Review

Immunotherapeutic Approaches for Renal Cancer

(cancer / immunotherapy / interferon / interleukin / intra-lymphatic / kidney / LAK / renal carcinoma / transfer factor)

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Renal carcinoma is no doubt one of the strangest urologic tumours. Its multifaceted symptoms can defy the most perceptive physician, for more often than not the symptoms do not suggest a renal pathology.

Its frequency and mortality are not negligible. In the United States, there are 27 600 new cases of renal cell carcinoma each year, and 11 300 die from this cancer. Indeed, 30% of the patients have already developed metastases at the time of the initial diagnosis or at the time of the first relapse. Considering the resistance to chemotherapy and/or radiotherapy of this tumour, the prognosis remains bleak.

The peak age of incidence is sixty years, and affected men outnumber affected women two to one. Among the risk factors, cigarette smoking and exposure to cadmium are the most frequently cited, whereas familial forms have been associated with genetic translocations between chromosomes 3 and 8 or chromosomes 3 and 11. Furthermore, phakomatoses, e.g. von Hippel-Lindau disease, whose gene has been linked to the *raf-1* oncogene on chromosome 3, are associated with this cancer.

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Abbreviations: BCG – bacillus Calmette-Guerin, BRM – biological response modifiers, CCNU – chloroethyl-cyclohexy-nitrosourea, CR – complete response, CTL – cytotoxic T lymphocytes, DTH – delayed type hypersensitivity, 5-FU – 5-fluoro-uracil, IFN – interferon, IL-2 – interleukin 2, LAK – lymphokine activated killer cells, LN – lymph node, MHC – major histocompatibility complex, MLTC – mixed lymphocyte tumour cell cultures, MRCC – metastatic renal cell carcinoma, NDV – Newcastle disease virus-modified, PBMC – peripheral blood mononuclear cells, PR – partial response; RCC – renal cell carcinoma, Renca – murine renal cell carcinoma line, SD – stable disease, TF – transfer factor.

Conventionally, renal carcinomas are classified following the cell type and growth pattern. Cell types comprise clear, spindle, and oncocytic cells, whereas growth patterns include acinar, papillary, and sarcomatoid varieties. However, this classification has been modified in order to reflect the different types of adenocarcinomas more precisely by including morphological, histochemical, and molecular criteria. Thus, five carcinoma types have been identified. They comprise clear cell, chromophobic, chromophilic, oncocytic, and collecting duct types. Each of these types has a unique pattern of growth, cell of origin, and cytogenetic characteristics.

Despite many efforts, treatment of metastatic renal cell carcinoma (MRCC) has been proved disappointing over the years. No single treatment protocol or programme for MRCC has been uniformly effective. Thus, most physicians usually rely on novel therapies, including biologic response modifiers, investigational anticancer agents, differentiation agents such as retinoic acid, vaccines, or gene therapy.

Conventional treatment

Surgery

Standard therapy for localized renal cell carcinoma (RCC) is radical nephrectomy that includes removal of the kidney together with Gerota's fascia, the ipsilateral adrenal gland, and the regional hilar lymph nodes. Partial nephrectomy has become more popular, especially for patients with small tumours, those at risk for bilateral tumours, and those in whom the contralateral kidney is at risk because of the presence of other systemic diseases, such as diabetes and hypertension.

However, one of the main problems associated with partial nephrectomy is the possibility of tumour relapse, many renal tumours being multicentric. Local recurrence rates are 4 to 10%, but lower rates have been reported when partial nephrectomy was carried out for small (< 3 cm) lesions and the contralateral kidney was normal.

Neverthess, the role of surgery in the management of metastatic disease, either at initial presentation or later in a patient's course, remains controversial. Indeed, even when extensive surgery is carried out, the prognosis of most patients remains poor. Nephrectomy in patients with widespread metastatic disease, as a way of potentially improving their response to systemic therapy, has also been controversial. Although many research protocols require such resection, the practice should be viewed as uncertain. Nonetheless, a patient who does respond to systemic therapy should be considered for nephrectomy.

Chemotherapy

Several drugs have been used with variable, but unimpressive results (Table 1). For instance, a regimen combining oxaliplatin, 5-fluorouracil (5-FU), and folinic acid in fourteen MRCC patients previously treated with immunotherapy didn't produce any objective response (Chaouche et al., 2000). Using a combination of weekly intravenous gemcitabine with continuous infusion of 5-FU, Rini and co-workers observed only seven partial and five minor responses in 39 patients. The duration of response for the partial responders was 2–14 months.

The antitumour activity of zeniplatin, a third-generation, water-soluble platinum compound, has been assessed by Aamdal and co-workers. Only four MRCC patients were treated, and none responded. The main toxic effects were leucopoenia, nausea, and vomiting. Unexpected and serious nephrotoxicity was also observed, and for this reason, the studies were terminated before the planned number of patients had been included. A possible explanation for the nephrotoxicity may be drug interactions, but no definite conclusions were drawn.

Docetaxel (Taxotere, RP56976), a semi-synthetic analogue of paclitaxel, with a broad range of *in vitro* antitumour activity, was also evaluated in a phase II study. Twenty patients entered into the study, but no objective response was seen, except for one patient who showed a mixed response (Mertens et al., 1994). Similarly, no results were observed in 16 patients treated with fotemustine (Lasset et al., 1993).

Obviously, chemotherapy alone produces rather poor clinical results, but numerous adverse side effects that severely impair the quality of life. The data obtained in the last ten years on 451 patients (Table 1) show that in 4.6% of the cases, only a clinical response is obtained, with a median survival ranging from 2.5 to 12 months. These numbers agree with those of Yagoda et al. (1995), who carried out an evaluation of the various chemotherapy regimens used in 4542 patients during 82 phase II trials in previous years.

Immunotherapeutic attempts

Cytokine therapy is based on observations suggesting that this cancer may be responsive to immunotherapy. Indeed, it is well known that renal cancer can elicit an immune response in the host leading to spontaneous remissions. Although rare, this phenomenon has led many investigators to study agents that can stimulate the body's immune system. The agents that have been studied most extensively are interferon (IFN), interleukins (ILs), cytokines, and cellular-based therapies, in various combinations.

Table 1. MRCC treated with chemotherapy only

Authors	No. of patients	CR (%)	PR (%)	MR (%)	SD (%)	Median survival months	Range	Drugs
Liu et al., 2001	17	1 (6)	3 (17)	0	0	10	3-16	tamoxifen, vinblastine, 5-FU
Chaouche et al., 2000	14	0	0	0	2	nd	nd	oxaliplatin, 5-FU, folinic acid
Rini et al., 2000	39	0	7 (17.9)	5	nd	nd	nd	gemcitabine, 5-FU
Hao et al., 2000	17	0	3 (17.6)	0	nd	8	3-11	hydroxyurea, vinblastine
Pyrhonen et al., 1999	81	0	2 (2.4)	nd	nd	9	nd	vinblastine
Ritchie et al., 1999	168	0	4 (7)	0	15 (27)	2.5	0.5-5	MAP
Lummen et al., 1998	14	0	0	0	nd	nd	-	titanocene
Henriksson et al., 1998	63	2 (3.1)	0	0	0	12	nd	tamoxifen
Aamdal et al., 1997	4	0	0	0	0	nd	nd	zeniplatin
Mertens et al.,1994	20	0	0	1	0	nd	nd	docetaxel
Lasset et al., 1993	14	0	0	0	4	nd	nd	fotemustine
Totals	**451**	**3 (0.6)**	**19 (4)**	**6 (1.3)**	**21 (4.6)**	**2.5-12**	**0.5-11**	
Yagoda et al. 1995*	4542	59 (1.3)	213 (4.7)	-	-	-	-	83 phase II trials

* patients treated with chemotherapy from 1985 to 1995
() – % of response; MR – minor response; MAP – medroxyprogesterone acetate

Recombinant IFN

Utilization of IFN α, β, or γ alone has produced responses in approximately 12 to 20 percent of the treated patients. IFNs display multiple activities, inter alia an important anti-proliferative activity against renal cell carcinomas *in vitro*, are stimulatory to cell-mediated immunity, and can modulate the expression of major histocompatibility complex (MHC) molecules. The patients' response has been seen in many anatomic areas. However, patients who had had prior nephrectomy, but with only one pulmonary metastasis, and otherwise in good health, display a higher response rate. Duration of the response is usually less than two years, but longer-lasting remissions have been noted in a few patients.

IFNs have been combined with other immune modifiers and/or chemotherapeutic agents, but these combinations produced no real improvement in larger-scale trials. Several trials have combined IFN with IL-2 and chemotherapy (e.g., fluorouracil), and have shown some encouraging preliminary results (Gebrosky et al., 1997).

Combined therapies

Because of the poor results of chemotherapy or immunotherapy when used alone, protocols combining the use of cytotoxic drugs and cytokines were devised.

Schmidinger and co-workers (2000a) treated thirty-seven patients with progressing MRCC, who had already received treatment with IFN or IL-2, vinorelbine (30 mg/m^2, i.v.) for 22 days, and IFN-α-2c (4 800 000 U, s.c.) three times a week. Partial response (PR) occurred in 8% of the patients, whereas stable disease, with a median duration 8 and a range 3 to 35+ months, was observed in 46%. Median overall survival was 15 (range 1–49) months. No major toxicity was observed. Furthermore, patients who failed first-line treatment with biological response modifiers (BRM) had a greater chance to enter PR or stable disease (SD) under combined, low-toxicity therapy using vinorelbine and IFN-α-2c.

Naglieri et al. (1998) studied the impact of the association doxorubicin and epirubicin with IL-2 and IFN, and they observed a better survival. Patients were randomized to receive either IL-2 and IFN-α or IL-2 and 4-epirubicin. In 38 patients, two complete and two partial responses were observed, whereas 21 patients had stable disease. The authors considered these results as *"encouraging"*.

In 1999, a randomized study using IFN and medroxyprogesterone acetate showed poor activity of the latter: only 12% of partial responses in 168 patients treated were noticed, with a median survival of 2.5 months (Ritchie et al., 1999).

13-cis retinoic acid has also been used in MRCC, often in association with IFN and IL-2. The recent observations of Casali et al. (1998) appear promising despite the limited number of patients. 13-cis-retinoic acid was administered at 1 mg/Kg/day, and IFN-α-2a s.c. at 3 x 10^6 U/day. All patients had been previously treated with chemotherapy in association with immunotherapy. The treatment was not discontinued until neoplastic progression occurred. Two partial responses and five stabilizations were noticed with mild side effects.

Treatment with 5-FU and IFN-α was also used (Gebrosky et al., 1997). Twenty-one patients with advanced RCC underwent treatment with continuous intravenous infusion of 5-FU, 200 mg/m^2/day, and subcutaneous injections of recombinant IFN-α-2b, 1 x 10^6 U/day. An objective response was observed in nine patients (43%): a complete response (CR) in four patients (19%) (two with lung, one with bone, and one with liver metastasis), and a partial response in five (24%). The mean survival rate was 44 months for the complete responders, 42 for the partial responders, and 20 for the non-responders. The overall mean duration of response was 23 months. Responders entered progression to disease at a mean of 14 months after the initial response to therapy. Mild, dose-dependent toxicity was related to 5-FU infusion. Nearly all toxicities subsided with the temporary cessation and/or dose decreasing of the 5-FU infusion. Results were considered promising, but additional investigations are warranted.

It is mention worth that using vinblastine and IFN, Paolorossi et al. (1995) obtained two PRs in 13 treated patients, whereas Lopez-Hanninen and co-workers (1995) concluded that the second-line outpatient chemo/immunotherapy regimen of s.c. r-IFN-α and i.v. 5-FU showed a limited, albeit significant efficacy in pre-treated patients with progressive MRCC.

It seems that if chemotherapy alone produces disappointing clinical results, those are improved when IFN is added. The data obtained in 487 patients, and reported in 12 studies in the last 10 years (Table 2), show 15.5 % of CR+PR, and a median survival ranging from 9 to 23 months when IFN is added to chemotherapy. These observations are in agreement with those of Bukowski (2000, 2001).

IL-2

Table 3 shows results observed in the last ten years in MRCC patients, based on the utilization of IL-2 administered using various techniques: i.v. (continuous or bolus), s.c., by inhalation and intralymphatically. In many studies, to IL-2 administration were added other immunomodulators such as IFN, LAK cells, and transfer factor (TF).

In the past ten years, forty-four studies have been published. The median survival of the 3823 treated patients ranged from a minimum of 8.6 to a maximum of 39.5 months; 2–12 months being the median survival in 785 MRCC patients who did not undergo immunotherapy, reported in three different studies (Table 3).

In 12 studies (1473 patients), the overall response rate was 14.2% (range 5.3–58%), with 2–9.1% of CR and 5–58% of PR. The median survival in 926 patients,

Table 2. MRCC treated with chemotherapy and IFN

Investigators	No. patients treated	CR (%)	PR (%)	MR (%)	SD (%)	Median survival (months)	Range	Drugs
Vaishampayan et al., 2001	21	0	1 (4.7)	0	7	9	4-18+	13-cis retinoic acid, IFN, paclitaxel
Miller et al., 2000	38	0	2 (5.2)	0	0	-	-	9-cis retinoic acid, IFN
Motzer et al., 2000	142	0	17 (12)	0	-	15	-	13- cis retinoic acid, IFN
Schmidinger et al., 2000	37	0	3 (8.1)	0	-	15	1-49	vinorelbine, IFN
Reese et al., 2000	24	1 (4.1)	5 (20.8)	0	0	21	6-57+	FUDR, IFN
Pyrhonen et al., 1999	79	0	16 (20.5)	-	-	16	-	vinblastine, IFN
Casali et al., 1998	11	0	2 (18.2)	0	5	-	-	13-cis retinoic acid, IFN
Creagan et al., 1998	31	0	2 (6)	0	-	-	-	cimetidine, leucovorin, IFN
Gebrosky et al., 1997	21	4 (19)	5 (24)	0	-	-	-	5-FU, IFN
Tsavaris et al., 1996	37	0	6 (16.2)	0	10	-	-	5-FU, leucovorin, IFN
Paolorossi et al., 1995	13	0	2 (15.3)	0	5	-	-	vinblastine, IFN
Lopez-Hanninen et al., 1995	33	1 (3)	2 (6)	0	nd	nd	nd	5-FU, IFN
Totals	**487**	**6 (1.2)**	**70 (14.3)**	**5 (1)**	**27 (5.5)**	**9-23**		

reported in five different studies, ranged from 10 to 30 months, and the percentage of survival, one year after the beginning of the IL-2-based immunotherapy (reported in another eight studies related to 1526 patients), ranged from 41 to 69%, 23–43% being the survival of 785 non-treated historical controls (Table 3). The addition of IFN or LAK cells to IL-2 improved the response rate and survival in some studies, but it was not confirmed by others (Table 3).

Vaccine therapy

The concept of tumour vaccines is not new. However, advances in gene transfer technology, tumour immunology, molecular biology, and methods of monitoring the antitumour response have allowed for novel, more specific approaches.

First-generation tumour vaccines were composed of whole inactivated cancer cells, or tumour lysates administered together with immune adjuvants such as bacillus Calmette-Guerin (BCG). Current strategies include tumour cells modified by the insertion of genes encoding molecules capable to stimulate a cytotoxic T-cell response, such as cytokine, allogeneic HLA, or tumour-associated antigen (TAA) genes, as well as co-stimulatory molecules. It now seems that activation of cellular immunity requires at least three synergistic signals, including presentation of specific tumour antigens, co-stimulatory signals (B7 molecules), and propagation of the immune response via cytokine release (Antonia and Seigne, 2000).

Although there is no certain proof for the presence of tumour-specific antigens in RCC, and Oosterwijk et al. (1986) have even challenged their existence, at least six TAA have been described by Ueda et al. (1981). The complexity of the problem is illustrated, for instance, by the studies of Brouwenstijn and co-workers.

The authors isolated from the peripheral blood of a patient with RCC several T-cell clones that were able to lyse an autologous RCC cell line, but not an autologous EBV-transformed lymphoblastoid cell line. Most of the cytotoxic T-lymphocyte (CTL) clones recognized HLA-A1-positive allogeneic RCC cell lines, indicating that HLA-A1 was the restricting element for these T cells. Furthermore, one CTL clone exclusively recognized the autologous tumour cells. HLA-A1-restricted CTL clones could be further divided into two subsets of T-cell clones, one susceptible to be blocked by an HLA-A1-specific monoclonal antibody, but not the other. The reactivity of the HLA-A1-restricted T-cells of one particular clone from this patient was further studied, and it was shown that it could react with several melanoma cell lines, thus suggesting that the expression of the antigen recognized by this CTL clone was not restricted to RCC. Strangely, tumour cell lines did not exclusively express this antigen, since primary cultures of proximal tubulus epithelium cells, adult mesangial cells, and normal breast epithelium cells were also lysed.

These data are in favour of the hypothesis that renal carcinoma cells are immunogenic by virtue of a broadly distributed antigenic structure that may serve as a target for CTL and may be a potential candidate for tumour vaccine development. However, the data also suggest that the recognized antigenic determinants are neither unique nor specific for the RCC (Brouwenstijn et al., 1998). Hereafter, we briefly review various vaccinotherapeutic approaches in metastatic or advanced RCC (Table 4).

Until tumour-associated antigens shared by most renal carcinomas have been identified, the best approach for vaccine therapy remains to be the use of autologous tumour cells taken from the primary tumour and/or the metastases. The cells could be cultured so that they could be used intact following radiation, or

Table 3. IL-2 based therapies in MRCC patients

Investigators	Drugs	Administration route	Patients' No.	Median response duration No. studies	Min	Max	Median survival No. studies	Min	Max
Pizza et al., 2001	nIL-2,IFN,TF,LAK	intralymphatic	122	1	22.5	-	1	30	-
Schimidinger et al., 2000b	rIL-2, IFNγ	sc	63	1	9.6	-	1	-	-
van Herpen et al., 2000	RIL-2,IFN,5-FU	iv(b)	52	1	8.3		1	16.5	-
Bukowski,1997	rIL-2+IFN	sc+iv(b,c)	1411	19	7	16	11	11	39.5
Bukowski,1997	rIL-2+LAK	iv(b,c)	461	6	9	17	2	13	20
Bukowski,1997	rIL-2	sc+iv(b,c)	1714	16	6.5	31	12	8.6	20
Total/range			**3823**	**44**	**6.5**	**31**	**29**	**8.6-30**	**20-39.5**

Investigators	Drugs	Aministration route	Patients' No.	CR(%)	PR(%)	CR+PR (%)	Median response (%)
Westermann et al., 2001	IL-2,IFN,GM-CSF	sc	10	0	2 (20)	2 (20)	-
Olencki et al., 2001	rIL-2,IFN,5-FU	iv(b)	25	0	7 (28)	7 (28)	-
Lissoni et al., 2001	rIL-2, EPO	sc	12	0	7 (58)	7 (58)	-
Pizza et al., 2001	nIL-2,IFN,TF,LAK	intralymphatic	122	11 (9.1)	13 (10.6)	24 (19.6)	22.5
Bordin et al., 2000	rIL-2+LAK	sc	92	2 (2)	19 (21)	21 (23)	25
Schimidinger et al., 2000b	rIL-2, IFNγ	sc	63	3 (2)	8 (5)	7 (11)	9.6
Negrier et al., 2000	rIL-2	iv(c)	281	-	-	15 (5.3)	-
van Herpen et al., 2000	rIL-2,IFN,5-FU	iv(b)	52	0(0)	6 (11.8)	6 (11.8)	8.3
Figlin et al., 1999	rIL-2 based	various	203	12 (6)	36 (18)	48 (23.6)	-
Negrier et al., 1998	rIL-2+IFN	iv(b,c),sc	425	-	-	46 (11)	-
Huland et al., 1997	rIL-2+IFN	inhaled	105	3 (3)	13 (12)	16 (15)	9.6
Negrier et al., 1989	rIL-2+LAK	iv	83	-	-	20 (24)	-
Total/range			**1473**	**2-9.1**	**5-58**	**210(14.2)**	**8.6-22.5**

Investigators	Drugs	Administration route	Patients' No.	Median survival	1 year	% 2 years	3 years	survival 4 years	5 years	7 years
Pizza et al., 2001	nIL-2,IFN,TF, LAK	intralymphatic	122	30	69	52	45	41	39	30
Bordin et al., 2000	rIL-2	sc	92	-	58	-	17	-	9	-
Negrier et al., 2000	rIL-2	iv(c)	281	10	41	22			8	-
Figlin et al., 1999	IL-2-based	various	203	18	61	40	31	-	-	-
Negrier et al.,1998	rIL-2+IFN	iv(b,c),sc	425	-	65	40	30	-	-	-
Huland et al., 1997	rIL-2+IFN	inhaled	105	11.8	58	27		-	-	-
Lopez-Hanninen et al., 1996	rIL-2+IFN	sc	215	20.5	54	32	19	-	-	-
Total/range			**1526**	**10-30**	**41-69**	**22-52**	**17-30**	**41**	**8-39**	**30**
Pizza et al., 2001	w/o immunotherapy		89	8	28	13	5	2	1	-
Elson et al., 1988	w/o immunotherapy		610	2-12	23					-
DeKernion et al., 1978	w/o immunotherapy		86	-	43	25	18	15	11	2
Total/range			**785**	**2-12**	**23-43**	**13-25**	**5-18**	**2-15**	**1-11**	**2**

b - bolus; c - continuous; EPO - erythropoietin; GM-CSF - granulocyte-macrophage colony-stimulating factor; iv - intravenously; n - natural; r - recombinant; sc - subcutaneous; w/o - without

after formalin buffer treatment (Drake et al., 1972; Pizza et al., 1980), in order to inactivate their possible active replication *in vivo*.

Dillman and co-workers established short-term cultures of autologous tumours from patients with renal carcinoma for their use in active specific immunotherapy. In eight years, they treated 69 kidney tumour samples that had been surgically excised, including 43 primary tumours and 26 metastatic lesions. Efforts were made to establish short-term tumour cell cultures to utilize them as autologous tumour cell vaccines. Before treatment, patients underwent a baseline delayed type hypersensitivity (DTH) skin test to detect reactivity to tumour antigens, and thereafter received, three times weekly at the beginning, and then five times monthly, s.c. injections of 10^6 irradiated tumour cells that were admixed with various adjuvants. Cell lines were established for 55/69 patients (80%), including 36/43 (84%) from primary tumours and 19/26 (73%) from distant metastases. Vaccines were prepared for 41 patients, but only 27 were found suitable to receive this treatment, which was well tolerated. The median follow up for 26 patients was over five years. Of 10 patients who had no evident disease at the time of treatment, nine were alive 1–8 years later, and 5/8 had a conversion of their DTH test. In 16 patients with measurable metastatic disease at the time of treatment, there were no objective tumour responses, and their median survival was five months.

Schwaab and co-workers tried to add IFN, both alpha and gamma, or BCG as an adjuvant to autologous tumour cells used as a vaccine. In a rather short three-month period, nine MRCC patients entered the protocol: three showed a mixed response, one disease progression, and five remained stable. Toxicity was mild.

Fenton et al. (1996) performed a prospective, randomized study to determine whether subcutaneous administration of IL-2, in combination with an autologous renal cell vaccine, was feasible and could potentiate antitumour immunity. Patients with metastatic renal cell carcinoma underwent surgical resection and an autologous tumour cell vaccine was prepared. The patients were vaccinated intradermally twice at one-week intervals with 10^7 irradiated tumour cells, together with BCG, and once with 10^7 tumour cells alone. The immune response was monitored by the DTH response to tumour cells, and compared to normal autologous renal cells. Sixteen patients received vaccine therapy. Four patients (two after receiving no IL-2, and two IL-2 at a high dose) developed specific cellular immunity for autologous tumour cells, measured by DTH responses. Two PR were observed, both in the patients who received IL-2 at a high dose. One responding patient was DTH positive, and one negative. A third patient, who was DTH (+) after vaccination with no IL-2 addition, had a dramatic PR after receiving IL-2 subcutaneously in a subsequent protocol. These data suggest that subcutaneously administered adjuvant IL-2 does not noticeably increase the immune response to autologous renal cell vaccines, as assessed by the development of a tumour-specific DTH response.

As regards B7 molecules, Schendel et al. (2000) have selected a well-characterized human RCC line for the development of a genetically engineered tumour cell vaccine to be utilized in a study using allogeneic molecules. The cell line was genetically modified by retroviral transduction to express B7.1 co-stimulatory molecules. The unmodified tumour cells were compared to B7.1-expressing tumour cells for their ability to induce an immune response to TAA in allogeneic peripheral blood mononuclear cells (PBMC) of two normal control donors having single MHC class I allele matches with the tumour cells. Primed PBMC, by the use of B7.1-modified tumour cells, showed a preponderance of $CD3^+CD8^+$ cytotoxic T lymphocytes that proliferated over extended periods of time in mixed lymphocyte tumour cell (MLTC) cultures. Strong cytolytic activity developed in the primed populations and included allogeneic CTL, with specificity for mismatched HLA-A, B and C molecules. Nonetheless, it was possible to isolate CTL clones that were able to lyse tumour cells, but not lymphoblastoid cells that expressed the corresponding allospecificities. Thus, induction of complex allogeneic responses does not seem to hinder the development of tumour-associated CTL *in vitro*. These results support the use of genetically modified allogeneic tumour cell lines for vaccination of partially MHC-matched RCC patients.

Recently, another approach has been proposed. It consists in inducing autologous tumour cells to produce cytokines (i.e., IL-2, IL-12, TNF, GM-CSF), either by culturing the cells *in vitro* and transfecting them with ILs' cDNA, or by direct intra-tumour administration of the cDNA in order to induce paracrine secretion of immunostimulatory IL-2 and thus create a tumour vaccine *in situ*.

Daniels and Galanis administered directly into patients' tumours a lipid complex containing the IL-2 cDNA. They observed a 14% objective response rate in a phase I/II clinical trial in 14 treated patients. The clinical response (PR/CR) was long-lasting. Application of PCR and immuno-histochemistry in post-treatment tumour biopsies detected the IL-2 plasmid, in addition to increased IL-2 expression in tumour cells and CD8 infiltration. Clinical trials employing higher doses of the plasmid in RCC patients with limited disease are ongoing.

This approach corresponds to an animal model. Using a novel cationic lipid delivery system, Bishop et al. (2000) delivered murine IL-2 cDNA directly into an established murine renal cell carcinoma line (Renca). Production of IL-2 within the tumour induced rejection of established tumours (62% on average), whereas control plasmid had little or no effect (17% on average). Surviving animals treated with IL-2-lipid were highly resistant to Renca re-

Table 4. Vaccine therapy in MRCC

Investigators	Trial phase	No. patients	Vaccine type	Adjuvant	Concomitant therapy	CR (%)	PR (%)	MR (%)	SD (%)	PD (%)	Median survival (mo)
Schwaab et al., 2000	II	9	Aut. TC	BCG	IFN-γ	0	0	3 (33)	5 (56)	1 (11)	5
Dillman et al., 2001		16	Aut.TC	no	no	0	0				
Daniels and Galanis, 2001	I/II	14	IL-2-cDNA				2 (14)				
Kugler et al., 1998	I	24	Hybrid-Aut-TC			2 (8)	2 (8)				
Simons et al., 1997	I	16	Aut.TC-GM-CSF*	no	no	0	1 (6)				
Chang et al., 1997	I/II	12	Aut.TC	BCG		2 (16)	1 (8)				
Fenton et al., 1996		17	Aut.TC	BCG,IL-2			3 (17)				
McCune et al., 1990		18	Aut.TC				3 (16)				69
Pizza et al., unpublished	I/II	9	Aut.+Achn*-IL-2	no	TF,IL-2	1 (11)	1 (11)		2 (22)	5 (55)	18
Totals		**135**				**5**	**13**	**3**	**7**	**6**	**5-69**

MR - mixed response; PD - progression disease; mo - months; * - allogeneic tumour cells

Vaccines in advanced RCC (stage T2-T4)

Investigators	Trial phase	No. patients	Vaccine type	Adjuvant	Concomitant therapy	% Relapse	Median survival	Months range survival	Median follow-up
Kirchner et al.,1995	prophylaxis	208	Aut-TC-NDV	no	IFN-α, IL-2	18 (9)	21+	2-64+	39
Dillman et al., 2001	prophylaxis	10	Aut.TC	no	no	0	nd	1-8	60
Repmann et al., 1997	prophylaxis	116	Aut.TC	no	no		P = 0.007 vs106 r. c.		
Galligioni et al., 1996	prophylaxis	60	Aut.TC	BCG	no		P = NS vs 60 r. c.		
Totals		**395**							

r.c. - randomized controls

challenge, but not to cross-challenge with a syngeneic mammary adenocarcinoma. A combination of IL-2 gene therapy with 5-FU treatment increased the antitumoral efficacy and survival of mice bearing primary and metastatic renal tumours (42% survival with IL-2-lipid, compared to 94% survival with IL-2-lipid plus 5-FU). These data suggest that rejection of primary and metastatic tumours may be obtained following intra-tumour delivery of a non-viral IL-2 gene therapy; the rejection rate is increased if the systemic administration of a conventional chemotherapeutic agent is combined.

Transfection, using autologous tumour cells, may be difficult to carry out for each patient, since it is difficult to establish a tumour cell line for each patient (Dillman et al., 2001). Thus, other approaches have been developed in order to obtain tumour cell vaccines.

Belldegrun et al. (1993) used two established allogeneic renal tumour cell lines to produce IL-2 by transfection with pertinent cDNA. All peripheral blood cytokine-producing cells showed an increased ability to kill tumour cells *in vitro*. We used a similar approach for inducing an RCC cell line (ACHN) (Pizza et al., 1999), and subsequently we treated 10 MRCC patients in disease progression, while they were receiving IL-2, IFN, and TF. We obtained one CR, and one PR, whilst the 18 months median survival was significantly higher compared to that of 64 historical controls (Pizza et al., unpublished data).

Since tumour cells often fail to demonstrate *in vivo* the required or *'hoped'* immunostimulatory properties, dendritic cell-based vaccines seem to gain popularity, as these cells can present TAA to the immune system, and hence circumvent the poor antigen-presenting qualities of the tumour cells. Dendritic cells can be *'loaded'* with TAA or other molecules, either by using their natural endocytotic capabilities or by genetic manipulation.

The reasons for the frequent failures of the vaccine-therapy approach are related to the incomplete understanding of the mode of function of the immune response elicited by the various procedures. Stimulation of an immune response against a certain antigen does not necessarily mean a protective activity against the same antigen. Indeed, some responses are not important for tumour rejection, while others can stimulate tumour growth. Thus, detection of a tumour antigen does not necessarily imply identification of the antigen relevant to tumour rejection. Furthermore, antigens may produce different types of response, according to the ability of the individual immune system. Therefore, establishing a correlation between immunization with certain antigens and the cascade of immune responses leading to a favourable clinical outcome may shed some light into the problem, and set the basis for vaccine therapy preventing tumour relapses.

It is of importance that the antigen, i.e. the target of the immune response, can be easily *'seen'* by the immune cells. It thus appears that the most important antigens are those expressed on the cell surface of the tumour cells. Consequently, in the absence of precise knowledge of the *"private"* pattern of antigenicity, the best strategy remains to be the use of autologous tumour cells.

It is worth mentioning that heterogeneity of tumour cells is a well-known phenomenon, not only among different metastatic lesions, but also among the same single metastasis or primary tumour (Pizza et al., 1980). These observations illustrate how problematic may be the choice of the appropriate antigens for inducing an immune response. Hence, the many studies using whole irradiated (Dillman et al., 2001) or formalin-treated (Pizza et al., 1999) autologous tumour cells.

A hybrid autologous/allogeneic cell vaccine has been used by Kugler and co-workers in patients with progressive MRCC. Eleven patients were vaccinated with this hybrid cell vaccine consisting of lethally irradiated allogeneic RCC tumour cells fused with MHC class I-matched and class II-unmatched activated allogeneic lymphocytes. These patients were then followed for a mean period of 11 months. Another 13 patients were vaccinated with a hybrid cell vaccine consisting of autologous tumour cells fused with allogeneic activated lymphocytes, and followed for a mean period of six months. Six of the 11 patients receiving the allogeneic vaccination showed an initial response, with two complete and two partial responses, whereas only three patients who received autologous vaccination responded to treatment.

Chang's team used an adoptive immunotherapy with vaccine-primed lymph node (LN) cells, secondarily activated with anti-CD3 and IL-2, following a rather complex protocol. Irradiated autologous tumour cells were admixed with BCG and used to vaccinate patients. Seven days later, draining lymph nodes were removed for lymphocyte activation with anti-CD3 monoclonal antibody, followed by expansion in the presence of IL-2. Vaccine-primed LN cells were expanded *ex vivo*, and a mean of 8.4×10^{10} cells per patient were intravenously injected, concomitantly with IL-2 administration. Twelve MRCC patients were studied. The activated LN cells of most patients developed minimal cytotoxicity towards the autologous tumour cells. In contrast, a majority of the activated LN cells showed highly specific release of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IFN-γ in the presence of autologous, but not allogeneic tumour cells. Two patients had a complete and two a partial tumour regression, confirming a relationship between an increase of the DTH reactivity to autologous tumour cells after therapy and the observed tumour regression.

In a phase I randomized double blind study, Simons and co-workers treated 16 patients with equivalent doses of autologous irradiated RCC cells, with or without *ex vivo* GM-CSF gene transfer. An objective partial response was observed in one patient receiving the gene-transduced vaccine, who displayed the largest DTH conversion.

Table 5. T3N0M0 RCC patients' survival after surgery and IFN administration

Investigators	Groups	No. pts.	Pts. alive at 5y (%)	Pts. with metastases (%)	Median time to progression (months)	Pts. dead (%)
Migliari et al.,1995	IFN group	30	23 (76)	5 (16)	24	4 (13)
	Control	32	16 (50)	15 (55)	24	15 (47)
Jeon et al., 1999	IFN group	10	6 (60)	4 (40)	17.5	4 (40)
	Control	8	5 (62)	2 (25)	11	2 (25)

Instead of treating advanced MRCC patients, other investigators tried to prevent the tumour relapses or metastases following nephrectomy using the primary autologous tumour cells for vaccination. In Table IV we summarize studies pertaining to 395 patients with advanced renal cancer, and treated with various vaccination techniques.

Kirchner et al. (1995) treated 208 patients of locally advanced renal cancer with autologous Newcastle virus-modified (NDV) tumour vaccines, in combination with low-dose cytokines, after surgical treatment. Compared to historical data, based on the natural course of patients with locally advanced renal-cell cancer, the results showed an improvement of the disease-free survival. In contrast, Galligioni et al. (1996) using autologous tumour cells admixed with BCG were unable to show any advantage in survival in 60 patients treated and compared to a similar number of randomized controls. Interestingly, Repmann et al. (1997) reported that in 116 patients who received adjuvant treatment with autologous tumour-cell vaccines, only two showed minor side effects not exceeding WHO-grade 1 (Miller et al., 1981).

Repmann and his co-workers immunized 116 patients with renal carcinoma cells, obtained from an autologous tumour after radical nephrectomy. The survival rate of these patients was compared to a historical control group of 106 patients from the same hospital, who had received an identical surgical treatment, but no adjuvant immunotherapy. A difference in the survival rate of the two groups was observed, that of the autologous-tumour-cell-treated group being significantly greater ($P = 0.0007$). Following the individual Robson stages, patients in Robson II and Robson III showed significantly different survival rates (respectively $P = 0.02$ and $P = 0.04$), compared with the same stages of the control group. Due to the short follow-up time in the group Robson I, and the limited number of patients in the group Robson IV, no significant differences were observed in these groups. See in *Table IV: Vaccines in MRCC and in advanced RCC (stage T2-T4).*

Preventative adjuvant immunotherapy in T3N0M0 and in advanced stage RCC

The role of adjuvant immunotherapy, after radical nephrectomy in T3N0M0 renal cell carcinoma, has been recently assessed in several studies.

Jeon and collaborators computed the five-year overall survival in T3N0M0 RCC to 35–50%. They studied several factors, including tumour size, nuclear grade, mean nuclear area, and expression of the p53 protein to determine which factor(s) affect(s) disease progression in 18 patients treated by surgery only or surgery and chemo-immunotherapy. Ten T3N0M0 RCC patients, after radical nephrectomy, were randomly assigned to receive treatment with either IFN-α alone or with IFN-α plus vinblastine. Eight T3N0M0 RCC patients who received only radical nephrectomy were considered as the control "surgery only" group, and their results were compared with the immunotherapy group.

Five years after surgery, six out of ten (60%) patients in the adjuvant immunotherapy group were alive, with no evidence of disease. Metastases were documented in four patients (40%), with a median interval to progression of 17.5 months, and all patients died for causes related to tumour progression. In the "surgery only" group, five years after radical nephrectomy, five out of eight patients (62.5%) were still alive with no evidence of disease. Two patients (25%) developed distant metastases and they both died from their tumour. The median progression interval was 11 months. There were no statistical differences concerning progression and survival rates between the two groups.

Similar observations were previously made by Migliari's team in 1995. They decided to explore the theoretical advantage of adjuvant chemo-immunotherapy in radically resected stage II and stage III RCC. A single-institution phase II study was undertaken to evaluate the efficacy and safety of IFN-α-2a, in combination with vinblastine in 30 patients with pT2-T3 N0M0 RCC. The results of 32 patients who received only radical nephrectomy and extended lymphadenectomy were analysed and compared with the group receiving chemo-immunotherapy. Twenty-three of 30 (76.6%) patients in this group were alive, with no evidence of disease five years after surgery. The median follow-up for the surviving patients was 67 months (range 60 to 72). Metastases were documented in five patients (16.6%), with a median interval to progression of 24 months, and four (13.6%) died from their tumour. Five years after radical nephrectomy, 16/32 patients (50%) were alive in the control group, with a median follow-up for the patients still alive of 62 months (range 60 to 68); fifteen patients developed distant metastases, two

had a local recurrence, and all (47%) died from their tumour. The median progression interval was 24 months. After stratification by pathological grade, site, laterality, and number of nodes found at lymphadenectomy, there were no statistical differences in risk of disease progression or mortality between the two groups.

Transfer factor (TF)

At the end of this review, we have reserved a separate chapter to TF, since it has become matter of controversy, and yet it is still used with unequivocal success by several investigators, including ourselves.

TF has shown its clinical usefulness in the treatment of a variety of tumours, e.g. non-small-cell lung cancer (Kirsh et al., 1984; Fujisawa et al., 1983, 1984a, 1984b, 1996; Busutti et al., 1987; Whyte et al., 1992; Pilotti et al., 1996), Epstein-Barr virus-related tumours, i.e. nasopharyngeal carcinoma (Prasad et al., 1997) and Burkitt's lymphoma (Nkrumah et al., 1987; Neequaye et al., 1990), cervical cancer (Wagner et al., 1987) and hormone-unresponsive prostate cancer (Pizza et al., 1996).

In a prospective, not randomized trial Montie et al. (1977) used TF for the treatment of ten MRCC patients, and they reported temporary stabilization of metastatic disease. The same group of investigators treated 60 patients with MRCC in five different immunotherapy protocols consisting of a) TF, b) association of TF and BCG, c) association of TF, BCG, chloroethyl-cyclohexy-nitrosurea (CCNU) and megestrol acetate (Megase), d) association of BCG, CCNU, and Megase, and e) BCG alone (Montie et al., 1982). While this non-specific immunotherapy of renal adenocarcinoma has been associated with documented regression of metastases, response rates were similar to those obtained with hormonal therapy alone. Nonetheless, because of these results, further clinical studies were undertaken.

Thirty-seven MRCC patients, compared to 27 historical controls, were treated with combined immunotherapy including direct lymphatic injection of IL-2 and LAK cells, intramuscular injection of IFN-α-2a (10^6 units bi-weekly) and TF (bi-monthly injections of 4 x 10^8 mononuclear cell equivalent obtained from pooled buffy coats of healthy blood donors). This regimen produced CRs or PRs of metastases in 34% of the treated patients and stabilized progression of the disease in an additional 8%. The median survival was, respectively, 26 and 27 months for synchronous and metachronous metastatic treated patients, versus 8 and 14 months for the control group ($P < 0.001$) (Corrado et al., 1991). While no side effects were noticed in the treated patients, the observed results were comparable to those obtained by intravenous injections of large amounts of IL-2 and LAK cells, a protocol which nonetheless produced severe adverse side effects. Similar results have been recently reported in 122 MRCC patients treated in a comparable way (Pizza et al., 2001) (Table 3).

However, despite over fifteen hundred reports proving activity *in vitro* and clinical usefulness in treating not only cancer, but also viral, fungal, and parasitic diseases, its total lack of toxicity, and the fact that no publication has ever challenged these claims (indeed, since animal models exist, it should be easy and inexpensive to document the failure to reproduce published evidence), TF has become controversial. The reason is simple: TF's characteristics (low molecular weight, undefined chemical structure, proteinaceous nature, but resistance to proteolytic enzymes and capability to survive oral administration), together with its unusual biological properties (non-species specificity, unconventional mode of action, e.g. transfer of antigen-specific information using infinitesimal amounts of active material) have generated fierce opposition. And the failure to unravel the molecular structure has led immunologists to doubt its very existence, following the precepts of today's consensual *good scientific behaviour practice* that one may sum up by: *discard facts rather than endanger the paradigm*, in other words deny anything missing a molecular explanation.

Thus, nearly fifty years after the first observations, TF remains an elusive and controversial entity, although biochemical studies have produced evidence that it is a small peptide, with possibly three ribonucleotides attached to it. However, its complete molecular identity is still elusive, and attempts to sequence the amino acids failed due to a blocked amino terminus. And yet, elementary logic tells us that ignorance of the chemical structure of a compound never curtailed its activity. For instance, when belladonna or aspirin were used as crude plant extracts, their properties and potency were the same as today when we know the precise structure of their active ingredients, i.e. scopolamine, atropine, I-hyosciamine or acetyl-salicylic acid.

But it seems that modern biomedical logic would rather that facts were dismissed and treatments inefficacious or toxic than incomprehensible. For it is more respectable to reject a fact than to be mixed up with a fluke. And yet, in the TF story, we are not dealing with a spooky phenomenon of the paranormal eliciting *"society's negative response, which leads individuals to suppress their experience for fear of rejection or ridicule"* (van Lommel et al., 2001), but with a clinically and experimentally well-documented reality.

Be that as it may, the clinical effects of TF deserve closer examination. For not only has this moiety the ability to instruct T lymphocytes to fight an intruder, but several publications suggest that it can also be used for prevention, i.e., as a vaccine soliciting the cell-mediated immunity that plays the most important role in combating neoplastic and viral diseases (Steele et al., 1980; Viza et al., 1986).

We are convinced that once its biochemical structure has been defined, the path to understanding its precise mode of action will be clear, consequently opening new avenues into the unknown in immunology and molecular biology. Recent work has already paved the way by partially unravelling the amino-acid sequence riddle, thus giving biochemical flesh to an elusive entity (Kirkpatrick, 2000).

Postface

Although several publications (Pizza et al., 1984, 1987, 1988; Lefesvre et al., 1987; Corrado et al., 1991) have shown since 1984 that it is possible to reduce the amount of the administered IL-2 without decreasing its efficacy, most clinicians clung to the high-dosage protocol, paying little attention to the alternative quasi-homeopathic amounts proposed by other investigators and, for that matter, to the patients' suffering. It seems that modern medical science has forgotten that catalytic processes are common in biochemistry, and that they require infinitesimal amounts of the catalyst to perform complex and sizeable reactions. Immunological interplay being part of life's chemistry, the range of IL-2 effective dose should have been fully investigated, particularly since it was published that 2 000–5 000 U of IL-2 trigger a remarkable cascade of immunological changes a few minutes after injection (Corrado et al., 1991). Indeed, the average standard treatment uses more than 500 x 10^6 U for a 3-month period vs. less than 10 000 U for the low-dose protocol, i.e. 50 000 times less!

In the past 15 years, we have treated 122 MRCC patients with intralymphatic injections of non-recombinant IL-2, LAK cells, IFN-α and TF. The results show a survival 3.5 times higher compared to that of historical controls. Furthermore, when the data were compared with those of similar studies, they showed an equivalent response rate (19.7%), but a better survival (45% at 3 years, 39% at 5 and 30% at 7), as well as a better median survival (28 months). Indeed, eleven years after the beginning of the treatment, the Kaplan-Meier curve shows a survival rate more than 25% (Pizza et al., 2001).

However, the most important advantage of this protocol is the fact that it is devoid of adverse side effects. In 1 692 intralymphatic administrations of 250–490 IL-2 units, in less than 2% of cases we observed chills, fever (less than 38°C), and/or hypotension. The 10 315 IL-2 inhalations caused dyspnoea, or hypertension (0.2%) in less than 2% of patients. Nonetheless, no medication was necessary, the side effects disappearing after discontinuing the IL-2 administration. Furthermore, the quality of life was not affected, and this is in agreement with the observations of Heinzer et al. (1999), who used inhaled IL-2.

In contrast, the IL-2 regimens currently employed in other centres induce severe adverse clinical side effects, as defined by the World Health Organization classification system (Miller et al., 1981). Thus, in "*recent studies, grade 3 and 4 toxicity has been observed in a substantial number of patients and 5.2–9.4% of patients died during the treatment periods, or within a month following the treatment, from causes unrelated to renal-cell carcinoma*" (Negrier et al., 1998).

Obviously, the scarcity of side effects in our patients is due to the low IL-2 dosage. In 14 years, we administered a total of 4 006 773 units of non-recombinant IL-2 to 122 MRCC patients. This amount is less than the amount of IL-2 that is usually administered in one day to one MRCC patient under the prevalent IL-2 protocols. A windfall but far from negligible result of our protocol is the substantial decrease of the cost of the treatment, i.e. from 7 000 € to 200 € for a 3-month period.

It is therefore obvious that long-term intra-lymphatic administration of IL-2 at a very low dose, together with LAK cells and TF, is not only safe, but it also produces significant clinical improvement and one of the best survival rates among those reported in the literature. Moreover, and most significantly, it offers a good quality of life, while it dramatically reduces the cost of treatment.

However, if today IL-2 appears to be the most effective form of immunotherapy for MRCC, further studies are warranted, since certain aspects of its mode of action are not totally understood. For instance, a faster progression of the disease is noticed when the treatment is interrupted.

A thorough evaluation requires a detailed study of the different immunotherapeutic agents, i.e. both natural and recombinant IL-2, administered with LAK intralymphatically together with or without inhaled and/or subcutaneous IL-2 at different doses. However, because of the relatively low incidence of this tumour and the need for investigation of several parameters, such study requiring the cooperation of several centres is complex and cumbersome. For example, although the cost of the LAK preparation is not high (less than 15 € per injection), it cannot be carried out on a routine basis, since it requires an important backup from a pathology laboratory. We would therefore like to suggest a simpler approach: evaluate the effect of intralymphatic injections of recombinant IL-2, together with subcutaneous and/or intranasal low-dose administration. This protocol should make the study easier, despite the fact that intralymphatic injection is not a routine practice, although better known today because of the use of lymphography.

It should be emphasized that the vaccination protocols, using both whole tumour cells *in vivo*, as well as dendritic cells expanded *in vitro* also hold great promise. In all probability, in the years to come they

will play an important role in future protocols of immunotherapy, most likely in association with existing and new immunostimulating agents. The fight against cancer is far from being over, and the research for the development of new tools is of the essence.

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