	CR	47+	47+
	P.M./67	NSCLC	nodes, bones	6	SD	14	CR	46.8+	46.8+
	F.A.M./55	breast	nodes	12	PR	24	CR	45.7+	45.7+
	B.A./80	NSCLC	controlateral lung, nodes	8	SD	6	CR	6.2	13
	F.L./68	colon	nodes	6	SD	14	CR	33.4+	33.4+
IL-2	D.M.L.A./80	C.U.P.	liver, bones	8	PR	22	CR	51.4+	51.4+
	P.A./70	lymphoma	nodes, peritoneum	6	PR	12	CR	15.5	50+

NSCLC: non-small cell lung cancer;
H&N: squamous cell carcinoma of the head and neck;
C.U.P.: carcinoma of unknown primary;
CR: complete response; PR: partial response; SD: stable disease;
+: censored observation.

Table IV. *Toxicity according to WHO criteria.*

	WHO grade															
	Arm A								Arm B							
	0		1		2		Total		0		1		2		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Hematological																
Leukopenia	52	93	4	7	0	0	56	100	52	93	4	7	0	0	56	100
Neutropenia	56	100	0	0	0	0	56	100	56	100	0	0	0	0	56	100
Thrombocytopenia	50	89	6	11	0	0	56	100	50	89	6	11	0	0	56	100
Anemia	52	93	4	7	0	0	56	100	51	91	5	9	0	0	56	100
Gastrointestinal																
Diarrhea	52	93	4	7	0	0	56	100	52	93	4	7	0	0	56	100
Oral	52	93	4	7	0	0	56	100	50	89	6	11	0	0	56	100
Triglycerides	56	100	0	0	0	0	56	100	46	82	10	18	0	0	56	100
Cutaneous	20	36	29	51	7	13	56	100	18	32	30	54	8	14	56	100
Fever	46	82	4	7	6	11	56	100	46	82	4	7	6	11	56	100
Flu-like syndrome	49	87	0	0	7	13	56	100	49	87	0	0	7	13	56	100

receptor-positive breast cancer patients who may have a relapse after 20 years. For example, Meloni *et al.* have been treating 11 patients with acute myelogenous leukemia in an advanced phase of the disease, with low-dose maintenance IL-2 for almost 10 years and have noted no major toxicity (23). In patients with a PR or SD after chemotherapy, IL-2 may stimulate the proliferation of active lymphocytes against MRD. In fact it has been demonstrated that, to maintain activity, lymphocytes require periodic stimulation with a specific antigen (24). Previous retrospective studies have demonstrated that prolonged survival of renal cell cancer patients treated with IL-2 subcutaneously is related to the significant improvement of immunological parameters, mainly $CD4^+$ count (25) and total lymphocyte count (26).

In the present study, an immediate benefit from therapy was observed in both arms, with an improvement of immunological parameters (lymphocyte and NK count, $CD4^+/CD8^+$ ratio). After one year of therapy, such benefit persisted only in patients treated with the combination of IL-2 and RA; however, this may be partially due to the decreased number of patients who continued to respond to therapy. Overall survival was similar in both arms.

The decrease in VEGF levels observed in both arms as a result of IL-2 therapy was greater in patients in arm B receiving a combination of IL-2 and RA. The role of RA in the inhibition of angiogenesis is demonstrated by the fact that clinically achievable doses of RA rapidly cause large- and small-vessel endothelial cells to become refractory to stimulation of migration, either by tumor-conditioned media or purified angiogenic factors (a-fibroblast growth factor (aFGF), bFGF, VEGF, platelet-derived GF, TGF beta-1 and IL-8) (27). In fact, the toxicity of retinoids observed during pregnancy is due to the strong inhibition of embryonic angiogenesis by retinoids. The present results suggest that retinoids, in association with IL-2, are effective inhibitors of angiogenesis and can be used for the management of certain diseases accompanied by aberrant angiogenesis, particularly that occuring during progressive growth of solid tumors (28). In previous studies, we found that the weekend plus 1-week interval was very important to avoid complications linked to retinoid therapy (dry skin, kielitis and elevated triglycerides). The results of this study demonstrate that the repeated administration of IL-2 and RA, even at low doses, can achieve long-term restoration of the immune function, and in accordance with previously published studies, 2 patients in arm A (3.5%) and 7 patients in arm B (14%) obtained a clear benefit from biotherapy, improving their response from SD and PR to CR. Even if these patients were selected and selection biases can be advocated, it is very unlikely that 3 patients with stage IV NSCLC would have had a median time to progression of 16 months, or that an 80-year-old patient with liver and bone metastases of a cancer of unknown primary would be responding after 51.4. months.

In these patients the reduction in tumor size paralleled the improvement of immunological parameters. This fact may suggest that the presence of a valid host immune defense function is necessary to achieve a complete tumor response (29). The intermittent administration of RA in association with IL-2 was well tolerated, as described previously. Based on pre-clinical information, we assumed that RA might improve IL-2 activity possibly *via* modulation of surface IL-2-specific receptors.

We conclude that both the association of subcutaneous IL-2 at a dose of $1.8x10^6$ I.U./day, together with an oral RA dose of 0.5 mg/Kg or IL-2 alone, in an intermittent schedule, repeated for a long-term period is feasible, has low toxicity and results in the improvement of biomarkers for patient outcome, such as long-term $CD4^+$ count and of markers for tumor outcome, such as the conversion of SD and/or PR to CR. Patients treated with IL-2 and RA had a significant benefit in all immunological parameters explored. The improvement became statistically significant after the sixth course of biotherapy, indicating that, to obtain a clear immunological benefit with low-dose IL-2, an intermittent long-term administration schedule is necessary. Our data provide evidence of a safe and efficacious approach for the administration of IL-2 and RA early after chemotherapy in a variety of solid tumors. This trial provides a reference for further investigation of immunotherapy after chemotherapy using a combination of cytokines; at present, we are conducting a randomized phase III study in which patients, after a response to chemotherapy, are randomized to IL-2 and RA or observation.

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Received January 11, 2005
Accepted May 6, 2005