
Targeted Cancer Therapy by Dendritic Cell Vaccine

Hiroyuki Abe, Touko Shimamoto,
Shinichiro Akiyama and Minako Abe

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55450>

1. Introduction

The unpredictability of efficacy and toxicity of treatment are limitations of current standard cancer treatments. The clinical results obtained by standard therapies suggest a need for a paradigm change in cancer treatment. In recent years, immune cell therapy has been in the spotlight with the expectation of opening the door to a new area of cancer therapy. Targeted cancer therapy, which selectively takes action against targets expressed in the tumor surface, seems to be promising.

The immune system can control various types of tumors. Antigen-non-specific innate immunity and antigen-specific adaptive immunity can reject tumors.

The identification of tumor antigens recognized by T cells has facilitated the development of immune cell therapy in clinical oncology. The dendritic cell based cancer vaccine aims to induce tumor specific effector T cells (cytotoxic T lymphocyte, CTL) that can reduce tumor mass as well as tumor specific memory T cells that can control tumor relapse.

In this text, immune cell target therapy in clinical oncology will be discussed and hopefully this will be helpful in daily clinical practice.

2. Role of natural killer cells

Natural killer (NK) cells are present in the peripheral blood and number approximately 10-15% in the lymphocyte fraction. NK cells are the most important innate immune cell because of their ability to directly kill target cells as well as produce immunoregulatory cytokines. NK cells are defined by the surface expression of CD56, a neural cell adhesion molecule and lacks

the T cell antigen CD3 [1]. The function of NK cells are direct cytotoxic activity against virus-infected cells and tumor cells [2]. There are two distinct subsets of human NK cells based on the density of surface CD56 expression [3]. Approximately 90% of human NK cells are CD56^{dim} and have high density expression of CD16, others are CD56^{bright} and CD16^{dim/neg}. The CD56^{bright} and CD56^{dim} NK cell subsets show an important difference in cytotoxic potential, capacity for cytokine production and response to cytokine activation (Table 1) [4].

	CD56 ^{bright}	CD56 ^{dim}
NK receptors		
FcγRIII (CD16)	-/+	+++
KIR	-/+	+++
CD94/NKG2	+	-/+
Cytokine receptors		
IL-2Rαβγ	++	-
IL-2Rβγ	++	++
CCR7	++	-
Adhesion molecules	++	-/+
Effector functions		
ADCC	-/+	+++
Natural cytotoxicity	-/+	+++
Cytokine production	+++	-/+

*KIR indicated killer immunoglobulin-like receptor; IL, interleukin; ADCC, antibody-dependent cellular cytotoxicity.

Table 1. Functional Differences in Natural Killer (NK) cell Subsets* [2]

NK cells can mediate antibody-dependent cellular cytotoxicity (ADCC) through membrane FcγRIII (CD16) expressed on the majority of NK cells. CD56^{dim} NK cells are more cytotoxic against NK-sensitive targets than CD56^{bright} NK cells and respond to IL-2 with increased cytotoxicity. It is of clinical importance to know that CD56^{bright} cells, after activation with IL-2, can exhibit similar or enhanced cytotoxicity against NK targets compared with CD56^{dim} cells [5-7]. In addition, more than 95% of all CD56^{dim} NK cells express CD16 (FcγRIII) and are capable of ADCC. On the other hand, 50% to 70% of CD56^{bright} NK cells lack expression of CD16 or have only low-density expression of CD16 and therefore function minimally in ADCC.

It is well known that major histocompatibility complex (MHC) class I molecules are critical for the inhibition of NK cell-mediated lysis of normal autologous cells [8, 9]. NK cells selectively lyse autologous cell that have lost MHC class I self-expression [10].

In humans, two families of paired inhibitory and activating NK receptors have been identified, killer immunoglobulin (Ig)-like receptor (KIR) family and the heterodimeric CD94/NKG2 C-

type lectin family. In this text, these receptors are not discussed in order to simplify the clinical application of NK cell therapy. Although the activating KIR and CD95/NKG2 receptors are important in mediating NK cytotoxicity, Natural Cytotoxicity Receptors (NCR) and the homodimeric NKG2D receptors may be important in mediating cytotoxicity against abnormal, MHC class I-deficient, or class I-negative targets. This biological information will result in the clinical application of NK cell-based therapies for cancer. Table 2 shows the incidence of MHC class I deficient or negative cancer cells.

Tumor cell	Incidence (%)
Uterine cervical cancer	90
Osteosarcoma	
Primary	52
Metastatic	88
Breast cancer	81
Pancreatic cancer	76
Prostate cancer	74
Colon cancer	
Primary	32
Metastatic	72
Sarcoma	62
Melanoma	
Primary	16
Metastatic	58
Head and neck cancer	49
Hepatoma	42
Renal cell carcinoma	38
Lung cancer	38
Ovarian cancer	37
Pharyngeal cancer	56
Urinary bladder cancer	25

Table 2. Incidence of MHC class I deficient or negative cancer cells [11]

It is presumed that the difference of MHC class I expression may be the difference in the clinical efficacy of NK cell based immune therapy. Because of the variety of cancer cells, it is not enough to kill all cancer cells by NK cell-based therapy. It is necessary to target a cancer specific antigen by cytotoxic T cells, which are activated by a Dendritic Cell (DC)-based cancer vaccine (Figure 1).

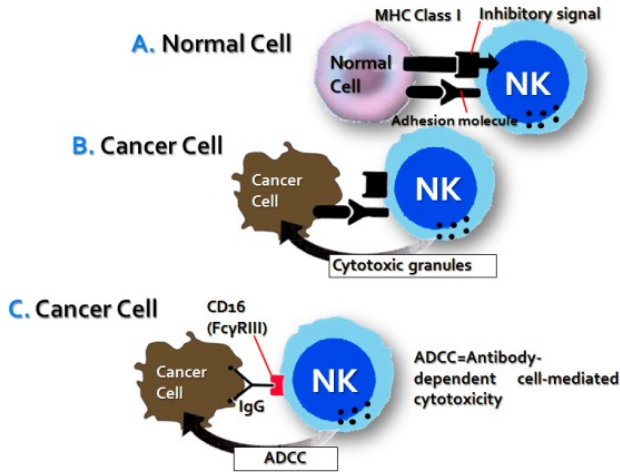


Figure 1. Cytotoxicity of NK cells [11]

3. Strategies using dendritic cell vaccine

Dendritic cells (DCs) are key regulators of both T- and B-cell immunity, because of their superior ability to take up, process and present antigens compared with other antigen presenting cells (APCs) [12]. DCs can also activate NK cells and natural killer T (NKT) cells [13]. Because of these functions, DCs can conduct all of the elements of the immune orchestra and they are therefore a fundamental target and tool for vaccines [14]. Cancer related antigens are a key factor implicated in the design of DC vaccine strategies. If a patient's own cancer cells are available for lysate, this will be used for the production of an individual DC-based vaccine, which is utilized for the optimally matched tumor surface antigen. In most instances, however, if a patient's own cancer cells are not available, then artificial cancer antigens are utilized for the production of a DC-based cancer vaccine.

A pilot project by the National Cancer Institute reported on the prioritization of cancer antigens to develop a well-vetted, priority-ranked list of cancer vaccine target antigens based on predefined and preweighted objective criteria [15]. Antigen prioritization involves developing a list of ideal cancer antigen criteria (Figure 2).

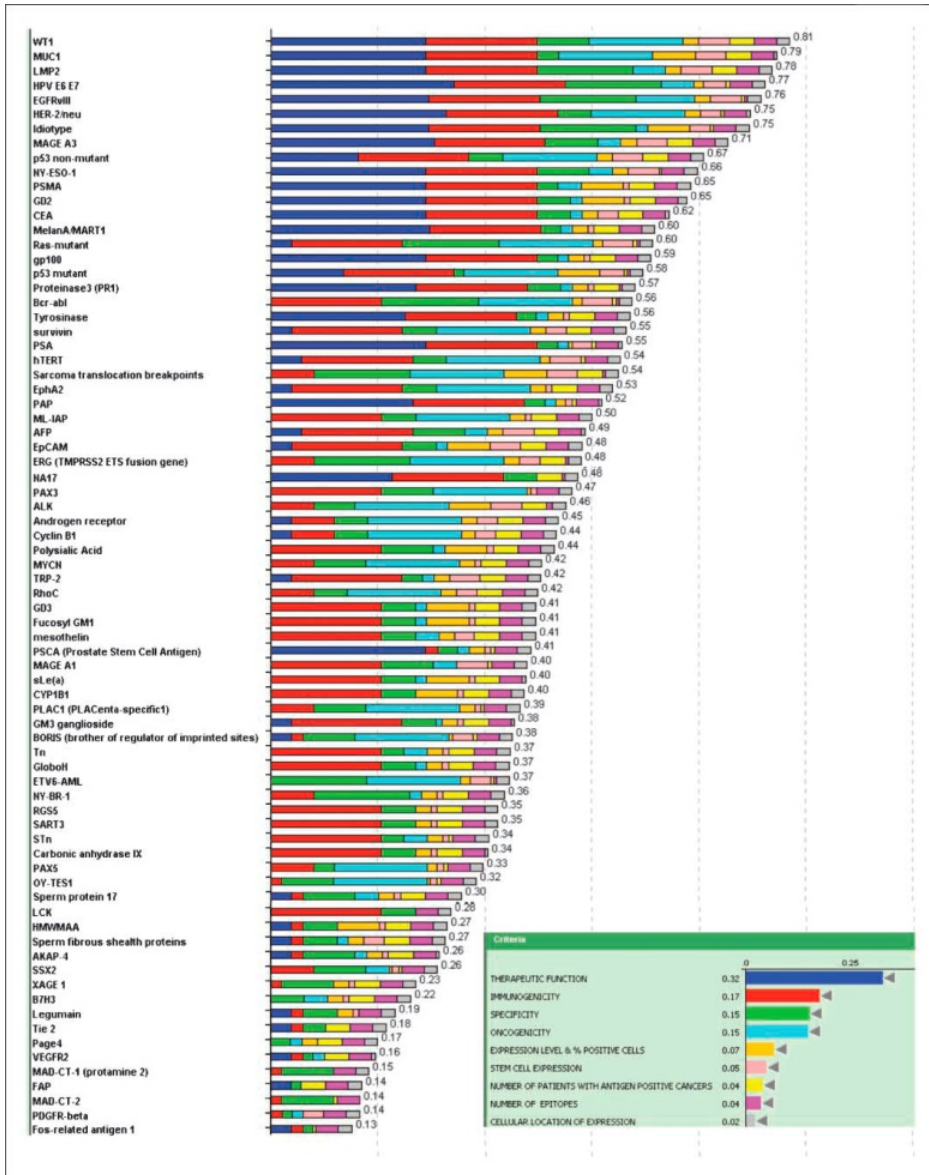


Figure 2. Cancer antigen pilot prioritization: representation of ranking based on predefined and preweighted criteria and subcriteria. Inset, the color used to designate each criterion and its relative weight. Number at the end of each bar, relative rank of that antigen. [15]

Among these, frequently used artificial tumor antigens are listed below. Some of these are restricted by HLA-A1 or HLA-A24, so that an HLA study is needed to select and match the

tumor antigens to the DCs (Table 3). Certain artificial tumor antigens cannot apply depending on HLA types on DCs and tumor cells.

HLA-A2 or HLA-A24 restricted	Non-restricted
WT1	MUC1
CEA	CA125
MAGE-1, MAGE-3	PSA
HER 2	NY-ESO-2
gp100	
NY-ESO-1	
PSMA	
SART-1, SART-3	
MART-1	
Melan-A	
HPV16-E7	

Table 3. Typical artificial tumor antigens.

WT1 peptide is a part of long chain of WT1 protein (Figure 3).

10	20	30	40	50
MGSDVRDLNA	LLPAVPSLGG	GGGCALPVSG	AAQWAPVLDL	APPGASAYGS
60	70	80	90	100
LGGPAPPAP	PPPPPPPHS	FIKQEPSWGG	AEPHEEQCLS	AFTVHFSGQF
110	120	130	140	150
TGTAGACRYG	PFGPPPSQA	SSGQARMFPN	APYIIPSCLES	QPAIRNQGYS
160	170	180	190	200
TVTFDGTPSY	GHTPSHHAAQ	FPNHSFKHED	PMGQQGSLGE	QQYSVPPPVY
210	220	230	240	250
GCHTPTDSC	GSQALLRTP	YSSDNLYQMT	SQLECYTWNQ	MNIGATLKG
260	270	280	290	
AAGSSSVK	TEGQSNHSTG	YESDNHTTPI	LCGAQYRIHT	HGVFRGIQDV
310	320	330	340	350
RRVPGVAPTL	VRSASETSEK	RPFMCAYPGC	NKRYFKLSHL	QMHSRKHTGE
360	370	380	390	400
KPYQCDFKDC	ERRFSRSDQL	KRHQRRHTGV	KPFQCKTCQR	KFSRSDHLKT
410	420	430	440	449
HTRHTGKTS	EKPFSCRWPS	CQKKFARSDE	LVRHHNMHQ	NMTKLQLAL

Figure 3. Amino acid sequence of WT1 protein (Total length 449, mass (Da) 49,188)

Wild sequence: RMFPNAPYL (126-135), HLA-A2 restricted

Modified sequence: CYTWNOMNL (235-243), HLA-A24 restricted

The sequence RMFPNAPYL is called wild type and is HLA-A2 restricted. Another sequence CYTWNQMNL is a modified type and is HLA-A24 restricted. WT1 is expressed on the cell surface of carcinomas such as esophageal, gastric, colorectal, pancreas, biliary tract, liver, breast, uterus, brain, lung (non-small cell), malignant melanoma, sarcoma, acute myeloid and lymphoid leukemia, salivary gland and prostate, etc.

Another important tumor antigen to be targeted is MUC1 which is the cell membrane associated protein. The sequence of this peptide is TRPAPGSTAPPAHGVTSAPDTRPAGSTAP and is HLA-non restricted (Figure 4). MUC1 is presented over the surface of cancer cells of the esophagus, stomach, colorectal, pancreas, biliary tract, breast, uterus, ovary, salivary gland, lung (adenocarcinoma), prostate and so on.

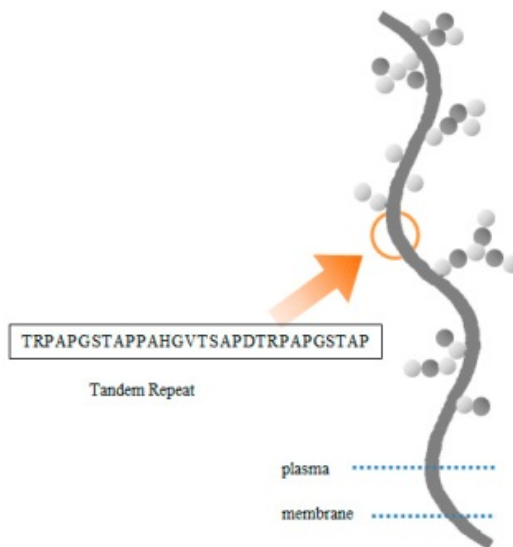


Figure 4. Sequence of MUC1 peptide.

Recently, new therapy strategies that focus on tumor associated antigens (TAAs) have been suggested as additional options to currently available treatments, due to their fewer adverse events and better tolerability. The establishment and maintenance of immune cell therapy for cancer relies on special TAAs, such as WT1 and MUC1 which have become primary targets for cancer vaccines [16, 17].

The high risk of metastatic recurrence suggests that cancer cell dissemination may occur early in most carcinomas, and therefore it seems that active immunotherapy may have a place among treatment modalities [18]. Among TAAs, above mentioned WT1 and MUC1 have received

particular attention as potential targets for vaccine-based immunotherapy, because with the exception of very few tissues such as the splenic capsule and stroma, they are not expressed in normal human tissues and become activated in a number of cancers [19-21]. Therefore vaccination is an effective medical procedure in clinical oncology, based on the induction of a long-lasting immunologic memory and characterized by mechanisms endowed with high destructive potential and specificity. These functions will elicit a persistent immune memory that can eliminate residual cancer cells and protect against relapses.

On this basis, vaccination strategies employing DCs have been regarded as a promising therapeutic approach, even for advanced cancer. DCs internalize the cancer antigen, process their protein and then displays them, as short peptides on their extra cellular surface in conjunction with major histocompatibility complex (MHC) class I and II molecules. DCs then migrate into corresponding lymph nodes, where they mature and present the antigen to naïve T lymphocytes. Helper T cells ($CD4^+$) recognize their cognate antigens (MHC class II molecules) on DCs, where $CD8^+$ cytotoxic T lymphocytes (CTLs) recognize foreign or cancer cells that display the complementary peptide-MHC class I molecule on their cell surface. Adapting single peptides for the development of vaccines is not an optimal approach. It has been shown that after a complete objective response to the NY-ESO-1 peptide vaccine, a NY-ESO-1 negative tumor later recurred, showing that single-target immunization approaches can result in the development of immune escape tumor variants [22]. Since MHC expression levels vary with tumor types and stages, it is difficult to eradicate cancer by administration of NK cells alone. So, it is rational to use NK cell together with cancer vaccine, which we call hybrid immune therapy. CTLs activated by DC-based vaccine target MHC expressing cancer cells, where NK cells attack cancers that do not express MHC. We have proposed WT1, MUC1, CEA, CA125 and HER2/neu as potential cancer antigens for DC-based cancer vaccine, according to the patient's primary lesions and the tumor markers [23-26]. It has been reported that WT1 and MUC1 are antigens with high immunogenicity and their targeted immunotherapy has confirmed their safety and clinical efficiency. However, there are few studies regarding cancer vaccines that simultaneously use WT1 and MUC1 as antigens [27].

Preparations of DCs are as follows; PBMC-rich fraction is obtained usually by leukapheresis using COM. TEC (Fresenius Kabi, Hamburg, Germany). PBMCs were isolated from the heparinized leukapheresis products by Fi-coll-Hypaque gradient density centrifugation [28]. These PBMCs are placed into 100mm plastic tissue culture plates (Becton Dickinson Labware, Franklin Lakes, NJ) in AIM-V medium (Gibco, Gaithersburg, MD). Following 30 min incubation at 37deg C, non-adherent cells are removed and adherent cells were cultured in AIM-V medium containing granulocyte-macrophage colony stimulating factor (GM-CSF, 500ng/ml, Primmune Inc., Kobe, Japan) and IL-4 (250ng/ml, R&D Systems Inc., Minneapolis, MN), to generate immature DCs [29]. The population of adherent cells remaining in the wells is composed of 95.6 +/- 3.3% $CD14^+$ cells. After 5 days of cultures, the immature DCs are stimulated with OK-432 (10 μ /mL) and prostaglandin E2 (50ng/mL, Daiichi Fine Chemical Co., Ltd., Toyama, Japan) for 24 hours to induce differentiation. Then, WT1, MUC1 and other antigens or proteins are pulsed onto the DCs in the same culture media are incubated for 24 hours. The concentrations usually used are shown (Table 4).

WT1	20µg/mL
MUC1, long peptide (30-mer)	20µg/mL
CEA peptides	20µg/mL
CA125 protein	500µg/mL
HER2	20µg/mL
Autologous tumor lysates	50µg/mL

Table 4. Concentrations of peptide using DC vaccine

To prepare the autologous tumor lysates, tumor masses were obtained by surgical resection exclusion and are homogenized. Aliquots of isolated tumor cells were then lysed by 10 cycles of repeated freeze in liquid nitrogen and thaw in a 37deg C water bath. The lysed cells were centrifuged at 14,000G for 5 min, and supernatants are passed through a 0.22µm filter (Millipore Corporation, Bedford, MA). Protein concentrations in the resultant cell-free lysates are determined using DC protein assay kits (Bio-Rad Laboratories, Hercules, CA). Aliquots (500µg/tube) are then cryopreserved at -135deg C until use [30]. Surface molecules are determined using flow cytometry. The cells defined as mature DCs are CD14⁺, HLA-DR⁺, HLA-ABC⁺, CD83⁺, CD86⁺, CD40⁺ and CCR7⁺. Vaccine quality control and FACS analysis are as follow; All vaccines are subjected to quality control evaluation, which involves assessing the total number of live DCs, monocyte-derived DC characteristics and percentage of viable cells. For vaccine to be deemed "adequate" 4x10⁷ viable DCs are required. The frozen DC cells are allowed to thaw quickly in a 37deg C water bath and are retrieved from the cryopreservation tube by rinsing with 0.02% albumin-containing FACS buffer cell Wash™ (Bioscience, San Hose, CA). The FACS analysis is performed for cell surface antigen detection. FITC-labeled anti-human CD14, CD40, CD80, HLA-A, B, C, PE-labeled anti-human CD11C, CD83, CD197 (CCR7⁺), HLA-DR and the FACS Calibur flow cytometer were used from DC Biosciences (Franklin Lakes, NJ).

4. Role of hyperthermia in DC vaccine therapy

Hyperthermia is widely used to enhance the efficiency of chemotherapy or radiation in patients with inoperable cancer [31]. It has been given much attention for the cellular response to heat stress with respect to the immune system in cancer. The anti-tumor immune response can be markedly enhanced by treatment with hyperthermia particularly in the fever range [32]. Immunological effects of mild hyperthermia are twofold. One is the effect on dendritic and other immune cells [33]. The other is the effect is on tumor cells. Protein or peptides derived from cancer which are chaperoned by heat-shock protein (HSP) are possible sources of antigens, transferred to antigen presenting cells for priming CD8⁺ T cell responses [34]. Human tumor-derived HSP70 peptide complexes (HSP70-PC) have the immunogenic potential to instruct DCs and cross-present endogenously expressed, nonmutated, tumor antigenic peptides. The cross-presentation of a shared human tumor Ag together with its exquisite

efficacy is an important new aspect for HSP70-based immunotherapy in clinical anticancer vaccination strategies, and suggest a potential extension of HSP70-based vaccination protocols from a patient-individual treatment modality to its use in an allogeneic setting [35]. The other studies support various clinical use of hyperthermia as part of an immunotherapeutic strategy in treating cancer [32, 36].

The mechanisms by which a tumor cell can escape CTL is critical for the design and modification of effective vaccine strategies against cancer. These mechanisms fall into four broad categories: (i) inadequate antigen presentation by tumor cells resulting in their poor sensitivity to lysis by CTL; (ii) inhibitory signals provided by the tumor microenvironment; (iii) inability of TAA-specific CTL to localize at a tumor site; and (iv) inability of the tumor microenvironment to sustain T cell function in vivo [37]. Therefore, adequate antigen expression by tumor cells is of crucial importance. Many research papers show the possible augmentation of MHC class I antigen presentation via heat shock protein expression by hyperthermia. It has been demonstrated that the cell surface presentation of MHC class I antigen is increased in tandem with increased heat shock protein 70 (HSP 70) [38]. It is clear that mild hyperthermia enhances both the expression of TAA on the surface of tumors and also increased presentation of TAA chaperoned by HSP on the dendritic cell. These findings are encouraging for usage of hyperthermia at the time of DC vaccination.

5. Clinical results in miscellaneous cancers

Indications for the DC-based cancer vaccine is summarized as follows:

1. Patients with advanced cancer refractory to standard treatments.
2. Cancer patients treated with standard treatments without satisfactory results.
3. Efficiency of current standard therapy is not expected, but there are some possibilities to improve quality of life and prolong survival time by use of DC-based vaccine.

Prevention of relapse or metastasis after surgery or other standard treatments.

In this communication, the results of the retrospective study of the DC-based vaccine are presented in cancers common in Japan. Most of these patients are called "cancer refugees" who are told that no further effective treatments are available. Patient evaluations included a medical history and physical examination which include measurement of performance status (PS), total protein, albumin, hemoglobin, WBC count, platelet count, blood urea nitrogen (BUN), creatinine, alkaline phosphatase, LDH, AST, ALT, bilirubin, HbA1c, tumor marker level and HLA. As an image marker, computed tomography (CT) scans or magnetic resonance image (MRI) as well as ultrasound studies were included. To be eligible, patients were required to have an ECOG PS of <3. They were also required to have adequate hematologic and hepatorenal functions as determined by the following parameters:

WBC counts of $\geq 2,500/\mu\text{L}$, platelet counts of $\geq 80,000/\mu\text{L}$, hemoglobin value of $\geq 9.0\text{g/dL}$, BUN $< 50\text{mg/dL}$, serum bilirubin level $< 5.0\text{mg/dL}$ and AST level $< 50\text{DIU/L}$.

Autologous DCs (1×10^7 cells) were administered intradermally at 14-day intervals, for a total of 6-8 times. Tolerable 1 to 5 KE doses of OK-432 (Chugai Pharmaceutical Co., Ltd., Tokyo, a streptococcus immunological adjuvant) was administered together with the DC vaccine. NK cells were simultaneously injected intravenously in some patients at 14-day intervals. Clinical response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 as follows: complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD). Adverse events were evaluated by grading the toxicity according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The most common advanced cancers that were refractory to standard treatments that were treated by our DC-based cancer vaccine were as follows: breast cancer, lung cancer, pancreatic cancer, and colorectal cancer (Figures 5-8). The most important factor for prolonged survival was good PS before entry into DC vaccine treatment. Patients in the better PS group had a significantly longer survival time compared to those in the poorer group. The overall survival (OS) based on our risk score was significantly better for patients with clinical response of CR, PR and SD compared to those with a response of PD group. Therapy was well tolerated during treatment and for 3 months after the final treatment. None of the patients experienced adverse events of grade 3 or higher during the treatment period. Grade 1 to 2 fevers and grade 1 injection site reactions, consisting of erythema, induration and tenderness lasting 1-5 days after injection occurred in most patients and did not result in any dosage modifications or delayed treatments. No signs of autoimmune disease (arthritis, rash, colitis, etc.) were observed either during or after therapy.

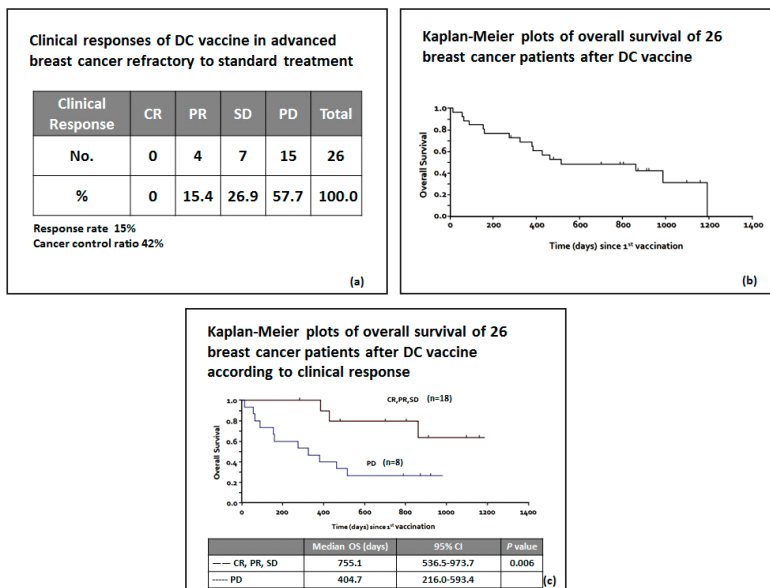


Figure 5. Clinical response of DC vaccine in advanced breast cancer refractory to standard treatment.

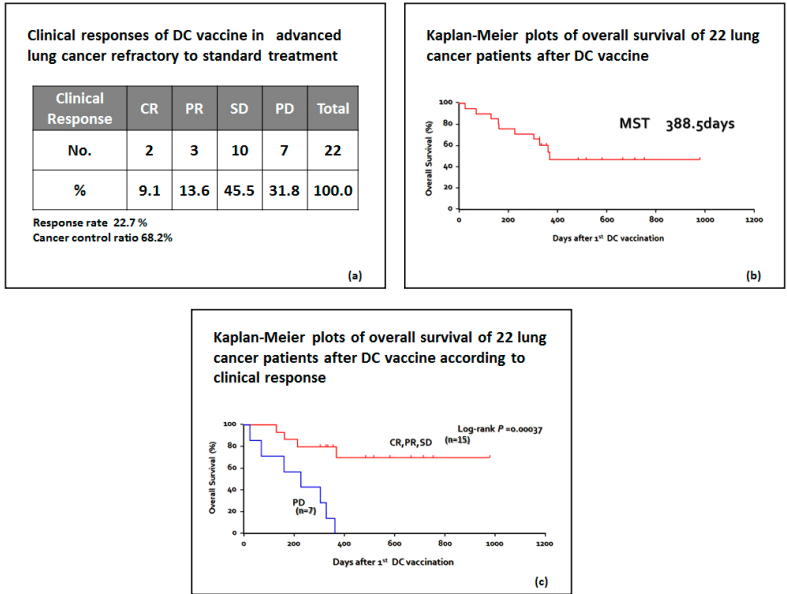


Figure 6. Clinical response of DC vaccine in advanced lung cancer refractory to standard treatment.

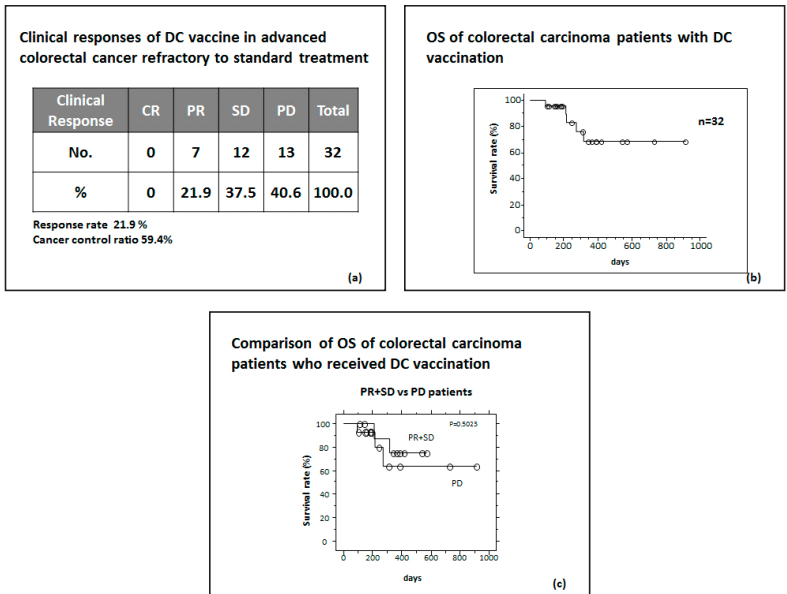


Figure 7. Clinical response of DC vaccine in advanced colorectal cancer refractory to standard treatment.

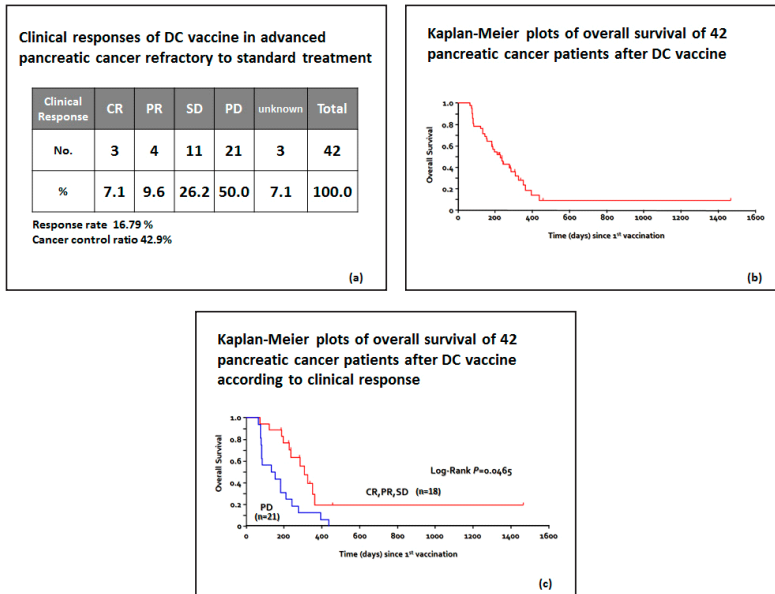


Figure 8. Clinical response of DC vaccine in advanced pancreatic cancer refractory to standard treatment.

The current results show that the DC-based vaccine is clinically applicable to any patient with a good PS at the initiation of DC-based therapy and can clinically benefit from continuing therapy beyond disease progression. Of the professional APCs, DCs are the most potent stimulators of T cell responses and play a crucial role in the initiation of primary immune responses [12]. Despite several immunotherapeutic approaches tested in colon cancer patients, only one has reported clinical results in a prospective randomized trial [39]. Preclinical data suggest that DC-based vaccines exert cytotoxic actions and that prolonged vaccine exposure is necessary for continued cancer suppression [40]. However, precisely when the full efficacy of antitumor vaccines will be realized, and when this approach will become routine therapy is difficult to predict. To date, most of peptide-based vaccines have targeted HLA class I-restricted peptides. However, there is increasing evidence that tumor-specific CD4⁺ T cells may also be important in inducing effective antitumor immunity. An ideal TAA is a protein that is essential for sustaining the malignant phenotype but which is not removed or down-regulated by the immune reaction. TAAs have been categorized according to their characteristics, such as therapeutic function, immunogenicity, oncogenicity, specificity, expression level, number of positive cells, and cancer stem cell expression.

It is important to understand the immunological mechanisms underlying the significant increase in cancer control ratios. Our results indicate that WT1 and/or MUC1-pulsed DC-based vaccination can have significant clinical benefits, even for advanced cancer patients that are refractory to standard therapies. These encouraging preliminary results suggest that WT1-and/or MUC1-pulsed DC-based vaccination strategies warrant further study as novel thera-

peutic approaches to patients with advanced carcinomas. The combination of cytotoxic therapy with immune stimulation against cancer has been studied preclinically for a variety of common tumor types and could be directly translated to clinical use [41-43]. The current result clearly supports the idea that quality specifications are of the highest priority and must be important considerations in any future vaccine-based study.

Moreover, the induction of memory T and B cells underlies immunological memory induced by vaccination [44]. The ability of memory T cells to confer protective immunity depends on the number and quality of the cells produced [45, 46]. In the current study patients with an outcome of SD lived for a relatively long time, which is unusual for other therapeutic modalities. This tendency may due to CTL proliferation and differentiation into effector memory CD8 cells.

Given the wide interest for vaccine intervention in treating miscellaneous cancers, our findings may help in guiding and designing future trials. Additional studies are necessary to identify appropriate targets for vaccine development in this new era of molecular-targeted agents for cancer treatment.

6. Discussion and conclusion

A better understanding of cancer molecular biology would enhance the design of novel therapies for cancer. Currently the scope of cancer immunotherapy is limited because most targeted antigens are restricted to a subset of patients. Molecular target DC vaccines evoke the power of each patient's immune system to help prevent recurrence and increase the long-term survival rate. If the patient's resected tumor is available, lysate is used as molecular antigens. Using this lysate, the vaccine induces an immune response against cancerous cells and creates immunologic memory. Because it is derived from the individual patient's tumor cells, this vaccine is a true targeted and personalized cancer therapy. When patient's own tumor cells are not available, integrating several candidates of peptides such as WT1, MUC1, CEA, CA125, Her2, PSA etc. can be used for the design of an anti-tumor vaccine which are restricted to the patient's HLA typing. Among these antigens, it is known that WT1 and MUC1 are the most important antigens expressed in cancer stem cells. Cancer stem cells form new tumors and may not be eliminated by chemotherapy or radiation. This has changed the perspective with regard to new approaches for treating cancer. Cancer stem cells are slow-dividing and inherently chemotherapy resistant. Eradication of these cancer stem cells may be necessary for the long-term success in cancer treatment. Using this strategy, a DC vaccine pulsed with WT1 and MUC1 and other tumor specific antigens would be used to eliminate cancer stem cells in individual patients. Hyperthermia is often used to activate immune system. There is evidence that when DCs take up HSPs together with the peptide they chaperone, the accompanying peptides are delivered into the antigen-processing pathways, leading to peptide presentation by MHC molecules. When DCs travel to the lymph nodes, T cells recognize the antigenic peptides and are specifically activated against cancer cells bearing these peptides [47]. Finally, the clinical results of molecular target cell therapy for cancers involving different organs, using

the DC vaccine and or combination with natural killer cells are discussed. The response rate and cancer control rate of advanced breast cancer, lung cancer, colorectal cancer and pancreatic cancer are 12% and 38%, 22.7% and 68.2%, 21.9% and 59.4%, 16.9% and 42.9%, respectively. Overall survival rates were more than that of expected in advanced cancer refractory to standard therapy. These findings of DC based vaccines suggest the usefulness for treating cancer patients. Given the wide interest for targeted vaccine intervention in treating miscellaneous cancers, our findings may help in guiding and designing future trials and the development of novel cancer treatment strategies.

Author details

Hiroyuki Abe*, Touko Shimamoto, Shinichiro Akiyama and Minako Abe

*Address all correspondence to: drabeqqq@yahoo.co.jp

Abe Cancer Clinic, Japan

References

- [1] Melder RJ, Whiteside TL, Vujanovic NL, Hiserodt JC, Herberman RB. A new approach to generating antitumor effectors for adoptive immunotherapy using human adherent lymphokine-activated killer cells. *Cancer Res.* 1988 Jun 15;48(12):3461-3469.
- [2] Farag SS, VanDeusen JB, Fehniger TA, Caligiuri MA. Biology and clinical impact of human natural killer cells. *Int J Hematol.* 2003 Jul;78(1):7-17.
- [3] Nagler A, Lanier LL, Cwirla S, Phillips JH. Comparative studies of human FcR3-positive and negative natural killer cells. *J Immunol.* 1989;143(10):3183-3191.
- [4] Miller JS, Oelkers S, Verfaillie C, McGlave P. Role of monocytes in the expansion of human activated natural killer cells. *Blood.* 1992;80(9):2221-2229.
- [5] Baume DM, Caligiuri MA, Manley TJ, Daley JF, Ritz J. Differential expression of CD8a and CD8b associated with MHC-restricted and non-MHC-restricted cytolytic effector cells. *Cell Immunol.* 1990;131: 352-365.
- [6] Rabinowich H, Pricop L, Herberman RB, Whiteside T.L. Expression and function of CD7 molecule on human natural killer cells. *J Immunol.* 1994;152(2):517-526.
- [7] Lee DM, Patel DD, Pendergast AM, Haynes BF. Functional association of CD7 with phosphatidylinositol 3-kinase: interaction via a YEDM motif. *Int Immunol.* 1996;8(8): 1195-1203.

- [8] Keever CA, Pekle K, Gazzola MV, Collins NH, Gillio A. NK and LAK activities from human marrow progenitors. I. The effects of interleukin-2 and interleukin-1. *Cell Immunol.* 1990;126(1):211-226.
- [9] Trinchieri G, Matsumoto-Kobayashi M, Clark SC, Sehra J, London L, Perussia B. Response of resting human peripheral blood natural killer cells to interleukin 2. *J Exp Med.* 1984;160(4):1147-1169.
- [10] Baume DM, Robertson MJ, Levine H, Manley TJ, Schow PW, Ritz J. Differential responses to interleukin 2 define functionally distinct subsets of human natural killer cells. *Eur J Immunol.* 1992;22(1):1-6.
- [11] Abe H, Akiyama S, Okamoto M. Clinical Cancer Immunotherapy: Molecular Targeting Immunotherapy. *J Integ Med.* 2008;1(1):38-46.
- [12] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature.* 1998 Mar 19;392(6673):245-252.
- [13] Kadowaki N, Antonenko S, Ho S, Rissoan MC, Soumelis V, Porcelli SA, et al. Distinct cytokine profiles of neonatal natural killer T cells after expansion with subsets of dendritic cells. *J Exp Med.* 2001 May 21;193(10):1221-1226.
- [14] Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. *Nat Rev Immunol.* 2005 Apr;5(4):296-306.
- [15] Martin A, Cheever, James P. Allison, Andrea S. Ferris, et al. The Prioritization of Cancer Antigens: A National Cancer Institute Pilot Project for the Acceleration of Translational Research. *Clin Cancer Res* 2009;15:5323-5337.
- [16] Akiyama S, Hamaya K, Ito Y, et al. Retrospective analysis of clinical responses in patients with gastroenterological cancer treated with dendritic cell vaccination. *Int J Integ Med.* 2011;3:12-24.
- [17] Akiyama S, Abe H. Successful treatment by hybrid immune therapy and high dose vitamin C therapy against hepatocellular carcinoma. *Int J Integ Med.* 2011;3:147-151.
- [18] Mittendorf EA, Peoples GE, Singletary SE. Breast cancer vaccines: promise for the future of pipe dream? *Cancer.* 2007;15:1677-1686.
- [19] Brieger J, Weidmann E, Fenchel K, et al. The expression of the Wilms' tumor gene in acute myelocytic leukemia as a possible maker for leukemic blast cells. *Leukemia.* 1994;8:2138-2143.
- [20] Menssen HD, Renkl HJ, Rodeck U, Maurer J, Notter M, Schwartz S, et al. Presence of Wilms' tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. *Leukemia.* 1995;9(6):1060-1067.
- [21] Ogawa H, Tamaki H, Ikegame K, Soma T, Kawakami M, Tsuboi A, et al. The usefulness of monitoring WT1 gene transcripts for the prediction and management of re-

- lapse following allogeneic stem cell transplantation in acute type leukemia. *Blood*. 2003 1;101(5):1698-1704.
- [22] Odunsi K, Qian F, Matsuzaki J, Mhawech-Fauceglia P, Andrews C, Hoffman EW, et al. Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer. *Proc Natl Acad Sci U S A*. 2007 31;104(31):12837-12842.
- [23] Sugiyama H. Cancer immunotherapy targeting Wilms' tumor gene WT1 product. *Expert Rev Vaccines*. 2005;4(4):503-512.
- [24] Mukherjee P, Ginardi AR, Madsen CS, Sterner CJ, Adriance MC, Tevethia MJ, et al. Mice with spontaneous pancreatic cancer naturally develop MUC-1-specific CTLs that eradicate tumors when adoptively transferred. *J Immunol*. 2000;165(6):3451-3460.
- [25] Nair SK, Hull S, Coleman D, Gilboa E, Lysterly HK, Morse MA. Induction of carcinoembryonic antigen (CEA)-specific cytotoxic T-lymphocyte responses in vitro using autologous dendritic cells loaded with CEA peptide or CEA RNA in patients with metastatic malignancies expressing CEA. *Int J Cancer*. 1999;82(1):121-124.
- [26] Larbouret C, Robert B, Navarro-Teulon I, Thèzenas S, Ladjemi MZ, Morisseau S, et al. In vivo therapeutic synergism of anti-epidermal growth factor receptor and anti-HER2 monoclonal antibodies against pancreatic carcinomas. *Clin Cancer Res*. 2007 Jun 1;13(11):3356-3362.
- [27] Ramanathan RK, Lee KM, McKolanis J, Hitbold E, Schraut W, Moser AJ, et al. Phase I study of a MUC1 vaccine composed of different doses of MUC1 peptide with SB-AS2 adjuvant in resected and locally advanced pancreatic cancer. *Cancer Immunol Immunother*. 2005 Mar;54(3):254-264.
- [28] Böyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest Suppl*. 1968;97:77-89.
- [29] Okamoto M, Furuichi S, Nishioka Y, Oshikawa T, Tano T, Ahmed SU, et al. Expression of toll-like receptor 4 on dendritic cells is significant for anticancer effect of dendritic cell-based immunotherapy in combination with an active component of OK-432, a streptococcal preparation. *Cancer Res*. 2004 Aug 1;64(15):5461-5470.
- [30] Nagayama H, Sato K, Morishita M, Uchimaru K, Oyaizu N, Inazawa T, et al. Results of a phase I clinical study using autologous tumour lysate-pulsed monocyte-derived mature dendritic cell vaccinations for stage IV malignant melanoma patients combined with low dose interleukin-2. *Melanoma Res*. 2003 Oct;13(5):521-530.
- [31] Feyerabend T, Wiedemann GJ, Jäger B, Vesely H, Mahlmann B, Richter E. Local hyperthermia, radiation, and chemotherapy in recurrent breast cancer is feasible and ef-

- fective except for inflammatory disease. *Int J Radiat Oncol Biol Phys.* 2001 Apr 1;49(5):1317-1325.
- [32] Calderwood SK, Theriault JR, Gong J. How is the immune response affected by hyperthermia and heat shock proteins? *Int J Hyperthermia.* 2005 Dec;21(8):713-716.
- [33] Srivastava P. Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. *Annu Rev Immunol.* 2002;20:395-425.
- [34] Binder RJ, Srivastava PK. Peptides chaperoned by heat-shock proteins are a necessary and sufficient source of antigen in the cross-priming of CD8+ T cells. *Nat Immunol.* 2005 Jun;6(6):593-599.
- [35] Noessner E, Gastpar R, Milani V, Brandl A, Hutzler PJ, Kuppner MC, et al. Tumor-derived heat shock protein 70 peptide complexes are cross-presented by human dendritic cells. *J Immunol.* 2002 Nov 15;169(10):5424-5432.
- [36] Ostberg JR, Repasky EA. Emerging evidence indicates that physiologically relevant thermal stress regulates dendritic cell function. *Cancer Immunol Immunother.* 2006 Mar;55(3):292-298.
- [37] Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. *Adv Immunol.* 2000;74:181-273.
- [38] Ito A, Shinkai M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T. Augmentation of MHC class I antigen presentation via heat shock protein expression by hyperthermia. *Cancer Immunol Immunother.* 2001 Dec;50(10):515-522.
- [39] Vermorken JB, Claessen AM, van Tinteren H, et al. Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. *Lancet.* 1999;353:345-350.
- [40] Sadanaga N, Nagashima H, Mashino K, Tahara K, Yamaguchi H, Ohta M, et al. Dendritic cell vaccination with MAGE peptide is a novel therapeutic approach for gastrointestinal carcinomas. *Clin Cancer Res.* 2001 Aug;7(8):2277-2784.
- [41] Meng Y, Carpentier AF, Chen L, Boisserie G, Simon JM, Mazeron JJ, et al. Successful combination of local CpG-ODN and radiotherapy in malignant glioma. *Int J Cancer.* 2005 Oct 10;116(6):992-927.
- [42] Najar HM, Dutz JP. Topical CpG enhances the response of murine malignant melanoma to dacarbazine. *J Invest Dermatol.* 2008 Sep;128(9):2204-2210.
- [43] VanOosten RL, Griffith TS. Activation of tumor-specific CD8+ T Cells after intratumoral Ad5-TRAIL/CpG oligodeoxynucleotide combination therapy. *Cancer Res.* 2007 Dec 15;67(24):11980-11990.

- [44] Pulendran B, Ahmed R. Immunological mechanisms of vaccination. *Nat Immunol.* 2011;12:509-517.
- [45] Gourley TS, Wherry EJ, Masopust D, Ahmed R. Generation and maintenance of immunological memory. *Semin Immunol.* 2004 Oct;16(5):323-333.
- [46] Hand TW, Kaech SM. Intrinsic and extrinsic control of effector T cell survival and memory T cell development. *Immunol Res.* 2009;45(1):46-61.
- [47] Li Z, Qiao Y, Liu B et al. Combination of imatinib mesylate with autologous leukocyte-derived heat shock protein and chronic myelogenous leukemia . *Clin Cancer Res.* 2005 11:4460-4468.

