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Combination Therapy with Tumor Cell-pulsed Dendritic Cells and Activated Lymphocytes for Patients with Disseminated Carcinomas

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Abstract. *This phase I study was performed to assess the safety and immune response of tumor cell-pulsed dendritic cell (DC) vaccine therapy against cancer patients with multiple metastases. DCs, generated from adherent cells of peripheral blood mononuclear cells (PBMCs) using interleukin-4 (IL-4) and granulocyte/monocyte colony-stimulating factor, were loaded with autologous necrotic whole tumor cells. Thereafter, the DCs were matured with culture supernatants of OK-432-stimulated PBMCs. Activated lymphocytes were also induced from non-adherent cells of PBMCs using OKT-3 and IL-2. Patients received a subcutaneous injection of DCs loaded with tumor cells every 2 weeks and received an intravenous injection of activated lymphocytes every 4 weeks. This combination therapy was named tumor-pulsed DC vaccine therapy. Tumor-pulsed DC vaccine therapy was continued as long as possible in 19 patients. No particular adverse reactions, except for low-grade fever, were found. The patients could be divided into two groups according to the survival time, i.e., 6 responders (long survival patients) and 13 non-responders (short survival patients). Based on the laboratory data of responders, eligibility criteria were determined. Using the eligibility criteria, a phase I/II study was recently performed with 15 patients. A delayed-type hypersensitivity reaction against tumor-pulsed DCs became positive in 13 of the 15 patients within 6 months after the therapy. This therapy was again safe, and no evidence of autoimmune disease was noted. The survival time of these 15 patients was significantly prolonged compared with that of the 13 non-responders of the phase I study ($p<0.0001$). This continuous tumor-pulsed DC vaccine therapy was well tolerated in patients with disseminated carcinomas.*

Although it has been shown that tumors possess antigenicity, which can induce specific immunity against tumors, immunotherapy targeting these antigenic molecules is successful only in limited cases (1). Recent advances in immunology have highlighted the importance of understanding the complex interactions between innate immunity and acquired immunity for the establishment of successful cancer immunotherapy. Among immune cells, the main players are dendritic cells (DCs) (2-4). Consequently, DC-based vaccine therapies with DCs loaded with various tumor-associated antigens (TAAs), such as tumor lysate (5), tumor-derived peptides (6), synthetic peptides (7), or tumor-derived RNA (8), are now under way. Synthetic peptides are very useful as antigen sources against known TAAs of target tumors. However, it is believed that the antigenicity of tumors is heterogeneous, and that some tumor cells do not contain the target TAAs (9, 10). Therefore, we used DCs loaded with whole tumor cells that contain both known and unknown TAAs (11). DC-based vaccine therapy is usually of 6-month duration (12, 13), however, in this clinical trial, the DC vaccine therapy was continued for as long as possible.

Materials and Methods

Patients. This phase I study included 19 inoperable cancer patients with multiple metastases, according to a protocol approved by the

Abbrevations: PBMCs, peripheral blood mononuclear cells; DCs, dendritic cells; TAAs, tumor-associated antigens; DHT, delayed-type hypersensitivity; ELISPOT, enzyme-linked immunospot; PR, partial response; SD, stable disease; PD, progressive disease.

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Table I. *Patient characteristics and adverse events (phase I study).*

Patient no.	Age (yrs)/ Gender	Site of primary tumor	Site of metastases	Previous therapy	Adverse reaction
1	38/Male	Rectum	Lung, bone	Chemotherapy	Low-grade fever
2	49/Male	Bile-duct	Lung, Pl, LN	Chemotherapy	None
3	65/Female	Pancreas	Pt, Liver	Chemotherapy	None
4	46/Female	Stomach	Pt	Chemotherapy	Low-grade fever
5	72/Female	Pancreas	Liver, Pt, skin	Chemotherapy	None
6	72/Male	Large intestine	Liver, LN	None	Low-grade fever
7	56/Female	Gall bladder	Liver, Pt	Chemotherapy	Low-grade fever
8	49/Male	Gall bladder	Liver	None	None
9	73/Male	Gall bladder	Liver, Pt	Chemotherapy	Low-grade fever
10	41/Male	Stomach	Pt, LN	None	None
11	49/Female	Rectum	Pt, bone	Chemotherapy	Eosinophilia
12	49/Male	Stomach	Pt	None	Low-grade fever
13	45/Female	Rectum	Pt, bone	None	None
14	54/Female	Pancreas	LN	None	Low-grade fever
15	73/Male	Lung	Lung, skin, LN	Chemotherapy	None
16	66/Male	Stomach	Pt	None	Low-grade fever
17	68/Male	Lung	Lung, LN	Chemotherapy	None
18	54/Female	Ovary	Pl, Pt	Chemotherapy	None
19	65/Male	Rectum	Lung, Pt	None	None

Pl: pleural membrane, LN: lymph node, Pt: peritoneum.

Kyushu University Ethics Committee, Japan. Inclusion criteria were: histologically confirmed cancer, not amenable to cure by any standard therapy; performance status of 0, 1 or 2 on the ECOG scale; a minimum estimated life expectancy of 3 months; adequate hematological, hepatic and renal function; age>18 years; presence of obtainable tumor cells. The clinical details of the patients are summarized in Table I. Based on data of the phase I trial, a phase I/II trial was recently performed with 15 patients who satisfied at least 2 of the 4 following eligibility criteria: absolute lymphocyte count more than 1,000/μl, serum total protein level more than 6 g/dl, hemoglobin more than 10 g/dl, and a positive PPD skin test. The clinical details of the patients are summarized in Table II.

Study design. Each patient received a subcutaneous injection of 2-30x10^6 mature DCs loaded with necrotic tumor cells into the left supraclavicular area, every 2 or 3 weeks. Intravenous injection of 1-5x10^8 OKT3/IL-2-activated lymphocytes was combined with the above DC vaccine every 4 weeks. This combination therapy has been named tumor-pulsed DC vaccine therapy. In principle, this tumor-pulsed DC vaccine therapy was continued for as long as possible in the outpatient clinic.

Preparation of DCs loaded with necrotic tumor cells. Peripheral blood mononuclear cells (PBMCs) were collected by leukapheresis with a COBE spectrum apheresis system (GAMBRO BCT, Inc, CL, USA). PBMCs were suspended at a cell density of 4x10^6 cells/ml in GMP-grade RPMI 1640 (Hy-Media; Nipro, Tokyo, Japan) supplemented with 1% human albumin, and 500 μl of the cell suspension were cultured for 4 h in 24-well culture plate. After non-adherent cells had been removed, the adherent cells were further cultured in Hy-Media containing 1% human albumin, 100 ng/ml of recombinant human granulocyte/monocyte colony-stimulating factor (GM-CSF; North China Pharmaceutical group Corporation-Gene Tech, China) and 50 ng/ml of recombinant human interleukin-4 (IL-4; Osteogenetics, Wuerburg, Germany) for 7 days. After 7 days, the cells were harvested as immature DCs.

Table II. *Patient characteristics and clinical outcome (phase I/II study).*

Patient no.	Age (yrs)/ Gender	Site of primary tumor	Site of metastases	Prognosis (Months)
1	40/Male	Rectum	Lung, bone	22, alive
2	49/Female	Rectum	Lung, Pt	14, dead
3	65/Male	Large intestine	Lung, Pt	16, alive
4	43/Male	Stomach	Bone, LN	11, dead
5	65/Female	Pancreas	Liver, Pt	10, dead
6	50/Female	Unknown	Liver, Pt	6, dead
7	55/Female	Pancreas	Pt, LN	15, alive
8	75/Male	Lung	Lung, Pl	7, alive
9	65/Male	Large intestine	Adrenal, LN	6, alive
10	45/Female	Stomach	Lung, Pt	5, dead
11	50/Male	Thymus	Lung, Pt	10, dead
12	55/Female	Stomach	Pt	6, alive
13	54/Female	Breast	Lung, Pt	6, alive
14	53/Female	Stomach	Pt, LN	6, alive
15	49/Female	Breast	Lung, Pt	4, alive

Pl: pleural membrane, LN: lymph node, Pt: peritoneum.

Tumor specimens were obtained from the tumor mass or malignant effusions by surgical biopsy or parasenthesis, respectively. The tumor specimens were minced mechanically without chemical digestion. Thereafter, they were resuspended in 2

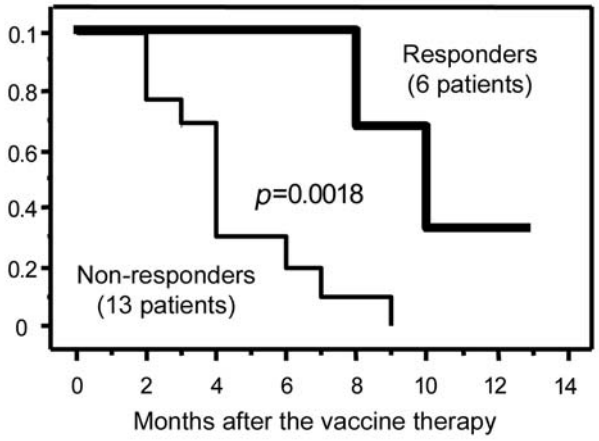

Figure 1. *Survival curves of the 19 patients enrolled in the phase I trial. According to the eligibility criteria described in Materials and Methods, 19 patients were divided into 6 suited patients (responders) and 13 unsuited patients (non-responders).*

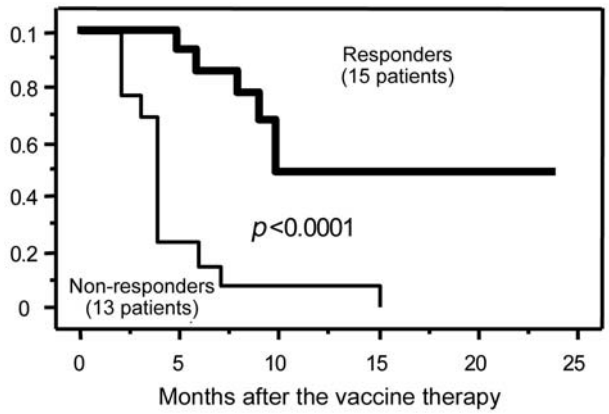

Figure 2. *Survival curves of the 15 patients who suited the eligibility criteria (phase I/II trial). The non-responders are the same as the non-responders of the phase I trial.*

ml of RPMI 1640 and lysed by 5 freeze and thaw cycles. The lysed cells (necrotic tumor cells) were used as a TAAs source.

Immature DCs were incubated with necrotic tumor cells overnight (DCs:tumor=5-10:1) and further cultured for 2 days in the medium containing 40% OK-432-induced PBMC culture supernatants to induce mature DCs (tumor cell-pulsed DCs). OK-432-induced PBMC culture supernatants were prepared by 1-day coculture of PBMCs (10^6/ml) of healthy volunteers and OK-432 (0.05 KE/ml) (14).

Preparation of activated lymphocytes. Non-adherent cells of patient's PBMCs were cultured for 2 weeks with Hy-medium containing 175 JRU/ml human recombinant IL-2 (Nipro) and immobilized monoclonal antibody to CD3 (10 μg/ml, OKT-3; Jansen-Kyowa, Tokyo, Japan).

Fluorescence-activating cell sorter (FACS) analysis. DCs ($1x10^5$) were suspended in 100 μl of diluted fluorescein-isothiocyanate or phycoerythrin-conjugated monoclonal antibodies (CD40, CD80, CD83, CD86, HLA-A, B and C, HLA-DR; Becton Dickinson, CA, USA) and assayed as described previously using a flow cytometer (FACS Caliber; Becton Dickinson) (14). The data were analyzed with CellQuest v3.2.1f1 (Becton Dickinson).

Measurement of serum tumor markers. Serum levels of tumor markers, including carcinoembryonic antigen (CEA), carbohydrate-antigen 19-9 (CA19-9), alpha fetoprotein (AFP) and DUPAN-2, were determined by enzymed-linked immunosorbent assay (ELISA).

Delayed-type hypersensitivity (DTH) reaction. One million tumor-pulsed DCs were injected intradermally into the forearm every 4 weeks. A positive DTH skin-test reaction was defined as >5 mm diameter erythema with induration 48 h after the DCs injection.

Enzyme-linked immunospot (ELISPOT) assay. Interferon-γ (IFN-γ)-producing PBMCs were assessed using an ELISPOT assay kit according to the manufacturer's protocol (Diaclone Research, Besancon, France) (15). Briefly, PBMCs ($5x10^4$ cells), together with tumor cell-pulsed or non-pulsed DCs (10^4 cells), were plated on nitrocellulose 96-well plates (Millipore, Bedford, MA, USA) coated with anti-IFN-γ antibody and incubated for 15 h at 37˚C. After removal of the cells, bound IFN-γ could be detected *via* a secondary biotinylated antibody. Streptavidine alkaline phosphatase binds to biotin and is detected *via* the BCIP/NBT substrate. The spots were counted with a stereomicroscope.

Clinical outcome. A partial response (PR) was defined as a decrease in all measurable tumor tissue of over 50% for at least 4 weeks without any new sign of disease. Stable disease (SD) was defined as a decrease in measurable tumor tissue of less than 50% and an increase of less than 25%. In this study, SD continuing for more than 6 months was named long SD.

Statistical analysis. The data were analyzed with a SAS statistical software package. Categorical variables were compared using Fisher's exact test. *P*-values less than 0.05 were considered statistically significant. The estimated probability of survival was demonstrated using the Kaplan-Meier method. The Mantel Cox log-rank test was used to compare curves between responders and non-responders.

Results

Phase I study. Nineteen patients, including 5 large intestinal cancer, 4 gastric cancer, 4 biliary tract cancer, 3 pancreatic cancer, 2 lung cancer and 1 ovarian cancer, were entered into this phase I trial. All of these patients were evaluated as progressive disease (PD) at the time of entry to the study (Table I). The patients received the tumor-pulsed DC vaccine therapy for as long as possible. No patient had to leave the study for safety reasons during this trial period of 1 year, because no severe adverse

Table III. *Change of serum tumor marker level by the vaccine therapy (phase I/II study).*

Evaluation[1]	Number of patients
Continuous decrease	0 (0%)
Transient decrease[2]	11 (73%)
No change	1 (7%)
Continuous increase	2 (13%)
Inability	1 (7%)

[1]A representative tumor marker was measured by ELISA every month after the vaccine therapy.
[2]Decrease lasted more than one month

Table IV. *Clinical outcome (phase I/II study).*

Response	Number of patients
CR	0 (0%)
PR	0 (0%)
SD	6 (40%)
Long SD[1]	8 (53%)
PD	1 (7%)

[1]SD continuing for more than 6 months.

Table V. *Immune response (phase I/II study).*

	Number of patients	
	DTH reaction (+)	DTH reaction (-)
ELISPOT assay (+)	9	0
ELISPOT assay (-)	0	4

The DTH reaction and ELISPOT assay were assessed at 3 months after the therapy.

reactions occurred. Low-grade fever, recovering within 24 h after the DC vaccine, was found in 8 patients. A transient increase of eosinophils was found on the first day after the third DC vaccine therapy in 1 patient, but recovered to the normal level within 3 days without treatment.

A Kaplan-Meier survival analysis indicated the presence of long survival patients who lived for more than 6 months. Based on the laboratory data common to these long survivors, the eligibility criteria for this tumor-pulsed DC vaccine therapy was determined as described in Materials and Methods. According to this eligibility criteria, 19 patients were divided into 6 suited patients (responders) and 13 unsuited patients (non-responders). The 6 responders showed a longer overall survival compared with the 13 non-responders ($p=0.0018$, Figure 1).

Phase I/II study. Using the above eligibility criteria, a phase I/II trial was again performed with cancer patients with multiple metastases. Fifteen patients, including 4 large intestinal cancer, 4 gastric cancer, 2 pancreatic cancer, 2 breast cancer, 1 lung cancer, 1 thymic cancer and 1 cancer of unknown origin were entered into this trial. Eleven patients had received prior second-line chemotherapy and 11 patients had received prior surgery. In addition, 3 patients had received prior radiotherapy. All of these patients were evaluated as PD at the time of entering the study (Table II). No particular adverse reactions, including autoimmune reactions, were found during this observation period (4-22 months). These 15 patients again showed a longer overall survival compared with the 13 non-responders who were treated in the above phase I study ($p<0.0001$, Figure 2). The 50% survival time of the 13 non-responders and the 15 patients was 3.0 months and 10.0 months, respectively. The serum levels of tumor markers were measurable in all the 15 patients and estimable in 14 of the 15 patients (Table III). In 11 patients (73%) there was a continued decrease for at least 1 month, while 2 patients (13%) and 1 patient (7%) showed a continuous increase and no significant change, respectively. Although neither CR nor PR was found, it is noteworthy that 14 patients showed SD and that 8 of the 14 SD patients maintained this SD for more than 6 months, *i.e.*, long SD (Table IV).

The patients' immune responses against tumor-pulsed DCs were evaluated by both the DTH skin reaction and IFN-γ ELISPOT assay before and after the DC vaccination. Both the DTH reaction and ELISPOT assay were assessed at 3 months after the tumor-pulsed DC vaccine therapy in 13 of the 15 patients (Table V). Nine of the 13 patients became positive for both the DTH reaction and ELISPOT assay, while the remaining 4 patients were negative for both. In 3 of the 4 negative patients, however, the DTH reaction became positive within 6 months after the therapy.

Most DCs induced from each patient were shown to develop high levels of MHC class II and costimulatory molecules CD80 and CD86, and showed the absence of CD14 (data not shown). However, the expression levels of these molecules were significantly low compared to the DCs induced from healthy volunteers' PBMCs, as previously reported (16).

Discussion

The initial purpose of this clinical trial was to evaluate the feasibility and toxicity of tumor-pulsed DC vaccine therapy against far-advanced cancer patients. Low-grade fever or eosinophilia were observed in only limited cases throughout the phase I trial and the phase I/II trial (Tables I and II). These adverse reactions did not require any particular treatment. As a result, the vaccine therapy did not need to be cancelled for adverse reactions. In the phase I/II trial, the maximum duration of treatment was in a patient who received 36 DC vaccinations in 22 months during the observation period. After the thirtieth vaccine therapy, edematous erythema without itching appeared at the injection site immediately after the intradermal injection of tumor-pulsed DCs for the DTH skin test. Erythema was accompanied with an increased serum IgE and was macroscopically similar to a type I allergic reaction. The erythema and IgE elevation recovered to normal within one hour and on the next day, respectively. Rheumatoid factor, anti-nuclear antibody and anti-thyroglobulin antibody in the sera were all negative throughout this trial period.

The second purpose of the study was to assess if tumor-pulsed DC vaccine therapy can induce some immune reactions against autologous tumor cells in patients. In order to evaluate the induction ability of tumor antigen-specific cytotoxic lymphocytes (CTLs) of this vaccine therapy, both the DHT skin test and IFN-γ ELISPOT assay were used as surrogate markers (17). In this study, the positive rate of the DTH reaction was significantly higher in responders than non-responders (data not shown). In this phase I/II study, both a positive DTH reaction and increased ELISPOT reaction were markedly induced by the vaccine therapy. Interestingly, the DTH reaction completely harmonized with the ELISPOT reaction (Table V). The high induction rate of positive DTH reaction and increased ELISPOT reaction indicates a potent CTL induction ability of this vaccine therapy.

The third purpose of the study was to find out the advantage of autologous tumor cells as an antigen source. As described above, necrotic tumor cells were used as the antigen source for induction of multiple CTLs against both known TAAs and unknown TAAs (11). For this purpose, the patient's HLA-A phenotype-binding synthetic peptides were first prepared as described in Materials and Methods. Next, PBMCs obtained from a patient in whom the ELISPOT reaction became positive were cultured together with known TAA peptide-pulsed DCs. If T cells which react to the TAA peptide exist in the PBMCs, they produce IFN-γ. For example, in this study, PBMCs from a patient treated by CEA, MAGE-1 and HER-2/neu-expressing tumor cells produced IFN-γ by co-culture not only with tumor-pulsed DCs, but also these peptides-pulsed DCs (data not shown). This data indicated that the tumor-pulsed DC vaccine therapy can elicit specific T cell responses against multiple TAAs.

Finally, we examined whether the tumor-pulsed DC vaccine therapy can prolong the survival time. Both the phase I and I/II trials showed the possibility that this therapy can prolong the survival time of far-advanced cancer patients (Figures 1 and 2). Before the efficacy for prognosis is evaluated, however, there are many problems that should be solved. For example, in the current clinical trials, the patient numbers were low and many types of carcinoma were targeted. Therefore, a phase II study is now under way to assess if this vaccine therapy can prolong the survival time.

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References

1 Morisaki T, Matsumoto K, Onishi H, Kuroli H, Baba E, Tasaki A, Kubo M, Nakamura M, Inaba S, Yamaguchi K, Tanaka M and Katano M: Dendritic cell-based combined immunotherapy with autologous tumor-pulsed dendritic cell vaccine and activated T cells for cancer patients: rationale, current progress, and perspectives. Hum Cell *16*: 175-182, 2003

2 Banchereau J, Paczesny S, Blanco P, Bennett L, Pascual V, Fay J and Palucka AK: Dendritic cells: controllers of the immune system and a new promise for immunotherapy. Ann N Y Acad Sci *987*: 180-187, 2003.

3 Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B and Palucka K: Immunobiology of dendritic cells. Annu Rev Immunol *18*: 767-811, 2000.

4 Lopez JA and Hart DN: Current issues in dendritic cell cancer immunotherapy. Curr Opin Mol Ther *4*: 54-63, 2002.

5 Asavaroengchai W, Korera Y and Mule JJ: Tumor lysate-pulsed dendritic cells can elicit an effective antitumor immune response during early lymphoid recovery. Proc Natl Acad Sci USA *99*: 931-936, 2002.

6 Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, Yong WH, Incardona F, Thompson RC, Riedinger MS, Zhang W, Prins RM and Black KL: Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. Cancer Res *61*: 842-847, 2001.

7 Celluzzi CM, Mayordomo JI, Storkus WJ, Lotze MT and Falo LD Jr: Peptide-pulsed dendritic cells induce antigen specific CTL-mediated protective tumor immunity. J Exp Med *183*: 283-287, 1996.

8 Boczkowski D, Nair SK, Snyder D and Gilboa E: Dendritic cells pulsed with RNA are potent antigen-presenting cells *in vitro* and *in vivo*. J Exp Med *184*: 465-472, 1996.

9 Velders MP, Nieland JD, Rudolf MP, Loviscek K, Weijzen S, de Visser KE, Macedo MF, Carbone M and Kast WM: Identification of peptides for immunotherapy of cancer. It is still worth the effort. Crit Rev Immunol *18*: 7-27, 1998.

10 Boland CR and Ricciardiello L: How many mutations does it take to make a tumor? Proc Natl Acad Sci USA *96*: 14675-14677, 1999.

11 Berard F, Blanco P, Davoust J, Neidhart-Berard EM, Nouri-Shirazi M, Taquet N, Rimoldi D, Cerottini JC, Banchereau J and Palucka AK: Cross-priming of naïve CD8 T cells against melanoma antigens using dendritic cells loaded with killed allogeneic melanoma cells. J Exp Med *192*: 1535-1544, 2000.

12 Geiger JD, Hutchinson RJ, Hohenkirk LF, McKenna EA, Chang A and Mule J: Treatment of solid tumours in children with tumor-lysate-pulsed dendritic cells. Lancet *356*: 1163-1165, 2000.

13 Chang AE, Redman BG, Whitfield JR, Nickoloff BJ, Braun TM, Lee PP, Geiger JD and Mule JJ: A phase I trial of tumor lysate-pulsed dendritic cells in the treatment of advanced cancer. Clin Cancer Res *8*: 1021-1032, 2002.

14 Kuroki H, Morisaki T, Matsumoto K, Onishi H, Baba E, Tanaka M and Katano M: Streptococcal preparation OK-432: a new maturation factor of monocyte-derived dendritic cells for clinical use. Cancer Immunol Immunother *52*: 561-568, 2003.

15 Versteegen J, Logtenberg T and Ballieux R: IFN-gamma-producing human lymphocytes by spot-ELISA. A method to detect lymphokine-producing lymphocytes at the single-cell level. J Immunol Methods *111*: 25-29, 1988.

16 Onishi H, Morisaki T, Baba E, Kuga H, Kuroki H, Matsumoto K, Tanaka M and Katano M: Dysfunctional and short-lived subsets in monocyte-derived dendritic cells from patients with advanced cancer. Clin Immunol *105*: 286-295, 2002.

17 Schuler-Thurner B, Schultz ES, Berger TG, Weinlich G, Ebner S, Woerl P, Bender A, Feuerstein B, Fritsch PO, Romani N and Schuler G: Rapid induction of tumor specific Type 1 helper cells in metastatic melanoma patients by vaccination with mature cryopreserved, peptide-loaded monocyte-derived dendritic cells. J Exp Med *195*: 1279-1288, 2002.

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