

IMMUNOTHERAPY OF METASTATIC KIDNEY CANCER

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From April 1986 to September 2000, 122 MRCC patients were treated by monthly intralymphatic injections (containing a mean of 573 IL-2 U and 26×10^6 LAK cells) and i.m. administration of IFN and TF; 71 patients also received a 3-day cycle of monthly IL-2 inhalations with a mean of 998 daily U. MRCC cases not treated by immunotherapy ($n = 89$) represent our historical controls. Adverse clinical side effects related to treatment were negligible. CR ($n = 11$) and PR ($n = 13$) were noticed in 24/122 patients. Of 24 responding patients, 17 resumed progression, whereas 7 remain in remission 11–69 months later. The overall median survival of treated patients (28 months) was 3.5-fold higher than the median survival of historical controls (7.5 months), and a Kaplan-Meier curve showed 25% survival 11 years after the beginning of immunotherapy. Apparently, the addition of IL-2 by inhalation improved survival. The present immunotherapy protocol appears to be efficacious, safe, devoid of adverse side effects, far less costly than others and able to offer a good quality of life to MRCC patients; if confirmed in a multicenter trial, it could set the basis for developing low-dose immunomodulatory treatments.

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Key words: immunotherapy; IL-2; lymphokine-activated killer cells; metastasis; transfer factor; interferon; renal cancer

The most promising systemic therapy for MRCC is immunotherapy with IL-2.¹ The original observations by Rosenberg *et al.*,² using i.v. administration of high-dose rIL-2 and LAK in MRCC patients showed encouraging clinical response rates and long-lasting remissions. These results have prompted many clinical trials using various administration routes: i.v. both in bolus and continuous or s.c. in combination or not with IFN and/or chemotherapeutic agents. In 1,411 MRCC patients, the overall rate of response was 20%.^{3,4}

Nonetheless, despite the lowering of the IL-2 dosage and the decrease of the 4% fatal outcome of the initial studies, thanks to the improved selection of patients and the clinical experience acquired,^{5,6} the persistence of more often than not severely adverse clinical side effects remains the most important hindrance for the wide use of IL-2.

Hence, being aware of the risk from a high IL-2 dose and of its ability to activate, at low doses, tumor mass rejection,^{7–9} we attempted to develop alternative protocols. An observation made in 1 MRCC patient, in whom injection of a metastasis with IL-2 and LAK cells was followed by regression not only of the treated tumor but also of several other abdominal lymph node metastases, prompted us to further investigate the low-dose effect using the intralymphatic route. Following intratumor low-dose injections of IL-2 into infiltrating bladder cancers or lymph node metastases of MRCC patients, both the injected and the non-injected distant metastases often disappeared.^{7,8} Thus, by administering very low doses of IL-2 and LAK cells into the lymphatic vessels of the foot, we observed encouraging clinical results with minimal side effects.^{10,11}

To boost the overall immune response^{12,13} and prevent opportunistic infections,¹⁴ patients also received i.m. TF injections. In our institution, TF is currently used as an adjuvant treatment of

certain tumors, *i.e.*, non-small-cell lung cancer^{14a} and hormone-unresponsive prostate cancer,¹⁵ with promising clinical results.

Following our preliminary observations, we report here results obtained in 122 MRCC patients treated with intralymphatic administration of non-recombinant IL-2 and LAK cells, IFN and TF.

MATERIAL AND METHODS

Patient population

From April 1986 to September 2000, 122 MRCC patients were treated. Their sex, age, appearance of metastasis from nephrectomy and organ involvement are reported in Table I. One hundred showed a performance status, according Karnofsky, of between 80 and 100, with 22 between 50 and 79. We further identified a subgroup of 99 patients by excluding 23 patients according to the following criteria: 8 because they were treated for <2 months; 1 because he was suffering from a known non-responsive histology (transitional-cell carcinoma), 4 because they were not nephrectomized and 10 because they were previously treated with rIL-2 elsewhere and, because of the adverse side effects, asked to enter into our protocol. These 10 patients are identified as group IL-2-R. Statistical analyses were carried out considering all groups. Four patients were not nephrectomized because of high surgical risk.

Only 118 patients underwent nephrectomy: 7 showed histological grade 4, 56 grade 3 and 20 grade 2. For 39 patients, we were unable to obtain grading and had only the pathologist's report stating "carcinoma of the kidney;" these nephrectomies were performed outside our hospital years before and slides were not available. All patients gave written informed consent for IL-2 treatment. The ethics committee of our hospital approved the written protocol. After evaluation of our results, the Emilia-Romagna Regional Therapeutic Committee, responsible for treatments, included "intralymphatic administration of therapeutic drugs" in the list of approved therapies.

Abbreviations: CD, cluster designation; CR, complete response; EBRT, external-beam radiotherapy; LAK cells, lymphokine-activated killer cells; MRCC, metastatic renal-cell cancer; MU, mega-unit; PB, peripheral blood; PBL, peripheral blood lymphocyte; PR, partial response; PROG, tumor progression; rIL-2, recombinant IL-2; TF, transfer factor; TNM, tumor, node, metastasis.

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TABLE 1—DATA ON TREATED PATIENTS

Patients	122
Sex	
Female	24 (19%)
Male	98 (81%)
Age (years)	18–85 (61.1 ± 11.8)
Patients with synchronous metastases	65 (53.2%)
Patients with metachronous metastases	57 (46.7%)
Appearance of metastasis from nephrectomy (months)	4–169 (54 ± 47)
Stage IV	122
Histological grading	
2	20 (16%)
3	56 (46%)
4	7 (6%)
?	39 (32%)
Site of metastases	
Lung	56 (45%)
Liver	4 (3.5%)
Bone	29 (24%)
Brain	4 (3.5%)
Lymph nodes	23 (19%)
Kidney	6 (5%)
Multiple organs	29 (24%)
Solitary metastasis	16 (13%)
>1 metastasis	106 (87%)
Months of immunotherapy	1–135
Months of follow-up	3–140

Historical controls

Eighty-nine nephrectomized MRCC patients not treated with immunotherapy represent our historical controls. All patients were treated and monitored in our institution; in 39 patients, the diagnosis was synchronous with that of a primitive kidney tumor and, in 50, metachronous, made at various post-nephrectomy time intervals. Of the 89 patients, 25% had bone metastases, 56% lung, 2.5% both brain and liver and 15% lymph node involvement. Certain patients underwent EBRT with 40–50 Gy conventional fractionation as complementary therapy.

Immunotherapy protocol

The current protocol consisted of 1 monthly intralymphatic administration of IL-2 and LAK cells, after 3 consecutive days of IL-2 inhalation; patients also received i.m. injections of TF monthly and IFN- α biweekly. The initial treatment cycle lasted 6 months, with restaging at 3 and 6 months. Persistence of disease or CR was followed by an additional 6-month cycle. In case of progression, treatment was discontinued, unless the patient wanted to pursue it for an additional cycle. All treated patients were analyzed statistically. Inclusion criteria were the confirmed histological diagnosis of renal cancer metastasis and written informed consent from the patient. Exclusion criteria were age <18 years; life expectancy <1 month; Karnofsky index <40; presence of acute viral, bacterial and/or autoimmune diseases; serum creatinine >0.2 g/l; cardiac infarction during the last 2 months; and cardiac failure requiring medication. We also excluded patients who needed cortisone medication, with the exception of 1 who underwent bilateral adrenalectomy and required daily cortisone (4 mg) and patients with brain lesions during EBRT. One patient with lung metastases, after bilateral nephrectomy necessitating renal dialysis, 8 hr 3 times a week, was also included.

IL-2 production and lymphocyte activation

IL-2 production and IL-2 activation (LAK cells) of PBLs were previously described.^{7,10} Briefly, 30 ml of PB were obtained from each patient and the lymphocytes separated by centrifugation on a Ficoll-Hypaque gradient. After 3 washes in RPMI-1640 (GIBCO BRL, Gaithersburg, MD), PBLs were suspended at 2×10^6 /ml of RPMI-1640 supplemented with 2% autologous, heat-inactivated

plasma (56°C for 20 min, then cleared by 15 min centrifugation at 800g) and incubated for 72 hr at 37°C in a CO₂ incubator with 20 U/ml IL-2 in 75 ml Falcon (Lincoln Park, NJ) plastic bottles. Immediately before incubation, 0.2 ml of the cell suspension was taken for sterility assessment on specific media for bacteria and mycoplasma following standard techniques. The IL-2-stimulating dose was chosen by assessing the *in vitro* effects of escalating doses, from 10–200 U/ml, on different CD leukocyte antigens expressed on the membrane of PBLs.

Since 20 U represents the lowest amount for obtaining, after 3 days of incubation, a statistically significant increase of surface lymphoid markers (CD4, CD16, HLA-DR and monocyte markers), this concentration was chosen for the preparation of LAK cells to be used for therapeutic purposes.¹¹ After incubation, IL-2-activated PBLs (LAK cells) were washed 3 times in saline and suspended in 2 to 3×10^7 cells/ml saline containing 250 to 10,000 IL-2 U; 2.5 U IL-2, thus determined,⁷ correspond to 1 pg human IL-2, as assessed using the ELISA kit Biotrak (Amersham, Aylesbury, UK).

Intralymphatic injection of IL-2 and LAK cells

Lymphatic vessels of the foot were localized according to the standard technique for lymphography and cannulated with a fine 27 G butterfly needle connected to a syringe with 5–10 ml of saline, containing 250 to 10,000 IL-2 U and 2 to 8×10^7 LAK cells. The syringe was connected to a mini-dose-injecting pump (0.5 ml/min). Injections were given over 10–20 min, in a dose-escalating fashion (*i.e.*, 250, 500, 1,000, 5,000, 10,000 U), until the appearance of minimal side effects (usually chills); they were performed at monthly intervals, alternating the foot. Patients were pre-medicated 1 hr before injection, using clorphenamine maleate (10 mg) and ranitidine hydrochloride (50 mg) (administered i.m.), to block the H1 and H2 receptors of lymphoid cells. In the presence of side effects, IL-2 administration was interrupted and a symptomatic treatment was immediately started. In a few cases, side effects did appear, starting with chills. The above-mentioned drugs were then re-administered. If chills persisted 15–20 min later, 200–500 mg of hydrocortisone were administered. If the blood pressure dropped to 90 mm Hg, dopamine hydrochloride was injected. Furthermore, with even minimal side effects, the next month's IL-2 injection was reduced to 250 U and LAK cells were derived from only 30 ml of blood.

IL-2 inhalations

From April 1992, in some patients with lung metastases for whom we could not administer IL-2 and LAK cells intralymphatically because of concomitant treatment with dicoumarol for the presence of thrombi in vena iliaca (we also inserted a filter for impeding thrombi dissemination), IL-2 was administered by inhalation according to the protocol of Huland *et al.*¹⁶ As results appeared hopeful, IL-2 inhalations were also offered to other patients: 250 IL-2 U in 5 ml of saline were administered 4 times a day for 3 consecutive days each month; inhalation lasted 15–20 min, and standard aerosol inhaler equipment was used. On the fourth day, patients received intralymphatic treatment.

IFN

Because of some favorable clinical reports¹⁷ and for a better expression of tumor-associated and histocompatibility antigens on the surface of tumor and lymphoid cells,¹⁸ 34 randomly chosen patients were biweekly injected i.m. with 10^6 units of commercially available recombinant IFN- α -2a that should, theoretically, produce 10–20 U/ml in the body fluid.

Transfer factor

From the beginning of the present protocol, MRCC patients treated with IL-2 also received monthly i.m. injections of 3.5 U TF. The latter was produced in our laboratories from mononucleated cells obtained from pools of 100 buffy coats of the hospital's

TABLE II - IL-2 TREATMENTS

Number of patients	Number of intralymphatic treatments		Number of LAK cells ($\times 10^6$)			Number of IL-2 U		
	Total	Mean per patient	Total	Mean per patient	Mean per injection	Total	Mean per patient	Mean per injection
122	1,858	15.2	49,250	403.7	26.5	1,065,113	8,730.4	573.3
Number of patients	Number of aerosol treatments		Number of cycles		Number of IL-2 U			
	Total	Per patient	Number of cycles	Per patient	Total	Per patient	Per aerosol day	Per aerosol
71	10,315	145.3	800	11.3	2,941,660	41,431.8	998.2	285.2

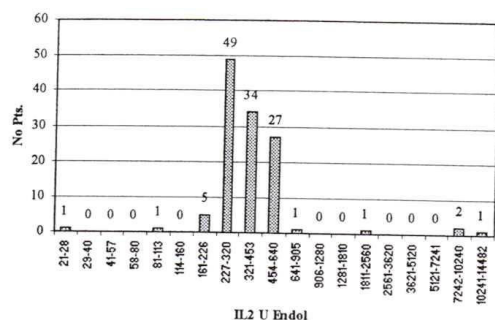


FIGURE 1 - Distribution of patients according to the mean dose of IL-2 U injected in the lymphatic vessels of the feet.

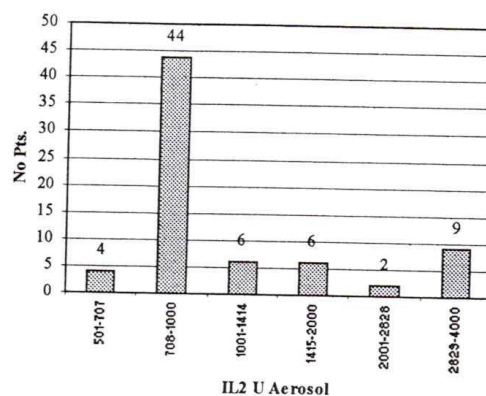
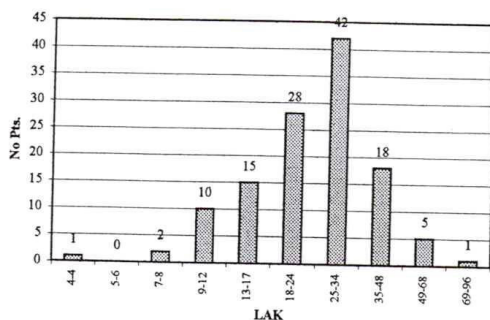


FIGURE 3 - Distribution of patients according to the mean dose of IL-2 administered by inhalation for a day cycle.

FIGURE 2 - Distribution of patients according to the mean dose of LAK cells ($\times 10^6$) injected in the lymphatic vessels of the feet.

blood bank donors. TF was extracted using the standard technique,¹⁹ slightly modified; it was stored lyophilized and solubilized in 5 ml of saline immediately before injection. One unit was obtained from 1×10^8 cells.

Evaluation of clinical response

Clinical response was evaluated by considering both the overall survival rate (Kaplan-Meier curves) and the response rate at the level of measurable metastases according to conventional parameters. Thus, CR is the complete disappearance of tumor in all sites the disease may be detected for at least 1 month without new lesions, PR is a decrease by 50% of the sum of the perpendicular diameters of all measured lesions in the absence of detectable tumor progression in any other site or the appearance (observed by CT scan or chest X-ray) of new lesions for 1 month and PROG is

a 50% increase of the sum of the perpendicular diameters of all measured lesions measured over the smallest sum observed (or over baseline status if no decrease occurs), the re-appearance of a lesion that had disappeared, worsening of detectable but non-measurable disease or the appearance of new lesions. Stable disease or no response was decided when the disease status was not modified following the criteria of complete or partial remission or disease progression.

At the beginning of treatment, patients were restaged every 4-8 weeks for the known metastatic site using standard X-rays (lung and bone) or ultrasonography (liver, kidney, lymph nodes). CT scan was done every 6-8 weeks for known brain metastases and every 3 months for unknown sites and very small lesions (<0.5 cm). Every 6 months, we performed total body scintigraphy for unknown bone lesions. Many patients in long-lasting remission often refused the too frequent examinations. In this case, we resorted to a total-body CT scan every 6 months and total-body scintigraphy every 8-12 months. Serum and urine biochemical parameters (Na^+ , K^+ , transaminases, bilirubin, creatinine, cholesterol) were evaluated monthly; electrocardiograms were recorded bimonthly. Blood pressure was measured twice, immediately before and at the end of IL-2 administration, if not required earlier for other reasons.

Concomitant treatment

In 57 patients, EBRT was used both as an adjuvant tumor reduction treatment and because of the possible synergy between EBRT and increased antigenicity of the tumor.²⁰ The dose range for radiation was 20-30 Gy for metastases situated in the mediastinum and inguinal or lumbar lymph nodes, 30-45 Gy for bone lesions and 40-50 Gy for brain lesions. Medication for pain was administered without drug restriction.

Statistical analysis

The patient sample showed a median age of 62 years, with the first quartile at 56 and the third at 69 years (Table I). The most important indicator was the right-censored survival curve, for which we used the Kaplan-Meier method.²¹ Clinical results were compared among subgroups of patients using Gehan's generalized Wilcoxon's test²² and Cox's *F*-test, which showed similar results.²³

RESULTS

LAK cells and IL-2

Table II shows the numbers of LAK cells and IL-2 U administered by intralymphatic injection or inhalation. During the 14-year period of the study, a total of 492.5×10^8 LAK cells were administered in 1,858 preparations to 122 patients. Each patient received a mean of 15.2 intralymphatic injections, with a mean total of 403.7×10^6 LAK cells during the treatment period and a mean of 26.5×10^6 cells per injection.

There were 1,065,113 total IL-2 U injected in lymphatic vessels, with a mean of 8,730 U/patient and 573 U/injection. Also, 71 patients received 10,315 IL-2 inhalations in 800 cycles, with a mean of 145 inhalations and 11.3 cycles/patient. A total of 2,941,660 IL-2 U were administered by inhalation, a mean of 41,431 U/patient and 998 U/day cycle. Each inhalation contained a mean of 285 IL-2 U in 5 ml saline and was administered over 15 to 20 minutes.

Figure 1 shows the distribution of patients according to the average dose of IL-2 administered in the lymphatic vessels. Most patients ($n = 110$) received between 227 and 640 U IL-2 per injection; 5 received 150–226 U, 3 received 7,000–14,000 U and 2 received 21 and 115 U. In contrast, a wider distribution was observed regarding the mean number of administered LAK cells (Fig. 2). Forty-two patients received between 25 and 34×10^6 cells per injection, 28 received between 18 and 24×10^6 cells and 18 received between 35 and 48×10^6 cells. Only 1 patient received $>69 \times 10^6$ cells and 1 received $<5 \times 10^6$ cells; 25 were injected with 9 to 17×10^6 LAK cells. Although most of the clinical responses were observed among patients injected with $>15 \times 10^6$ LAK cells, no clear correlation has been established between these numbers and tumor regression.

Figure 3 shows the distribution of patients following the dose of IL-2 inhaled during each day cycle. Forty-four patients received between 708 and 1,000 IL-2 U, 12 received between 1,001 and 2,000 IL-2 U and 11 received between 2,001 and 4,000 IL-2 U. Regarding the duration of treatment, 15 patients were treated for <4 months, 39 for 5–12 months, 34 for 13–32 months, 23 for 33–60 and 11 for 61–120 months.

Side effects

Table III shows the number of adverse clinical side effects during the 10,315 IL-2 inhalations and 1,858 intralymphatic administrations. In only 7% of cases we observed a decrease of blood pressure >20 mm Hg, requiring medical intervention, or mild

fever. This happened when the IL-2 dose ranged between 500 and 14,000 U. In 1 patient, we noticed dyspnea with bronchospasm, treated by i.v. administration of 200 mg hydrocortisone; however, hospitalization of the patient in the intensive care unit was not necessary and because he was an outpatient, he was regularly discharged a few hours after the episode. The IL-2 treatment was pursued, but in the subsequent session the lowest dose (250 U) was used both by inhalation and intralymphatically. Most patients were treated as outpatients. No side effects were observed in relation to TF injections. Regarding IFN administration, we sometimes noticed a transient flu-like syndrome or a mild increase of body temperature. No medication was necessary.

Clinical response

The entire group of patients underwent immunotherapy for 1 to 135 months, with a follow-up of 3–140 months (Table I). We observed 11 CRs (9.1%) and 13 PRs (10.6%), mostly at the lung and lymph node sites (5 and 3, respectively, for CR; 6 and 2, respectively, for PR). Four of the clinical responses were noticed in bone (Table IV). Stable disease was noted in 27 patients (22%) and progression in the remaining 71 (58%). Looking at single tumor lesions, the most responsive organ site, i.e., that with the highest regression rate, was the lymph node with CR+PR in 9/23 patients (39%). Except for the kidney (3 responses in 6 patients treated), a similar response rate was observed in the remaining organs (lung 14/56, 25%; liver and brain 1/4, 25% each; bone 4/29, 14%). Regarding performance status, 20 PR+CR (83%) were observed among patients with performance status >80 and 4 (16%) in patients with performance status between 50 and 79 ($p < 0.005$ Fisher's exact test).

Table IV shows the number of months necessary for a clinical response and the duration of treatment: it ranges from 1–39, with a mean of 9 ± 9 for PR, and 1–50, with a mean of 14 ± 17 for CR.

Table V illustrates the type and organ site of the clinical response for each patient, the number of days necessary for obtaining a regression, its duration, the site of relapse, the duration of follow-up and the status of the patient. Two long-lasting CRs have been observed at the lung level (1,668 and 1,591 days); the longest, and still ongoing, PR is 1,984 days. The longest CR was observed in patient 10010, who at the beginning underwent partial nephrectomy for a primary tumor on the left kidney and 2 apparently metastatic lesions on the right. Relapses were observed in both kidneys 2,734 days later; surgical removal of the remaining half-kidneys obliged the patient to undergo permanent renal dialysis.

Table VI depicts the behavior of the clinical response: of the 11 patients in CR, 8 went in progression (73%) after 3–90 months (mean 34 ± 31) and 3 are still in remission after 10–69 months (mean 38 ± 29). Of the 13 in whom we first noticed a PR, 9 progressed (69%) in 7 \pm 4 months and 4 remained in PR after 22 ± 29 months (range 11–59).

The most responsive metastases considering the organ involved were 2 kidney lesions, which subsequently relapsed. Of

TABLE III—CLINICAL ADVERSE SIDE EFFECTS OBSERVED DURING IL-2 ADMINISTRATION

Adverse side effects	Intralymphatic (IL2 500–10,000 U)	Intralymphatic (IL2 250–490 U)	Inhaled (IL2 250 U)
Chills	18%	1%	0%
Fever ($<38^\circ\text{C}$)	21%	2%	0%
Nausea and vomiting	8%	0%	0%
Cutaneous rash	2%	1%	0%
Hypotension (>20 mm Hg)	7%	2%	0%
Hypertension (>20 mm Hg)	0%	0%	0.2%
Pain (inguinal lymph nodes)	4%	0%	0%
Dyspnea	1%	0%	2%
Number of patients evaluated	21	101	71
Number of IL-2 administrations	166	1,692	10,315

TABLE IV - NUMBER, PERCENTAGE AND SITES OF CLINICAL RESPONSE, INCLUDING MEAN, RANGE AND MEDIAN OF MONTHS NECESSARY FOR OBSERVATION AND DURATION OF IMMUNOTHERAPY

Site	CR	PR	Stable	PROG	CR + PR	Patients	Type (number of patients)	Months to clinical response			Months of immunotherapy		
								Mean \pm SD	Median	Range	Mean \pm SD	Median	Range
Lung	5 (45%)	6 (46%)	—	—	11 (46%)	Group 99	CR (9)	14 \pm 17	6	1-50	66 \pm 37	6	13-135
Liver	1 (9%)	1 (8%)	—	—	2 (8%)		PR (13)	9 \pm 9	7	1-39	39 \pm 31	7	5-113
Lymph node	3 (27%)	2 (15%)	—	—	5 (21%)		Stable (17)	11 \pm 12	4	2-46	11 \pm 12	4	2-46
Bone	1 (9%)	3 (23%)	—	—	4 (17%)	All 122	PROG (60)	13 \pm 14	6	1-67	28 \pm 25	6	2-119
Kidney	1 (9%)	1 (8%)	—	—	2 (8%)		CR (11)	14 \pm 16	9	1-50	64 \pm 37	9	13-134
Totals	11 (9.1%)	13 (10.6%)	27 (22%)	71 (58%)	24 (100%)		PR (13)	9 \pm 9	7	1-39	39 \pm 31	7	5-113
Mean \pm SD (months)	14 \pm 17	9 \pm 9	7 \pm 10	12 \pm 14	—		Stable (27)	7 \pm 10	3	2-46	7 \pm 10	3	2-46
Range (months)	1-50	1-39	2-46	1-67	—		PROG (71)	12 \pm 14	6	1-67	26 \pm 23	6	2-119

the 4 bone-regressed metastases, 1 was still in remission 365 days later when the patient died from heart infarction; the remaining 3 patients presented new bone metastases 245 to 1,410 days later (Table V). In contrast, patients in whom we noticed regression of lung (4/13) or lymph node metastases (2/5) remain in remission: only 3/13 and 1/5 relapsed in the same organ between 103 and 517 days. For the remaining regressed lesions that subsequently resumed progression, organ incidence rates in liver, bone, brain and lymph nodes were 10% to 30% (Table V).

We observed regressions not only of small tumors but also of very important tumor loads. For instance, as shown in the chest X-rays of a 53-year-old woman (patient 10,088, Table V, Figs. 4,5), the 1 to 2 cm lung metastases went in CR 314 days after the beginning of immunotherapy and remained in remission for 1,668 days. An example of a more important tumor load regression is the case of a 80-year-old woman (patient 67,188) who experienced, 1 year after starting immunotherapy, a CR of multiple lung metastases (Fig. 6) and a PR of massive liver metastases, the latter treated with 2 injections of alcohol within a 3-month period (Figs. 7,8). Also of interest is a 62-year-old man (patient 10,074) with 3 lung metastases who went into remission following 3 months of immunotherapy. He relapsed 1,591 days later at the abdominal lymph node level.

Survival

Survival was measured from the beginning of therapy to the date of death or to the last date that the patient was known to be alive. Of 122 patients, 56 died and 66 had censored survival times. Survival curves observed in the 122 treated patients and the 89 historical controls are shown in Figure 9. The median survival of treated patients (28 months) was 3.5 times higher than that of controls (7.5 months).

Figure 10 shows the survival of group 99 and group 122 in comparison with the 10 patients in group IL-2-R treated with rIL-2. The curves are quite similar, without a statistically significant difference.

No difference in survival was noticed following the classification of patients with synchronous or metachronous disease.

Better survival was observed when the evaluation took into account the addition of inhaled IL-2 to the standard intralymphatic treatment. Indeed, a statistically significant difference ($p < 0.05$ Wilcoxon's test, Cox's test and log rank test) was observed in both groups (groups 99 and 122, Fig. 11), with a clearly different median (26 vs. 48 months), though survival at 6 years appeared similar (34% vs. 38%). Long survivors of the group treated only intralymphatically were censored at the beginning of IL-2 inhalation, so their survival time was computed only for the period of the intralymphatic injections and they were not included in the IL-2-inhalation group.

Figure 12 illustrates survival according to histological grading. As expected, the G2 and G3 patients show a better survival rate than G4 patients (Wilcoxon $p < 0.05$). No difference was observed between G2 and G3 and/or all groups and/or unknown histological grading.

IFN and survival

IFN does not appear to improve survival. Indeed, 28/34 treated patients died compared to 59/88 non-treated patients (Fisher's exact test $p = NS$).

EBRT and clinical response

Table VII shows the clinical results observed from both EBRT-treated and non-treated patients. Some patients were radiated at multiple sites and on different dates during follow-up. Fifteen patients (24%) were radiated for lung metastases with a total dose of 20-30 Gy. Also, 6, 2 and 5 patients were radiated for lymph node, pleural and brain metastases with 30, 30 and 40-50 Gy,

TABLE V—CLINICAL RESPONSE

Patient	Clinical response	Site of response	Days from immunotherapy	Length of remission (days)	Site of relapse	Follow-up from immunotherapy (days)	Status
10,712	CR	Liver + lung	610	517	Lung	1,413	Alive
67,154	CR	Lung	80	315+	No	395	Alive
10,088	CR	Lung	314	1,668	Brain	2,793	Dead
10,010	CR	Kidney	99	2,734	Kidney	3,654	Alive
10,719	CR	Lymph node	925	1,064+	No	1,989	Alive
10,312 ^{1,2}	CR	Lymph node	775	2,095+	No	2,870	Alive
10,082 ¹	CR	Lung	174	134	Bone	458	Dead
10,030 ³	CR	Bone	193	1,410	Bone	2,123	Dead
66,684	CR	Lung	1,532	249	Bone	1,781	Alive
10,074	CR	Lung	105	1,591	Lymph node	1,696	Dead
10,064 ³	CR	Lymph node	30	103	Lymph node	475	Dead
10,006	PR	Lymph node	303	146	Liver	811	Dead
67,188	PR	Lung/liver	273	79+	No	352	Alive
10,008 ³	PR	Lymph node	170	82	Pleural	151	Dead
66,897	PR	Lung	162	74	Brain	328	Dead
66,778	PR	Lung	1,176	337	Lung	1,460	Alive
10,602	PR	Lung	131	1,984+	No	1,816	Alive
10,625	PR	Lung	206	259	Lymph node	1,480	Dead
10,718	PR	Lung	156	230	Lung	1,676	Alive
10,047	PR	Bone	228	401	Bone	3,045	Alive
66,803	PR	Bone	299	365+	No	664	Dead ⁴
67,036	PR	Lung	297	280+	No	577	Alive
10,075	PR	Bone	230	245	Bone	245	Dead
66,971	PR	Kidney	37	127	PROG	154	Dead

¹10,312: (i) rIL-2 progressed twice in 3 months, (ii) IL-2n (natural IL-2) PR then CR and no relapse in 6 years. 10,082: (i) IL-2n PROG, (ii) rIL-2 + 5-fluoro-2'-deoxyuridine (FUDR), CR then PROG.—²Only treatment with IL-2n is reported.—³Also intralesional injection in some superficial lesions.—⁴Heart infarction.

TABLE VI—MEAN DURATION AND RANGE OF CLINICAL RESPONSE AND ITS EVOLUTION

Response	Type	Months	Range
CR (n = 11)	PROG (n = 8, 73%)	34 ± 31	3–90
	CR (n = 3, 27%)	38 ± 29	10–69
PR (n = 13)	PROG (n = 9, 69%)	7 ± 4	2–13
	PR (n = 4, 31%)	22 ± 29	11–59

Number of patients treated, 122.

respectively. No significant statistical difference in either clinical response or survival rate was observed between EBRT-treated and non-treated groups. Among the 24 patients who showed a clinical response (CR+PR), 13 were radiated. Among them, clinical response was noticed in 5 cases at the radiated sites (4 bone, 1 lung). Subsequently, 3/4 bone lesions resumed progression at the same site; the fourth patient (patient 66,803) died of heart infarction without noticeable progression. The fifth patient (patient 10,625) resumed progression in the mediastinal lymph node.

DISCUSSION

Long-term survival in MRCC is extremely poor. Because of the variability of the data reported in the literature, it was necessary to evaluate the results of IL-2 treatment in as many reliable studies as possible, without pretending to be exhaustive. This was necessary to compare the results observed using the intralymphatic route. In particular, it was of interest to examine the various injection routes of IL-2 (s.c., i.v., in bolus or continuous, inhaled), as well as the use of LAK cells and IFN. It was also important to consider the response rate, median survival and survival in long-term follow-up. A survey of the non-treated historical controls was also necessary. Although the number of treated patients using the intralymphatic route was relatively small, the comprehensive analysis of the summarized reports renders, in our opinion, the data comparable.

The clinical response in 79 studies published so far, including 3,586 MRCC patients treated with IL-2-based therapies, is 19.6%,

the median survival ranges between 8.6 and 20 months and the median response duration ranges between 6.5 and 17 months (Table VIII).³ Furthermore, survival 3 years after the beginning of treatment is between 19% and 31%.^{24–29} 5–18% being the survival of non-treated patients (Table VIII).^{30,31} Sporadic long-term survivors are observed only among patients who underwent complete remission.^{29,32}

In the past 14 years, we have treated 122 MRCC patients with intralymphatic injection of non-recombinant IL-2, LAK cells, IFN and TF. Our results, compared with those reported in the literature and with our historical controls, show a similar response rate (19.7%) but better survival (45% at 3 years, 39% at 5 and 30% at 7), as well as better median survival (28 months) (Table VIII, Fig. 9). The Kaplan-Meier curve shows a survival rate of >25% of patients 11 years after the beginning of treatment (Fig. 9).

Of no less importance is the fact that the protocol is practically devoid of adverse side effects. Indeed, in 1,692 intralymphatic administrations of 250–490 IL-2 U, we observed in <2% of cases chills, fever (<38°C) and/or hypotension. The 10,315 IL-2 inhalations caused in <2% of cases dyspnea or hypertension (0.2%) (Table III). No medication was necessary, the side effects disappearing after discontinuing IL-2 administration. Furthermore, quality of life was not affected, and this is in agreement with the observations of Heinzer *et al.*³³ using inhaled IL-2. In contrast, the IL-2 regimens currently in use in other centers induce severe adverse clinical side effects, as measured using the World Health Organization classification system.³⁴ Grade 3 and grade 4 toxicity levels have been observed in a substantial number of patients, and 5.2–9.4% of patients have died during the treatment periods or within a month following treatment from causes unrelated to renal-cell carcinoma.²⁶

The scarcity of side effects is due to the low IL-2 dosage. In 14 years, we have administered a total of 4,006,773 U non-recombinant IL-2 to 122 MRCC patients. This is less than the amount of IL-2 usually administered in 1 day to 1 MRCC patient under the current rIL-2 protocols. Of almost equal importance is the fact that the lowered IL-2 dosage results in a substantial decrease in the cost

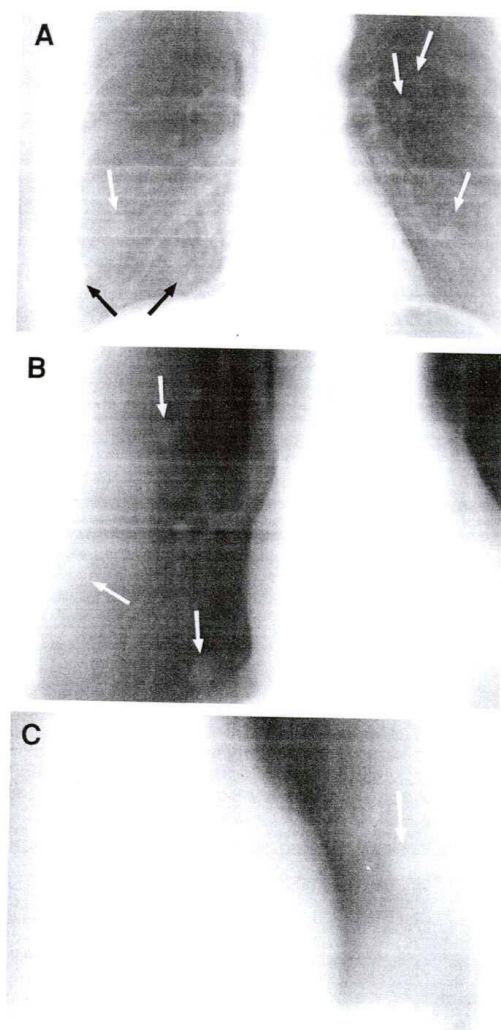


FIGURE 4 – Bilateral lung metastases (arrows).

of treatment, *i.e.*, from US \$6,000 to US \$200 for a 3-month period.

The duration of treatment in the present study, up to 135 months, is longer than in any other IL-2 protocol. Most patients refused to discontinue IL-2 administration; hence, they continued the treatment when in CR or PROG. We restricted the duration of the protocol in only the first 15 patients. Indeed, when we started observing relapses, we considered its continuation pertinent, both for preventing additional relapses and for slowing down tumor progression. Progression appeared to be faster in patients in whom we interrupted the IL-2 than in those in whom administration was continued.

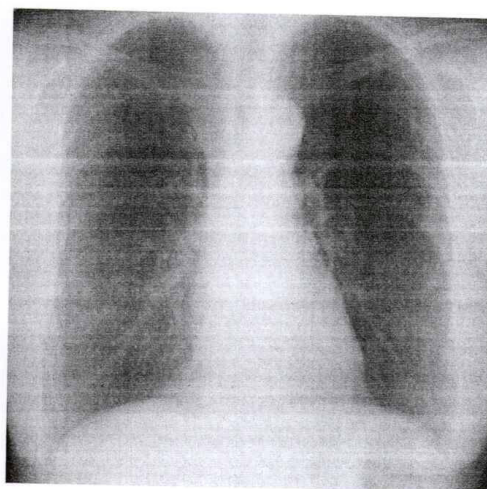


FIGURE 5 – Bilateral lung metastases completely disappeared 10 months after the beginning of immunotherapy.

All patients referred to the Bologna center and meeting the inclusion criteria were consecutively treated. Although the number of patients of known grade 4 was low ($n = 7$, 6%), 56 (46%) were grade 3 and the survival of the 39 patients of unknown grade was better than that of grade 4 but worse than that of grades 2 and 3; it is possible to speculate, among the unknown histology group, the presence of an additional number of patients with grade 4. As reported,^{4,35} a better correlation was found between high performance status and clinical response. At any rate, the selection bias both for low-grade tumor and performance status, although not confirmed, cannot be excluded.

TF administration has been previously explored in MRCC patients, both as a sole treatment¹² and in combination with chemotherapy and/or bacillus Calmette-Guérin (BCG).³⁶ Using strict response criteria for measurable disease in 60 treated patients, objective responses were seen in 14% to 22% of cases. Further trials are warranted using more sophisticated immunotherapy.³⁶

IFN- α -2a, as sole treatment or in combination with chemotherapy, has also been extensively evaluated with contrasting results. The latest studies show a response rate of 13%, with significantly improved survival (1 to 2 years) compared to patients treated with medroxyprogesterone.³⁷ IFN- γ has also been used, and a randomized study showed a response rate of 5%.³⁸ Nonetheless, these data were not confirmed by another randomized study, which showed no difference from the placebo group.³⁹ Similarly, in our patients, the beneficial role of IFN was not established: no significant improvement of survival was observed in treated compared to non-treated patients.

On the contrary, inclusion of very low doses of IL-2 by inhalation appears to significantly improve survival rates compared to non-IL-2 inhalers (Wilcoxon $p < 0.05$). One could speculate that the simultaneous use of 2 different routes for low-dose IL-2 administration might produce better results.

Administration of LAK cells alone in the bloodstream of patients who underwent surgery for liver cancer resulted in a significant decrease of tumor relapse and increased survival in a 6-year follow-up.⁴⁰ It is plausible that the sole injection of

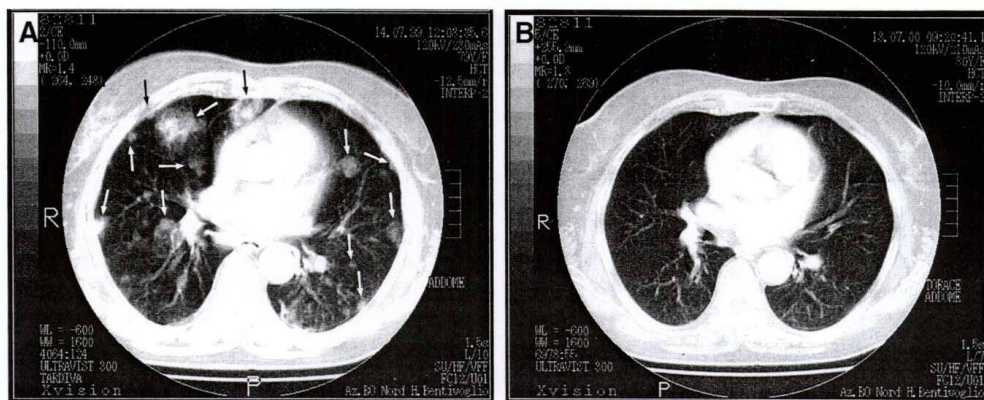


FIGURE 6—An example of an important tumor load regression is the case of this 80-year-old female patient (code 67,188) who experienced 1 year after starting immunotherapy CR of more than 15 lung metastases (arrows) measuring 1 to 4 cm in diameter. PR was also observed in liver metastases.

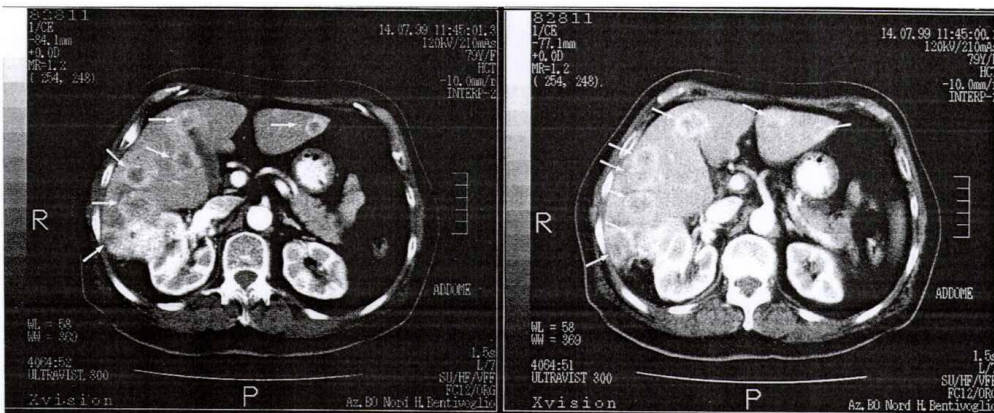


FIGURE 7—Seven of the 17 metastases present in the various layers of the liver before immunotherapy: the sum of their perpendicular diameters was 69.1 cm.

LAK cells in lymphatic vessels could activate a similar clinical response.

The usefulness of EBRT is not evident. Although the data of the 57 EBRT-treated patients are heterogeneous, no apparent advantage was noticed in either clinical response or survival rate compared to patients who were not EBRT-treated. The aim for EBRT was, for some patients, pain palliation; for others, we hoped to obtain a synergy with the immunotherapy by increasing the antigenicity of the radiated tumor cells.²⁰ Although our study was not carried out in a randomized fashion, by reviewing retrospectively our results from a heterogeneous sample of patients, our data confirm the absence of efficacy of EBRT as an adjuvant treatment to IL-2 immunotherapy in MRCC and are in agreement with those of Redman *et al.*⁴¹ and Ryan *et al.*⁴² though others have reported encouraging results.⁴³

Following our first observation,¹⁰ some phase I studies with intralymphatic rIL-2 have been initiated.^{44,45} However, the authors used high doses of rIL-2 and observed severe clinical side effects, similar to those seen when an easier route of administration (s.c. or i.v.) was used. For this reason, intralymphatic injections were quickly abandoned.

In conclusion, it appears that long-term intralymphatic administration of non-recombinant IL-2 at very low doses together with LAK cells and simultaneous i.m. administration of TF is safe and produces clinical improvement and a survival rate among the best of those reported in the literature. Moreover, it offers good quality of life to MRCC patients and markedly reduces the cost of treatment. We believe that IL-2 today is the most effective form of MRCC immunotherapy and should be further studied since some aspects of its mode of action are not completely understood. For



FIGURE 8 – The only remaining metastasis after 1 year of immunotherapy: the sum of perpendicular diameters was 15 cm. This shows a regression of >75% with respect to the beginning of treatment, taking into account the parameters established for PR. In addition, the lesion appears mostly necrotic.



FIGURE 9 – Overall survival of treated patients ($n = 122$) vs. historical controls patients ($n = 89$).

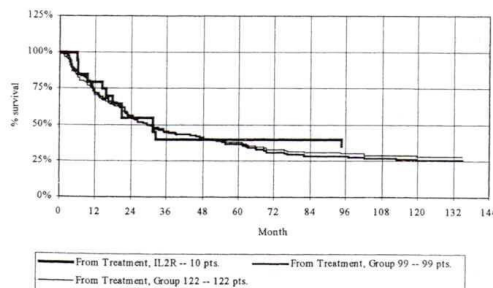


FIGURE 10 – Survival of patients according to groups (all $n = 122$, $n = 99$, group IL-2R $n = 10$).

instance, a faster progression of the disease was observed when the treatment was interrupted.

Be that as it may, a thorough evaluation of the present approach needs a detailed study of the different immunotherapeutic agents used, *i.e.*, both natural and recombinant IL-2 and LAK cells

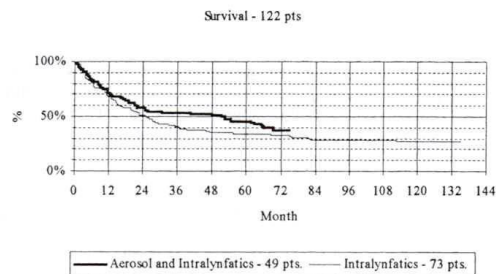


FIGURE 11 – Survival of patients ($n = 122$) treated only with intralymphatic IL-2 in comparison with patients treated with intralymphatic and inhaled IL-2. Patients treated by the intralymphatic and inhalation protocol were censored at the beginning of the IL-2 inhalation treatment (inhalation, 37 censored and 12 dead; intralymphatic injection, 40 censored and 33 dead). Survival was evaluated using Wilcoxon's, Cox's and the log rank tests with the same results ($p < 0.05$).

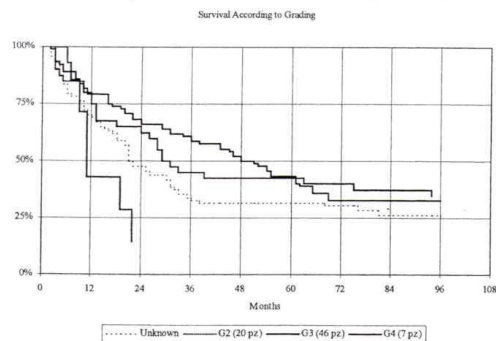


FIGURE 12 – Survival of patients ($n = 99$) according to histological grading (Wilcoxon's test: G2 vs. G3, NS; G3 vs. G4, $p < 0.05$; G2 vs. G4, $p < 0.05$).

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