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Active Specific Immunotherapy with an Autologous Virus-modified Tumour Cell Vaccine in Human Renal Cell Carcinoma (RCC)

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DESPITE ALL efforts, there is still no adjuvant or palliative treatment of choice available in renal cell carcinoma (RCC) after nephrectomy. The 5 year recurrence rate for locally advanced non-metastatic RCC is 50%, and even for T2-tumours 10% of patients relapse within 5 years after surgery. For metastatic RCC, the prognosis is even worse. Five years after surgery nearly all patients will die of progressive disease [1].

We report the results of our still ongoing prospective study of locally advanced and metastatic RCC treated by radical tumour nephrectomy, combined with active specific immunotherapy (ASI). The tumour tissue was prepared under sterile conditions in a laminar air flow bench according to the technique recently described by Schirrmacher and associates of the German Cancer Research Centre in Heidelberg, Germany [2]. After removing fat, blood and necrotic parts, tissue was minced through a sieve and digested in Medium 199 (Gibco), containing collagenase, hyaluronidase and deoxyribonuclease I (Sigma), for approximately 30 min in order to achieve a single cell suspension. Erythrocytes were removed via a lysation buffer containing ammonium chloride. Viability and percentage of RCC cells in the cell suspension were assessed by the trypan blue exclusion test. The suspension was adjusted to a concentration of 1.5×10^6 cells/ml and frozen rapidly in a CO_2 freezer. Afterwards the vials were stored in liquid nitrogen. Microbiological testing for contamination with bacteria or fungi was performed routinely in each case [3].

For vaccination, vials were thawed rapidly in a 37°C waterbath, washed and incubated with 32 HAU Newcastle Disease Virus (NDV). After removing the non-adherent viruses by washing, the cells were irradiated with 100 Gy and injected strictly intracutaneously (i.c.) together with 450 000 IU (75 000 Cetus U) recombinant interleukin-2 (rIL-2). As controls, we also injected i.c.: 1.5×10^6 unmodified irradiated RCC cells, NDV alone and rIL-2 alone. Vaccination was performed 4, 8 and 24 weeks after nephrectomy. The local delayed type hypersensitivity reaction was measured 72 h after injection.

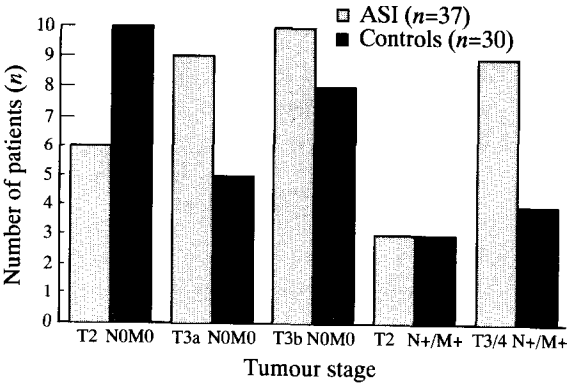

Figure 1. Distribution of tumour stages. ASI, patients treated with active specific immunotherapy.

Between 1990 and 1993, 37 patients were vaccinated, of these 25 patients had non-metastatic and 12 had metastatic RCC at the time of nephrectomy. The mean follow-up is 20 months for non-metastatic and 26 months for metastatic disease, ranging from 8 to 41 months.

ASI patients were compared to a control group of 30 patients who underwent tumour nephrectomy alone in the same department between 1990 and 1993 and did not receive ASI. Control patients with locally advanced RCC ($n = 21$) received no further treatment after surgery. Control patients with metastatic disease ($n = 9$) were treated with interferon-alpha (IFN) and/or vinblastine (VBL).

In both groups, ASI and controls, the distribution according to sex was similar: the male to female ratio was approximately 1.6 to 1. This is a normal distribution for this malignancy [4]. Because most of the patients were matched into pairs, distribution of tumour size, tumour stage, age and sex was similar in both ASI and controls (Figure 1).

For non-metastatic disease, the 2 year recurrence rate was 20.0% in the ASI group, and 30.4% in the control group. Although the populations are small, there was a trend towards longer disease-free survival in the vaccinated ASI patients (Figure 2).

For metastatic RCC patients, the 2 year survival rate was 50% in the ASI group and 28% in the control group. However, there

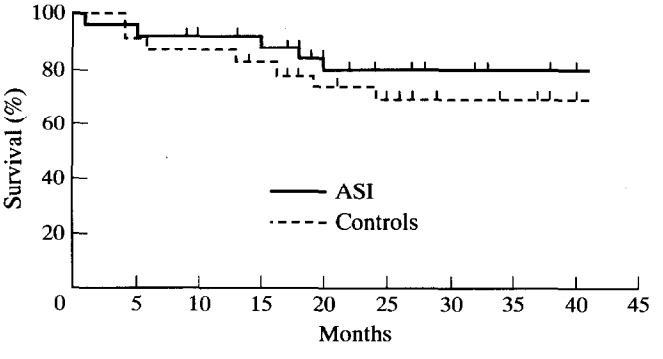

Figure 2. Disease-free survival of patients with non-metastatic renal cell carcinoma (RCC).

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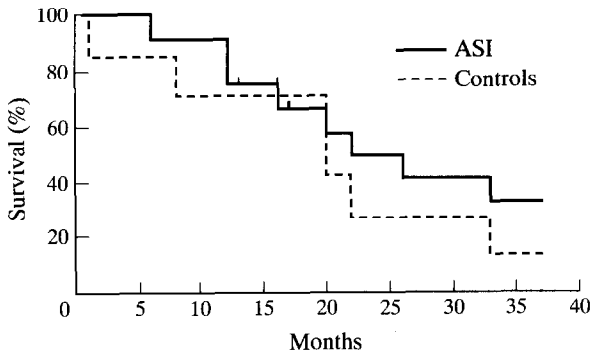

Figure 3. Survival of patients with metastatic renal cell carcinoma (RCC).

was no notable difference in long term survival between both groups (Figure 3).

According to the data presented, patients with locally advanced non-metastatic tumours undergoing ASI after nephrectomy show a tendency towards a lower recurrence rate compared to patients receiving no further treatment after surgery. However, in metastatic disease, there was no notable difference in survival between patients receiving ASI compared with patients receiving other treatment regimes or no palliative treatment at all after tumour nephrectomy. Therefore, tumour nephrectomy combined with ASI alone in patients with metastatic RCC is insufficient and shows no improvement compared to other palliative treatment regimes.

In future, the preparation techniques need to be optimised, and *in vitro* expansion of RCC cells has to be used in order to obtain enough vials for ASI vaccination, even in patients with small or fibrotic and necrotic tumours. The identification of tumour-associated antigens in RCC is one of the major goals for developing more effective adjuvant and palliative treatment regimes. The prolongation of immunostimulation after vaccination might improve the activation, maturation and response of cytotoxic lymphocytes involved in immunological tumour control. For this purpose, new adjuvants are being developed [5]. Non-specific stimulation of the immune system by cytokines, e.g. interferons and interleukins, may be helpful for increasing immunoreactivity in RCC patients undergoing ASI treatment.

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Ondansetron for the Control of Dacarbazine-induced Emesis

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THE MAJORITY of anti-emetic trials have addressed the problem of emesis induced by cisplatin-containing chemotherapy regimens, and it has been demonstrated that ondansetron provides effective prophylaxis. The addition of dexamethasone to ondansetron further improves anti-emetic control [1, 2]. Even if dacarbazine is a commonly used chemotherapeutic agent with high emetogenic potential [3], very few studies concerning the control of dacarbazine-induced emesis have been reported [4, 5]. The following open study was performed to assess the anti-emetic efficacy of the 5-HT3-receptor antagonist, ondansetron, in patients receiving chemotherapy including dacarbazine.

19, chemotherapy-naive, advanced colorectal cancer patients, median age 64 years, median ECOG performance status 1, entered a phase II clinical trial of dacarbazine 500 mg/m^2 followed after 2 h by the nitrosourea, fotemustine, 100 mg/m^2 day 1, every 4 weeks [6]. At the first course of therapy, 18 patients received ondansetron 8 mg orally 1 h prior to chemotherapy and then after 6 and 12 h. Ondansetron 8 mg orally three times daily was given on days 2 and 3. All patients were treated on an outpatient basis. Emesis was evaluated as follows: complete protection = no emetic episodes; major protection = one–two episodes; minor protection = three–four episodes; no protection ≥five episodes. Nausea was graded as follows: zero = none; one = mild, induced by certain odours or flavours; two = moderate, food intake compromised; three = food intake impeded.

Results of protection from acute emesis (any emetic episode occurring within 24 h of chemotherapy) with ondansetron were: 8 (44.4%) and 7 (38.8%) patients had complete and major emetic protection, respectively. No patient experienced more than five emetic episodes. Nausea was absent in 8 patients (44.4%) or mild in 6 cases (33.3%). Side effects reported with ondansetron were mild, and consisted of constipation in 4 patients and headache in 4 patients. Of note is the fact that the only patient (1/19) not receiving ondansetron experienced severe gastrointestinal toxicity (>15 emetic episodes).

The encouraging results observed in this small number of patients suggest that ondansetron is a safe, effective and well-tolerated anti-emetic agent that can be recommended in the prevention of nausea and vomiting from dacarbazine-containing chemotherapy.

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