Original Articles

Allogeneic Gene-Modified Tumour Cells in Metastatic Kidney Cancer. Report II

(vaccine / gene-modified / immunotherapy / IL-2 / MRCC / renal cancer)

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Abstract. In a limited study, comprising only ten patients, we have previously reported that allogeneic irradiated RCC-cell-line cells, engineered to produce IL-2 (ACHN-IL-2), admixed with autologous metastatic formalin-treated tumour cells were used to vaccinate MRCC patients in progression of disease and also receiving IL-2 immunotherapy. The cells, admixed to autologous TC, were administered subcutaneously. We now report an extended study on thirty patients and one hundred thirty-one controls. Patients received 4–20 injections (mean 10 ± 4), containing an average of $92 \times 10^6 \pm 45 \times 10^6$ ACHN-IL-2 transfected cells (a minimum of 25×10^6, and a maximum of 200×10^6). Autologous TC, admixed to allogeneic, were also administered by 4–16 s.c. injections (mean 7 ± 3), i.e. a total of 12×10^6–160×10^6 cells. Vaccination was administered during 73–1451 (307 ± 316) days, and the follow-up continued for 1122 ± 1240 days (106–5137). Throughout this period, the patients continued receiving the previously set immunotherapy treatment. No adverse side effects related to the treatment were noticed. One complete and four partial tumour responses were observed, as well as nine cases of stable disease. Thirteen patients died in the treated group (43%) and 63 (44%) in the control group. Responding patients resumed progression in 4–11 months and died 18 and 36 months after beginning the vaccine therapy. The Gehan Wilcoxon's test showed a significantly ($P < 0.01$) better survival in the vaccinated patients compared to that of the controls. Thus, we confirm, in an increased number of patients and an extensive follow-up, that our vaccination protocol is safe, devoid of adverse side effects, and promising.

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Abbreviations: ATC – autologous tumour cells, CR – complete response, IL-2 – interleukin-2, i.m. – intramuscularly, LAK – lymphokine-activated killer cells, MRCC – metastatic renal cell cancer, PB – peripheral blood, PBL – peripheral blood lymphocytes, PR – partial response, PROG – tumour progression, PS – performance status, pt(s) – patient(s), s.c. – subcutaneous, sd – standard deviation, TAA – tumour-associated antigens, TNM – tumour, node, metastasis, U – unit, y – year(s).

It is well known that patients suffering from metastatic renal cell cancer (MRCC) have a poor prognosis and the treatment represents a very difficult challenge in urological oncology (Hrushsky and Murphy, 1977; Elson et al., 1988). It is also commonly accepted that, because the response rate is in average 20% (Bukowski et al., 1997, 2000; Vogelzang et al., 1998; Pizza et al. 2001, 2002), the most promising therapy for MRCC is immunotherapy. However, since 80% of patients go in progression, new therapeutic approaches are needed; an "old" tool is now emerging reshaped: tumour vaccination.

The concept of tumour vaccines is not new and although no definite proof is offered concerning the

Table 1. Age, sex, histology grading, anatomical site of metastases, duration of immunotherapy and follow-up of treated patients and controls

Parameter	No. treated pts		No. control pts	
Patients	30		131	
Sex F.	7	(23.3%)	3	(28.2%)
Sex M.	23	(76.7%)	94	(71.8)
Age (years)	35-70	(53±9)	20-82	(63±11)
Pts with synchronous metastases	9	(30%)	63	(48.1%)
Pts with metachronous metastases	21	(70%)	68	(51.9)
Months of appearance of metastases following nephrectomy	6-148	(42±34)	1-169	(49±45)
Performance status (Karnofsky)				
PS 40-70	6	(20%)	15	(11,5%)
PS 80-100	24	(80%)	116	(88,5%)
Stage IV	30		131	
Histology grading:				
G1	0		1	(0.8%)
G2	5	(18.5%)	24	(18.3%)
G3	19	(70.4%)	45	(34.4%)
G4	3	(11.1%)	10	(7.6%)
G?	3	(10%)	51	(38.9%)
Site of metastases:				
Bone	21	(70.0%)	64	(48.9%)
Brain	6	(20.0%)	12	(9.2%)
Kidney	6	(20.0%)	17	(13.0%)
Liver	4	(13.3%)	22	(16.8%)
Lung	20	(66.7%)	79	(60.3%)
Lymph nodes	14	(46.7%)	55	(42.0%)
Muscle	5	(16.7%)	10	(7.6%)
Pancreas	2	(6.7%)	5	(3.8%)
Pleura	1	(3.3%)	10	(7.6%)
Renal loggia	5	(16.7%)	7	(5.3%)
Skin	4	(13.3%)	5	(3.8%)
Suprarenal gland	8	(26.7%)	18	(13.7%)
Miscellaneous	4	(13.3%)	14	(10.7%)
Multiple organ involvement	2	(90.0%)	89	(68.2%)
Pts with solitary metastasis	3	(10.0%)	42	(29.5%)
Pts with > 1 metastasis	30	(100.0%)	100	(76.3%)
Months of concomitant immunotherapy	4-166	(37±45)	0-113	(19±24)
Months of follow-up	4-185	(43±48)	4-114	(22±25)

presence of tumour-specific antigens in kidney cancer cells (Ueda et al., 1981; Oosterwijk et al., 1986), many different approaches have been suggested with some encouraging observations (Belldegrun et al., 1993; Pizza et al., 1999, 2003; Schwaab et al., 2000; Dillman et al., 2001). We report here a continuation of our previous studies describing observations obtained with 30 patients and a larger group of control patients. The treatment was administered following a 3-year clinical experimental protocol authorized by the Ministry of Health and the members of the local Ethics Committee.

Material and Methods

Transfected allogeneic cell line (ACHN)

The ACHN tumour cell line from ATCC, American Tissue-Type Culture Collection, established from a kidney cancer was obtained from the Emilia-Romagna and Lombardia Regions Experimental Institute of Zooprophylaxis. The transfection and its ability to produce IL-2 after irradiation have been already reported (Pizza et al. 1999; 2003). Briefly, a human-IL-2 expression vector, pcDNA-I Neo-IL-2 (7683 bp length), has been prepared by insertion of IL-2 cDNA (683 bp length) in *Hind*III/*Bam*HI sites of the plasmid of the polylinker pcDNAINeo (InVitrogen™, Invitrogen S.R.L., San Donato Milanese, Italy). The amount of 10^7 ACHN cells has been transfected with 10 μg of pcDNA-hIL2 using the $CaPO_4$ technique (Graham and Vanderb, 1973; Cavallo et al., 1993). Resistant clones were isolated, and IL-2 production was evaluated using the CTLL-2 line (Gillis and Watson, 1981), or the PHA- (Difco, Detroit, MI) and IL-2-conditioned human blasts (Pizza et al., 1984). Cells were irradiated with a 60-cobalt bomb at 100 cGy/min to a total of 40–60 Gy. They were unable to replicate, but the *in vitro* IL-2 production appeared to continue for 32 days, with a mean concentration of 230 pg/day/10^6 cells as assessed using the ELISA kit "Biotrak"® (Amersham, Life Science, Little Chalfont, UK) (Pizza et al., 2003). Tumorigenesis in nude mice was negative and their *in vitro* ability to replicate nil during an observation period of 60 days. At the end of this period all cells were dead.

Autologous metastatic tumour cells (ATC)

ATC were obtained from patients' metastases during surgery carried out mainly because of pathologic bone fractures as described by Pizza et al. (2003). Briefly,

Table 2. Stage and grade of disease, Karnofsky's index, months from nephrectomy of treated patients

Patient number	Patient code	Karnofsky's PS	Grading	Stage	Months from nephrectomy
1	10010	100	3	4	0
2	10033	100	3	2	6
3	10602	100	3	2	9
4	10627	100	3	?	68
5	66684	100	3	2	36
6	66778	100	3	4	148
7	66942	100	4	2	33
8	66955	90	?	3	92
9	66988	100	2	4	17
10	67024	70	2	4	38
11	67025	80	3	3	22
12	67139	70	3	3	42
13	67152	70	3	3	25
14	67177	100	3	?	36
15	67202	100	?	4	0
16	67222	90	3	2	15
17	67229	100	4	2	76
18	67250	80	3	4	0
19	67261	80	?	2	30
20	67283	60	3	2	16
21	67297	60	3	4	0
22	67299	100	3	4	0
23	67322	100	2	3	23
24	67338	80	4	4	0
25	67358	90	3	2	27
27	67364	50	3	2	42
27	67402	90	3	3	0
28	67410	90	2	4	0
29	70028	100	2	3	79
30	70037	90	3	4	0

"Stage" is referred to the time of nephrectomy. "*Zero months from nephrectomy*" means metastasis synchronous with the kidney tumour.

tumour samples were immediately processed under sterile conditions, washed 3–4 times with saline, the necrotic areas discarded, then passed through a metal mesh (49G), gently washed again to obtain a tumour cell suspension. Subsequently, cell samples were suspended in a formalin buffer (1v/25v) and left at room temperature overnight. They were further washed 3 times, suspended in saline at a concentration of 5×10^6/ml, and stored in 1–2 ml aliquots at +4°C (Pizza et al., 1980). A few days later, two samples underwent sterility tests, slides were prepared for histological examination, and the cell types were counted.

Patients' selection and controls

Inclusion criteria were MRCC in progression of disease in spite of continuing immunotherapy (Pizza, et al., 2001), confirmed histological diagnosis, and patient's written informed consent. The exclusion criteria were: age less than 18 years, life expectancy less than one month, Karnofsky's index less than 40, presence of acute viral, bacterial and/or autoimmune diseases, serum creatinine > 0.2 g/litre, cardiac infarction during the last 2 months, cardiac failure requiring medication. Patients who needed cortisone medication were also excluded. Thirty nephrectomized stage IV and in progression of disease in spite of the immunotherapy treatment MRCC patients entered the vaccine protocol. All patients were treated and monitored in our Institution. Their sex, age, appearance of metastasis from the date of nephrectomy and organ involvement, grade and performance status (PS), according to Karnofsky, are reported in Tables 1–3. One hundred thirty-one, stage IV nephrectomized MRCC patients, treated with the same protocol of immunotherapy and in progression of disease, represent our controls. All patients were treated and monitored in our Institution. Their sex, age, appearance of metastasis from nephrectomy and organ involvement, grade and PS according to Karnofsky are reported in Tables 1 and 3.

Vaccine treatment protocol

The protocol consisted of s.c. injections every 10–14 days for 4–6 times, during a 45–60 days cycle of 10^7 allogeneic transfected and irradiated ACHN-IL-2 cells admixed to $5–10 \times 10^6$ ATC suspended in one millilitre of saline. The duration of a treatment cycle was initially intended for 45–60 days, with disease restaging at 1, 2, and 4 months from the beginning of the vaccine therapy. Persistence of disease or CR was followed by an additional cycle. In case of disease progression, the treatment was discontinued, unless the patient wanted to pursue it for an additional cycle. Treatment was administered mostly on an outpatient basis. Because of the absence of adverse side effects in the previous set of treated patients, no premedication was administered. The injection site was the inguinal region, usually the same in each patient. The previously administered immunotherapy (Pizza et al., 2001) remained a concomitant treatment.

Evaluation of the clinical response

The clinical response was evaluated by considering both the overall survival rate (Kaplan-Meier curve) and

Table 3. Percentages of various performance status indices, grade and stage of treated patients and controls

Parameter	Patients	Controls
Performance status (Karnofsky) (%)		
50	1 (3%)	0 (0%)
60	2 (7%)	10 (8%)
70	3 (10%)	5 (4%)
80	4 (13%)	25 (19%)
90	6 (20%)	38 (29%)
100	14 (47%)	53 (40%)
Total	30 (100%)	131 (100%)
Grading (known)		
1	0 (0%)	1 (1%)
2	5 (19%)	24 (30%)
3	19 (70%)	45 (56%)
4	3 (11%)	10 (13%)
Total	27 (100%)	80 (100%)
Stage (known)		
I	0 (0%)	5 (5%)
II	10 (36%)	18 (19%)
III	7 (25%)	22 (23%)
IV	11 (39%)	51 (53%)
Total	28 (100%)	96 (100%)

the response rate at the level of measurable metastases according to conventional parameters as already described (Pizza et al., 2003). Every six months, we performed total body scintigraphy for the detection of new bone lesions. The serum and urine biochemical parameters (Na^+, K^+, transaminases, bilirubin, creatinine, cholesterol) were evaluated monthly, and electrocardiograms were carried out bi-monthly.

Lymphocyte Stimulation Test (LST)

Twenty-seven treated patients agreed to donate periodically 30 ml of blood for *in vitro* studies, i.e. lymphocyte stimulation in the presence of tumour antigens (Pizza et al., 1980; 2003). Briefly, peripheral blood lymphocytes (PBL) were collected on F-H gradient, washed 3 times with RPMI-1640 culture medium. The amount of 5×10^5 lymphocytes was incubated in 5% CO_2 humidified atmosphere for 6 days in 0.2 ml of medium supplemented with 10% autologous heat-inactivated (30 min at 60°C) serum in the presence of ACHN and, when available, formalin-treated ATC at four different ratios: 50 : 1, 10 : 1, 2 : 1, 1 : 1. Triplicate cultures were also prepared in the presence of 1 μg of PHA-P (Difco). The lymphocyte response was evaluated by methyl-^{3}H-thymidine incorporation, which was added 24 h before harvesting (1 μCi/ml; spec. act. 20 μCi/mM, Amersham Biosciences, Little Chalfont, UK) with a harvester cell system on paper glass filter disks. Cultures were made in triplicate (Falcon 3040 BD, Milan, Italy). The results were evaluated as reported in the statistical analysis LST section.

Statistical analysis

Survival. As regards survival, the most important indicator considered was the right-censored survival curves. For its evaluation we used the Kaplan-Meier method (Kaplan and Meier, 1985). The survival of treated patients was compared to that of 131 control patients. For the comparison, Wilcoxon's test was used (Kalbfeisch and Prentice, 1980).

LST. For the statistical evaluation of LST we designed a general linear mode analysis (GLM) for assessing the effect of independent variables on the dependent ones. The latter are the logarithms of the DPM values obtained in the presence of certain mitogens. DPM is distributed in lognormal mode. The independent variables considered were: control DPM (logarithm), day after vaccination (linear), before/after vaccination (classificatory variable with 2 values), autologous or AB serum (classificatory variable with 2 values), patient (classificatory variable with many values for eliminating the individual influence on the model), sample (classificatory variable with 4 values; for the PHA analysis these were the concentrations of 0.1, 1 and 3 μg/l and the use of ConA30, for the tumour antigens cells the ratios mitogen : lymphocytes = 1 : 1, 1 : 2, 1 : 5 and 1 : 50), mitogen tumour antigen (autologous/ACHN, classificatory variable with 2 values). The mitogens used are the following: PHA at 3 days, with concentration of 0.1, 1 and 3 μg/l, and ConA30. PHA was used on day 5 as internal control for the other mitogens and at a concentration of 1 μg/l. For each mitogen three different conditions were analysed: the comparison of values preceding vaccination to the values after vaccination, without considering the days after vaccination, since these values were not present before; comparison of the data preceding vaccination, without considering the variables before or after and the days number; comparison of the values observed after vaccination, without considering the variables before or after. At the beginning, the statistical model considered all the above reported independent variables; the non-significant ones were eliminated from the successive evaluations. At the end of the study, we obtained a model with only significant independent variables. All observed results are reported.

Results

Preliminary evaluations

Because it is an open retrospective study, we performed some preliminary evaluations on the homogeneity and comparison of treated patients and control groups. The assessed variables were: Karnofsky index, grading, stage, age of appearance of metastases, months to appearance of metastases from nephrectomy, number of metastatic sites (Table 4 – Basic statistics). Only the age of patients at the time of diagnosis of metastases,

Table 4. Basic statistics performed for confrontation of the patients and controls

Variable	Mean pats	Mean cont.	t-value	df	p	N° pats	N° cont.	SD pats	SD cont.	F-ratio variances	p variances
Karnofsky	88.000	89.083	-.427	159	.669	30	131	14.715	11.990	1.506	.126
Grading	2.925	2.800	.887	105	.375	27	80	.549	.663	1.458	.279
Stage	2.833	2.374	1.447	159	.149	30	131	1.147	1.647	2.061	.025
Age metast.	53.03*	63.12*	-4.68*	151*	**.000***	30*	123*	9.159*	10.88*	1.741*	.028*
Mo.s to met	41.904	49.089	-.670	86	.504	21	67	33.867	45.245	1.784	.148
N.met.sites	3.333*	2.427*	3.13*	159*	**.002***	30*	131*	1.470*	1.419*	1.071*	.762*

pats – treated patients, cont. – control patients, df – degrees of freedom, N° – valid number, SD – standard deviation, stage and grading at the time of nephrectomy, Age metast. – age at the time of metastasis, * – significant statistic difference (Wilcoxon's P paired test), Mo.s to met – months from nephrectomy to the appearance of metastases on metachronous patients, N.met.sites – number of metastases

Group	Synchronous	Metachronous	Total
Patients	9	21	30
Controls	63	68	131
Total	72	89	161

$\chi^2 = 2.54$, not significant; P = 0.11, not significant

and the number of metastatic sites appeared significantly different. Both were in favour of the control group, thus rendering the invalidation of the analysis impossible in case the treated group showed better results.

Treatment

Thirty MRCC patients were treated. Their sex, age, stage, grade, time of appearance of metastases from date of nephrectomy or of progression and organ involvement are reported in Tables 1–3. The number of injections and days of vaccine treatment are reported in Table 5. Three hundred four injections were administered (5–20 for each patient with a mean of 10 ± 4), containing 2778×10^6 ACHN-IL-2 transfected cells (with a mean of $92.6 \times 10^6 \pm 45.1 \times 10^6$ per patient), and a minimum of 25×10^6 and maximum 200×10^6 each. As regards ATC, 204 injections were administered (3–16 for each patient with a mean of 7 ± 3), containing 1891×10^6 cells (with a mean of $63.6 \times 10^6 \pm 34.4 \times 10^6$ per patient), a minimum of 16×10^6 and a maximum 160×10^6 each. The length of the administration period was 73–1451 days, with a mean of 307 ± 316. The entire follow-up was of 1122 ± 1240 days (106–5137), during which patients continued the concomitant immunotherapy treatment administered previously. No early or late adverse side effects were noticed during the entire observation period.

In vitro studies

Twenty-seven patients accepted to donate 30 ml of peripheral blood for evaluating their cell-mediated immune reactivity (CMI) before and after vaccination. The results are reported in Tables 6 and 7. The values observed before vaccination, both as regards the response to PHA or tumour antigens, are always less than those observed after vaccination, thus confirming a positive stimulatory activity of the vaccination on the CMI ($P < 0.0001$). It is worth mentioning here that these values, although increased during the period immediately preceding vaccination, subsequently showed, at the end of the vaccine administration, a decreasing trend ($P < 0.01$). The values observed using the ACHN as mitogen are always higher than those observed using ATC, probably because of a diminished antigenicity of the autologous versus the allogeneic tumour cells ($P < 0.0001$).

Clinical results

Clinical results of treated patients, type, site and organs of the response, as well as its duration and evolution are shown in Table 8. Six treated patients had a performance status between 50–79 and 24 between 80–100. In a similar range, the number of control patients was respectively 15 and 116. No early or late adverse side effects were noticed. During the observation period we noticed 1 complete response (CR) (lung and lymph-nodes sites), 4 partial responses (PR) (1 liver, 1 bone and 2 in lung sites), 9 patients in stable disease (SD) condition, with duration respectively of 346, 137 ± 80, 569 ± 871 days (Table 8). Sixteen patients

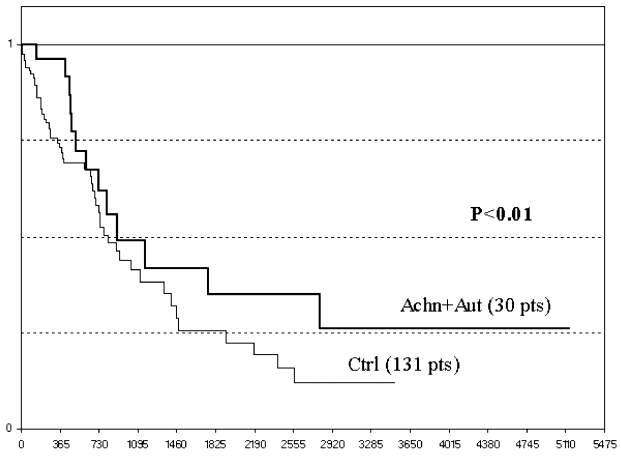

Fig. 1. Kaplan-Meier survival-curve of treated patients and controls

Table 5. Numbers of vaccine cells administered and follow-up for each patient

Patient's number	Patient's code	Days of vaccination	N° ACHN administr.	N° ATC administr.	ACHN x 10^6	ATC x 10^6	Follow-up days
1	10010	142	5	5	25	50	2806
2	10033	212	13	10	130	100	5137
3	10602	761	10	9	90	90	3611
4	10627	724	13	13	130	130	2203
5	66684	1451	19	11	190	103	3343
6	66778	382	16	16	160	160	2861
7	66942	512	9	8	90	68	725
8	66955	176	7	7	35	70	438
9	66988	1097	8	5	80	39	1760
10	67024	454	17	4	135	24	1168
11	67025	171	9	7	75	61	739
12	67139	402	13	7	106	70	469
13	67152	239	8	6	56	60	599
14	67177	520	8	8	56	80	611
15	67202	540	4	4	40	40	845
16	67222	86	8	5	80	38	238
17	67229	224	14	2	140	10	393
18	67250	98	8	8	80	80	128
19	67261	648	11	11	110	70	746
20	67283	392	20	2	200	12	455
21	67297	74	4	4	40	40	140
22	67299	216	14	10	140	100	912
23	67322	154	8	4	70	16	357
24	67338	240	8	4	80	40	481
25	67358	323	13	3	120	30	806
27	67364	99	8	8	80	80	517
27	67402	163	9	7	70	70	430
28	67410	218	7	6	70	60	308
29	70028	232	8	7	70	70	334
30	70037	73	5	3	30	30	106
Total	**-**	**11023**	**304**	**204**	**2778**	**1891**	**33666**
Mean ± standard deviation		**307 ± 316**	**10.1 ± 4.2**	**6.8 ± 3.2**	**92.6 ± 45.1**	**63 ± 34.4**	**1122 ±1240**

N° ACHN (ATC) administr. – number of ACHN (ATC) administrations

showed progression in 241 ± 190 days. Of the responding patients, 2 (PR) are still in remission and only one with stable disease progressed (Table 8). Thirteen treated patients died for causes linked to tumour progression and 17 are still alive. The survival curve (according to Kaplan-Meier) of treated patients and controls is reported in Fig. 1. Survival time was measured from the beginning of therapy to the last date that patients were known to be alive. The patients had a mean age of 63 (min. 52, max. 71). Of the 131 controls (the survival was measured from the beginning of progression during the administration of immunotherapy), 52 died, and 79 had censored survival times. The I and III quartile of the patients' group are 11.8 and 22.5 months, versus 4.8 and 22.4 months in the control group. From the beginning of the vaccine therapy, the median survival of the treated patients is 18.9 months versus 12.2 for the controls. Despite the difference in the number of treated patients with respect to the controls, Wilcoxon's test for paired data showed a significant ($P < 0.01$) improvement in survival in the vaccinated group, compared to that of the control.

Discussion

With an increased number of patients and an extended follow-up, the present report confirms our previous observations on ten MRCC patients. However, despite the increased number of patients and controls, the present study has obvious limitations. For instance, we

Table 6. General linear model basic statistics performed for the evaluation of data

Mitogen	Situation	Control	Days	Before/ after	Individual	Serum (b)	Mitogen	Sample
PHA 3dd	All	0.01 (+)	—	NS	< 0.0001	< 0.0001	—	< 0.0001 (1)
	Before	NS	—	—	< 0.0001	< 0.001	—	< 0.0001 (1)
	After	0.05 (+)	< 0.01 (-)	—	< 0.0001	< 0.0001	—	< 0.0001 (1)
PHA 5dd	All	< 0.05 (+)	—	NS	0.01	NS	—	—
	Before (a)	(NS)	—	—	(< 0.0001)	(<0.0001)	—	—
	After	NS	< 0.01 (-)	—	< 0.0001	< 0.05	—	—
Mitogen	All	<0.0001 (+)	—	0.01 (2)	< 0.0001	< 0.0001	NS	NS
	Before	<0.0001 (–)	—	—	< 0.0001	< 0.0001	NS	NS
	After	<0.0001 (+)	NS	—	< 0.0001	< 0.0001	< 0.05 (3)	< 0.05 (4)

"—" independent variables discarded, NS – independent variables discarded because not statistically significant; (a) this model should not be used because the matrix obtained is singular; (b) for the serum, when significant, it is always observed that AB < AUT; (+) positive correlation; (-) negative correlation; (1) the values are always in the following concentration order: PHA 0.1 µg/l < ConA30 < PHA 1 µg/l <= PHA 3 µg/l; (2) the values observed before vaccination are lower than those observed after vaccination; (3) the values observed using the ACHN as mitogen are always lower than those observed using ATC; (4) the values of the samples are always in the following order of mitogen : lymphocytes ratio: 1 : 50 < 1 : 5 < 1 : 2 < 1 : 1.

Table 7. General linear model basic statistics performed for the evaluation of final data

Final model		Number of		Analysis of the final model described		
Mitogen	Situation	Patients	Observations	DF Mod/ DF Err	F	P
PHA 3dd	All	22	92	27 / 64	14.41	< 0.0001
	Before	17	51	20 / 30	14.97	< 0.0001
	After	7	41	12 / 28	26.08	< 0.0001
PHA 5dd	All	14	27	14 / 12	7.36	< 0.001
	Before (a)	12	14	12 / 1	—	—
	After	5	13	6 / 6	33.71	< 0.001
ACHN/ATC	All	27	237	29 / 207	21.99	< 0.0001
	Before	24	128	25 / 102	24.23	< 0.0001
	After	9	109	14 / 94	21.5	< 0.0001

(a) This model should not be considered because the matrix observed is singular.

DF Mod – number of freedom degrees of the model
DF Err – number of freedom degrees of the error
F – value of the distribution

Clinical response	Number of patients	Days to the clinical response			Duration of the clinical response		
		Mean	SD	Median	Mean	SD	Median
CR*	1	346.0	–	346	324.0	–	324
PR**	4	137.3	80.4	147	142.5	80.0	135
No change	9	569.6	871.5	272	–	–	–
PROG	16	241.6	190.7	124	308.0	190.5	295
Totals	30	329.5	533.2	170.5			

CR* – lung+lymph nodes; PR** – liver, bone, n. 2 lung; No change – kidney, n. 4 lung, suprarenal gland, muscle, bone, local relapse; PROG – 12 bone (n. 3 plus brain, n. 2 plus lung), n. 3 lung (n. 1 plus brain), skin plus liver

ignore whether tumour regression was mediated by the use of allogeneic or autologous antigens or by the synergy of both. Furthermore, although we noticed an increase of CMI during the vaccination period, we cannot assert that tumour regression was in correlation with some known immune responses, e.g. LST in PBL. In fact, in some patients we observed increased CMI and progression of disease. In addition, the role of the various cytokines produced by the ACHN line is not yet clearly understood, and the survival observed, because of the variability of response in MRCC patients, could be different in a larger cohort.

Table 8. Evolution of the clinical response

N. clin. resp.	Clin. resp.	Evolution of clinical response to date			
		PR	STAB	PROG	DEC
1	CR			1	
4	PR	+2		2	
9	No change		+8		1
16	PROG			7	9
30	**Total**	**2**	**8**	**10**	**10**

No change: refers to patients treated after surgical removal of metastases but apparently remaining tumour-free.

Be that as it may, it is worth stressing that the patients underwent vaccine therapy following the failure of the IL-2 treatment, which represents one of the best protocols that can be offered to MRCC patients in progression of disease. Under this treatment, the median survival reported in the literature ranges between 9 and 17 months (Bukowski, 1997, 2000). Moreover, a recent review describes some of the various vaccine therapy approaches of the last ten years in 126 MRCC patients with a response rate of 11% (Pizza et al., 2002).

One should not underestimate the complexity of the vaccine therapy and of the heterogeneity of the antigenic sites expressed on the surface of tumour cells. This problem has been described by Brouwenstijn and coworkers (1998). Their cellular immunity studies in RCC corroborated the notion that renal carcinoma cells are immunogenic because of a broadly distributed antigenic structure that may serve as target to cytotoxic T cells, and may thus be a potential candidate for tumour vaccine development. However, the authors confirmed that the recognized antigenic determinants are neither unique nor specific for the RCC.

Some additional advantages of our protocol lie in the very low cost and the absence of adverse side effects. And one should not underestimate that the response rate and median survival observed in our patients is among the highest reported in the literature for this pathology (Bukowski, 1997, 2000; Pizza et al., 2002).

In conclusion, the clinical results reported here, the high compliance of the patients to the protocol, and the low cost of the proposed techniques are promising and warrant further investigation. The reported data and the high compliance of the patients should justify, in our opinion, a prospective controlled study, comprising on the one hand patients treated with ATC+ACHN-IL-2 and on the other only ACHN-IL-2-treated patients. The feasibility of such a study is under active evaluation.

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