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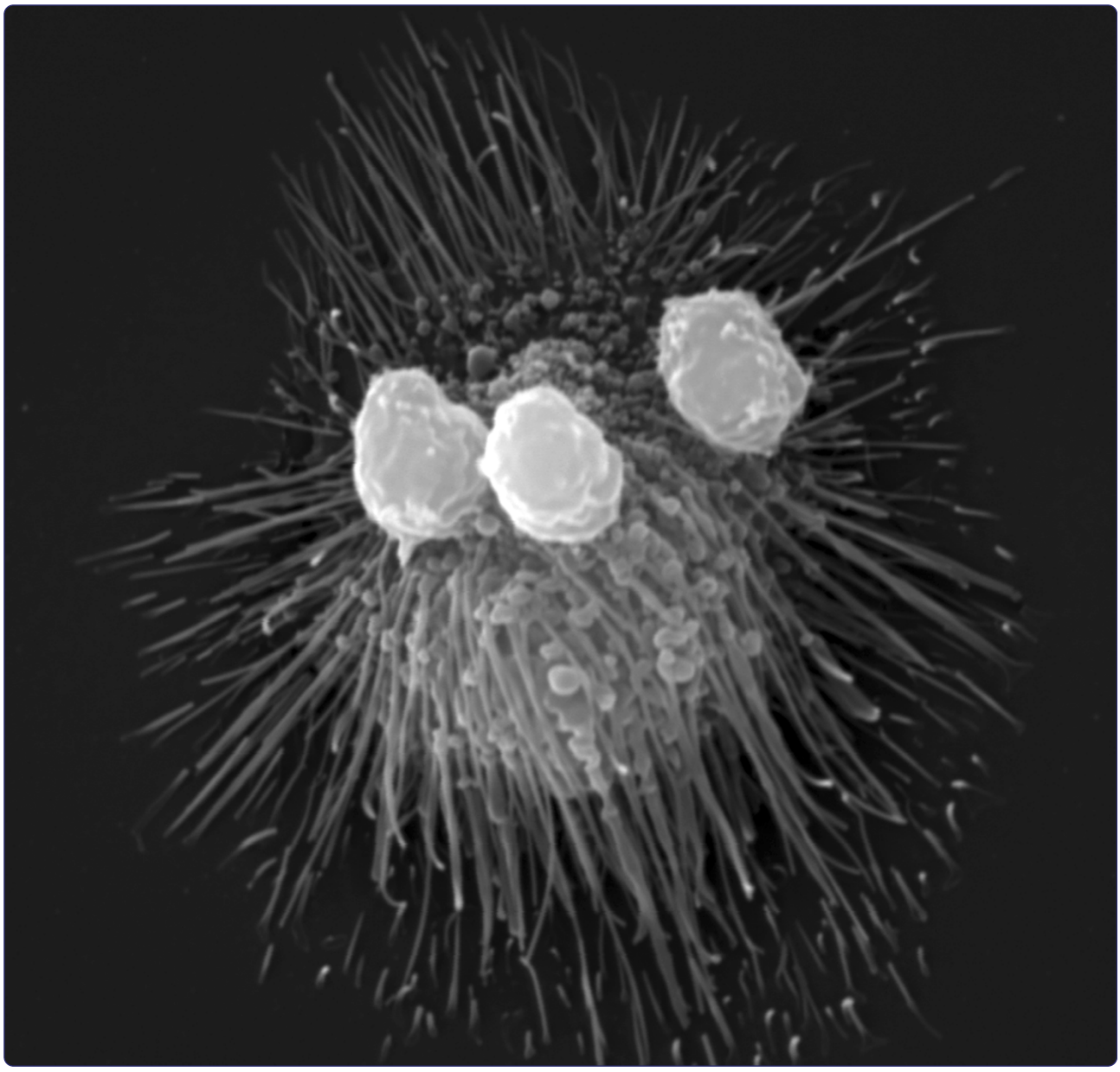

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RESEARCH ARTICLE Open Access

A pilot study of autologous tumor lysate-loaded dendritic cell vaccination combined with sunitinib for metastatic renal cell carcinoma

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Abstract

Background: Sunitinib, a tyrosine kinase inhibitor currently in use for the treatment of metastatic renal cell carcinoma (mRCC), has been reported to modulate immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) in addition to exerting anti-angiogenic effects. We conducted a clinical trial of dendritic cell (DC)-based immunotherapy together with sunitinib in mRCC patients in an effort to enhance immunotherapeutic efficacy by inhibiting immunosuppressive cells.

Methods: Patients aged ≥20 years with advanced or recurrent mRCC who underwent nephrectomy were eligible for this study. Autologous tumor samples were obtained by surgery under aseptic conditions and used for preparing autologous tumor lysate. About 4 weeks after surgery, leukapheresis was performed to isolate peripheral blood mononuclear cells (PBMCs). DCs were generated from adherent PBMCs in the presence of recombinant human granulocyte macrophage colony-stimulating factor (GM-CSF) (500 IU/ml) and recombinant human IL-4 (500 IU/ml). Autologous tumor lysate was loaded into mature DC by electroporation. Eight patients were enrolled in the study and received sunitinib at a dose of 50 mg p.o. daily for 28 days followed by 14 days of rest. Tumor lysate-loaded DCs were administered subcutaneously every two weeks, with concomitant sunitinib.

Results: No severe adverse events related to vaccination were observed. Sunitinib decreased the frequencies of MDSCs in peripheral blood of 5 patients and of Tregs in 3. Tumor lysate-reactive CD4 or CD8 T cell responses were observed in 5 patients, 4 of whom showed decreased frequencies of Tregs and/or MDSCs. The remaining 3 patients who failed to develop tumor-reactive T cell responses had high levels of IL-8 in their sera and did not show consistent reductions in MDSCs and Tregs.

Conclusions: DC-based immunotherapy combined with sunitinib is safe and feasible for patients with mRCC.

Trial registration: UMIN000002136

Keywords: RCC, Sunitinib, Dendritic cell, Lysate

Background

Renal cell carcinoma (RCC) accounts for 2–3% of all adult cancers. Approximately 20–30% of patients present with metastatic disease. Although surgery is the primary curative therapy for localized RCC, the prognosis for patients with advanced metastatic disease is poor, with a 5-year survival rate of <10% [1,2]. Since the first receptor tyrosine kinase inhibitor (TKI) sorafenib was approved for the treatment of cytokine-refractory metastatic RCC (mRCC), many agents have become available for the treatment of this disease. However, many tumors acquire resistance to these agents by mutating the target genes or activating other pathways that bypass the site of inhibition. This occurs rapidly, often within several months [3]. Therefore, development of other modalities such as immunotherapy is still needed for the treatment of mRCC.

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RCC appears to be one of the most immune-sensitive cancers. This has encouraged the use of immunomodulating treatments such as cytokine-based therapy using IL-2 and/or interferon-α (IFN-α) [4,5]. Nonetheless, nephrectomy is still recommended for patients with mRCC [6], because cytoreductive therapy was shown to provide overall survival benefit in patients treated with IFN-α [7]. Although it is still controversial whether cytoreductive therapy also contributes to the efficacy of TKIs [8], nephrectomy reduces the tumor burden, alleviates symptoms and allows more information on histology to be acquired. In addition, we can utilize the resected tumor as a source of autologous materials, such as tumor lysates, for the production of autologous tumor vaccines. It has been reported that adjuvant treatment with autologous tumor lysate vaccine resulted in a significantly improved overall survival in pT3 stage RCC patients [9]. Antigen-specific vaccination with dendritic cells (DCs) has also been conducted, but with only limited success so far [10-15], possibly due to functionally-defective T cell responses in the tumor microenvironment.

It is well accepted that the tumor microenvironment imposes different degrees of immunosuppression allowing the tumor to evade immune responses [16]. These include the delivery of negative costimulatory signals to T cells (via PD-L1, B7-H4) and production of immunosuppressive factors (eg. IL-10, TGF-β, IDO and others). Recently, promising immunotherapeutic strategies have emerged from our understanding of immunoinhibitory pathways termed "immune checkpoints", which are crucial for maintaining self-tolerance and modulating the duration and magnitude of physiological immune responses. Tumors utilize such immune checkpoints as a resistance mechanism to escape anti-tumor immune responses [17]. Hence, immune checkpoint blockade is a promising approach to activating antitumor immunity. The antibodies that block CTLA-4- and PD-1-dependent interactions have been successfully applied for the treatment of mRCC [18-21].

In addition, different regulatory cell populations, such as MDSCs or Tregs, are involved in this process. The accumulation of MDSCs as well as the suppression of T-cell function in mRCC patients has been reported [22,23]. TKIs such as sunitinib and sorafenib were approved some time ago and are now the mainstay for the treatment of mRCC [24-26]. In addition to its anti-angiogenic effects, sunitinib has been demonstrated to modulate immunosuppressive MDSCs in human [27] and mouse [28]. It has also been reported that sunitinib reverses type-1 immune suppression and decreases Tregs in renal cell carcinoma patients [29]. Furthermore, sunitinib, unlike sorafenib, does not inhibit specific T cell responses [30]. Therefore, sunitinib appears to be a promising molecular target drug for combination therapy together with cancer vaccines for mRCC.

Here, we report the results of a clinical trial in which we evaluated the safety and feasibility of DC-based vaccination combined with sunitinib for mRCC patients and tested whether sunitinib enhances immune responses by reducing immunosuppressive cells.

Results

Patients

Eight patients (5 men and 3 women) with a median age of 68 yr (range, 55–75) were enrolled in this study (Table 1). Two patients were categorized into the MSKCC poor risk group and the other six as having an intermediate risk. One patient (#1808) had unclassified RCC, while the other seven had clear cell RCC. Two patients, #1802 and #1803, received sunitinib or IFN-α and radiation for bone metastasis, respectively, before surgery.

DC Vaccine combined with sunitinib

DCs were successfully generated from all 8 patients (Table 2). Final concentrations of tumor lysate per 10^7 DCs ranged from 0.44 to 1.33 mg (mean value, 0.90 mg). Flow cytometric analysis of the harvested tumor lysate-loaded DCs revealed a phenotype characteristic of mature DCs with high expression of CD40, CD80, CD83, CD86, HLA-ABC, HLA-DR, and CCR7 (Figure 1 and Table 3). While there were some differences in the fluorescent intensities of these molecules among patients' DCs (Additional file 1), the phenotype of these DCs were quite comparable. None of the DC preparations was microbially contaminated. Each patient was given 1×10^7 DCs at each time point, with the exception of one patient (#1823) who received 0.5×10^7 DCs (Table 2). Patients received 6 vaccinations and sunitinib at a dose of 50 mg p.o. daily for 28 days followed by 14 days of rest, according to the schedule (Additional file 2). Vaccination was well-tolerated and no severe vaccination-related toxicity or autoimmune manifestations were observed in any patient.

Frequencies of MDSCs and Tregs in peripheral blood

MDSCs in peripheral blood were evaluated by two criteria (percent of $CD14^-CD15^+$ or $CD33^+HLA\text{-}DR^-$ cells within the $Dye780^-CD45^+$ population) (Additional file 3). In individual patients, decreased percentages of MDSCs were observed in 5 of the 8 patients (#1802, #1803, #1806, #1814, and #1823) by both criteria (Figure 2A and Table 4) compared to pretreatment baseline. No marked changes were observed in patients #1808, #1812 and #1817. Sunitinib significantly reduced the average percentage of $CD14^-CD15^+$ MDSCs in 8 patients from 0.62 ± 1.20% (mean ± SD) at the baseline to 0.083 ± 0.17% at the 6th DC injection ($p = 0.0039$, Wilcoxon signed-rank test); the average percentage of $CD33^+HLA\text{-}DR^-$ MDSCs in 8 patients did not change (2.57 ± 2.86% at the baseline and 3.17 ± 6.73%

Table 1 Patients' characteristics

Patient ID	Age/Sex	Stage	Meta site	MSKCC	Histology	Grade	Prior treatment
1802	72/F	pT3aN2M1	Lung, LN	Poor	Clear cell	2 > 3	Sunitinib
1803	72/M	pT3bN0M1	Liver, lung, bone	Poor	Clear cell	3 > 2	IFN-α, radiation
1806	72/F	pT4N1M1	Lung, LN	Intermediate	Clear cell	3	no
1808	75/M	pT3aN2M1	Lung, LN, bone	Intermediate	Unclassified	3	no
1812	61/M	pT1bN1M1	LN	Intermediate	Clear cell	2 > 1> > 3	no
1814	55/M	pT3aN0M1	Lung	Intermediate	Clear cell	2	no
1817	64/F	pT3bN1M1	Lung, LN, bone	Intermediate	Clear cell	3 > 2	no
1823	57/M	pT1N0M1	Lung, pleura	Intermediate	Clear cell	2 > 3	no

MSKCC, Memorial Sloan Kettering Cancer Center risk criteria; LN, lymph node.

after sunitinib treatment) ($p = 0.23$, Wilcoxon signed-rank test). For Tregs, the percentages of $CD25^+Foxp3^+$ cells within the $Dye450^-CD3^+CD4^+$ population (Additional file 3) were found to be decreased relative to the baseline in patients #1802, #1803 and #1814, but not in patients #1806, #1808, #1812, #1817 and #1823 (Figure 2B). However, there was no statistical difference ($p = 0.273$, Wilcoxon signed-rank test).

DTH reactions and tumor-reactive T cell responses

DTH testing was performed in all 8 patients to detect tumor lysate-reactive responses. Three patients (#1802, #1814 and #1823) had positive DTH reactions (Table 4). Tumor lysate-reactive $CD4^+$ and $CD8^+$ T cell responses in all patients were further investigated in vitro using the IFN-γ secretion assay at different time points after vaccination. Data from an individual patient #1802 are shown in Additional file 3. Before vaccination, the percentage of $CD8^+$ IFN-γ^+ T cells after simulation with EP-DCs or unloaded DCs was essentially identical (1.6%-vs-1.4%, respectively). However, after vaccination, a higher percentage of $CD8^+$ IFN-γ^+ T cells was observed on stimulation with EP-DCs (2.9%) than with unloaded DCs (1.5%). Similarly, a higher percentage of $CD4^+$ IFN-γ^+ T cells was observed on stimulation with EP-DCs (4.5%) than with unloaded DCs (3.0%). These T cell responses fluctuated during the course of treatment and no statistically significant difference in the increase of IFN-γ^+ T cells after vaccination was detected. Figure 2C shows the percentage of tumor lysate-reactive IFN-γ^+ cells (both $CD4^+$ as well as $CD8^+$ T cells) for all 8 patients. When the percentages at any point after vaccination are elevated 3-fold higher than those at the baseline (mean value of the percentages at days 0 and 14), the tumor-reactive T cell responses are considered to be positive. By this criteria, the induction of tumor lysate-reactive $CD4^+$ T cell responses were detected in patients #1802, #1803, #1814 and #1823; patients #1802, #1812, #1814 and #1823 had tumor-reactive $CD8^+$ T cell responses (Table 4). The T cell responses were detected even at the time of registration in Patients #1808 and 1812.

Concentration of IL-8 in the sera

To search for biomarkers predicting responsiveness to combination therapy with sunitinib and DC-based immunotherapy, we analyzed concentrations of IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, TNF-α, and TNF-β in sera from the 8 patients before and during treatment. With the exception of IL-8, which was present at different levels in all patients, serum cytokines were barely detectable. Patients #1806, #1808, #1817 and #1823 had greatly elevated levels of >60 pg/ml IL-8 during treatment (Figure 2D and Table 4), whereas patients #1802, #1803, #1812, and #1814 had basal levels <60 pg/ml.

Table 2 Quality and quantity of tumor lysate-loaded DCs

Patient ID	Tumor lysate used for EP (mg)	DCs used for EP ($x10^7$)	Tumor lysate (mg)/ 10^7 DCs	Number of DCs injected	Viability (%)
1802	15	29.5	0.51	$1x10^7$	82.9
1803	15	18.3	0.82	$1x10^7$	82.1
1806	20	17.1	1.17	$1x10^7$	92.8
1808	7	16.0	0.44	$1x10^7$	83.6
1812	20	20.1	1.00	$1x10^7$	89.0
1814	20	21.5	0.93	$1x10^7$	91.7
1817	20	20.0	1.00	$1x10^7$	87.5
1823	20	15.0	1.33	$0.5\ x10^7$	92.4

EP, electroporation.

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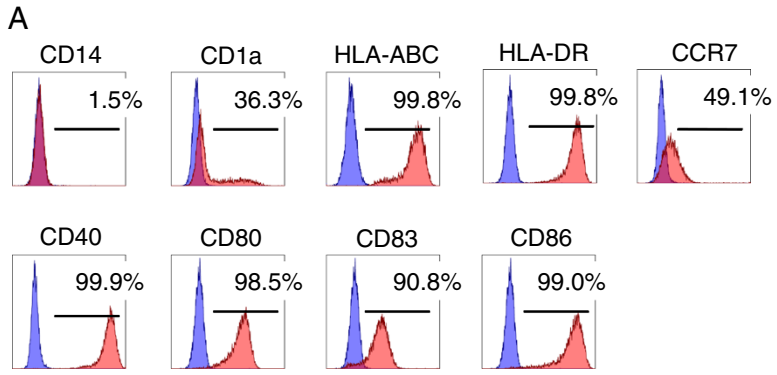

Figure 1 Surface phenotype of DCs; specific mAb staining (red) and isotype control mAb staining (blue).

Clinical responses

The follow-up period ranged from 100 to 1140 days (Table 4). Except for one patient who died of a brain hemorrhage due to hypertension, patients remained alive during the trial with a median overall survival (OS) of 346 days and median progression-free survival (PFS) of 164 days. One patient achieved a complete response (CR), another patient had a partial response (PR), 3 had stable disease (SD) and 2 had progressive disease (PD) according to the RECIST criteria (Table 4). Patient #1814 who achieved the CR was one of three patients who had developed DTH, as well as $CD4^+$ and $CD8^+$ T cell responses. In this patient, the percentages of both MDSCs and Tregs decreased during treatment. In the CT scan, the size of the mass in the left lung decreased from 17.9 mm to 8.2 mm in diameter after 6 immunizations and had disappeared after 10 (Additional file 4). The other patient who had a DTH reaction, #1802, also had $CD4^+$ and $CD8^+$ T cell responses, as well as decreased MDSCs and Tregs, and low IL-8. She manifested SD in spite of multiple tumor metastases in the lung (Additional file 4). Her quality of life was markedly improved by a reduction of the pleural effusion (Additional file 4). As shown in Additional file 4, the tumor volume was decreased and pleural effusion was reduced in patient #1823, who develop also positive DTH, $CD4^+$ and $CD8^+$ T cell responses (Table 4). Patient #1812 was defined as PD when target lesion, supraclavicular lymph node metastasis, was enlarged by 30.3% in size. Therefore, he received surgery to resect the metastatic lymph node and no recurrence was observed with no further treatment.

Safety

The most common adverse events were hand-foot syndrome, stomatitis, peripheral edema and other skin disorders (Table 5). Sunitinib-related severe adverse events were hypertension and hematological and laboratory abnormalities. They were managed with interruption of sunitinib and were reversible in most cases, except for a fatal hypertensive intracranial hemorrhage in patient #1806 who had no brain metastasis. No severe adverse events related to DC therapy were observed.

Discussion

Here we report a clinical trial of DC-based immunotherapy combined with sunitinib in mRCC patients. We evaluated the safety and feasibility of this approach. In the course of treatment, one patient developed cerebral hemorrhage due to hypertension. However, no severe vaccination-related toxicity or autoimmunity was observed in any of the 8

Table 3 The surface phenotype of DCs

Patient ID	% Expression								
	CD14	CD1a	HLA-ABC	HLA-DR	CCR7	CD40	CD80	CD83	CD86
1802	7.8	51.6	98.7	99.7	10.6	99.9	96.4	60.1	96.3
1803	1.4	79.6	99.2	99.9	38.7	99.4	99.1	94.1	99.5
1806	1.5	36.3	99.8	99.8	49.1	99.9	98.5	90.8	99
1808	5.2	53.3	98.8	99.5	43.1	99.4	96.7	75.6	98.7
1812	0.6	83	99.9	99.7	10.3	99.8	99.2	87.4	97.9
1814	0.6	66.5	99.4	99.4	58.3	99.6	98.7	91.5	99.2
1817	1.4	34.7	99.7	98.3	50.3	99.8	96.7	61.8	98
1823	0.8	50.4	99.7	99.6	30.5	99.7	99.3	95.4	99.3

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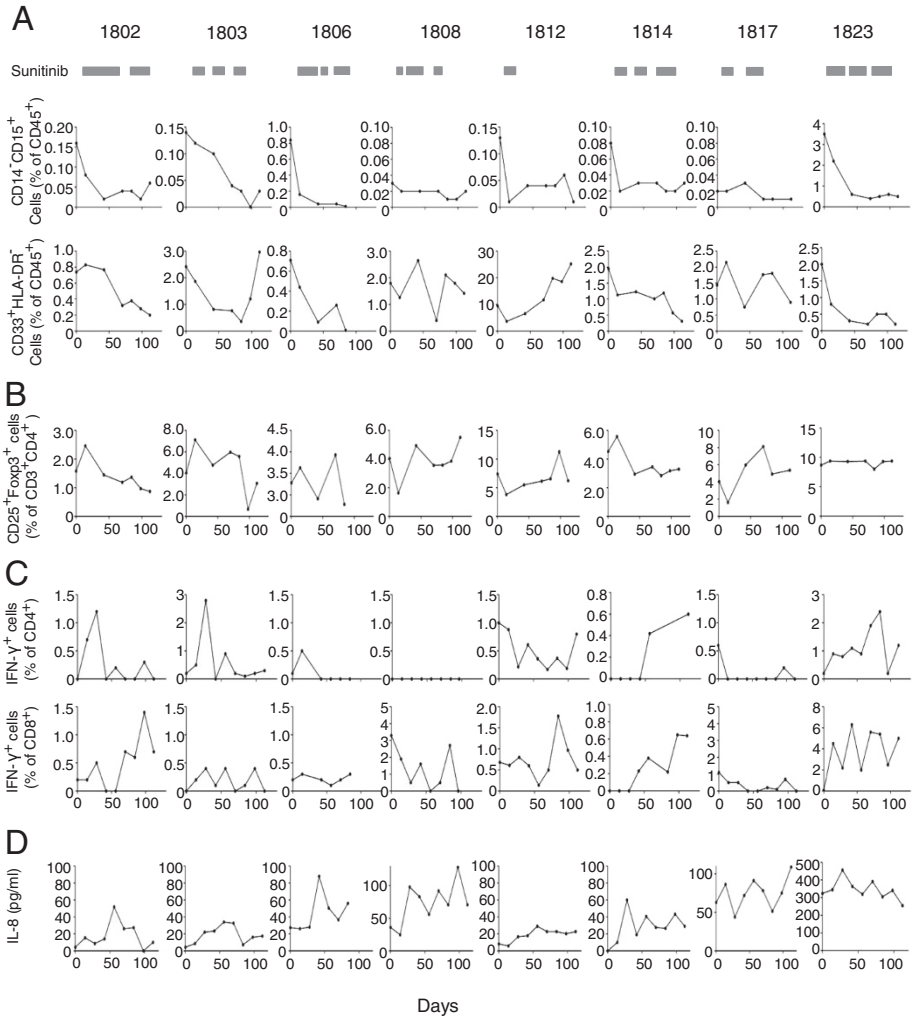

Figure 2 Immunomonitoring. A. Percentages of MDSCs by two criteria. **B**. Percentages of Tregs. **C**. Changes of tumor-reactive IFN-γ^{+} cells (% of CD4^{+} or CD8^{+} T cells). Assay was performed as described in Methods section. **D**. The concentration of IL-8 in sera measured by a cytofluorometry-based ELISA system at different time points during treatment of the 8 patients.

patients treated. Sunitinib decreased the frequencies of peripheral blood MDSCs and/or Tregs. Vaccination with tumor lysate-loaded DCs induced tumor-reactive CD4^{+} and/or CD8^{+} T cell responses. The treatment showed some clinical benefits in patients possibly linked to successful control of immunosuppressive cells and induction of T cell responses. This was particularly notable in patient #1814 where lung metastases disappeared. However, there is a possibility that these clinical responses are solely due to sunitinib rather than vaccine-induced immune response, since the DC was given concurrently with sunitinib which is an active drug for the treatment of RCC.

Consistent with previous reports [27,29], we observed reduced percentages of MDSCs during sunitinib treatment, but only in 5 of 8 patients (Figure 2A and Table 4). Of these 5, 4 developed increased tumor-reactive T cell responses. However, the very low number of patients included in this study and the fluctuations in magnitude of T cell responses during the course of treatment make it difficult to conclude the relationship between MDSC and T cell responses. Regarding mechanisms underlying the modulation of MDSCs by sunitinib, it has been shown that this agent inhibits STAT3 signaling. This induces apoptosis in murine MDSCs, where STAT3 is a critical factor responsible for their expansion [31,32]. On the other hand, GM-CSF accumulating in the tumor expands MDSCs to promote sunitinib-resistance due to preferential STAT5 activation, which cannot be suppressed by sunitinib [33]. Thus, to understand the different sensitivity of MDSCs to sunitinib in different mRCC patients, the STAT3 or STAT5 activation status in the MDSCs and expression of cytokines such as GM-CSF in the tumor would need to be investigated.

A decreased percentage of Tregs after sunitinib treatment was also observed, although only in 3 of the 8

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Table 4 Immune responses and clinical outcomes in 8 patients

ID	No. DC injection	DTH	CD4 T cell response	CD8 T cell response	MDSCs*	Tregs*	IL-8[†]	Change in Target Lesions (%)	Clinical Response[‡]	PFS[§](d)	OS[§](d)	Prognosis
1802	6	+	+	+	decreased	decreased	low	−25.4	SD	173	339	Dead
1803	6	-	+	-	decreased	decreased	low	0	SD	200	353	Dead
1806	6	-	-	-	decreased	no change	high	−18.4	N.A.[¶]	100	100	Dead
1808	6	-	-	-	no change	increased	high	−5.4	SD	155	193	Dead
1812	6	-	-	+	no change	no change	low	30.3	PD[#]	101	1140[‖]	Alive
1814	12	+	+	+	decreased	decreased	low	−100	CR	347	1127[‖]	Alive
1817	6	-	-	-	no change	increased	high	−27.8	PD[**]	88	206	Dead
1823	12	+	+	+	decreased	no change	high	−35.3	PR	342[‖]	342[‖]	Alive

PFS, progression free survival; OS, overall survival; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
*Compared to the baseline.
[†]High or low is defined as more or less than 60 pg/ml in sera.
[‡]4wks after last injection.
[§]From the registration (days).
[¶]Withdrawn from the study by sudden hypertensive cerebral hemorrhage.
[‖]A censored case due to the termination of the study.
[#]After surgical removal of target lesion (LN metastasis), no recurrence was observed.
[**]Though target lesion became smaller, accumulation of pleural effusion was increased.

patients (Figure 2B and Table 4). The mechanism underlying regulation of Tregs by sunitinib remains unclear. It has been proposed that the reduction of Tregs by sunitinib may be an indirect effect of the downregulation of MDSCs and/or increases in IFN-γ production [27]. In our case, reduced frequencies of Tregs were observed in 3 of the 5 patients who did show reduced MDSCs. No reduction of Tregs was seen in a further 3 of 3 patients in whom there was no reduction of MDSCs. Nevertheless, the number of patients was too small to lead to any conclusion.

To identify biomarkers for predicting outcome of combination sunitinib and DC-based immunotherapy, we tested a wide range of cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, TNF-α, and TNF-β) in sera from patients before and during treatment. We found IL-8 in all patients, with 4 having highly elevated levels (>60 pg/ml) during treatment. IL-8 is a member of the CXC family of chemokines and is a potent proangiogenic factor [34]. Renal cell carcinoma has been shown to produce IL-8, and IL-8 expression is known to cause mRCC resistance to sunitinib [35,36]. IL-8 angiogenic signaling is thought to functionally compensate for the inhibition of VEGF/VEGFR-mediated angiogenesis. Further, the secretion of IL-8 from cancer cells may have a variety of effects on the tumor microenvironment, because the IL-8 receptors CXCR1 and CXCR2 are expressed on cancer cells, endothelial cells, neutrophils and tumor-associated macrophages. It has been shown that production of IL-8 by tumors induces Treg migration into tumors [36]. IL-8 produced by tumor cells may also recruit MDSCs into tumor sites. Therefore, high IL-8 expression may contribute to shaping the immunosuppressive environment in the tumor and inhibiting tumor-reactive T cell responses. In this study, no reduction of IL-8 was achieved by sunitinib (Figure 2D). Therefore, targeting IL-8 signaling may be required for improving this cancer vaccine.

Cancer immunotherapy based on the regulation of immunosuppressive cells, soluble factors, and signaling pathways are now considered essential element of the treatment of cancer [37]. Similar effects are also achieved by molecular targeted therapy, which primarily aims to inhibit molecular pathways that are crucial for tumor cell growth and survival. Importantly, such small molecule inhibitors may also modulate the immune system, which raises the possibility that targeted therapy might be effectively combined with immunotherapy to improve clinical outcomes [38]. This may indeed be the case in our small pilot study. A reduction of immunosuppressive cells by sunitinib likely contributed to stimulating anti-tumor immune responses induced by tumor lysate-loaded DC vaccines.

Initially 15 patients were planned to be included in this study; we terminate the study with 8 patients reproted here, because other TKIs, pazopanib and axitinib, and mTOR inhibitors, temsirolimus and everolimus, are now available for the RCC treatment in addition to sunitinib and sorafenib. A new pilot study is currently underway to determine the better combination of these molecular target drugs with DC-based immunotherapy. Though our

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Table 5 Adverse Events and Laboratory abnormalities

	Grade					
Adverse Events, Regardless of Causality	All	1	2	3	4	5
General disorders						
Fatigue	2		2			
Pyrexia	2	1	1			
Insomnia	1		1			
Gastrointestinal disorders						
Dyspepsia	2		2			
Dysgeusia	2	1	1			
Diarrhea	2	2				
Nausea	1	1				
Esophagitis	1	1				
Respiratory, thoracic and mediastinal disorders						
Cough	1		1			
Musculoskeletal and connective tissue disorders						
Back pain	3	1	2			
Metabolism and nutrition disorders						
Hypothyroidism	4		4			
Skin and subcutaneous tissue disorders						
Hand-foot syndrome	8	2	6			
Stomatitis	4	2	2			
Peripheral Edema	4	3	1			
Anal diseases	3		3			
Skin ulceration	1		1			
Pruritus	1		1			
Trichophytosis	1		1			
Rash	1	1				
Vascular disorders						
Hypertension	3		1	1		1*
Hematological and other laboratory abnormalities						
Anemia	3		1	2		
Leukopenia	3			3		
Neutropenia	3			3		
Lymphocytopenia	3			3		
Thrombocytopenia	3			3		
Increased creatinine	2			2		

*Intracranial hemorrhage.

study has some limitations in that this is a single institution study and sample size was only 8 patients, our results support the notion that immunotargeted therapy represents an appropriate future direction for developing successful treatment of mRCC.

Conclusions

This pilot study of DC-based therapy together with sunitinib for mRCC patients has documented the safety and feasibility of this approach. The reduction of both MDSCs and Tregs was achieved by sunitinib in patients whose serum IL-8 levels were not excessive. Autologous tumor lysate-loaded DCs in combination with sunitinib induced both $CD4^+$ and $CD8^+$ T cell responses in mRCC patients.

Methods

Patient selection

A pilot study of DC-based immunotherapy combined with sunitinib in mRCC patients was conducted. The primary endpoints were the safety and feasibility of this approach; the secondary endpoints were to obtain immunological proof of concept and preliminary data for anti-tumor effect, overall survival (OS) and progression-free survival (PFS). Patients aged ≥20 years with advanced or recurrent mRCC who underwent nephrectomy were eligible for this clinical study of DC therapy combined with sunitinib. To be included, patients had to have an Eastern Cooperative Oncology Group performance status (PS) of 0, 1 or 2, normal kidney, liver, and bone marrow function, and at least 1 measurable cancer lesion assessed by computed tomography. Patients positive for anti-adult T-cell leukemia-associated antigen or anti-human immunodeficiency virus antibody, other primary cancers, uncontrolled infection, active enterocolitis, severe heart disease, severe drug allergy, cryoglobulinemia, or autoimmune disease, were excluded from the study. Those receiving systemic steroid therapy, who were pregnant or lactating, or who had brain metastasis and hypertension were also excluded. The research protocol was approved by the Ethical Committee of our institution and was registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) (Unique trial number: UMIN000002136) on July 2, 2009. Written informed consent was obtained from each patient before they entered the study. The study was performed in accordance with the Declaration of Helsinki.

Generation of DCs

About 4 weeks after surgery, patients underwent leukapheresis to isolate peripheral blood mononuclear cells (PBMCs) using a Fresenius AS.TEC204 with the C4Y white blood cell set. Approximately 5 $\times 10^9$ PBMCs from each patient were allowed to adhere to tissue culture flasks in AIM-V medium (Invitrogen, Carlsbad, CA) at 37°C. After one hour, nonadherent cells were removed by washing with warm medium. To generate immature DCs, adherent PBMCs were cultured in AIM-V for 5 days in the presence of recombinant human granulocyte macrophage colony-stimulating factor (GM-CSF) (500 IU/ml; Berlex Laboratories, Montville, NJ) and recombinant human IL-4 (500 IU/ml; CellGenix Technologie Transfer GmbH, Freiburg, Germany). Immature DCs were then matured by adding GM-CSF (250 IU/ml), recombinant human IL-4 (250 IU/ml), tumor necrosis factor (TNF-α)

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(0.01 μg/ml; CellGenix Technologie Transfer GmbH), prostaglandin E2 (PGE2) (1 μg/ml; Sigma, St. Louis, MO) and zoledronate (5 μM; Novartis, Basel, Switzerland) for a further 2 days [39].

Preparation of tumor lysates and electroloading of dendritic cells

Autologous tumor samples were obtained by surgery under aseptic conditions. Tumor tissues were minced with a scalpel in phosphate-buffered saline (PBS). The samples were then lysed by six freezing and thawing cycles, sonicated and centrifuged to produce tumor lysate. Finally the supernatant was filtered using 0.22-μm pore-size filters. The quantitation of total protein was performed using BCA Protein Assay Kit (Pierece Biotechnology, Rockford. IL, USA) according to the manufacturer's instruction. Colorimetric changes were detected by VersaMax microplate reader (Molecular Device Japan, Tokyo, JAPAN) at the wavelength of 562 nm with Softmax Pro software (Molecular Device Japan). Autologous tumor lysate was loaded into mature DCs using a MaxCyte GT electroporation-based system (MaxCyte Inc, Gaithersburg, MD) according to the manufacturer's instructions [40]. Tumor lysate-electroporated DCs, designated EP-DCs, were cryopreserved with 1 ml of autologous serum containing 10% DMSO and stored in liquid N_2 until use.

Immunization schedule

After leukapheresis, patients received sunitinib at a dose of 50 mg p.o. daily for 28 days followed by 14 days of rest. Two weeks after leukapheresis, patients received $1x10^7$ EP-DCs subcutaneously in the deltoid region; DC injection was repeated biweekly six times in total, extended to 12 for one long-surviving patient. For immunomonitoring, peripheral blood was drawn before DC therapy, at each treatment time point and 4 weeks after the last treatment. PBMCs were isolated by density gradient centrifugation using Lymphoprep (Axis-Shield, Oslo, Norway) and stored in liquid N_2 until use. Adverse events were graded according to National Cancer Institute-Common Terminology Criteria for Adverse Events version 4.0. Clinical responses were assessed by computed tomography and classified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, version 1.1 [41].

IFN-γ secretion assay

PBMCs (1×10^6) from each time point and EP-DCs (1×10^5) were thawed and resuspended in AIM-V medium supplemented with 10% heat-inactivated pooled human serum (complete medium), and co-cultured in a 24-well plate at 37°C in a 5% CO_2 atmosphere for 2 days. Recombinant human IL-2 (Chiron, Emeryville, CA) was then added every 2–3 days to a final concentration of 50 IU/ml for another 12 days. The cultured PBMCs were harvested and used as responder cells, as described below. The IFN-γ secretion assay was carried out according to the manufacturer's protocol (Miltenyi Biotec, Bergisch Gladbach, Germany) [42]. Briefly, 1×10^6 responder cells were stimulated with 1×10^5 EP-DCs or mature DCs without electroporation (unloaded DCs) in complete medium for 4 hr at 37°C in a 5% CO_2 atmosphere. The cells were then washed and suspended in 100 μl of cold PBS, and treated with a mouse anti-IFN-γ antibody (IFN-γ catch reagent) (2 μl) for 5 min on ice. The cells were then diluted in complete medium (1 ml) and placed on a slowly rotating device (Miltenyi Biotec) to allow IFN-γ secretion at 37°C in a 5% CO_2 atmosphere. After incubation for 45 min, the cells were washed with cold PBS and treated with Fixable viability dye eFluor 450 (eBioscience, San Diego, CA), PE-labeled anti-IFN-γ (detection reagent), Alexa Fluor 647-labeled anti-human CD3 (Biolegend, San Diego, CA), PC5-labeled anti-human CD8 (Beckman Coulter, Fullerton, CA), and PECy7-labeled anti-human CD4 (Biolegend) mAbs. After incubation for 10 min at 4°C, the cells were washed and analyzed on a Gallios Flow Cytometer (Beckman Coulter).

Tregs and MDSCs

Analysis of Treg percentages in patient PBMC was carried out on thawed samples. Cells were stained in fluorescence-activated cell sorting (FACS) buffer (1× PBS with 2% heat-inactivated fetal bovine serum and 0.02% sodium azide). Nonspecific antibody binding was blocked by pretreatment with Clear Back (Human Fc receptor blocking reagent, MBL, Nagoya, Japan). Cells were stained with Dye450, Alexa Fluor 647-labeled anti-CD3, Alexa Fluor 488-labeled Foxp3, PE-Cy5-labeled CD4, and PE-labeled CD25 Abs according to the instructions for use of the Human Treg Flow Kit (Biolegend). MDSCs were also analyzed by FACS on thawed patient PBMC stained with Dye780, ECD-labeled CD14 (Beckman Coulter), FITC-labeled CD15 (Biolegend), PE-Cy5-labeled CD33 (Biolegend), and PE-labeled HLA-DR (BD Biosciences) Abs for 30 min at 4°C. Cells were washed in buffer and then fixed in 1% paraformaldehyde and analyzed by flow cytometry.

Delayed-type hypersensitivity (DTH)

EP-DCs or unloaded DCs were injected intradermally into different forearms. DTH reactions were evaluated 24 and 48 hours after the 6th injection of DCs and considered to be positive when a skin reaction (>10 mm diameter of erythema) was triggered by EP-DCs but not unloaded DCs.

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TH1/TH2 cytokine quantification

Amounts of IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, TNF-α, and TNF-β in patients' sera were quantified by a cytofluorometry-based ELISA system (Flowcytomix, Bender Medsystems GmbH, Austria). Standard curves for each cytokine were generated using the reference cytokine concentrations supplied by the manufacturer. Cytokines in sera from patients at different time points were estimated according to the manufacturer's instructions. Raw data of the FC bead assay were analyzed by FlowCytomixPro2.3 software.

Statistical analysis

The statistical analyses of immunological parameters and prognostic factors (PFS or OS) were performed using Wilcoxon signed-rank test and Kaplan-Meier method, respectively, with JMP software, version 9.0.3 (SAS Institute Inc., Cary, NC, USA).

Additional files

Additional file 1: The mean fluorescent intensity (MFI) of the surface expression of immunological molecules. Tabular data.

Additional file 2: Schedule for DC vaccination combined with sunitinib in this clinical trial. Supplementary figure.

Additional file 3: Data from an individual patient. Supplementary figure.

Additional file 4: Computed tomography (CT) images. Supplementary figure.

Abbreviations

RCC: Renal cell carcinoma; mRCC: Metastatic RCC; TKI: Tyrosine kinase inhibitor; MDSCs: Myeloid-derived suppressor cells; Tregs: Regulatory T cells; PBMCs: Peripheral blood mononuclear cells; GM-CSF: Granulocyte macrophage colony-stimulating factor; DCs: Dendritic cells; TNF-α: Tumor necrosis factor α; PGE2: Prostaglandin E2; OS: Overall survival; PFS: Progression-free survival; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease; RECIST: Response Evaluation Criteria in Solid Tumors; PBS: Phosphate-buffered saline.

Competing interests

Department of Immunotherapeutics is an endowed department supported by financial contributions from Medinet Co. Ltd. (Yokohama, Japan). Dr. Kazuhiro Kakimi received research support from Medinet Co. Ltd. The costs of the entire DC culture production and part of the immunological assays were covered by Medinet Co. Ltd. The study sponsors had no involvement in study design; collection, analysis, and interpretation of data; writing the report; and the decision to submit the report for publication. No potential conflicts of interest were disclosed by the other authors.

Authors' contributions

Conceived and designed the study: HM, YE, YH and KK. Performed the clinical study: HM, YE, HK, TN, HF, MS, TH, and KK. Analyzed the data: HM and KK. Wrote the paper: HM, YE, YH and KK. All authors read and approved the final manuscript.

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References

1. Motzer RJ, Bander NH, Nanus DM: **Renal-cell carcinoma.** *N Engl J Med* 1996, **335:**865–875.
2. Cohen HT, McGovern FJ: **Renal-cell carcinoma.** *N Engl J Med* 2005, **353:**2477–2490.
3. Abe H, Kamai T: **Recent advances in the treatment of metastatic renal cell carcinoma.** *Int J Urol* 2013, **20:**944–955.
4. Klapper JA, Downey SG, Smith FO, Yang JC, Hughes MS, Kammula US, Sherry RM, Royal RE, Steinberg SM, Rosenberg S: **High-dose interleukin-2 for the treatment of metastatic renal cell carcinoma.** *Cancer* 2008, **113:**293–301.
5. Negrier S, Escudier B, Lasset C, Douillard J-Y, Savary J, Chevreau C, Ravaud A, Mercatello A, Peny J, Mousseau M, Philip T, Tursz T: **Recombinant human interleukin-2, recombinant human interferon alfa-2a, or both in metastatic renal-cell carcinoma.** *N Engl J Med* 1998, **338:**1272–1278.
6. Fujioka T, Obara W, the Committee for Establishment of the Clinical Practice Guideline for the Management of Renal Cell C, the Japanese Urological A: **Evidence-based clinical practice guideline for renal cell carcinoma: the Japanese urological association 2011 update.** *Int J Urol* 2012, **19:**496–503.
7. Flanigan RC, Mickisch G, Sylvester R, Tangen C, Van Poppel H, Crawford ED: **Cytoreductive nephrectomy in patients with metastatic renal cancer: a combined analysis.** *J Urol* 2004, **171:**1071–1076.
8. Biswas S, Kelly J, Eisen T: **Cytoreductive nephrectomy in metastatic clear-cell renal cell carcinoma: perspectives in the tyrosine kinase inhibitor Era.** *Oncologist* 2009, **14:**52–59.
9. May M, Brookman-May S, Hoschke B, Gilfrich C, Kendel F, Baxmann S, Wittke S, Kiessig S, Miller K, Johannsen M: **Ten-year survival analysis for renal carcinoma patients treated with an autologous tumour lysate vaccine in an adjuvant setting.** *Cancer Immunol Immunother* 2010, **59:**687–695.
10. Azuma T, Horie S, Tomita K, Takahashi T, Tanaka Y, Kashiwase K, Nieda M, Takeuchi T, Ohta N, Shibata Y, Hirai H, Kitamura T: **Dendritic cell immunotherapy for patients with metastatic renal cell carcinoma: University of Tokyo experience.** *Int J Urol* 2002, **9:**340–346.
11. Matsumoto A, Haraguchi K, Takahashi T, Azuma T, Kanda Y, Tomita K, Kurokawa M, Ogawa S, Takahashi K, Chiba S, Kitamura T: **Immunotherapy against metastatic renal cell carcinoma with mature dendritic cells.** *Int J Urol* 2007, **14:**277–283.
12. Berntsen A, Trepiakas R, Wenandy L, Geertsen PF, thor Straten P, Andersen MH, Pedersen AE, Claesson MH, Lorentzen T, Johansen JS, Svane IM: **Therapeutic dendritic cell vaccination of patients with metastatic renal cell carcinoma: a clinical phase 1/2 trial.** *J Immunother* 2008, **31:**771–780.
13. Gitlitz BJ, Belldegrun AS, Zisman A, Chao DH, Pantuck AJ, Hinkel A, Mulders P, Moldawer N, Tso C-L, Figlin RA: **A pilot trial of tumor lysate-loaded dendritic cells for the treatment of metastatic renal cell carcinoma.** *J Immunother* 2003, **26:**412–419.
14. Höltl L, Zelle-Rieser C, Gander H, Papesh C, Ramoner R, Bartsch G, Rogatsch H, Barsoum AL, Coggin JH, Thurnher M: **Immunotherapy of metastatic renal cell carcinoma with tumor lysate-pulsed autologous dendritic cells.** *Clin Cancer Res* 2002, **8:**3369–3376.
15. Soleimani A, Berntsen A, Svane IM, Pedersen AE: **Immune responses in patients with metastatic renal cell carcinoma treated with dendritic cells pulsed with tumor lysate.** *Scand J Immunol* 2009, **70:**481–489.
16. Rabinovich GA, Gabrilovich D, Sotomayor EM: **Immunosuppressive strategies that are mediated by tumor cells.** *Annu Rev Immunol* 2007, **25:**267–296.

17. Pardoll DM: **The blockade of immune checkpoints in cancer immunotherapy.** *Nat Rev Cancer* 2012, **12:**252–264.
18. Yang JC, Hughes M, Kammula U, Royal R, Sherry RM, Topalian SL, Suri KB, Levy C, Allen T, Mavroukakis S, Lowy I, White DE, Rosenberg SA: **Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis.** *J Immunother* 2007, **30:**825–830.
19. Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, Xu H, Yao S, Pons A, Chen L, Pardoll DM, Brahmer JR, Topalian SL: **Durable cancer regression Off-treatment and effective reinduction therapy with an anti-PD-1 antibody.** *Clin Cancer Res* 2013, **19:**462–468.
20. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M: **Safety, activity, and immune correlates of anti–PD-1 antibody in cancer.** *N Engl J Med* 2012, **366:**2443–2454.
21. Brahmer JR, Tykodi SS, Chow LQM, Hwu W-J, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM: **Safety and activity of anti–PD-L1 antibody in patients with advanced cancer.** *N Engl J Med* 2012, **366:**2455–2465.
22. Zea AH, Rodriguez PC, Atkins MB, Hernandez C, Signoretti S, Zabaleta J, McDermott D, Quiceno D, Youmans A, O'Neill A, Mier J, Ochoa AC: **Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion.** *Cancer Res* 2005, **65:**3044–3048.
23. Mirza N, Fishman M, Fricke I, Dunn M, Neuger AM, Frost TJ, Lush RM, Antonia S, Gabrilovich DI: **All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients.** *Cancer Res* 2006, **66:**9299–9307.
24. Hiles JJ, Kolesar JM: **Role of sunitinib and sorafenib in the treatment of metastatic renal cell carcinoma.** *Am J Health Syst Pharm* 2008, **65:**123–131.
25. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA: **Sunitinib versus interferon Alfa in metastatic renal-cell carcinoma.** *N Engl J Med* 2007, **356:**115–124.
26. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R, Bukowski RM: **Sorafenib in advanced clear-cell renal-cell carcinoma.** *N Engl J Med* 2007, **356:**125–134.
27. Ko JS, Zea AH, Rini BI, Ireland JL, Elson P, Cohen P, Golshayan A, Rayman PA, Wood L, Garcia J, Dreicer R, Bukowski R, Finke JH: **Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients.** *Clin Cancer Res* 2009, **15:**2148–2157.
28. Ozao-Choy J, Ma G, Kao J, Wang GX, Meseck M, Sung M, Schwartz M, Divino CM, Pan P-Y, Chen S-H: **The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies.** *Cancer Res* 2009, **69:**2514–2522.
29. Finke JH, Rini B, Ireland J, Rayman P, Richmond A, Golshayan A, Wood L, Elson P, Garcia J, Dreicer R, Bukowski R: **Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients.** *Clin Cancer Res* 2008, **14:**6674–6682.
30. Hipp MM, Hilf N, Walter S, Werth D, Brauer KM, Radsak MP, Weinschenk T, Singh-Jasuja H, Brossart P: **Sorafenib, but not sunitinib, affects function of dendritic cells and induction of primary immune responses.** *Blood* 2008, **111:**5610–5620.
31. Xin H, Zhang C, Herrmann A, Du Y, Figlin R, Yu H: **Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells.** *Cancer Res* 2009, **69:**2506–2513.
32. Kujawski M, Kortylewski M, Lee H, Herrmann A, Kay H, Yu H: **Stat3 mediates myeloid cell–dependent tumor angiogenesis in mice.** *J Clin Invest* 2008, **118:**3367–3377.
33. Ko JS, Rayman P, Ireland J, Swaidani S, Li G, Bunting KD, Rini B, Finke JH, Cohen PA: **Direct and differential suppression of myeloid-derived suppressor cell subsets by Sunitinib is compartmentally constrained.** *Cancer Res* 2010, **70:**3526–3536.
34. Waugh DJJ, Wilson C: **The interleukin-8 pathway in cancer.** *Clin Cancer Res* 2008, **14:**6735–6741.
35. Huang D, Ding Y, Zhou M, Rini BI, Petillo D, Qian C-N, Kahnoski R, Futreal PA, Furge KA, Teh BT: **Interleukin-8 mediates resistance to antiangiogenic agent Sunitinib in renal cell carcinoma.** *Cancer Res* 2010, **70:**1063–1071.
36. Eikawa S, Ohue Y, Kitaoka K, Aji T, Uenaka A, Oka M, Nakayama E: **Enrichment of Foxp3+ CD4 regulatory T cells in migrated T cells to IL-6– and IL-8–expressing tumors through predominant induction of CXCR1 by IL-6.** *J Immunol* 2010, **185:**6734–6740.
37. Chen DS, Mellman I: **Oncology meets immunology: the cancer-immunity cycle.** *Immunity* 2013, **39:**1–10.
38. Vanneman M, Dranoff G: **Combining immunotherapy and targeted therapies in cancer treatment.** *Nat Rev Cancer* 2012, **12:**237–251.
39. Takahara M, Miyai M, Tomiyama M, Mutou M, Nicol AJ, Nieda M: **Copulsing tumor antigen-pulsed dendritic cells with zoledronate efficiently enhance the expansion of tumor antigen-specific CD8+ T cells via Vγ9γδ T cell activation.** *J Leukoc Biol* 2008, **83:**742–754.
40. Weiss JM, Allen C, Shivakumar R, Feller S, Li L-H, Liu LN: **Efficient responses in a murine renal tumor model by electroloading dendritic cells with whole-tumor lysate.** *J Immunother* 2005, **28:**542–550.
41. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J: **New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).** *Eur J Cancer* 2009, **45:**228–247.
42. Desombere I, Meuleman P, Rigole H, Willems A, Irsch J, Leroux-Roels G: **The interferon gamma secretion assay: a reliable tool to study interferon gamma production at the single cell level.** *J Immunol Methods* 2004, **286:**167–185.

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