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Pizza et al., unpublished	I/II	9	Aut.+Achn*-IL-2	no	TF,IL-2	1 (11)	1 (11)		2 (22)	5 (55)	18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