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-