Abstract. Aim: This study aimed to determine the long-term survival of 30 dendritic cell (DC) vaccinated patients with metastatic renal cell cancer. Patients and Methods: Patients were treated with a therapeutic vaccination of pulsed DCs in one of three clinical phase I/II trials during the years 1999 - 2003. Results: Patients were followed up until 191 months (mean 65 days after DC therapy), when all patients had died. Total response to treatment was 40% with partial remission in 3%, mixed response in 7% and stable disease in 30%. The progressive disease rate was 60%. Long-term survival ranged from 3 to 191 months, with a mean survival of 39 months. Interestingly, patients who were treated previously with another form of immunotherapy showed a significantly improved probability of surviving. Mean long-term survival from the beginning of DC therapy was 21 months (1 to 75 months). Conclusion: Patients treated with DC vaccination seem to have a benefit in long-term survival.

Renal cell cancer constitutes 2-4% of all malignant tumours (1) and has an incidence of about 8-9 persons per 100,000 each year, which is increasing (2). The age at which individuals are diagnosed with renal cell cancer peaks at an age between 50 and 70 years. More men than women are affected (3). Approximately 85% of renal cell carcinomas are adenocarcinomas. At the time of diagnosis, 30% of patients have metastases, mostly in lung, liver and bones (4).

Because of poor response to radiation therapy or chemotherapy, several studies have been initiated to find alternative therapeutic options. In addition to immunotherapy with cytokines, combination of cytokine and chemotherapy, allogeneic blood stem cell transplantation (5) and vaccination with dendritic cells (DCs), newer proposed therapies involve with the inhibition of tyrosine kinases or tumour angiogenesis factors. These newer therapeutic options offer positive response rates up to 40% (6, 7). Nevertheless, immunotherapy still is an important therapeutic option (8) for these patients.

DCs are known to be the most potent stimulators of T lymphocytes, and are important in the anti-tumour immune response (9). DCs obtained from peripheral blood mononuclear cells (PBMCs) have been used in several studies to increase the cytotoxic activity of immunological effector cells in vitro and in vivo (10).

The low expression of active antigens of renal cell carcinomas is one of the main reasons for the low response of the systemic immune defense. Defective systemic co-stimulators seem to be inhibited by the tumour cells. By vaccination with DCs, the immune system is stimulated to produce a strong immune response. DC treatment is well tolerated. Severe side effects from using DC vaccines have not been reported (11-13).

This paper describes three clinical phase I/II trials that were carried out on patients with metastatic renal cell cancer between 1999 and 2003. The common factor between these trials was the vaccination with autologous or allogeneic DCs fused in vitro with tumour-specific material.

Long-term survival of these patients was determined from the day of first diagnosis until the point of death.

Patients and Methods

The three clinical phase I/II trials treating patients with metastatic renal cell cancer with DC vaccination were all carried out between 1999 and 2003.

In the first trial, which took place between 1999 and 2000, 15 patients received 5 DC vaccinations in total over a period of 7 weeks: 11 patients were vaccinated with tumour lysate pulsed autologous DCs, and 4 patients received unpulsed DCs. The DCs were delivered intranodally or subcutaneously. Further details are given elsewhere (14).

Twelve patients were enrolled in the second trial between 2000 and 2001: 4 of these patients were vaccinated with allogeneic DCs fused with autologous tumour cells and 8 patients received allogeneic DCs fused with HLA-matched allogeneic tumour cells.
All 12 patients were vaccinated by intradermal injections on 3 appointments during a period of 8 weeks (15).

In the last trial, which took place between 2002 and 2003, 10 patients were treated with tumour-specific human telomerase reverse transcriptase (hTERT)-pulsed DCs. These were injected five times over a period of 7 weeks both delivered equally intradermally and intratumourally by using computed tomography (CT) guidance. One patient received only intradermal injections. Further details are given in (16, 17).

The common feature of these studies was a progressive metastatic renal cell carcinoma, Karnofsky score 60%-100%, age >18 years.

Exclusion criteria included a time lapse of less than 28 days to previous chemotherapy or cytokine treatment, severe heart disease, severe psychiatric disease, active hepatitis A, B or C and HIV infection.

From the initial 37 patients studied over these 3 clinical trials, only 30 were included in the final analysis. This was because 3 patients participated in 2 of the trials and 1 patient in all 3 clinical trials using differently pulsed DCs. The data of 2 other patients could not be used because they did not participate in the further investigations. Patient characteristics, as well as pretreatment information, are given in Table I.

All patients received intradermal, intranodal, subcutaneous or intratumoural injection of loaded DCs.

Determination of clinical outcome was based mainly on comparison of computed tomography scans before and after treatment during follow-up until day 65 (on average) in each of the studies.

Response rates were defined as follows: CR (complete remission), tumour reduction to 0%; PR (partial remission), tumour reduction to 1-50%; MR (mixed response), tumour reduction to 50-75%; SD (stable disease), tumour mass of 75-125%; PD (progressive disease), >125% tumour mass.

Long-term survival of the patients was calculated as from the time of first diagnosis until point of death. For probability of survival calculations, the Kaplan-Meier method was used and was presented graphically by SPSS Version 11.5.1 (SPSS Inc., Chicago, IL, U.S.A.).
Figure 3. Long-term survival in months from the day of first diagnosis of patients with previous IL-2 and/or interferon alpha therapy.

$p = 0.05$

Figure 4. Long-term survival in months from the day of first diagnosis of patients with previous DC therapy.

$p = 0.12$

Figure 5. Long-term survival in months from the day of first diagnosis dependent on TNM stage at first diagnosis.

$p = 0.05$

Figure 6. Comparison of long-term survival in months from the day of first diagnosis of patients treated with differently pulsed DC.

$p = 0.78$
Results

All participants of these studies were treated with differently pulsed DC vaccinations between 1999-2003 in three clinical phase I/II trials (14-17). Four of the patients had been vaccinated with more than one form of fused DC via their participation in more than one of the three trials described.

The primary endpoint of the analysis was the long-term survival from day of first diagnosis. Long-term survival ranged from 3 to 191 months, with a mean survival of 59 months (Figure 1). In follow-up investigations until day 65 after DC therapy, a response rate of PD=60% (18 patients), SD=30% (9 patients), MR=7% (2 patients), PR=3% (1 patient) was observed. Complete Remission was not detected in any of the patients. The patients that had SD after DC vaccination demonstrated the longest survival with a mean of 88 months and a range between 25 to 164 months (Figure 2).

In comparison to untreated patients, those with a previous cytokine therapy (IL-2 and/or interferon alpha) had a significant \( p=0.05 \) increase in survival time (Table II and Figure 3). By analysing the long-term survival from day of first diagnosis after treatment with DC vaccination, those patients who took part in more than one of the described three clinical trials had no significant \( p=0.12 \) higher probability of survival than those who participated in only one trial (Table III and Figure 4). One patient, who took part in all three clinical trials, and had a previous cytokine therapy, had a survival by day of first diagnosis (tumour stage III) of 191 months. In regard to patient survival, a comparison between the different DC vaccinations detected no significant differences (Figure 6). Long-term survival from the beginning of DC vaccination ranged from 1 to 75 months, with a mean survival of 21 months (Figure 7).

Discussion

This analysis concentrated on the long-term survival from day of first diagnosis until point of death after vaccination with DCs in three clinical phase I/II trials carried out between 1999-2003. These studies concerned the vaccination of pulsed DCs fused with different tumour constitution parts. Vaccination of tumour cell pulsed DCs supports the immunological response by circumventing the tumour natural lacking expression of antigenetic active antigens. The outcome was a long-term survival between 3 and 191 months from the day of diagnosis (Figure 1), depending on the tumour stage at the time of first diagnosis (Figure 5), which seems to be a good predictor of long-term survival as described by Ficcaro et al. (18). The mean survival was 59 months.

These data show that patients with SD after DC vaccination show the longest survival, with a mean of 88 months and a range between 25 to 164 months (Figure 2). The observation of an elongated SD after vaccination with DCs has also been confirmed by other studies, such as that of Berntsen et al. (19).

The toxicities of the treatment with vaccinated DC are minor. Neither systemic or autoimmune side-effects have been described yet (13).

By showing a high response rate in tumour remission, IL-2 and interferon alpha are still the most important immune therapeutic strategies (20). Nevertheless, the side-effects...
effects, such as capillary leak syndrome, forbid a high intravenous single application of IL-2.

Hilles and Kolesar described the inhibition of tyrosine kinases by giving sunitinib and sorafenib as second line therapy, which showed a superior long-term survival (21).

In this context, a significantly higher response rate for sunitinib compared with interferon alpha is described by Motzer et al. (22).

Dutcher et al. (23) showed no higher response rates for temsirolimus compared with interferon alpha.

In the present analysis, a higher response rate of combined therapies is demonstrated. Participates with a previous cytokine therapy (IL-2 and/or interferon alpha) had a significant \( p=0.05 \) benefit in survival (Table II, Figure 3) in comparison to untreated patients.

By analysing the long-term survival after treatment with DC vaccination, those patients who took part in more than one of the three described clinical trials had no statistically significant \( p=0.12 \) higher probability of survival than those treated in only one trial (Table III, Figure 4). However, one patient who took part in all three clinical trials, with a tumour stage 3 on day of first diagnosis, showed a long-term survival of 191 months. The value of this analysis is limited by the low number of cases, but supports recent reports that show that current months. The value of this analysis is limited by the low number of cases, but supports recent reports that show that current

Acknowledgements

We kindly acknowledge the support of the Deutsche Krebshilfe, Bonn, Germany, and the expertise of our clinical teams at our departments.

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