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# Original Articles

## IMMUNOLOGICAL EFFECTS OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR AND AUTOLOGOUS TUMOR VACCINE IN PATIENTS WITH RENAL CELL CARCINOMA

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ABSTRACT

Purpose: Biological therapy for renal cell carcinoma (RCC) uses agents that mobilize immune effector cells which are able to recognize and destroy cancer. We evaluated the effects of weekly then monthly autologous tumor vaccine combined with daily granulocyte macrophage-colony stimulating factor (GM-CSF) in patients with RCC as a method of stimulating antigen presenting cells.

Materials and Methods: Eligible patients with pathological stage II to IV RCC were entered into this pilot study. Autologous tumor vaccine (0.5 to $1 \times 10^7$ irradiated tumor cells) admixed with 250 $\mu$g GM-CSF per vaccine was given subcutaneously weekly for 4 weeks and then monthly for 4 months. GM-CSF (125 $\mu g/m^2$) was given subcutaneously for 13 days after vaccine injection 1 and injections 4 to 8. Treatment related tumor specific CD4 and CD8 positive T cell precursors were assessed.

Results: A total of 22 patients were entered into this study. Patients were stratified by bulk of disease (group 1, 9 patients with micrometastatic disease, and group 2, 13 patients with macrometastatic disease). In general treatment was well tolerated. Of 9 patients in group 1 7 remained disease-free after nephrectomy. In group 2, 6 patients had stable (46.2%) and 7 patients had progressive disease (53.8%). Statistically significant treatment related increases in CD4 ($p = 0.028$) and CD8 ($p = 0.018$) positive tumor specific T cell precursors were observed for the entire group of patients. Changes in CD4 and CD8 positive precursors correlated significantly with each other ($p = 0.0001$). This correlation was seen in the 2 patient subpopulations as well (group 1 $p = 0.003$, group 2 $p = 0.013$). Patients with minimal disease, and with changes in CD4 and CD8 positive tumor specific T cell precursors greater than the median appeared to have an improved time to progression as well as a survival benefit.

Conclusions: GM-CSF and autologous vaccine can be given safely in combination to patients with renal cell cancer. We observed treatment related changes in tumor specific circulating lymphocyte populations.

KEY WORDS: granulocyte-macrophage colony-stimulating factor, cancer vaccines; carcinoma, renal cell; immunity

Renal cell carcinoma (RCC) represents the ninth leading site of cancer in the United States (31,000 new cases estimated for 2003) and is the 10th leading cause of cancer related mortality (12,500 deaths estimated in 2003). Metastatic disease develops in approximately 40% of patients.[1] Less than 10% of these patients survive for 2 years and virtually all patients die within 5 years. Systemic therapies using hormones or cytotoxic agents have not demonstrated survival advantage. No single combination cytokine therapy for metastatic RCC is clearly superior and a number of standard treatments are practiced. Recent reviews of RCC therapy found significant overall survival in patients treated with interleukin-2 and/or interferon-$\alpha$.[2,3] Identification of RCC specific T cell responses along with clinical responses to biotherapy and spontaneous regressions in patients with metastatic RCC strongly suggest a role for the host immune system in this disease.[4–8]

The feasibility of antitumor vaccination via autologous tumor vaccine has been demonstrated[9,10] and biological effects have been observed.[11,12] However, immunization with autol-

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***Editor's Note:*** **This article is the first of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 1292 and 1293.**

ogous tumor has produced few clinical responses suggesting that an additional stimulus might be needed to improve clinical outcome. Lymphocyte proliferation and activity are modulated through a complex cytokine network. In a recent study of autologous RCC vaccine with rIFN-$\gamma$ and rIFN-$\alpha$, we found further evidence that cytokines might be crucial in the initiation of appropriate immune responses.[13] Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) stimulates the proliferation and activation of T cells, macrophages and dendritic cells. In the present pilot study, we investigated the feasibility and immunological effects of autologous tumor vaccine combined with rhGM-CSF in patients with RCC.

## MATERIALS AND METHODS

*Patients.* Patients with pathological tumor stage II and IIIa or stage IIIb and IV RCC were eligible. The 1997 American Joint Committee on Cancer international TNM staging system was used to classify pathological tumor stage. Recent review articles quote a 5-year survival rate for pathological T1 tumors of 91% and for T2 tumors of only 74%.[14] In addition, the estimated 5-year disease-free survival is significantly decreased in patients with microvascular invasion or extent of tumor into the renal vein or inferior vena cava (T3b), compared to patients with extent of tumor into the adrenal gland or perinephric fat (T3a).[14] To account for the possible impact of tumor mass on immunological parameters and to adjust for the previously mentioned prognostic implications, patients were divided into group 1 (micrometastatic disease), patients at high risk for recurrence (pathological tumor stages II and IIIa), and group 2 (macrometastatic disease), pathological tumor stages IIIb and IV as well as patients with documented metastases before treatment start.

Patients who received previous treatment with chemotherapy, radiotherapy, hormone therapy or biological therapy were eligible for study as long as they had not been treated for at least 4 weeks. Previous GM-CSF was permitted as long as it had been used as treatment for bone marrow suppression only, the patient had fully recovered and treatment had been completed for at least 4 weeks. Patients had to have a Karnofsky performance status of greater than 70% and adequate organ function as demonstrated by a white blood cell count of greater than 4,000 cells per dl, platelets greater than 100,000 cells per dl, BUN less than 30 and creatinine less than 2.0 mg/dl. Liver function tests were required to be less than 2 times the upper limit of normal unless abnormalities were due to the disease itself. Patients were ineligible for the study if they had significant heart disease (New York Heart Association class 3 or 4), a history of autoimmune disease, active tuberculosis or a history of second malignancy (other than cervical carcinoma in situ or nonmelanoma skin cancer within the last 5 years). The trial protocol was reviewed and approved by the Norris Cotton Cancer Center's Scientific Review Committee and the Dartmouth Medical School Institutional Review Board. The protocol and vaccine were prepared under Investigational New Drug Application #BB4990. All patients were required to provide informed written consent before they were registered.

*Vaccine preparation.* Tumor cell preparation was in accordance with Investigational New Drug Application BB4990 following a previously reported protocol.[11] Briefly, nephrectomy specimens were placed in sterile media (Hank's Balanced Salt Solution [Cellgro Mediatech, Herndon, Virginia] and 50 $\mu$g/ml of gentamicin [Gibco/Life Technologies, Rockville, Maryland]) and subsequently minced. All work with the tumor cell suspension was performed under strict sterile conditions in accordance with good manufacturing procedures. Single cell suspensions were produced by mincing tissue that was filtered, centrifuged and washed (mechanically dissociated cells). The remaining tissue was digested with 0.4% collagenase type V, 0.02% deoxyribonuclease I (Sigma, St. Louis, Missouri) and RPMI-1640 (Cellgro-Mediatech) at 37C for approximately 60 minutes. The digest was then filtered and cells were washed (enzymatic cell preparation). Mechanical and enzymatic cell preparations were stored at −140C in freeze media made up of 90% pooled human AB serum (NABI, Boca Raton, Florida) and 10% DMSO (Sigma). For the vaccine preparation, autologous tumor cells were thawed, washed and irradiated (10,000 cGy, Irradiator Model 81-14R, J. L. Shepard & Associates, Glendale, California).

*Treatment plan.* Patients in both groups were treated with the same dose and schedule. The administration of the autologous vaccine was consistent with a previously reported treatment regimen.[11] The treatment schedule for GM-CSF was based on a previously reported regimen suggesting survival benefit in patients with surgically resected metastatic melanoma.[15] Treatment was divided into 2 phases, an induction phase and a consolidation phase. The induction phase consisted of vaccination with 0.5 to 1.0 $\times$ $10^7$ irradiated autologous tumor cells admixed with 250 $\mu$g rhGM-CSF (donated by Immunex, Inc., Seattle, Washington) administered intradermally weekly for 4 weeks. In addition, 125 $\mu$g/m$^2$ rhGM-CSF was given subcutaneously for 6 consecutive days starting the day after each of the first 2 induction vaccinations. The consolidation phase was 1 to 4 cycles monthly depending upon availability of autologous tumor cells. This phase consisted of the same vaccine as the induction phase with 125 $\mu$g/m$^2$ rhGM-CSF given subcutaneously for 13 consecutive days starting the day after each consolidation vaccine. If not enough tumor cells were available for all planned vaccines, patients still underwent treatment with rhGM-CSF alone at the scheduled vaccination time for a total of 4 cycles during the consolidation phase.

No decrease in the number of tumor cells in the vaccine was permitted. The dose of rhGM-CSF was decreased by 50% for grade 3 or greater skin reactions for the subsequent treatment and by 75% if necessary for persistent toxicity. RhGM-CSF was discontinued if toxicity persisted despite dose reductions.

*Measurement of precursor frequencies.* To evaluate the induction of tumor specific CD4 positive and CD8 positive peripheral blood T cells during the course of treatment a dye dilution proliferation assay was performed as previously described.[12] T cell precursor frequencies were measured 2 weeks before the first vaccine and 2 weeks after the last vaccine. The dye dilution proliferation assay is a multiparameter flow cytometric approach which uses PKH-26, a lipophilic dye stably incorporated into the cell membrane. When excited at 488 nm the dye fluoresces, giving cells a bright, homogenous label which is detected in a flow cytometer's FL2 channel (585 $\pm$ 42 nm). With each cell division the dye distributes equally between the daughter cells and the PKH-26 fluorescent intensity is decreased by ½. With mathematical deconvolution of the fluorescence histograms providing information about the proportion of cells in each of the daughter generations, information can be derived about the precursor frequency of cells in the original population that responded to the specific stimulus.

Briefly, peripheral blood mononuclear cells (PBMC) were isolated and labeled with PKH26 dye (Sigma) at a final concentration of 1 $\times$ $10^{-6}$ M. PBMCs before and after treatment were then incubated with the antigens or mitogens of interest. In this trial conconavalin A (10 $\mu$g/ml, Sigma) was used as a positive control and PBMCs alone served as a negative control. Autologous tumor cells were used to assess for individual and tumor specific treatment effects. PBMCs were incubated with the antigens in tissue culture plates for 10 days. On day 10 the contents of each well were harvested, and cells were washed and counted. They were subsequently stained for flow cytometric analysis with mIg$G_1$-FITC con-

trol, or αCD4-FITC or αCD8-FITC (Dako, Carpinteria, California). Flow cytometric data files were analyzed using ModFitLT software (Verity Software House, Topsham, Maine), allowing deconvolution of the PKH-26 (FL-2) fluorescence intensity to determine the proportion of cells having undergone 0 to 10 cell divisions.

*Delayed-type hypersensitivity skin testing.* Delayed-type hypersensitivity (DTH) skin testing using mechanically dissociated irradiated autologous tumor cells was performed on each patient as an in vivo correlate to T cell precursor frequency and as a clinical measure of enhanced immune responsiveness resulting from treatment. Testing was performed before (within 2 weeks of commencing therapy), during and after treatment. Each test involved planting a standard panel of antigens (candida, mumps, tuberculin, tetanus). A skin test was considered positive when a reaction of greater than 5 mm skin induration was observed.

*Evaluation of clinical response.* Initial pretreatment evaluation included a routine history and physical examination, computerized tomography of the chest, abdomen and pelvis, chest x-ray and detailed blood tests. Radiologic evaluation was repeated every 8 to 12 weeks. Clinical end points in this study were time to progression (TTP), interval from study registration until documented objective disease progression, and overall survival (OS), the time from registration until death. Objective responses to treatment were also assessed in patients with measurable metastatic disease.

*Statistical analysis.* Statistical considerations before the trial assumed a 0.05 level of significance and a power of 0.9 in paired t tests to determine the differences in precursor frequencies. A sample size of 11 patients was required to be able to detect a mean difference of 1 standard deviation in each disease group. Changes in precursor frequencies were analyzed using the Wilcoxon signed-rank test. Median time to progression and survival (in months) were calculated using the Kaplan-Meier method. Correlation of clinical and immunological end points was performed by comparing the TTP and OS of patients who had precursor frequency changes greater than the median after treatment with patients who had precursor frequency changes less than the median.

## RESULTS

*Patients.* A total of 22 patients were entered into this pilot study. Patient characteristics are reported in table 1. Nine patients had T2 or T3a tumors and were classified into group 1. Patient 3 had an enlarged, likely reactive lymph node on post-nephrectomy computerized tomography. There were 13 patients who presented with tumor stage IIIb or IV consistent with macrometastatic disease (group 2). The most common sites of metastases were lung, bone, lymph nodes and brain. The number of metastases ranged from 0 to 4 per patient (M0 to M4). Patient 13 presented with bilateral renal tumors and was treated with a right radical and left partial nephrectomy. Patient age ranged from 19 to 76 years at nephrectomy. The time from nephrectomy to first vaccination ranged from 1 to 40 months. Median followup for group 1 was 23 months and for group 2 was 12.8 months. Time to progression and survival data were documented from the first vaccination. Median number of vaccinations received was 8 (range 6 to 8, mean 7.7). The tumor-cell viability ranged from 8% to 88% and the median was 63% before irradiation. This viability was determined in a standard trypan-blue preparation after the final wash.

*Toxicity.* Treatment was generally well tolerated. Side effects of grades I and II toxicities included erythema at injection site, fever, chills, fatigue and hypothyroidism. Two patients experienced side effects consistent with grade III toxicity (table 2). Seizures developed in 1 patient after 6 vaccine courses and treatment was discontinued. Further evaluation demonstrated new brain lesions and the seizure activity was believed to be secondary to inflammatory reactions at the metastatic sites as a result of treatment. An episode of congestive heart failure requiring hospitalization was seen in 1 patient while on treatment. This episode was believed to be secondary to underlying cardiac disease. The same patient experienced grade 3 hyperglycemia and was medically treated while completing all 8 courses of therapy.

*Induction of tumor specific T cell precursors.* CD4 and CD8 positive T cell precursor frequencies were measured before and after treatment to assess the treatment effect on tumor specific immune responses (table 3). Of the 22 patients entered into the trial 17 had T cell precursor data available. T cell precursor frequencies represented the percentage of tumor specific T cells within a lymphocyte population that proliferated in response to an antigen. The mean percentage (±SD) of tumor-specific CD4 positive T cell precursors was 0.46 (±0.11) before and 1.20 (±0.35) after completion of treatment. The mean change was 0.74 (±0.30). Similarly, the

TABLE 1. *Patient demographics*

| Pt No. — Pt Age | Histology | Pathological Tumor Stage | Best Response While on Study | DTH-Response Pre/post | Mos TTP | Mos Survival From Study Start |
|---|---|---|---|---|---|---|
| | | | *Group 1* | | | |
| 3 — 62 | Clear cell | T3aN1M0 | No evidence of disease | −/+ | 32.8 | 42.3+ |
| 7 — 52 | Clear cell | T3aN0M0 | Progressive | −/− | 8 | 14.1 |
| 8 — 42 | Clear cell | T3aN0M0 | No evidence of disease | +/+ | 27.4+ | 27.4+ |
| 9 — 19 | Not available | T3aN0M0 | No evidence of disease | −/− | 22.8 | 22.8 |
| 12 — 50 | Clear cell | T2N0M0 | No evidence of disease | −/− | 38+ | 38+ |
| 15 — 76 | Clear cell | T3aN0M0 | No evidence of disease | Not available | 29+ | 29+ |
| 19 — 50 | Clear cell | T3aN0M0 | No evidence of disease | −/+ | 23+ | 23+ |
| 20 — 60 | Clear cell | T3aN0M0 | No evidence of disease | Not available | 16+ | 16+ |
| 22 — 67 | Clear cell | T3aN0M0 | No evidence of disease | −/− | 3+ | 3+ |
| | | | *Group 2* | | | |
| 1 — 48 | Not available | T4N0M4 | Stable | −/− | 2 | 7 |
| 2 — 57 | Clear cell | T2N1M2 | Stable | −/+ | 36.6 | 36.6 |
| 4 — 55 | Clear cell | T3aN1M1 | Progressive | −/− | 4.1 | 35 |
| 5 — 72 | Clear cell | T3bN0M1 | Stable | −/− | 2.5 | 52+ |
| 6 — 40 | Not available | T2N2M1 | Stable | +/+ | 19.8 | 19.8 |
| 10 — 73 | Clear cell | T3aN1M1 | Stable | −/− | 4.1 | 8 |
| 11 — 73 | Papillary | T3bN1M0 | Stable | −/+ | 28.8 | 28.8 |
| 13 — 70* | Not available | T2N0M1 | Progressive | −/− | 2.98 | 10.6 |
| 14 — 47 | Clear cell | T3bN2M1 | Progressive | Not available | 4.6 | 13.3 |
| 16 — 45 | Clear cell | T3aN1M1 | Progressive | −/− | 4.1 | 10 |
| 17 — 50 | Clear cell | T3bN0M4 | Progressive | −/− | 1.9 | 5.5 |
| 18 — 43 | Clear cell | T2N1M1 | Progressive | +/+ | 2.5 | 12.8 |
| 21 — 74 | Clear cell | T3aN0M1 | Progressive | −/− | 4 | 9 |

* Bilateral renal tumors treated with right radical and left partial nephrectomy.

TABLE 2. *Toxicities*

| Pt No. | Toxicity Type | Respective Grade |
|---|---|---|
| 1 | Seizure (brain metastases) | 3 |
| 2 | None | — |
| 3 | Erythema, fatigue, chills, hypothyroidism | 1,1,1,2 |
| 4 | Erythema, fatigue | 1, 1 |
| 5 | Pain lt arm | 1 |
| 6 | Fatigue, rt upper quadrant discomfort | 1, 1 |
| 7 | None | — |
| 8 | None | — |
| 9 | None | — |
| 10 | Fatigue, congestion (small bowel perforation 7 days off study) | 1, 1 |
| 11 | Cramps | 1 |
| 12 | None | — |
| 13 | Congestive heart failure, hyperglycemia | 3, 3 |
| 14 | None | — |
| 15 | None | — |
| 16 | None | — |
| 17 | None | — |
| 18 | Increased BUN + alkaline phosphatase, fatigue, skin rash | 1,1,1,1 |
| 19 | Increased BUN + aspartate transaminase, sore throat, backache | 2,1,1,1 |
| 20 | Injection site reaction, dry skin, back spasm | 1,1, 1 |
| 21 | Leg edema, back spasm | 1, 1 |
| 22 | None | — |

mean percentage of tumor-specific CD8 positive T cell precursors was 0.40 (±0.14) before and 1.42 (±0.69) after treatment with a mean change of 1.02 (±0.69). There was a statistically significant increase in tumor-specific CD4 positive ($p = 0.028$) and CD8 positive T cell precursors during the course of treatment ($p = 0.018$). There was a significant positive correlation in the increase in CD4 and CD8 positive T cell precursors ($p = 0.0001$). Statistical analysis was limited in the 2 patient groups because of the small sample size. However, we were able to demonstrate that the increase in CD4 and CD8 positive T cell precursors correlated with each other in group 1 as well as in group 2 ($p = 0.003$ and $p = 0.013$, respectively).

*DTH-responses.* DTH-responses were assessed before, during and on completion of treatment. Two patients in group 1 and two patients in group 2 showed a negative DTH response before treatment and a positive response at the completion of the trial. An additional 3 patients showed a positive DTH response before the trial.

*Clinical responses.* As expected there was a difference in time to disease progression as well as in survival time between the 2 groups (figs. 1 and 2). In the micrometastatic group to date, 6 of 9 patients remain free of disease after nephrectomy. Three patients had recurrence at 8, 23 and 33 months after treatment, respectively, and 1 of them died secondary to progressive disease 14 months after treatment. Median time to progression was greater than 32.9 months. Median time of survival was not reached. All remaining patients are still under close followup.

In the macrometastatic disease group, the time to disease progression ranged from 2 months to more than 52 months. Median time to progression was 4.1 months. The overall survival rate for the macrometastatic group ranged from 5 months to more than 53 months after treatment with a median survival of 13.1 months. Of 13 patients 8 have died secondary to progressive disease. In this group 6 patients had stable disease while on protocol (46.2%) and 7 patients showed progressive disease (33.8%).

The T cell precursor frequencies were analyzed in a Wilcoxon signed-rank test and time to progression as well as survival time were calculated with the Kaplan-Meier method to identify correlations between the induction of T cell precursors and clinical responses. There appeared to be an advantage in time to progression as well as in survival time for patients with micrometastatic disease with percent changes greater than the median in CD4 and CD8 positive tumor specific precursors (figs. 3 and 4). This observation was not statistically significant.

## DISCUSSION

Immunotherapeutic approaches to renal cell carcinoma have earned a prominent position in the treatment of advanced disease. Strategies involving cytokines and autologous tumor vaccine have shown some clinical efficacy.[2,3,11] Repmann et al recently reported a significant benefit in 5-year survival in patients treated with adjuvant autologous tumor cell lysate.[16] In addition, Mulders et al demonstrated that immunosuppressed tumor infiltrating lymphocytes could be activated with autologous dendritic cells pulsed with autologous tumor lysate.[17] Su et al reported expansion of tumor specific T cells in patients vaccinated with tumor RNA-transfected dendritic cells.[18] Finally, Simons et al demonstrated the bioactivity of a GM-CSF gene-transduced autologous tumor vaccine.[19] In this context we investigated the tumor-specific effects on the immune system of patients with advanced RCC when treated with a combination of GM-CSF and autologous tumor vaccine. Tumor specific CD4 positive and CD8 positive precursors were assessed before and after treatment. We have shown that autologous tumor vaccine with adjuvant GM-CSF is feasible and in general well tolerated.

TABLE 3. *Precursor frequencies before and after vaccine*

| | CD4 Before Vaccine | CD4 After Vaccine | CD8 Before Vaccine | CD8 After Vaccine |
|---|---|---|---|---|
| Group 1 pt No.: | | | | |
| 3 | 1.00 | 2.90 | 0.89 | 1.56 |
| 7 | 0.10 | 0.14 | 0.00 | 0.00 |
| 8 | 0.69 | 2.33 | 0.42 | 1.28 |
| 9 | 0.10 | 0.06 | 0.00 | 0.01 |
| 15 | 0.70 | 0.64 | 1.31 | 1.05 |
| 19 | 1.61 | 3.10 | 0.35 | 12.13 |
| Mean (SE) | 0.70 (±0.23) | 1.53 (±0.57) | 0.50 (±0.21) | 2.67 (±1.91) |
| Group 2 pt No.: | | | | |
| 1 | 0.15 | 4.69 | 0.07 | 1.87 |
| 2 | 0.04 | 0.46 | 0.00 | 0.26 |
| 4 | 0.43 | 0.18 | 0.00 | 0.35 |
| 6 | 0.29 | 0.03 | 1.79 | 0.62 |
| 10 | 0.21 | 0.02 | 0.00 | 0.21 |
| 11 | 0.28 | 0.56 | 0.05 | 0.76 |
| 13 | 0.20 | 0.11 | 0.05 | 0.05 |
| 14 | 0.43 | 0.18 | 0.00 | 0.35 |
| 16 | 0.46 | 1.12 | 0.44 | 0.69 |
| 27 | 1.17 | 3.14 | 1.24 | 2.83 |
| 28 | 0.03 | 0.10 | 0.00 | 0.00 |
| Mean (SE) | 0.34 (±0.09) | 1.02 (±0.46) | 0.35 (±0.18) | 0.74 (±0.26) |

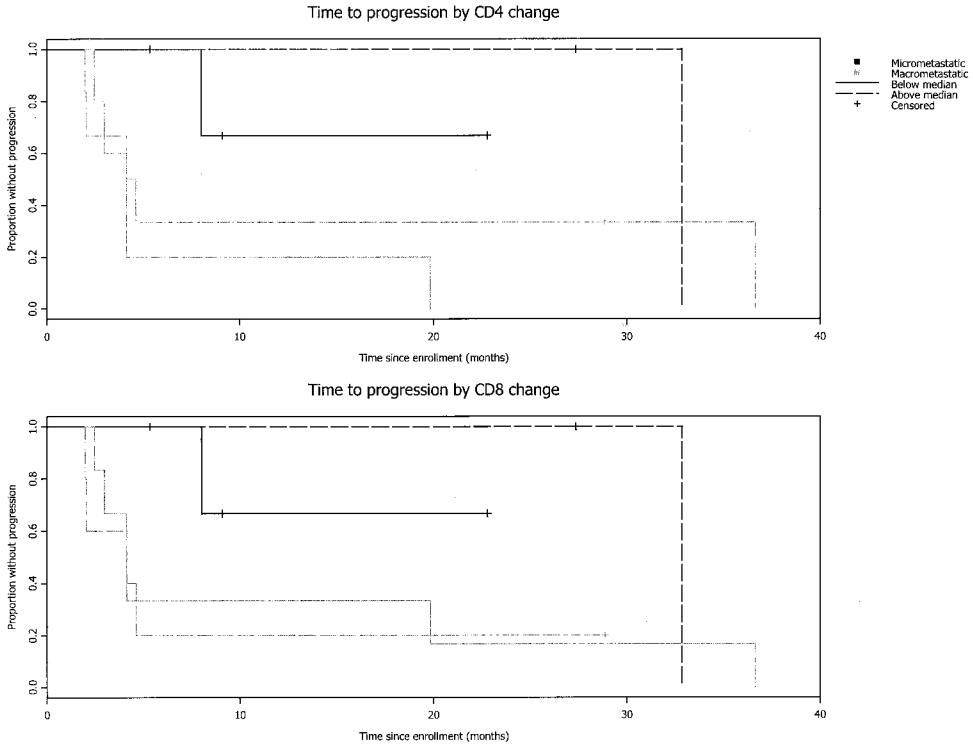


FIG. 1. Time to progression. Proportion of patients without evidence of progressive disease by months. Solid line represents patients with macrometastatic or bulky disease. Broken line represents patients with minimal residual or micrometastatic disease. +, censored patients.

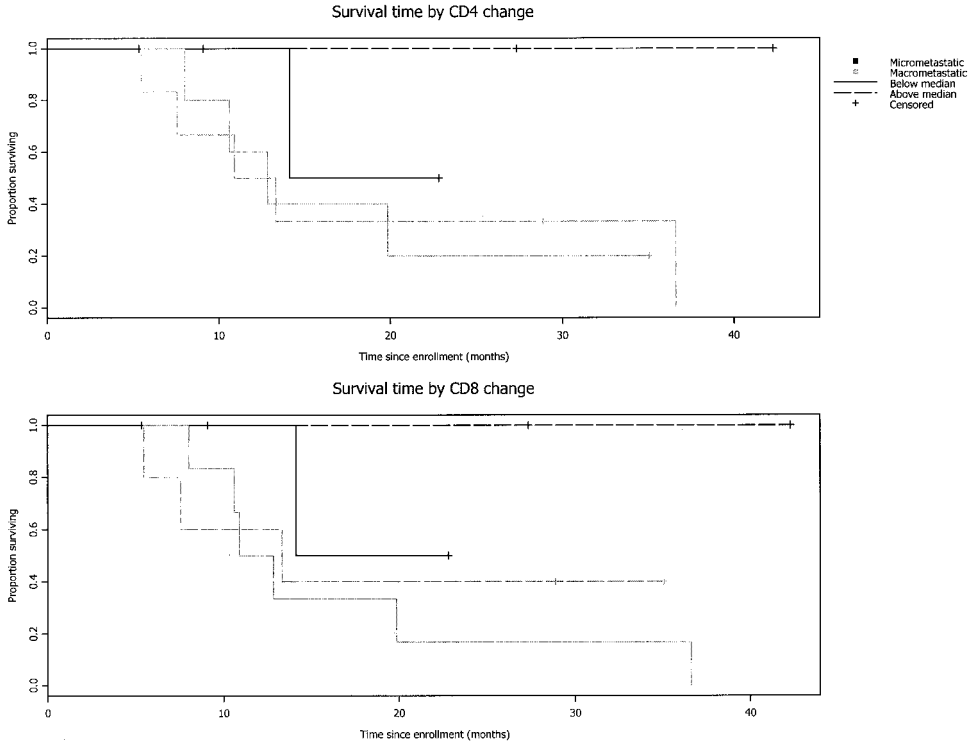


FIG. 2. Survival time. Proportion of patients alive by months. Solid line represents patients with macrometastatic or bulky disease. Broken line represents patients with minimal residual or micrometastatic disease. +, censored patients.

We recently demonstrated the immunological effects of autologous vaccine and interferons in patients with RCC.[12] We suggested that a certain subpopulation of patients showed a state of improved immune readiness resulting in improved outcomes with immunotherapy. Following this hypothesis we identified the induction of tumor specific T cell precursors throughout treatment to monitor effects on the immune system in the present study. We demonstrated the induction of tumor specific T cell precursors during the course of treatment for CD4 and CD8 positive T cells. This change was statistically significant. We further showed that the increases in CD4 and CD8 positive T cell precursors correlated with each other. This correlation was also statistically significant, even in the 2 subgroups with fairly small

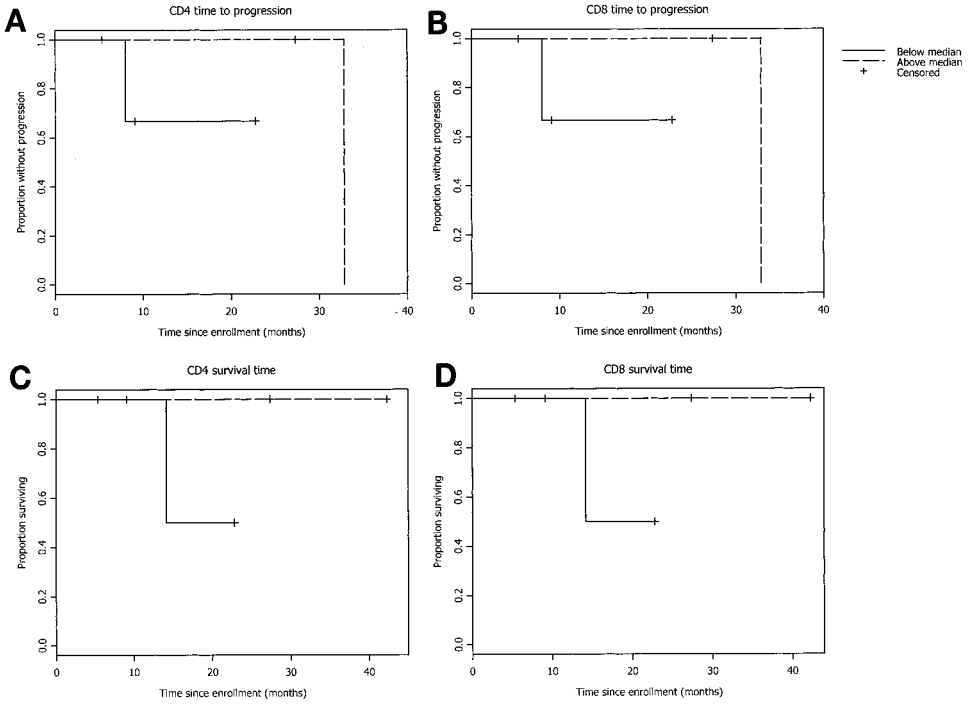


FIG. 3. Evaluation of CD4 (*A* and *C*) and CD8 (*B* and *D*) tumor specific precursors by time to progression (*A* and *B*) or survival (*C* and *D*) for patients with minimal residual or micrometastatic disease. Solid line represents patients with induction of CD4 or CD8 precursors below median. Broken line represents patients with induction of CD4 or CD8 precursors above median. +, censored patients.

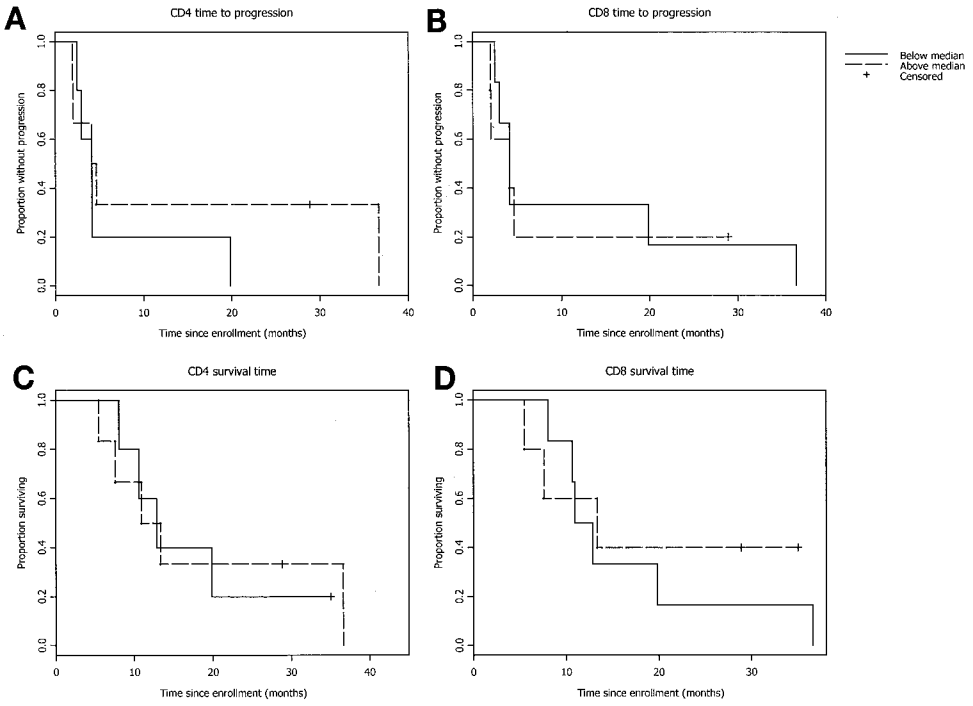


FIG. 4. Evaluation of CD4 (*A* and *C*) and CD8 (*B* and *D*) tumor specific precursors by time to progression (*A* and *B*) or survival (*C* and *D*) for patients with macrometastatic or bulky disease. Solid line represents patients with induction of CD4 or CD8 precursors below median. Broken line represents patients with induction of CD4 or CD8 precursors above median. +, censored patients.

patient numbers. These data suggest a significant impact of this treatment combination on the immune system.

GM-CSF has shown clinical benefit in solid tumors.[15] However, its role as a single agent in advanced RCC has been limited so far.[20,21] To our knowledge the assessment of immunological parameters has been limited in these trials. GM-CSF has a wide variety of nonspecific immune stimulatory effects. We report the induction of tumor-specific, autologous T cell precursors and hypothesize that these findings are the synergistic result of the combination of GM-CSF with autologous vaccine. Future clinical trials will have to determine the impact of either treatment component separately.

Dillman et al described DTH-conversion as a valid tool for the assessment of successful treatment with autologous tumor vaccines. They suggested a survival benefit for patients with DTH-conversion.[22] They report a DTH-conversion rate

of 25% to 30%.[23] We reported a DTH conversion of 4 of 22 patients (18%). However, patient numbers were too small to perform any statistical analyses, and the interpretation of the clinical efficacy of this treatment regimen based on DTH-response is limited.

The clinical end points of this study were TTP and OS. One complete response was seen in a patient with pulmonary metastases and 7 of 9 patients in the micrometastatic group are still free of progressive disease. It should be noted that the patient with a complete response had recurrence on completion of treatment. This finding might be an indication of immune escape either due to the heterogeneity of tumor antigen expression or via immune suppressor pathways.[14] Prophylactic treatment with adjuvant cytokines might be considered based on these observations. As was expected TTP and OS differed among the 2 groups. We correlated TTP and OS with the percent change in precursor frequencies in an attempt to identify patients who might benefit from immunotherapeutic regimens. There appeared to be an advantage in TTP and OS for patients with micrometastatic disease that showed changes in CD4 and CD8 positive precursors greater than the median. This observation was not statistically significant. However, the identification of patient subpopulations that will benefit significantly from immunotherapeutic treatment strategies is an aspect of utmost importance in this malignancy.[14] These observations will have to be verified in a larger patient population.

## CONCLUSIONS

We report the results of a pilot study that assessed immunological effects of combined treatment with GM-CSF and autologous tumor vaccine in patients with advanced RCC. We demonstrated a tumor specific treatment effect on the immune system, specifically CD4 and CD8 positive T cell precursors. A distinct subpopulation of patients with micrometastatic disease and induction of T cell precursors appeared to have a survival advantage and an improved TTP. Future trials will have to focus on the identification of patients who will benefit from immunotherapy. The data presented in this study will be beneficial in this context.

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