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ORIGINAL ARTICLE

EORTC QLQ-BM22 and QLQ-C30 quality of life scores in patients with painful bone metastases of prostate cancer treated with strontium-89 radionuclide therapy

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Abstract
Purpose Approximately 80 % of patients with prostate cancer will develop bone metastases, which often lead to bone pain and skeletal-related events. Sr-89 is an established alternative for the palliation of bone pain in prostate cancer. We aimed to assess the effect of Sr-89 radionuclide therapy on quality of life (QOL) in prostate cancer patients with painful bone metastases.
Materials and methods Thirteen patients received a single intravenous injection of Sr-89 at a dose of 2.0 MBq/kg. All patients underwent QOL evaluation prior to Sr-89 treatment and 1, 2, and 3 months afterward using the Japanese version of the EORTC QLQ-BM22, EORTC QLQ-C30, a VAS, and face scale. We also evaluated PSA and ALP response and toxicity of the Sr-89 therapy.
Results The pain characteristics subscale of the EORTC QLQ-BM22 was significantly reduced from 1 month onward compared with the baseline. The functional interference and psychosocial aspects subscales were significantly higher than baseline from 2 months onward. At 2 months, VAS indicated a significant reduction in pain as compared to the baseline. Sr-89 therapy caused a non-significant reduction in PSA and ALP levels. No patients had leukocyte toxicity, and one patient had grade 3 platelet toxicity.
Conclusion Sr-89 radionuclide therapy can provide not only reduced pain characteristics but also better psychosocial aspects and functional interference in patients with painful bone metastases of prostate cancer.

Keywords Prostate cancer · Bone metastases · Quality of life · Strontium-89

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Introduction

Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of male cancer death in the world, accounting for 14 % (903,500) of the total new cancer cases in 2008 [1]. Bone is the most common site for metastasis in prostate cancer, and many patients will develop bone metastases during the natural course of their disease. It is most commonly seen in the advanced stages and significantly decreases the patient's quality of life (QOL). Therefore, symptom control and improved QOL are primary goals of treatment in palliative oncology, the aim of which is to simply reduce severe pain while minimizing untoward side effects and complications [2].

Radiation therapy can be effective for the treatment of localized disease, but the hemibody irradiation used to treat widespread bone metastases may cause severe bone marrow toxicity [3, 4]. Sr-89 is a pure beta-emitting radioisotope that is concentrated in the areas of high osteoblastic activity.

Previous clinical trials of bone metastasis largely focused on skeletal-related events as objective end points. However, QOL is also a priority for all patients with cancer [5, 6]. Bone metastases trials have traditionally used the European Organisation for Research and Treatment of Cancer Quality of Life Group core questionnaire (EORTC QLQ-C30 version 3) [7] in cancer clinical trial research, and it has been well established as a QOL tool for cancer patients in general [8, 9]. In response to the need for a comprehensive module to evaluate bone metastasis-specific QOL, the EORTC developed a Quality of Life Questionnaire for Patients with Bone Metastases (EORTC QLQ-BM22). This module has been developed to be used together with the EORTC QLQ-C30, a QOL scale for cancer patients, or with its shortened version, EORTC QLQ-C-15-PAL [6].

To aim of this study was to evaluate the effect of Sr-89 radionuclide therapy on the QOL of prostate cancer patients with painful bone metastases. This is the first assessment using the EORTC QLQ-BM22 and QLQ-C30 for patients undergoing Sr-89 radionuclide therapy.

Materials and methods

Patients

The patients had prostate cancer with painful bone metastases, as diagnosed by ^{99m}Tc-MDP bone scan, and had not received any Sr-89 therapy previously. All patients gave written informed consent to participate in the study, in accordance with institutional regulations. Ethical approval for conducting this research was obtained from the institutional ethics committee.

Inclusion criteria were persistent pain in spite of analgesic treatment and life expectancy of more than 3 months. The main exclusion criteria were disseminated intravascular coagulation, significantly degraded renal function, or serious bone marrow suppression [platelet (PLT) count $<50{,}000/mm^3$, white blood cell (WBC) count $<2{,}000/mm^3$, leukocytes count $<1{,}000/mm^3$, hemoglobin <8.0/dL]. Cancer stages were evaluated according to the staging system of the American Joint Committee on Cancer, 7th edition [10].

Study design and treatment

We conducted a 12-week, prospective, single-arm, open-label pilot study to assess the effect of Sr-89 radionuclide therapy on QOL in prostate cancer patients with painful bone metastases.

Thirteen patients were consecutively enrolled in this study. The patients received a single dose of 2.0 MBq/kg of Sr-89. The Sr-89 solution was administered over 60 s using an i.v. catheter, which was then slowly flushed with saline solution. QOL and pain response assessments and blood tests were conducted prior to and at 1, 2, and 3 months after Sr-89 injection.

Quality of life assessments

The Japanese versions of the EORTC QLQ-C30 and QLQ-BM22 [11] were self administered by patients or completed with the aid of an individual not related to the patient and blind to the clinical results.

EORTC QLQ-C30 version 3.0

The EORTC QLQ-C30 version 3.0 is a questionnaire developed to assess the QOL of cancer patients. The questionnaire consists of five functional scales (physical, role, emotional, cognitive, social), symptom scales, and one global scale. All measures are scored from 0 to 100, with the same direction as QoL-E, but higher scores on the symptom scales indicate more severe problems.

The EORTC QLQ-BM22

The EORTC QLQ-BM22 addresses disease symptoms related to bone metastasis or its diagnosis; side effects, complications, and other issues related to treatment of bone metastases; and additional QOL dimensions that are relevant across diagnosis and treatment modalities in the management of bone metastases. This 22-item module is composed of four subscales, painful sites (PS) and pain characteristics (PC) on the symptom scale and functional interference (FI) and psychosocial aspects (PA) on the functional scale.

Pain assessment

The visual analog scale (VAS) and face scale are commonly used as global parameters of QOL. A VAS is a 10-cm line with descriptors at each end (e.g., good to bad, none to severe). The score is measured as the distance of the mark from one end of the line [12]. VAS has been described as a simple and clearly rated scale for subjective experiences. A face scale has five face figures considered adequate for a five-stage evaluation.

PSA and ALP response and toxicity

Each month, serum prostate-specific antigen (PSA) and serum alkaline phosphatase (ALP) were measured, physical examinations were conducted, WBC and PLT counts were monitored, and adverse events were assessed.

Adverse events were scored using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (NCI-CTCAE v. 4.0).

Statistical analysis

Analysis of variance was used to make comparisons between the means. All analyses were performed with Statview 5.0 (SAS Institute, Cary, NC, USA). Values are reported as means ± standard error, unless otherwise specified. p values <0.05 were considered statistically significant. To determine the statistical significance of changes in QOL scores and pain assessment scales, the Wilcoxon signed-rank test was used before and after Sr-89 injection.

Results

Characteristics

Table 1 lists the baseline characteristics of the 13 patients who underwent Sr-89 therapy. All patients had hormone therapy, and 10 patients (76.9 %) had castration-resistant prostate cancer before Sr-89 therapy. Eight patients (61.5 %) received external-beam radiation therapy to a symptomatic site of bone metastasis prior to the Sr-89 treatment, with a median time of 32.5 months (range, 9–149 months) from external radiation therapy treatment to the Sr-89 treatment. Three patients (23.1 %) received chemotherapy prior to the Sr-89 treatment, with a median time of 4 months (range, 3–12 months) from last chemotherapy to the Sr-89 treatment. Three patients reduced the analgesics dosage within 3 months after Sr-89 treatment. No patients increased the analgesics dosage during the study period.

According to the Eastern Cooperative Oncology Group scale, performance status prior to Sr-89 treatment was reported by the physician to be 0 or 1 in 3 patients (23.1 %) and 2 or more in the remaining10 patients (76.9 %).

Quality of life analysis

In the QLQ-C30, the mean score for global QOL was 29.9. The baseline value of the five scales of functioning ranged from 30.6 (role functioning) to 54.9 (emotional functioning). The highest baseline symptom score in the QLQ-C30 was for fatigue, followed by pain and constipation. Three items on the functional scale were significantly higher after Sr-89 therapy than at the baseline, and six items on the symptom scale decreased significantly after treatment compared with the baseline (Fig. 1).

Figure 2 shows the results of QLQ-BM22. For the symptom scale, a high score represents a high level of problems. PS was lower than the baseline at 1, 2, and 3 months, but there were no significant changes. PC, however, was significantly reduced from 1 month onward compared with the baseline (1 month, $p = 0.0170$; 2 months, $p = 0.01$; 3 months, $p = 0.0098$). For the functional scale, a high score represents a high level of functioning. Both FI and PA were significantly increased after Sr-89 treatment compare with the baseline (FI: 1 month, $p = 0.0423$, 2 months, $p = 0.0460$, 3 months, $p = 0.0258$; PA: 2 months, $p = 0.0091$, 3 months, $p = 0.0006$).

The level and extent of bone pain was rated from 0 to 10 according to a VAS. Bone pain was significantly decreased at 2 months ($p = 0.0393$) and 3 months ($p = 0.0445$) (Fig. 3a). Although the bone pain as assessed using the face scale was reduced every month after Sr-89 therapy, there were no significant changes compared to the baseline (Fig. 3b).

PSA and ALP response

Overall, there were no significant changes in serum PSA and ALP after Sr-89 therapy (Fig. 4). Only 4 of 13 patients (30.8 %) had a lower PSA after treatment, whereas ALP was reduced in 10 of 13 patients (76.9 %).

Toxicity

Figure 5 shows WBC and PLT counts following Sr-89 therapy. No patient had leukocyte toxicity in this study. The PLT counts remained below the baseline level at 1, 2, and 3 months after Sr-89 therapy. One patient (7.7 %) had grade 3 platelet toxicity and required platelet transfusion 3 months after treatment.

Discussion

Bone metastasis of malignant tumors is recognized in about 80 % of autopsy cases, and bone can be a metastatic site for all types of cancer. However, owing to recent progress in various therapy modalities, including the third-generation bisphosphonate, radiotherapy, and chemotherapy, the alleviation of bone-related events and prolonged survival have been achieved in patients with advanced cancer. In cases of prostate cancer, breast cancer, and multiple myeloma, longer life expectancy has been reported even in the disease stage with bone metastasis. Therefore, as physicians consider various therapeutic strategies and measures, long-term QOL must be considered as well.

There was no QOL scale targeting patients with bone metastasis, however, until the EORTC QLQ-BM22

Table 1 Baseline patient characteristics ($n = 13$)

Age	
Median	74
Range	60–87
TNM	
T1–2	3
T3–4	10
N0	7
N1	6
M0	0
M1	13
Pre-treatment performance status	
Median	2
Range	1–3
Post-treatment performance status	
Median	1
Range	0–2
Prior hormone therapy	
GnRH analog	13 (100 %)
Anti-androgen	12 (92.3 %)
CRPC	10 (76.9 %)
Prior radiotherapy	8 (61.5 %)
Prior chemotherapy	3 (23.1 %)
Baseline PSA	
Median	170
Range	9.2–3160

GnRH gonadotropin-releasing hormone, *CRPC* castration-resistant prostate cancer, *PSA* prostate specific antigen

evaluation module was developed recently. This module was translated into Japanese, and the preciseness of the translation was confirmed by back translation. The expression and appropriateness of each question in the Japanese version were evaluated by a structured interview and further modified based on the results. The official Japanese version of EORTC QLQ-BM22 was completed after final consultation with EORTC-QLQ [11], and it was first used in this clinical study.

The QLQ-BM22 is divided into symptom and functional scales [5, 6]. The purpose of the PS subscale is to specifically identify the painful site(s); it is possible not only to judge whether the pain is local or systemic but also to make a site-by-site evaluation of pain alleviation accompanied a change in therapy. With the PC subscale, it is possible to obtain information useful for a chronological evaluation of the therapy by assessing whether the pain is persistent or intermittent. The FI subscale allows a detailed evaluation of the pain in activities of daily life, ranging from rest to body movement to violent body movement, to investigate the limitations on daily living caused by the pain. Finally, the PA subscale comprehensively evaluates the QOL components, which cannot be evaluated with EORTC QLQ-C30 or QLQ-C15-PAL, by evaluating psychological and social worries, such as concerns about disease status and health conditions.

Currently, international field study was to test the reliability, validity, and responsiveness of the EORTC QLQ-BM22 module to assess health-related quality of life in

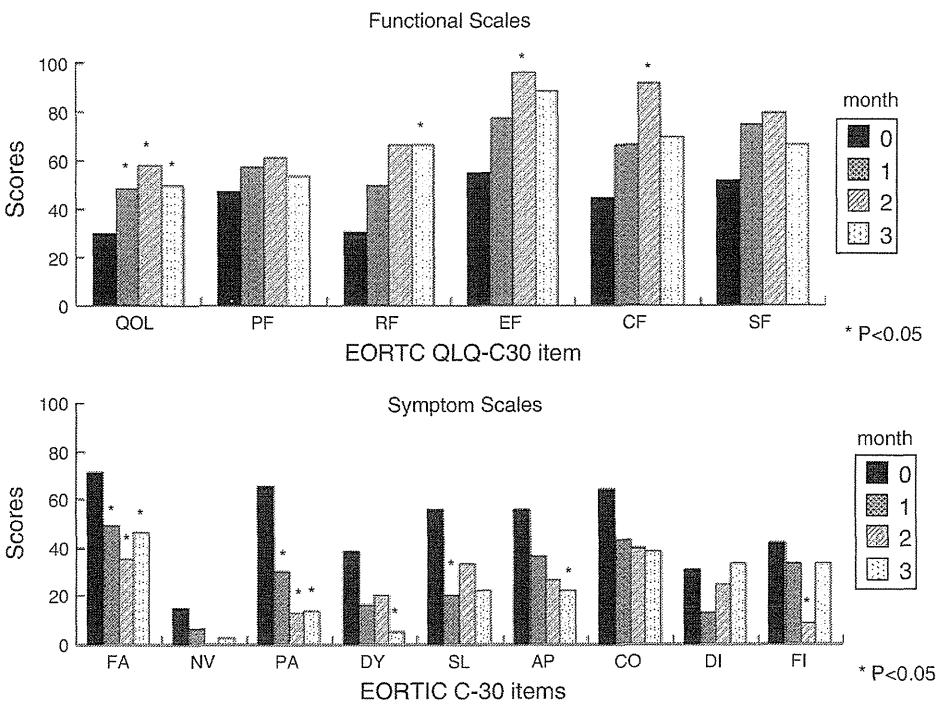

Fig. 1 Evaluation of quality of life in 13 prostate cancer patients with bone metastasis pain treated with Sr-89 therapy using the EORTC QLQ-C30, in which a high score represents a higher response level. Thus, a high score on the functional scale represents a high (healthy) level of functioning, but a high score on the symptom scale represents a high level of problems. QOL, overall quality of life; *PF* physical functioning, *RF* role functioning, *EF* emotional functioning, *CF* cognitive functioning, *SF* social functioning, *FA* fatigue, *NV* nausea and vomiting, *PA* pain, *DY* dyspnoea, *SL* insomnia, *AP* appetite loss, *CO* constipation, *DI* diarrhea, *FI* financial difficulties

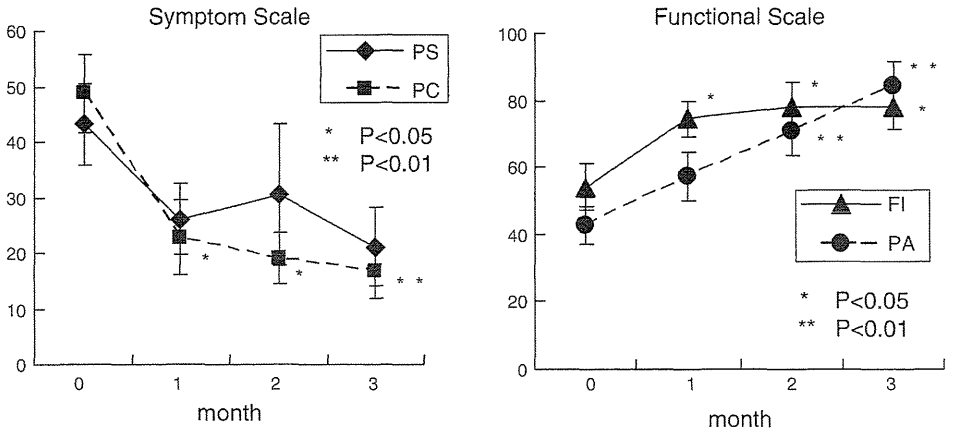

Fig. 2 Evaluation of quality of life in 13 prostate cancer patients with bone metastasis pain treated with Sr-89 therapy using the EORTC QLQ-BM22. PS: painful sites subscale. *PC* pain characteristics subscale, *FI* functional interference subscale, *PA* psychosocial aspects subscale

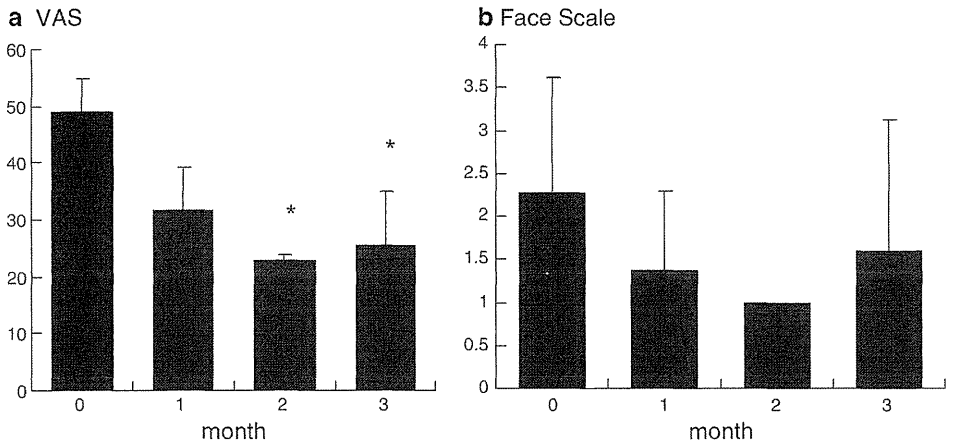

Fig. 3 Evaluation of pain level and extent in 13 prostate cancer patients with bone metastasis pain treated with Sr-89 therapy using **a** a visual analog scale (VAS), and **b** a face scale

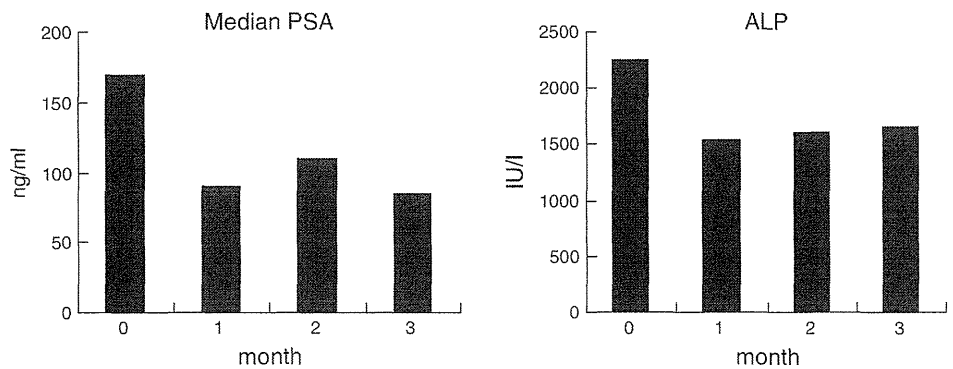

Fig. 4 Median PSA and ALP levels in 13 prostate cancer patients with bone metastasis pain treated with Sr-89 therapy ($p > 0.05$ at all time points)

patients with bone metastases. Patient feedback from the debriefing questionnaire demonstrated that the majority of patients did not have any difficulties or confusion with the items, and did not find the questionnaire items upsetting. Only 1 % of responses were missing for all 4 scales at baseline and follow-up, and the majority of patients were

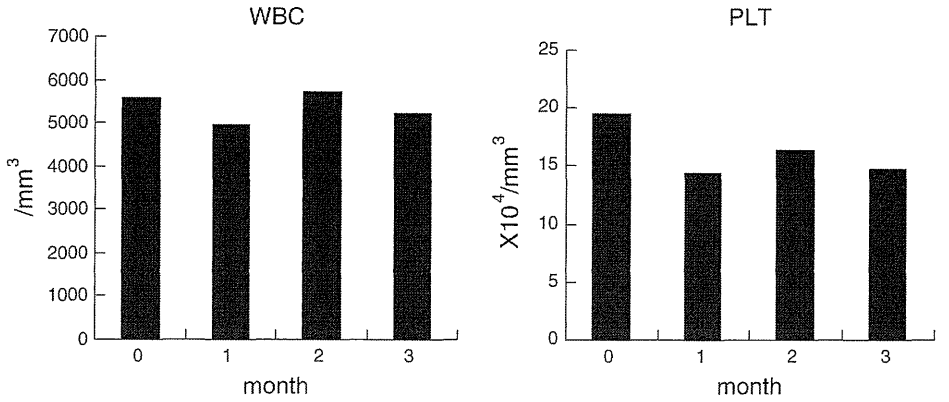

Fig. 5 White blood cell (WBC) and platelet (PLT) counts in 13 prostate cancer patients with bone metastasis pain treated with Sr-89 therapy ($p > 0.05$ at all time points)

able to complete both the QLQ-BM22 and QLQ-C30 within 15 min. Results confirmed the validity, reliability, cross-cultural applicability, and sensitivity of the QLQ-BM22 [13].

Conventional pain evaluation modules include the VAS, which assesses the extent of pain as a linear scale; the verbal rating score, which expresses the extent of pain with words; and the face scale, the patient is looking at an illustration of five faces and choosing the one that best matches their level of pain. However, these conventional pain evaluation modules are problematic because: [1] site-by-site evaluation of pain is not feasible; [2] the chronological factor—whether the pain is persistent or intermittent—is not included in the evaluation; and [3] psychological and social worries caused by the pain cannot be evaluated.

According to the VAS, the patients reported that their bone pain was significantly improved at 2 and 3 months after Sr-89 therapy as compared with the baseline. Analysis based on the face scale showed that pain improvement reached the maximum at 2 months after Sr-89 therapy.

In the functional scale of the EORTC QLQ-C30, QOL was significantly improved at 1, 2, and 3 months after the Sr-89treatment; emotional and cognitive functioning significantly improved at 2 months; and role functioning was significantly improved at 3 months. In the symptom scale, fatigue and pain were significantly improved at all time points, and dyspnoea, insomnia, appetite loss, and financial difficulties were significantly improved at one time point.

Based on the evaluation with EORTC QLQ-BM22, on the symptom scale PC was significantly improved at all time points after Sr-89 treatment. On the functional scale, FI was significantly improved at all time points and PA was significantly improved 2 and 3 months after the treatment. These findings indicate that treatment with Sr-89 is not only useful for conventional pain alleviation, but it is also capable of contributing to functional QOL of a patient.

Internal radiation therapy using Sr-89 is a relatively convenient method not requiring a special preparation or divided dosage, and applicable with minimal patient restraint. It should be regarded as a new approach to pain relief, rather than an alternative to analgesics and external-beam radiation therapy. It is expected that the radiopharmaceuticals will be used more widely in the future, as it is a method of treatment for patients, whose QOL may be enhanced by relieving not only pain in the advanced stage but also due to malignant diseases. In addition, Sr-89 might be more effective in patients with periosteal extension, metastases in non-load-bearing sites, and high levels of bone turnover markers [14].

In this study, serum PSA was decreased by treatment with Sr-89, but not significantly so. Turner et al. [13] did find that PSA 'response' occurred in 30 patients (37 %) over 4 months after Sr-89. Yoshimura et al. confirmed 'tumoricidal effect' of Sr-89 using magnetic resonance imaging and Tc-99 m methoxyisobutylisonitrile (MIBI) [15]. Thus, Sr-89 and Ra-223 are expected to have an oncological effect as "bone-seeking radiopharmaceuticals," and their contribution to prolonging survival duration is highlighted by the onco-niche concept [16].

This study had several limitations. First, this study had a small sample size of only 13 patients with only a single arm, although a prospective design was adopted. Second, the follow-up period after Sr-89 therapy was short (3 months). Careful and prolonged monitoring of hematologic parameters after Sr-89 therapy is required [17]. Third, it is difficult to accurately determine the effect of Sr-89, because almost half of the patients received external-beam radiation therapy and/or chemotherapy prior to Sr-89 therapy. Finally, the efficacy of repeated-dose administration and the durable effect of Sr-89 were not evaluated here and require further investigation.

Pain is said to be the fifth vital sign, and it has been recognized as a very important aspect of evaluating the

conditions of patients in clinical settings. However, pain is also quite subjective, and it is difficult to quantify the extent of pain patient's experience. Using the EORTC QLQ-BM22 module, this study showed for the first time that Sr-89 therapy improves the comprehensive QOL of prostate cancer patients with bone metastasis pain. This radionuclide therapy can provide not only reduced pain characteristics but also better psychosocial aspects and functional interference in this patient population.

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FULL PAPER

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Keywords: phase I trial; vaccine therapy; renal cell carcinoma; peptide vaccine; vascular endothelial growth factor receptor

Phase I clinical trial of human vascular endothelial growth factor receptor 1 peptide vaccines for patients with metastatic renal cell carcinoma

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Background: It is well known that renal cell carcinoma (RCC) represents one of the most immune-responsive cancers. Although the lack of defined antigens in RCC has hindered more specific vaccine development, research regarding vaccination therapy has been of special interest for the treatment of RCC for more than 30 years.

Methods: To evaluate the safety of the vascular endothelial growth factor receptor 1 (VEGFR1) peptide vaccination and its clinical outcomes, data from 18 metastatic RCC (mRCC) patients treated with VEGFR1 vaccine were collected. Toxicity assessments were performed. Clinical outcomes included assessment using CT scanning, magnetic resonance imaging or X-ray examination in accordance with the WHO Response Evaluation Criteria in Solid Tumors.

Results: No patient showed any toxicities of grade 3 or greater. Of the 18 patients, 2 patients showed a partial response during treatment. Stable disease for more than 5 months was observed in eight patients with a median duration of 16.5 months (4–32 months). At the time of the analysis in this study, six patients were alive with a median follow-up of 30 months (26–36 months).

Conclusion: These results suggest that VEGFR1 peptide vaccine is safe and is recommended for further trials for patients with mRCC.

Renal cell carcinoma (RCC) is the most common type of kidney tumours in adults, responsible for ~80% of cases. It is well known that where the tumour is confined to the renal parenchyma, the prognosis is relatively good and 5-year survival rate is 70–80%. In such cases, initial treatment is most commonly a radical or partial nephrectomy and remains the mainstay of curative treatment (Rini *et al*, 2008).

In the era of molecular targeted agents, new immunotherapy trials such as vaccination with tumour lysate, loaded autologous dendritic cells have shown some benefits, although the complicated procedures of dendritic cell vaccine and its low response rates have hampered the wide prevalence of dendritic cell treatment. So far, a large number of tumour-associated antigens and their peptides recognised by MHC class I-restricted cytotoxic T lymphocytes (CTLs) have been identified in various malignancies (Kawakami *et al*, 1994a,b; Fisk *et al*, 1995; Robbins *et al*, 1996; Correale *et al*, 1997) and used in clinical trials (Finn and Lotze, 2001; Slingluff *et al*, 2001; Ramanathan *et al*, 2005; von Mehren, 2005). In search for RCC-associated antigen peptides available to induce CTLs, we have also identified three carbonic anhydrase 9 (CA9) antigen-derived peptides and have shown their ability to induce human leukocyte antigen (HLA)-A24-restricted, CA9-specific CTLs (Shimizu *et al*, 2003; Uemura *et al*, 2006).

Similarly, peptide vaccine therapy has been attempted since the identification of epitope peptides. However, because of their minimal clinical responses, several mechanisms of immune evasion of tumours have been implicated as issues to improve cancer immunotherapy. For instance, the antitumor effect of CTLs

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induced by peptide vaccine was suspected to be inhibited because of tumour cell heterogeneity, and also the downregulation or loss of HLA or antigen proteins (Khong and Restifo, 2002; Ryschich *et al*, 2005). The vascular endothelial growth factor receptor 1 (VEGFR1) peptide is an immunogenic peptide derived from VEGFR1 restricted with HLA-A2 and A24, which are common HLA-A allele in Japanese population. VEGFR1 is an important factor in tumour angiogenesis (Shibuya, 1995; Dvorak, 2002; Li *et al*, 2002) and in the growth of RCC. In this study, a phase I clinical trial of VEGFR1 peptide vaccines was carried out in patients with metastatic RCC (mRCC) to assess toxicity, induction of immune responses and clinical usefulness.

PATIENTS AND METHODS

Patient eligibility. Patients with mRCC and pathologically confirmed clear cell carcinoma were candidates for this study. All patients had progressive disease after standard cytokine therapy, such as IL-2 and/or IFN-α and molecular targeted therapy (sorafenib and sunitinib). Patient eligibility included the following: HLA-A2 and/or A24 positivity; age 20 to 80 years; an Eastern Cooperative Oncology Group performance status of 0 or 1; granulocyte count $\geqslant 3000$ per mm^3; haemoglobin $\geqslant 10\,g\,dl^{-1}$; platelets $\geqslant 100\,000$ per mm^3; bilirubin and creatinine equal to or less than the institutional normal limits; life expectancy $\geqslant 12$ weeks; measurable or evaluable disease; no immunotherapy, chemotherapy or radiotherapy within 4 weeks (washout for 4 weeks); and negative serological tests for hepatitis B, hepatitis C and HIV. Patients with serious illness or an active secondary malignancy were excluded. Other exclusion criteria also included existence of immunosuppressive or autoimmune disease, or receipt of immunosuppressive agents (e.g., steroids). All patients were informed of the investigational nature of the study, and signed informed consent in accordance with the institutional guideline was obtained. This study was approved by the Kinki University institutional review board, and all subjects provided written informed consent before commencing study-related procedures. Each patient underwent a complete pretreatment clinical evaluation, including a clinical history, physical examinations with assessment of performance status, laboratory studies, and measurements of radiographic studies.

Patient demographics. From May 2007 to November 2009, 18 patients with cytokine-refractory and tyrosine kinase inhibitor (TKI) failure mRCC were enroled at the Kinki University Hospital. All patients previously underwent radical nephrectomy of the primary tumour and had clear cell carcinoma. The characteristics of the patients are summarised in Table 1. The median age of the patients was 66 years (range, 44–78 years). All patients had a performance status of 0 and distant metastases at the time of enrolment as shown in Table 1.

Peptides. The good manufacturing practice (GMP)-graded VEGFR1-770 peptide restricted with HLA-A0201 (TLFWLLLTL) and the GMP-graded VEGFR1-1084 peptide restricted with HLA-A2402 (SYGVLLWEI) were synthesised by the American Peptide Company (Sunnyvale, CA, USA) according to a standard solid-phase synthesis method and were purified by reversed-phase high-performance liquid chromatography. Human leukocyte antigen-A0201-restricted CMV peptide (NLVPMVATV) and HIV peptide (ILKEPVHGV), and HLA-A2402-restricted CMV peptide (QYDPVAALF) and HIV peptide (RYLRDQQLL) were used for CTL response measurements.

Study design and treatment protocol. This study was a non-randomised, open-label, phase I clinical trial with dose escalation of the VEGFR1-770/1084 peptide for patients with mRCC. Two VEGFR1 peptide vaccines were derived from VEFGR1 restricted with HLA-A0201 or A2402. In this study, the patients were administered either HLA-A0201-restricted peptide or A2402-restricted peptide. The primary endpoint of this trial was the safety of vaccination. The secondary endpoints were immunological responses, clinical outcomes, and the determination of the

Table 1. Patient demographics of the peptide vaccination

Pt	Age	Sex	Clinical stage at Nx	Histological type	Metastases	Previous treatment	PS
A2-1	66	F	pT3N0M0	Clear cell carcinoma	Lung/LN	Nx/IFN	0
A2-2	61	M	pT1bN1M0	Clear cell carcinoma	Lung/LN	Nx/IFN	0
A2-3	65	M	pT2N0M1	Clear cell carcinoma	Pancreas	Nx/IL-2/IFN	0
A2-4	44	M	pT1bN0M0	Clear cell carcinoma	Lung/LN	Nx/IFN/So	0
A2-5	77	M	pT3N0M0	Clear cell carcinoma	LN/liver	Nx/IL-2/IFN/So	0
A2-6	53	F	pT2N0M1	Clear cell carcinoma	Lung	Nx/IL-2/IFN/So	0
A2-7	66	M	pT3N1M1	Clear cell carcinoma	Lung/bone	Nx/IL-2/IFN/So	0
A2-8	69	M	pT2N0M1	Clear cell carcinoma	Lung	Nx/IL-2/IFN/Su	0
A2-9	73	M	pT3N2M1	Clear cell carcinoma	Lung/pancreas	Nx/IFN/So/Su	0
A24-1	49	M	pT1bN0M0	Clear cell carcinoma	Lung/LN	Nx/IFN/So	0
A24-2	74	M	pT3N0M1	Clear cell carcinoma	Lung/LN	Nx/IL-2/IFN	0
A24-3	75	M	pT3N2M0	Clear cell carcinoma	Lung/LN	Nx/IL-2/IFN	0
A24-4	61	M	pT3N0M1	Clear cell carcinoma	Adrenal/brain	Nx/IL-2/IFN	0
A24-5	49	M	pT3N2M0	Clear cell carcinoma	Lung/LN	Nx/IL-2/IFN/So	0
A24-6	69	M	pT3N0M1	Clear cell carcinoma	Lung/liver/pancreas	Nx/IL-2/IFN/So	0
A24-7	78	F	pT3N2M0	Clear cell carcinoma	Lung/bone	Nx/IL-2/IFN/Su	0
A24-8	61	M	pT3N2M1	Clear cell carcinoma	Bone	Nx/IL-2/IFN/So	0
A24-9	69	M	pT1bN0M1	Clear cell carcinoma	Lung/LN	Nx/IFN/So/Su	0

Abbreviation: F = female; IFN = interferon-α; IL-2 = interluekin-2; LN = lymph node; M = male; Nx = nephrectomy; PS = performance status; Pt = patient; So = sorafenib; Su = sunitinib.

optimal dose of peptide. The dose was escalated as 0.5, 1.0, and 3.0 mg per body of the vaccinated peptide. The washout period of the previous treatment was 4 weeks. The VEGFR1 peptide vaccine was emulsified with incomplete Freund's adjuvant (Montanide ISA-51VG; Seppic, Paris, France) and subcutaneously administered into the site of the upper arm on days 1, 8, 15, and 22 in a 28-day treatment cycle. After one course of treatment, the safety of the peptide was evaluated (Figure 1). If the patient was evaluated favourably, the administration of VEGFR1 vaccine was continued every 2 weeks until death, intolerance, marked disease progression, major violations, or patients' withdrawal of consent.

Clinical assessment. All patients were followed up until death due to disease, intolerance, or self-withdrawal. Toxicity assessments were done at least every 2 weeks using the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0. Clinical and laboratory assessments were checked at each visit. Clinical outcomes included assessment using CT scanning, magnetic resonance imaging, bone scintigram, or X-ray examination in accordance with the WHO Response Evaluation Criteria in Solid Tumors (RECIST). Radiological evaluation was performed at 2 to 4 weeks after one course of vaccination and every 3 months thereafter. Overall survival (OS) was estimated from the date of the initial vaccination to the date of death using the Kaplan–Meier method.

Immunological monitoring. To measure CTL responses against peptide, an enzyme-linked immunospot (ELISPOT) assay was performed. The detail method of our ELISPOT assay was described elsewhere (Miyazawa *et al*, 2010; Okuno *et al*, 2011). Peripheral blood mononuclear cells (PBMC) were obtained from patients at pre- and post vaccine treatment at the end of each course, and then frozen and stored in liquid nitrogen until their use. The frozen PBMCs derived from the same patient were thawed at the same time, cultured with respective peptide and IL-2 (Novartis, Emeryville, CA, USA), and were collected after 2 weeks. Following CD4+ cell depletion, IFN-γ ELISPOT assay was performed with peptide-pulsed or HIV-pulsed (as the control) HLA-A0201-positive T2 cells (ATCC, Rockville, MD, USA) and HLA-A2402-positive TISI cells (IHWG Cell and Gene Bank, Seattle, WA, USA) using the Human IFN-γ ELISpot PLUS kit (MabTech, Cincinnati, OH, USA) and the MultiScreen-IP 96-plate (Millipore, Bedford, MA, USA). The plates were analysed by the automated ELISPOT reader, ImmunoSPOT S4 (Cellular Technology Ltd, Cleveland, OH, USA). All ELISPOT assays were performed in triplicate. The number of peptide-specific spots was calculated by subtracting the spot number in the wells of the control cells from that in the well with the peptide-pulsed T2 or TISI cells. The peptide-specific T-cell response was classified into four grades (−, +, ++, and +++) according to the algorithm flowchart described in our previous report (Kono *et al*, 2012). Sensitivity of our ELISPOT assay was estimated as an approximately average level among participating laboratories in the ELISPOT panel of the Cancer Immunotherapy Consortium in 2009 and 2011. Peptide-specific CTL precursors in PBMCs were detected using IFN-γ release assay as previously described (Hida *et al*, 2002; Maeda *et al*, 2002; Suzuki *et al*, 2002). The data were considered positive (specific CTL induction) when the level of IFN-γ production in response to each corresponding peptide was significantly higher ($P<0.05$) than that in response to HIV control peptide. All experiments were performed in four different wells and in duplicates.

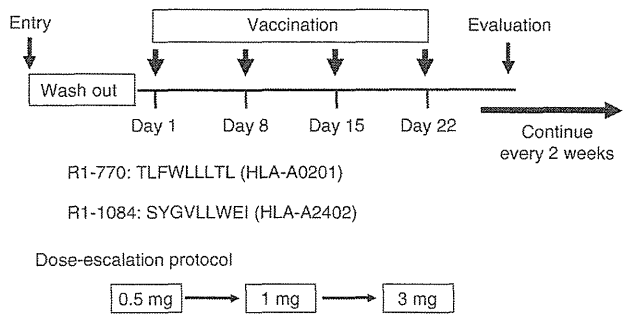

Figure1. Treatment protocol. VEGFR1 vaccine was administered subcutaneously on days 1, 8, 15, and 22 in a 28-day treatment cycle. After evaluation, vaccination was continued every 2 weeks. The amino acid sequences of the two VEGFR1 vaccine are shown. The dose administered was designed in a dose-escalation manner.

RESULTS

Safety. The overall toxicity of the 18 patients is summarised in Table 2. No patient showed any toxicities of grade 3 or greater. Of the 18 patients, 10 patients developed grade 1 or 2 local skin reaction at the injection sites with induration, redness, and swelling. Four patients experienced grade 1 fever but no medication was required. Grade 1 fatigue was observed in one patient with HLA-A2-restricted peptide vaccine. Grade 1 headache was noted in one patient with HLA-A2-restricted peptide vaccine and three patients complained of rashes, which were resolved without any medications. In this study, no case revealed any vascular adverse events, such as hypertension, bleeding, and thromboembolism, and no hepatic or renal toxicities were also found during vaccination. In addition, no dose-limiting toxicities were observed in this trial.

Cellular immune responses. The positive CTL responses after the first course of vaccination were found in five of the six patients (83%) receiving 0.5 mg per body vaccination, five of the six patients (83%) receiving 1 mg per body, and three of the six patients (50%) receiving 3 mg per body, respectively (Table 3). Two patients were not evaluated on CTL induction. Overall, of the 18 patients, 15 patients (83%) who received at least one course of vaccination revealed positive CTL responses. Peptide-specific delayed-type hypersensitivity reactions were observed in six patients. The representative data from the IFN-γ ELISPOT assay and CTL responses in the patient who received peptide vaccine derived from VEGFR1 restricted with HLA-A2 before and after the treatment are shown in Figure 2.

Clinical outcomes. Of the 18 patients, 2 patients showed a partial response (PR) during treatment (Table 3). One of the two PR

Table 2. Overall vaccine-associated adverse events

	Grade				
Toxicity	**1**	**2**	**3**	**4**	**Total**
Local reaction[a] (A2/A24)	4 (2/2)	5 (0/5)	0	0	9 (2/7)
Induration (A2/A24)	3 (1/2)	5 (0/5)	0	0	8 (1/7)
Redness (A2/A24)	2 (1/1)	0	0	0	2 (1/1)
Swelling (A2/A24)	1 (0/1)	0	0	0	1 (0/1)
Fever (A2/A24)	4 (2/2)	0	0	0	4 (2/2)
Fatigue (A2/A24)	1 (1/0)	0	0	0	1 (1/0)
Headache (A2/A24)	1 (1/0)	0	0	0	1 (1/0)
Rash (A2/A24)	3 (2/1)	0	0	0	3 (2/1)

[a]Injection site of vaccine overall toxicities ($n = 18$).

Table 3. Clinical and immunological outcomes of the peptide vaccination

			CTL			AEs				
Pt	Peptides (mg)	No. of vaccination	After one course	Two or more courses	DTH response	G1	G2	G3/4	Best clinical response	Survival
A2-1	0.5	12	+	+ +	–	–	–	–	PD	CD
A2-2	0.5	38	+	+ + +	–	+	–	–	PR	CD
A2-3	0.5	43	+ + +	+ + +	+	–	–	–	PD	Alive (192 weeks)
A2-4	1	28	+ +	+ + +	–	+	–	–	SD	Alive (169 weeks)
A2-5	1	33	+ + +	+ + +	–	–	–	–	PD	CD
A2-6	1	42	–	+ +	+	–	–	–	SD	CD
A2-7	3	36	+ + +	+ + +	+	–	–	–	PD	Alive (161 weeks)
A2-8	3	7	–	ND	–	–	–	–	PD	CD
A2-9	3	28	+ +	+ + +	–	–	–	–	SD	CD
A24-1	0.5	26	+	+ +	–	–	–	–	PD	CD
A24-2	0.5	33	+ +	+ + +	+	+	+	–	SD	Alive (266 weeks)
A24-3	0.5	12	–	–	–	+	–	–	PD	CD
A24-4	1	28	+	+ +	–	–	–	–	SD	CD
A24-5	1	12	+ +	+ + +	–	+	–	–	SD	CD
A24-6	1	7	–	ND	–	–	–	–	SD	Alive (216 weeks)
A24-7	3	27	+	+ + +	+	–	–	–	SD	CD
A24-8	3	55	–	+	–	+	–	–	PR	Alive (195 weeks)
A24-9	3	42	–	+	+	–	–	–	SD	CD

Abbreviations: AEs = adverse events; CD = cancer death; CTL = cytotoxic T lymphocyte; DTH = delayed-type hypersensitivity; ND = not determined; PD = progressive disease; PR = partial response; SD = stable disease.

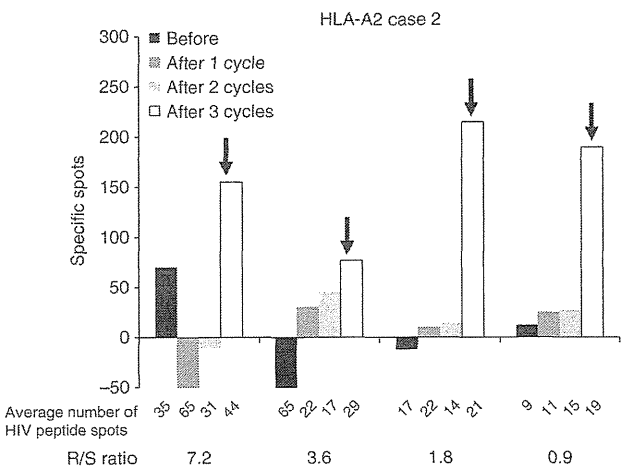

Figure 2. Changes of CTL response after vaccination. Significant induction of CTL was observed by VEGFR1 peptide vaccine.

patients, who had multiple metastases in lung and regional lymph nodes, showed shrinking of pulmonary lesions and maintained PR for 5 months (Figure 3A). However, this patient developed pancreas metastasis and died of cancer 18 months after initiation of vaccine treatment. The rest of the two had multiple bone metastases resistant to IL-2/IFN/sorafenib treatment. This patient achieved PR with shrinking of the bone metastatic lesions, which were evaluated with both CT scan and bone scintigram, and, at present, is stable continuing vaccine treatment for 36 months (Figure 3B). Stable disease for more than 5 months was observed in eight patients with a median duration of 16.5 months (4–32 months). The remaining eight patients had no clinical responses. At the time of the analysis in this study, 12 patients died due to RCC and 6 patients were alive with a median follow-up of 30 months (26–36 months). Median OS of the patients with the HLA-A2-restricted peptide was 85 weeks and that of those with HLA-A24-restricted peptide was 45 weeks. The entire OS curve is shown in Figure 4, indicating a median OS of 70 weeks.

DISCUSSION

Renal cell carcinoma is the most common type of kidney tumours in adults, which accounts for 2–3% of all adult cancers, and ~20–30% of patients present with metastatic disease. Although a radical or partial nephrectomy is the primary curative therapy for patients with localised RCC, the prognosis for patients with advanced mRCC is poor, with a 5-year survival rate of < 10% (Schrader *et al*, 2006). As RCC is one of the most immunoresponsive cancers in humans, immunotherapy remains a basis of promising treatment strategies. Until recently, the standard therapy for mRCC has been cytokine-based immunotherapy with IL-2 and/or IFN-α with only few durable complete remissions.

Recently, molecular targeted agents, such as TKIs and mTOR inhibitors, have been introduced to the treatment of mRCC, which have improved the outlook for mRCC in clinical responses, especially in progression-free survival. However, they have not yet demonstrated improved OS remarkably. Under these circumstances, several clinical trials of peptide-based vaccine treatment

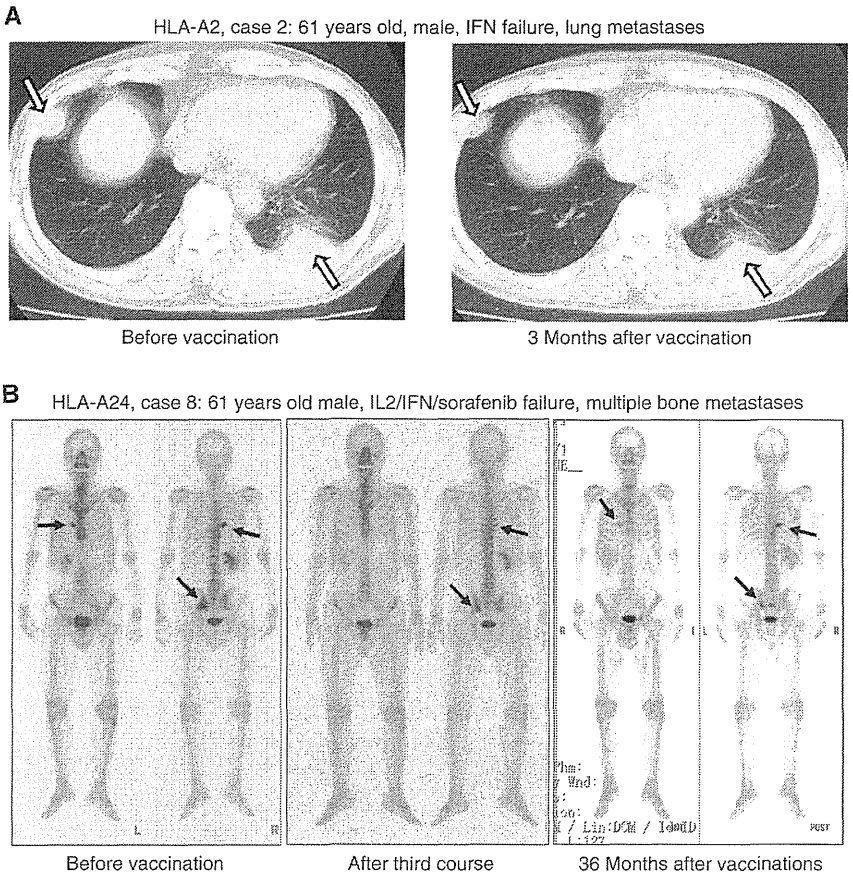

Figure 3. Instance of VEGRF1 vaccine responder. (A) The patient had lung metastases resistant to IFN treatment. Shrinking of lung metastases was noted. (B) The patient had multiple bone metastases resistant to IL-2/IFN/sorafenib treatment. He achieved PR with shrinking of the bone metastatic lesions, and, at present, is stable continuing vaccine treatment.

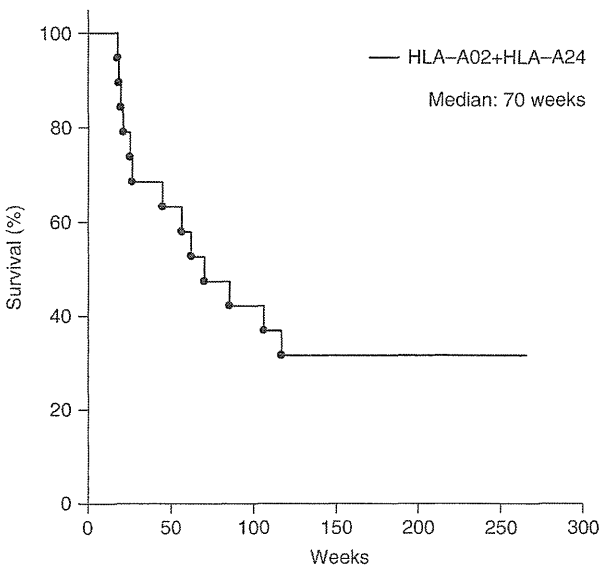

Figure 4. The entire overall survival curve is indicated with a median OS of 70 weeks.

have been done in recent years. The RCC vaccines are explored in metastatic and adjuvant settings. To date, they are clinically effective only in a minority of patients and, generally, are still considered experimental. Much attention has been given to CA9 that is overexpressed in ~90% of all RCC types.

In addition to CA9-derived peptides, several new RCC-associated antigens and derived MHC class I-restricted ligands were recently introduced in some clinical trials (Iiyama *et al*, 2007; Suekane *et al*, 2007; Patel *et al*, 2008). Recently, Walter *et al* (2012) reported the outcomes of phase I/II clinical trials using multiple tumour-associated peptides (TUMAPs) called IMA901. They treated a total of 96 HLA-A02-positive patients with mRCC with IMA901 in two consecutive studies. In the phase I study, they showed that the T-cell responses of the patients to multiple TUMAPs were associated with better disease control. The randomised phase II trial demonstrated that a single dose of cyclophosphamide administration before IMA901 immunotherapy reduced the number of T-regulatory cells and confirmed that immune responses to multiple TUMAPs were associated with longer OS. A randomised phase III study to determine the clinical benefit of treatment with IMA901 is now ongoing (NCT 01265901). IMA901 multipeptide vaccine is considered one of the most available peptide vaccines in practical clinic, such as sipuleucel T, in the near future.

Vascular endothelial cells have crucial roles in the growth and progression of tumours and stably express HLA molecules. Vascular endothelial growth factor receptor 1, which is a functional molecule associated with neovascularisation, is highly expressed in newly induced tumour blood vessels, but not in normal vessels. It is an important factor in tumour angiogenesis (Shibuya, 1995; Dvorak, 2002; Li *et al*, 2002) and in the growth of RCC. The VEGFR1 peptide we used in this study is an immunogenic peptide

derived from VEGFR1 restricted with HLA-A2 and A24, which are common HLA-A allele in the Japanese population. In this study, we administered peptide vaccines in a dose-escalation manner (0.5, 1.0, 3.0 mg per body, respectively). The primary endpoint was to evaluate the safety and toxicity of VEGFR1 peptide vaccination. All patients had only minor adverse events and, therefore, this vaccine treatment is considered well tolerated and acceptable for mRCC patients. One of the secondary objectives of this investigation was to evaluate vaccine-induced specific immune reactions. As a result, the specific CTL responses against the vaccinated peptide were observed in 15 (83%) of the 18 patients, clearly demonstrating that CTL against VEGFR1 could be induced by a vaccination of the VEGFR1 peptide, although no obvious correlations of CTL responses and clinical outcomes were found in this study. A similar result is shown in patients with advanced gastric cancer (Masuzawa *et al*, 2012). Further investigations are required to confirm this problem, because a relatively small number of patients ($n = 18$) was enroled in this phase I study. The other secondary endpoint was the determination of the optimal dose of peptide. The specific CTL responses against the peptide were observed equally in 0.5, 1, and 3.0 mg peptide group, respectively, and no dose-limiting toxicities were found in this study. On the basis of these results, 3 mg of peptide vaccine may be safe and available in future clinical trials. Clinically, two patients showed a clinical response during treatment. One of the patients who has PR with multiple bone metastases, who was evaluated with both CT and bone scan, was considered to have PR according to RECIST v1.0. When RESIST v1.1 was used for evaluation, this patient was not considered to have PR, because apparent reduction of soft tissue metastases in bone is needed not only for bone scan but also for CT scan. However, the patient is stable and is continuing the vaccine treatment. Therefore, we evaluated the patient who achieved PR as shown in Figure 3B. Stable disease for more than 5 months was observed in eight patients, with a median duration of 16.5 months (4–32 months). The remaining eight patients had no clinical responses. As a result, 12 patients died due to RCC and 6 patients remained alive, with a median follow-up of 30 months (26–36 months) so far.

In conclusion, treatment of mRCC patients with VEGFR1 peptide vaccines was well tolerated, and induction of antigen-specific immunity was observed in two-thirds of the patients. In the era of molecular targeted therapy, the observed clinical outcomes of the peptide vaccine therapy in this study are encouraging. Further well-designed clinical trials, including optimally selected patients, will be required in the use of peptide vaccines for mRCC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Review Article

Role of vaccine therapy for renal cell carcinoma in the era of targeted therapy

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Abbreviations & Acronyms
AE = adverse event
APC = antigen presenting cells
BCG = bacillus Calmette–Guérin
CA = carbonic anhydrase
CA9 = carbonic anhydrase IX
CPA = cyclophosphamide
CR = complete response
CTLA = cytotoxic T lymphocyte antigen
CTL = cytotoxic T lymphocytes
DC = dendritic cell
DFS = disease-free survival
GM-CSF = granulocyte-macrophage colony-stimulating factor
GMTV = genetically modified tumor vaccines
HLA = human leukocyte antigen
HR = hazard ratio
IFN = interferon
IL-12 = interleukin-12
IL-2 = interleukin-2
KHL = keyhole limpet hemocyanin
LMI = large multivalent immunogen
MHC = major histocompatibility complex
MR = minor response
mRCC = metastatic renal cell carcinoma
mTOR = mammalian target of rapamycin
MUC = mucin
MVA = modified vaccinia virus Ankara
N/A = not applicable
NK = natural killer
OS = overall survival
PADRE = Pan-DR-binding peptide
PD = progressive disease
PFS = progression-free survival
PR = partial response
RCC = renal cell carcinoma
RECIST = Response Evaluation Criteria in Solid Tumors
SD = stable disease
TAA = tumor-associated antigens
Th1 = T helper 1
Th2 = T helper 2
Treg = regulatory T cell
TRIST = TroVax Renal Immunotherapy Survival Trial
TUMAP = tumor-associated peptides
VEGF = vascular endothelial growth factor
VHL = von Hippel-Lindau
WT1 = Wilms tumor 1

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Abstract: Renal cell carcinoma is the most common malignant tumor originating from the kidney. Compared with other solid tumors, it does not respond to traditional management modalities, such as chemotherapy and radiotherapy. However, it is well known that renal cell carcinoma represents one of the most immune-responsive cancers and several immunotherapeutic strategies have been investigated in the management of renal cell carcinoma with variable degrees of success. The development of immunotherapy with α-interferon or high-dose interleukin-2 is the best established treatment, and is associated with durable disease control. Although the lack of defined antigens in renal cell carcinoma has hindered more specific vaccine development, research regarding vaccination therapy has been of special interest for the treatment of renal cell carcinoma for more than 30 years. At present, there are three types of cell-based vaccines in renal cell carcinoma treatment: autologous tumor-cell vaccines, genetically modified tumor vaccines and dendritic cell-based vaccines. A further type is peptide-based vaccination with tumor-associated antigens as possible targets, such as carbonic anhydrase IX, survivin and telomerase that are overexpressed in renal cell carcinoma. In the present article, we review data from completed clinical trials of vaccine therapy, and discuss future trials to assess the current knowledge and future role of vaccine therapy for renal cell carcinoma in the era of recently developed targeted therapy.

Key words: autologous tumor cell vaccine, dendritic cell vaccine, genetically modified vaccine, peptide vaccine, renal cell carcinoma.

Introduction

Since the end of the 19th century, and even before, medical scientists have been attempting to utilize the power of the host's immune system to cure cancer.[1] The vaccine therapy developed by Coley in the 1890s is now considered a type of non-specific immune response, which was induced by lipopolysaccharides composed of the bacteria administered, and then cytokines, such as tumor necrosis factor, were produced to elicit antitumor activities. At present, this treatment strategy still exists and is supported by the small but significant number of patients with metastatic cancer, especially mRCC, that have durable disease control, which is designed to manipulate the immune system.

RCC is the most common type of kidney tumor in adults, responsible for approximately 80% of cases. It is well known that when the tumor is confined to the renal parenchyma, the prognosis is relatively favorable, and the 5-year survival rate is 70–80%. In such cases, initial treatment is most commonly a radical or partial nephrectomy, and remains the mainstay of curative treatment. However, the survival rate is lowered considerably when the patient with RCC has regional or distant metastases. It is resistant to conventional treatment modalities, such as chemotherapy or radiotherapy, although some cases respond to immunotherapy, such as IFN or IL-2, which shows limited effects. Therefore, the treatment for patients with mRCC still remains challenging, and multidisciplinary treatment modalities are required to those with mRCC.

Recently, the management of mRCC has drastically changed with the arrival of VEGF and mTOR pathway-targeting agents. However, complete and durable responses are rare with those agents that target VEGF or mTOR, which requires sequential therapy to maintain

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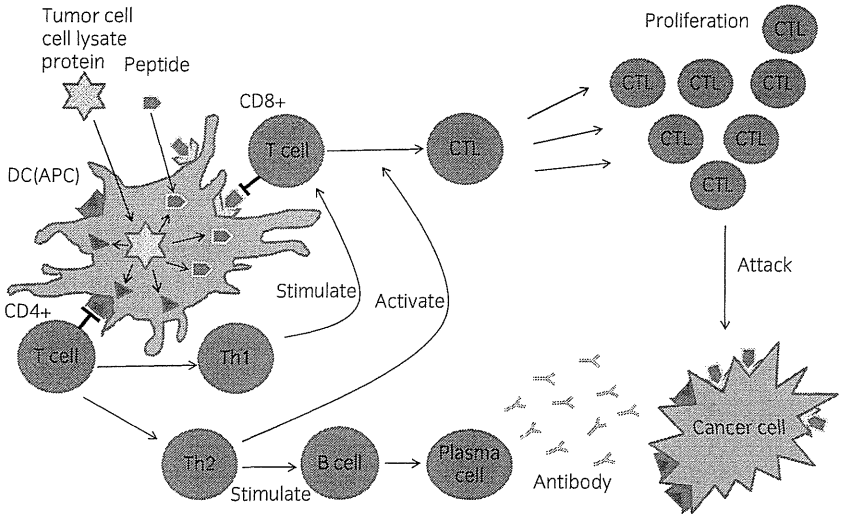

Fig. 1 Schematic presentation of events in the process of tumor vaccine therapy. Peptides presented by MHC class I complexes are recognized by $CD8^+$ T cells. Peptides presented by MHC class II complexes on APC activate $CD4^+$ T cells. This interaction leads to a proliferation of the cytotoxic T lymphocytes, which will attack cancer cells. Th1, T helper 1; Th2, T helper 2. , MHC class I; , MHC class II; , Class I peptide; , Class II peptide.

clinical benefits. The concept and subsequent development of therapeutic tumor vaccines for patients with mRCC has been under investigation for decades with various results.[2–4] The final achievement of newly developing curative RCC vaccines is to stimulate the immune system of the host to recognize and to attack existing tumor cells. RCC vaccines are clinically tried in the metastatic and adjuvant setting. To date, they are only clinically effective in a minority of patients and are still considered experimental. However, the recent USA FDA approval of Provenge (sipuleucel-T) as the first active cellular immunotherapy in advanced prostate cancer and ipilimumab (Yervoy), an anti-CTLA antibody, in advanced melanoma patients has led to a renaissance of immunotherapy approaches.

In the present review, the PubMed database was searched using a combination of the search items "renal cell carcinoma" or "kidney cancer" and "vaccine" or "vaccination therapy" or "vaccine therapy". Another search combined the items "dendritic cells" or "active immunotherapy" or "peptide vaccine" or "genetically modified tumor vaccine" or "autologous tumor cell vaccine" with "kidney cancer" or "renal cell carcinoma". The search was concentrated on articles published in English, and cross-references were used for search completion. Subsequent references were identified from the reference lists of retrieved articles. All articles on the title and abstracts were screened, and selected for the present review. A total of 84 articles were relevant to use. With those articles, we provide an overview of the current role and future options of vaccine therapy for RCC and information on completed clinical studies.

Mechanisms of tumor vaccination, TAA for RCC, and types of therapeutic vaccines

The action of tumor vaccines is shown in Figure 1. The basis for the immune recognition of tumor cells is the presence of TAA. These antigens are glycoproteins expressed by tumor cells, generally at higher expression levels, at altered points during cell differentiation or in mutated forms. This pattern of expression allows the immune system to detect abnormal cell characteristics, which can be used for immune-mediated attack to a malignant cell. The best-studied antigens belong to a class of normal cell differentiation antigens that become overexpressed during malignant transformation. The action of tumor vaccines comprises immunization by genetically modified or irradiated tumor cells, antigen-loaded DC (or tumor–DC cell fusion), and non-cell-based tumor cell lysates and peptides derived from TAA. In general, tumor antigens are presented to the immune system by APC that acquire antigen by uptake of dying tumor cells or circulating proteins.[5] DC are the most potent APC, and process proteins into shorter peptide fragments that are linked to MHC for presentation to T cells.[6] Peptides presented by MHC class I complexes are recognized by $CD8^+$ T cells, which are capable of differentiating into CTL, and can mediate tumor regression with the release of IFN-γ and the production of lytic enzymes, such as perforin and granzyme B[7]. Peptides presented by MHC class II complexes on APC activate $CD4^+$ T cells, which can differentiate into Th1 cells that provide help for the generation of cytotoxic $CD8^+$ T cell responses or Th2 cells that provide help for the generation of B cell and antibody responses.[8] A subset of $CD4^+$ T cells that mediate immune suppression (regulatory T cells) are also activated after exposure to tumor antigens.[9] Although the precise mechanisms that regulate anti-tumor immunity are not completely resolved, there is a report that shows how this process can be used to activate effective therapeutic responses against established tumors in murine tumor models of RCC.[10]

At present, only a few potentially interesting TAA have been identified in RCC, as compared with other immunoresponsive tumors, such as melanoma. Early RCC vaccines were primarily cell-based tumor vaccines in which tumor

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Table 1 Clinical trials with autologous tumor cell vaccine in renal cell carcinoma

Investigator	Year	No. patients	Phase	Stage	Vaccine	Adjuvant	Clinical outcomes
Adjuvant setting							
Galligioni *et al.*[19]	1996	120	2	I to III	Autologous irradiated tumor cells	BCG	5-year DFS 63%
Repmann *et al.*[22]	1997	222 (116 vaccines)	2	I to III	Autologous tumor cells	None	Significantly improved OS; significantly better OS in Robson II and III, not in I and IV
Jocham *et al.*[21]	2004	379	3	pT2-3b pN0-3	Autologous irradiated tumor cell lysate	None	5-year PFS 77.4% (control 67.8%, $P = 0.0204$)
May *et al.*[23]	2010	692	Matched-pair analysis	pT2-3 pNx-2 M0	Autologous irradiated tumor cell lysate	None	Significantly improved OS (HR = 1.28, $P = 0.030$) and in subgroup with pT3 tumors (HR = 1.67, $P = 0.011$)
Metastatic setting							
Kurth *et al.*[16]	1987	33	2	mRCC	Autologous ($n = 22$) and allogeneic ($n = 4$) irradiated tumor cells	Corynebacterium parvum	8 objective response Trend to better OS (insignificant statistically)
Schwaab *et al.*[20]	2000	14	2	mRCC	Autologous irradiated tumor cells	BCG, IFN-α and β	5 SD, 3 MR
Dillman *et al.*[24]	2004	25	2	11 II to IV 14 mRCC	Autologous irradiated tumor cells	BCG, IFN-β, GM-CSF and CPA	No objective response Median PFS reached more than 7 years
Dudek *et al.*[25]	2008	31	2	IV	Autologous LMI	None, CPA, CPA + IL-2	5 SD 4 SD 1 PR, 3 SD

cells provided sources of unknown TAA for immunization. The tumor antigen preparation can be based on whole tumor cells, tumor-derived cellular lysates, whole apoptotic or necrotic tumor cells, or tumor-derived total RNA, mRNA or DNA.[11,12] This approach aims to stimulate a polyclonal T cell immunoresponse against a broad range of tumor-derived epitopes, thereby reducing the possibility that tumors escape immune surveillance and destruction. There are three types of cell-based RCC vaccines: autologous tumor cell vaccines, GMTV and DC-based vaccines.[13] Another type is the peptide-based vaccines with tumor antigens. Studies of the most promising vaccine therapy in development for RCC are described here.

Autologous tumor cell vaccines

Cell-based vaccines are basically comprised of non-viable autologous tumor cells or some form of preparation that provides antigens to activate an immune response. Autologous tumor vaccine is based on the knowledge that RCC themselves express TAA that will induce CTL responses. It has already been recognized that additional treatment must be used to enhance the immune response necessary for a strong therapeutic effect. For this reason, traditional adjuvants, such as incomplete Freund's adjuvant, IL-2, IL-12, GM-CSF and BCG, have been used.[14,15] Several studies reported various results, in which toxicities were relatively mild.[16–21]

Clinical trials using autologous tumor cell vaccines in the metastatic and adjuvant setting are shown in Table 1.

As far as studies in the adjuvant setting are concerned, the first report on vaccine therapy in the advanced setting in localized and surgically resected RCC was reported by Galligioni *et al.* in 1996.[19] A total of 120 RCC patients (stages T3a-bN0M0 or T2-3N1M0) were randomized in a phase II trial to receive either adjuvant BCG-activated vaccine therapy or no adjuvant vaccine treatment. Vaccination comprised of three intradermal administrations of 10^7 autologous irradiated tumor cells mixed with/without 10^7 colony-forming units of BCG. A total of 38 out of 54 treated patients showed a significant response to autologous tumor, but not to normal renal cells, 1 month after vaccination. The 5-year PFS was enhanced, with 63% for the treated patients and 72% for the control group after a median follow up of 61 months.[19]

After Galligioni's report, Repmann *et al.* showed the results of a non-randomized trial evaluating the outcome of 116 patients treated with an autologous tumor cell vaccination in the adjuvant setting compared with 106 control patients in 1997.[22] Significantly prolonged OS was observed in the vaccine group ($P = 0.0007$). Patients at Robson stages II and III showed significantly improved survival rates ($P = 0.02$ and 0.04, respectively). However, there was no significant difference for patients at stages I and IV, possibly because of the short follow up and the limited number of patients.

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In 2004, Jocham *et al.* reported a phase III trial showing significant benefit for RCC patients by adjuvant vaccination therapy.[21] In this randomized trial, PFS of patients with RCC at stage T2-3bN0-3M0 after nephrectomy followed by an adjuvant autologous tumor cell vaccination (Reniale; Vaccentis AG, Zurich, Switzerland) was significantly prolonged compared with control patients. After 5 years and 70 months, respectively, the HR for PFS were 1.58 (95% CI 1.05–2.37) and 1.59 (95% CI 1.07–2.36) in favor of the vaccine group ($P = 0.0204$), respectively. PFS rates at these time-points were 77.4 and 72.0% in the vaccine group and 67.8 and 59.3% in the control group, respectively ($P = 0.0204$). However, the results of that study have been questioned and have raised criticism because of some methodological pitfalls.

In 2007, a second updated intention-to-treat analysis including a higher number of patients ($n = 477$) reported a significantly prolonged PFS ($P = 0.0476$), but not OS ($P = 0.1185$), after vaccination. In a per-protocol analysis ($n = 352$), both PFS ($P = 0.024$) and OS ($P = 0.0356$) were significantly enhanced after vaccine treatment with Reniale.[26]

In 2010, May *et al.* confirmed the efficacy of adjuvant therapy with Reniale. They reported the results of a 10-year survival analysis of patients treated with Reniale in the retrospectively designed matched-pair adjuvant setting.[23] The study group comprised 692 patients with complete follow up (stages pT2–3, pNx–2, M0). Adjuvant treatment with autologous vaccination therapy resulted in a significantly improved overall survival in pT3 stage RCC patients, suggesting benefit, especially in this subgroup.

With regard to trials for metastatic RCC, one of the first trials using autologous tumor cell vaccination in the metastatic setting was carried out by Kurth *et al.* Of the 33 patients with metastatic disease, eight patients had objective responses with a median survival of 32 months compared with the overall survival of 17 months. Although the results were not statistically significant, a favorable trend was observed and toxicity was minimal.[16]

The results of two trials using irradiated tumor cells in 14 patients with mRCC were published by Schwaab *et al.* in 2000 (applied in addition to BCG and IFN-α and IFN-β)[20] and Dillman *et al.* in 2004 (in addition to BCG, IFN-β, GM-CSF and cyclophosphamide).[24] Although five patients in the trial by Schwaab featured SD and a further three patients had MR, in the trial by Dillman, no objective responses were observed.

In 2008, Dudek *et al.* have investigated the safety and tolerability of autologous LMI in stage IV RCC.[25] LMI results from preparation of immobilized autologous tumor cell plasma membrane on 5-μm diameter silica beads and is used to augment a tumor-specific CTL response. A total of 31 patients received LMI monotherapy every month (group 1) or were randomized to treatment with LMI in combination with cyclophosphamide (group 2) or LMI, cyclophosphamide and IL-2 (group 3). Low-dose cyclophosphamide was applied to downregulate suppressor T cell activity and to enhance immune response. Clinical outcomes were five SD in group 1, four SD in group 2, and one PR and three SD in group 3. Although a favorable clinical response was observed, there was no validated tool to establish immune response monitoring.[25]

Although devitalized tumor cell vaccines have proven to be safe, there has been a lack of data to support any significant clinical benefit from this type of vaccine therapy. However, especially in the adjuvant setting, favorable results have been shown to warrant further research.

Genetically modified tumor cell vaccines

To increase the immunogenic response, tumor cell-based vaccines using autologous or allogeneic tumor cells have been genetically modified. By incorporating genes encoding immunostimulatory cytokines or costimulatory molecules of the B7 family, such as GM-CSF, CD80, IL-2, IL-12 and IFN-γ, into tumor cells, several GMTV have been designed.[13,27–29] GMTV strategy is based on the idea that local cytokine secretion can elicit T cell and NK cell activation, and can also induce inflammatory responses against tumors.[30]

There are two basic strategies for using GMTV. One utilizes autologous tumor cells transfected with a costimulatory gene.[13,30] The second strategy utilizes genetically modified cells from well-established RCC cell lines, which dissolves the limitation that only limited amounts of tumor cells are available and lifelong immunization is required with autologous tumor cells.

Clinical trials using GMTV are shown in Table 2. In 1997, Simons *et al.* reported the first phase I trial showing safety as well as bioactivity of an autologous GM-CSF GMTV in mRCC patients.[27] They used a replication-defective retroviral vector to transfer the GM-CSF gene into irradiated autologous tumor cells, and an inclination towards increased delayed-type hypersensitivity with increased macrophage, eosinophil, neutrophil and T cell infiltration in the injection site was observed in the vaccine group. However, no significant difference in clinical response was achieved.

In 2002, Antonia *et al.* reported the results of GMTV using costimulatory B7.1 (CD80) gene in combination with IL-2. Of the 13 patients, two PR and two SD were observed. Three out of these four patients showed delayed-type hypersensitivity skin test reaction.[28] Pizza *et al.* evaluated the clinical efficacy of irradiated allogeneic GMTV producing IL-2 mixed with formalin-treated autologous tumor cells for mRCC patients after failure of IL-2 treatment.[29] Of the 30 mRCC patients, one CR and four PR were observed, and nine cases had SD. Although it is not clear that these clinical

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Table 2 Clinical trials with genetically modified tumor cell vaccine in renal cell carcinoma

Investigator	Year	No. patients	Phase	Stage	Vaccine	Adjuvant	Clinical outcomes
Simons *et al.*[27]	1997	16	1	mRCC	Autologous irradiated GM-CFS	None	1 PR No significant statistical difference
Wittig *et al.*[31]	2001	5	1/2	mRCC	Autologous irradiated tumor cells transfected with GM-CFS and IL-7	Oligonucleotides	1 CR, 1 PR, 2 SD
Antonia *et al.*[28]	2002	13	1	mRCC	Autologous irradiated B7.1 gene	IL-2	2 PR, 2 SD
Tani *et al.*[32]	2004	6	1	mRCC	Autologous irradiated tumor cells transfected with GM-CSF	None	1 SD, 1 MR
Pizza *et al.*[29]	2004	30	2	mRCC	Allogeneic irradiated IL-2-producing tumor cells with autologous formalin-treated tumor cells	None	1 CR, 4 PR, 9 SD
Fishman *et al.*[33]	2008	39	2	mRCC	Autologous irradiated B7.1 transduced tumor cells	IL-2	1 CR, 2 PR, 25 SD

benefits are a result of a response elicited by the use of allogeneic or autologous tumor antigens, the fact remains that there was some useful achievement in the patients.

Recently, Fishman *et al.* investigated the use of irradiated B7.1-transduced, cultured autologous tumor cells plus subcutaneous IL-2 in a non-randomized trial. This trial could not show a higher rate of tumor regression; however, one CR, two PR and 25 SD were noted.[33] Further investigations with regard to GMTV therapy in clinical trials are required to verify the aforementioned relatively small number of trials.

DC-based vaccines

The recent trend in cell-based vaccine therapy has been directed to DC-based vaccines. DC are potent APC derived from CD34+ bone marrow cells and CD14+ monocytes. These cells are naturally found in peripheral tissues and migrate into the lymphoid organs to induce T cell immune response.[34,35] Infiltration of DC into primary tumor lesions has been associated with improved survival in a wide range on malignancies. Major steps have been taken towards the development of culture methods to differentiate and expand DC populations. DC pulsed in culture with various TAA from tumor cells or tumor cell lysate transferred back to the patient play a major role to present antigens to native T cells and induce primary immune responses.[30] Monocyte-derived DC are mostly used for clinical applications. However, isolation procedures of peripheral blood mononuclear cells, the differentiation towards DC and maturation are very different between the published clinical studies.[36]

Clinical trials using DC-based vaccines are summarized in Table 3. In 2009, meta-analysis by Van Poppel *et al.* on recently published trials using DC vaccines was reported. In this article, 37% of all patients (95 out of 256 patients) achieved a clinical response (4 CR, 12 PR and 79 SD).[57] Höltl *et al.* showed specific immune responses in the trial using autologous DC loaded with autologous or allogeneic tumor cell lysate and keyhole limpet hemocyanin.[37] A total of 35 patients with mRCC were enrolled in this trial, and 10 achieved a clinical response (2 CR, 1PR and 7 SD). Furthermore, an association between immune response and clinical response was detected.[39]

In 2006, Wierecky *et al.* reported the results of DC vaccine therapy pulsed with HLA-A2-binding peptide mucin 1 for mRCC patients. In this phase I trial ($n = 20$), immune or clinical responses were found in six patients (clinically, 1 CR, 2 PR and 2 MR) during treatment duration of 14 months.[49] A significant correlation between clinical and immune response was noted ($r = 0.791$) with higher induction of immune response in the case of SD or reduction of metastatic lesions ($P = 0.046$). In another phase I/II trial with an autologous tumor cell lysate-pulsed DC vaccine, Kim *et al.* showed five SD and one PR at a median follow up of 17.5 months in nine patients with mRCC.[52] Except for one patient, all patients showed an antigen-specific lymphocyte proliferation response after the first cycle, and patients with PR or SD had higher responses by day 42. Similar findings have been published suggesting the value of DC vaccines.[50,51,54] In these trials, an association between clinical and immunological responses was observed. Recently, Wei *et al.* reported vaccine therapy using hybrids of DC fused with tumor cells (dendritomas) in 10 patients. They concluded that their therapy is safe and effective when administered alongside escalating doses of IL-2. Immune, as well as clinical, responses (1 PR and 3 SD) were achieved.[53] In another trial by Oosterwijk-Wakka *et al.* using DC pulsed with autologous tumor cell lysate in combination with IL-2, no regression of metastases was noted. Although a measurable immunological response was not induced, there was extended disease stabilization.[58]

In 2009, Schwaab *et al.* reported clinical outcomes and immune response after DC vaccine therapy in combination with IL-2 and IFN-α in 18 mRCC patients.[55] The overall clinical response rate using RECIST was 50%, and three

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Table 3 Clinical trials with dendritic cell vaccine in renal cell carcinoma

Investigator	Year	No. patients	Phase	Antigen	Adjuvant	Clinical outcomes
Höltl *et al.*[37]	1999	4	1/2	Tumor lysate + KHL	None	1 PR
Märten *et al.*[38]	2002	15	1/2	Tumor lysate + KHL	None	1 PR, 7 SD
Höltl *et al.*[39]	2002	35	2	Tumor lysate + KHL	None	2 CR, 1 PR, 7 SD
Märten *et al.*[40]	2003	12	1/2	Tumor cells	None	4 SD
Oosterwijk *et al.*[41]	2003	12	1	Tumor lysate + KHL	IL-2	8 SD, No measurable immune response
Gitlitz *et al.*[42]	2003	14	1	Tumor lysate	None	1 PR, 3 SD
Su *et al.*[43]	2003	15	1	Tumor RNA	None	N/A
Arroyo *et al.*[44]	2004	5	1	Tumor lysate + KHL	None	3 SD
Pandha *et al.*[45]	2004	5	1/2	Tumor lysate	KHL	2 SD
Avigan *et al.*[35]	2004	13	1	Tumor cells + KHL	None	5 SD
Dannull *et al.*[46]	2005	11	1/2	Tumor RNA	Treg depletion	N/A
Barbuto *et al.*[47]	2005	22	1/2	Tumor cells	None	2 PR, 14SD
Höltl *et al.*[48]	2005	22	1/2	Tumor lysate + KHL	CPA	2 MR, 3 SD
Wierecky *et al.*[49]	2006	20	1/2	MUC1 peptide + PADRE	IL-2	1 CR, 2 PR, 5 SD
Matsumoto *et al.*[50]	2007	3	N/A	Tumor lysate + KHL	None	1 SD
Bleumer *et al.*[51]	2007	6	1/2	CA 9 peptide + KHL	None	No clinical response
Kim *et al.*[52]	2007	9	1/2	Tumor lysate + KHL	None	1 PR, 5 SD
Wei *et al.*[53]	2007	10	2	Dendritomas†	IL-2	1 PR, 3 SD
Avigan *et al.*[54]	2007	20	1/2	Tumor cells	None	2 PR, 8 SD
Schwaab *et al.*[55]	2009	18	2	Tumor lysate	IL-2, IFN-α2a	50% clinical response rate
Soleimani *et al.*[56]	2009	17	1/2	Telomerase and survivin/ allogeneic tumor cell lines	IL-2	No CR, specific T cell response in SD

†Hybrids of DC with tumor cells.

patients achieved CR. The median time to progression was 8 months, and the median survival had not been reached within a median follow up of 37 months. Soleimani *et al.* also evaluated DC vaccine therapy for 27 mRCC patients in phase I/II clinical trials.[56] In the first trial, HLA-A2$^+$ patients were treated with autologous DC pulsed with telomerase and survivin, whereas HLA-A2$^-$ patients were administered with autologous DC pulsed with allogeneic tumor cells. In the second trial, immune responses in HLA-A2$^-$ patients were evaluated during vaccine therapy to identify potential response biomarkers. As a result, tumor lysate specific T cell response was induced, and predominant Th1 response with tumor lysate-specific IFN-γ T cell responses before and during vaccine therapy was found to correlate to disease stabilization. However, serum concentrations of cytokines were comparable in both SD and PD patients during treatment. The authors concluded that the future of DC-based vaccines might be a combination with current cancer treatment regimens to attenuate regulatory T cells and to expand effector T cells.

These reported studies show that DC-based vaccines are safe in mRCC patients and feasible to induce antigen-specific immune response, and clinically achieve tumor regression in several patients. However, the results should be viewed with caution, because of the relatively small number of patients enrolled in these trials and the multiplicity of vaccination strategies used.

Peptide-based vaccines

With regard to vaccine design, the use of restricted antigens is more relevant to tumor vaccine therapy than the use of tumor cells or cell lysates. Tumor cell and tumor lysate vaccines contain unknown antigens including normal self-proteins, which might result in unexpected host immune responses. Other disadvantages of tumor cell or lysate vaccine are the *ex vivo* preparation of cells and the limitation of tumor materials as the source of antigens. In contrast to these obstacles of using these types of vaccines, synthetic peptide-based vaccines have several advantages, such as easy production, stability, safety, no tumor tissue required and cost effectiveness.[59] However, despite these advantages, only a limited number of clinical studies using peptide-based RCC vaccines have been reported to date. Clinical trials with peptide-based vaccines in the metastatic and adjuvant setting are summarized in Table 4.

Studies in the metastatic setting

CA9 antigen is a tumor-associated glycoprotein expressed in a variety of malignancies, such as cervical, colorectal, esophageal and lung cancers.[69–73] Approximately 90% of any type of RCC and 99% of clear cell RCC express CA9, whereas CA9 expression in normal tissues including kidney tissue is limited. Therefore, CA9 antigen is a suitable target

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