European Journal of Cancer (2013) 49, 852–859

Available at www.sciencedirect.com

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journal homepage: www.ejcancer.info

Impact of dendritic cell vaccines pulsed with Wilms' tumour-1 peptide antigen on the survival of patients with advanced non-small cell lung cancers

Hidenori Takahashi [a,h], Masato Okamoto [b], Shigetaka Shimodaira [c], Shun-ichi Tsujitani [d], Masaki Nagaya [a], Takefumi Ishidao [e], Junji Kishimoto [f], Yoshikazu Yonemitsu [g,*], The DC-vaccine study group at the Japan Society of Innovative Cell Therapy (J-SICT)

[a] *Seren Clinic Tokyo, Tokyo, Japan*
[b] *Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan*
[c] *Cell Processing Center, Shinshu University Hospital, Nagano, Japan*
[d] *National Center for Global Health and Medicine, Tokyo, Japan*
[e] *Research and Development Division at Tella Inc., Tokyo, Japan*
[f] *Center for Clinical and Translational Research, Kyushu University Hospital, Fukuoka, Japan*
[g] *R&D Laboratory for Innovative Biotherapeutics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan*

Available online 11 December 2012

KEYWORDS
Non-small cell lung cancer
Dendritic cell vaccine
Wilms' tumour-1
Overall survival

Abstract ***Purpose:*** Dendritic cell (DC)-based vaccines have been expected to serve as new therapeutic approaches for advanced non-small cell lung cancers (NSCLCs); however, their clinical outcomes have not been fully elucidated. We report a single-centre clinical study analysing factors affecting the survival of patients with advanced NSCLCs who received DC vaccines pulsed with or without Wilms' tumour protein-1 (WT1) peptide.

Methods: Among 62 patients with previously treated inoperable or postoperatively relapsed NSCLCs who met the inclusion criteria, DCs from 47 (76%) patients who showed HLA-A2402/0201/0206 were pulsed with one or more corresponding WT1 peptide antigens. DC vaccines were intradermally injected biweekly.

Results: Clinical responses based on response evaluation criteria in solid tumours (RECIST) were found in 31 (50%) patients at 3 months after the first DC vaccine (complete response: 1 (1.6%), partial response: 4 (6.5%), stable disease: 26 (41.9%)). Median survival time was

* *Corresponding author:* Address: R&D Laboratory for Innovative Biotherapeutics, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel./fax: +81 92 642 4777.
E-mail addresses: bzr06263@nifty.com (H. Takahashi), yonemitu@med.kyushu-u.ac.jp (Y. Yonemitsu).
[h] Address: Seren Clinic Tokyo, 2-10-2 Shiroganedai, Minato-ku, Tokyo 108-0078, Japan. Tel.: +81 3 3449 6095; fax: +81 3 3449 6097.

0959-8049/$ - see front matter
http://dx.doi.org/10.1016/j.ejca.2012.11.005

27 months (82% in 1 year and 54% in 2 years) from initial diagnosis, and that was 12 months (48% in 1 year and 22% in 2 years) from the first DC vaccination. Importantly, multivariate analyses revealed that only two factors, blood haemoglobin and the use of WT1 peptides, significantly affected the overall survival of patients from both initial diagnosis and first vaccination. ***Conclusions:*** This study is the first to suggest that DC vaccines pulsed with WT1 may hold a significant impact to prolong the overall survival of patients with advanced NSCLCs.

1. Introduction

Lung cancer is the most commonly diagnosed malignancy, and it is the leading cause of death in males among developed countries. Non-small-cell lung cancers (NSCLCs) account for approximately 85% of all lung cancer cases.[1] The prognosis of patients with NSCLCs remains unsatisfactory: usually less than 12–13 months of median survival time (MST) despite the use of available therapeutics.[2–7] Therefore, the development of new and preferably less invasive therapeutics against NSCLSs has been much desired.

Since the first report regarding tumour-associated antigens (TAAs),[8] a number of clinical trials using synthetic peptides encoding TAA, namely 'cancer peptide vaccines', have been performed worldwide.[9] These studies demonstrated the safety of and possible immune responses against administered peptides, however, the clinical outcome, including the antitumour effect as well as the prolongation of survival, has been largely limited.[10] Although the exact cause of such limited clinical efficacy has been undetermined, the dysfunction of professional antigen-presenting dendritic cells (DCs) in a tumour-bearing host has been suggested as a possible mechanism by which a tumour can escape immune surveillance.[11]

To overcome such dysfunction of DCs in tumour-bearing individuals *in vivo*, cell-based vaccines have been developed to manipulate and activate TAA-pulsed autologous DCs *ex vivo*.[12–15] A number of reports have demonstrated early promising results against advanced malignancies,[16] including NSCLCs,[17–20] however, the clinical efficacy, as well as factors affecting it, have been still largely undetermined.

Therefore, we here retrospectively analysed the clinical data of inoperable or postoperatively relapsed NSCLC patients who were vaccinated with OK-432-activated DCs[21,22] with or without Wilms' tumour gene-1 (WT1) peptide antigen, a potent TAA,[23,24] before moving to a well-controlled prospective trial.

2. Patients and methods

2.1. Patients

A total of 62 NSCLC patients who had been initiated on DC vaccines (usually 1×10^7 cells/dose) between January 2007 and May 2011 at Seren Clinic Tokyo were retrospectively examined. All patients were diagnosed as inoperable due to primary tumour spread or postoperative relapse and agreed to be involved in this study with written informed consent. Patients were eligible in this study if they met the safety criteria, including a white blood cell (WBC) count of 2500 cells/μl or more, haemoglobin (Hb) of 8.0 g/dl or more, platelet count of 100,000 cells/mm^3 or more and no serious organ dysfunction in the cardiovascular and respiratory systems. Here we did not use the overexpression of the WT1 as an inclusion criterion in this study, because overexpression of the WT1 gene was sufficiently frequent and detected by RT-PCR in 96%[25] or by immunohistochemistry in 72%[26] of patients with *de novo* NSCLCs. Patient follow-up was done by routine phone calls or letters to family and/or the patients' primary care physicians. This study was approved by the Institutional Review Board.

2.2. DC vaccine

2.2.1. Preparation of DCs

DCs were prepared as previously described.[27] Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from the leukapheresis products by Ficoll–Hypaque gradient density centrifugation. These PBMCs were placed on tissue-culture plates, and the adherent cells were cultured in medium containing granulocyte–macrophage colony-stimulating factor (500 ng/mL) and interleukin-4 (250 ng/mL) to generate immature DCs. Five days later, DCs were stimulated with OK-432 (10 μg/mL) and prostaglandin E2 (50 ng/mL) for 24 h. DCs were then pulsed with autologous tumour lysate (50 μg/mL) or, if that was not available, with peptide antigens according to the HLA-A pattern. The DCs were cryopreserved and kept until the day of administration. The phenotype $CD14^{-/low}$/$HLA\text{-}DR^+$/$HLA\text{-}ABC^+$/$CD80^+$/$CD83^+$/$CD86^+$/$CD40^+$/$CCR7^+$ was taken to define mature DCs.

2.2.2. Tumour lysate

Autologous tumour lysates were prepared as previously described.[27] Briefly, tumour cells were isolated by depleting lymphocytes using monoclonal antibodies against CD2, CD14 and CD19 conjugated to immunomagnetic beads (Dynal, Oslo, Norway) according to the instructions. Aliquots of the isolated tumour cells were then lysed by 3-freeze/thaw cycles. Aliquots (500 μg/tube) after passing through a 0.22-μm filter were then stored at –135 °C until use.