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Progress reports on immune gene therapy for stage IV renal cell cancer using lethally irradiated granulocyte-macrophage colony-stimulating factor-transduced autologous renal cancer cells

Abstract There is no effective treatment for patients with stage IV renal cell cancer (RCC), although the introduction of new therapy is imminent. Cancer gene therapy is currently considered to be one of the most promising therapeutic modalities in the field of cancer treatment. Based on the results of animal studies, vaccination using autologous granulocyte-macrophage colony-stimulating factor-transduced renal cancer cells appears promising. Before initiating a clinical study using an ex vivo gene-transduced autologous cell vaccine-based immunogene therapy for RCC in Japan, in 1992 we initially planned a Japanese version of a clinical protocol in collaboration with a US group. In 1993, the original protocol was refined. We performed five preclinical qualification studies using RCC nephrectomy specimens from patients in 1997, and the results showed that preparation of RCC cells for autologous vaccines at the Clinical Cell Technology Facility, Research Hospital of the Institute of Medical Science, University of Tokyo, was feasible. Subsequently in August 1998, the Ministry of Health and Welfare and the Ministry of Education, Science, Culture, and Sport approved our clinical protocol. We have recruited two patients with stage IV RCC to our study so far. Here we report the background to the initiation of cancer gene therapy in Japan.

Key words Granulocyte-macrophage colony-stimulating factor · Retrovirus vector · Stage IV renal cell cancer · Clinical trial · Replication-competent virus

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Introduction

In 1990, Blaese et al. of the USA first administered gene therapy using a retroviral vector to a patient with severe combined immune deficiency due to adenosine deaminase

(ADA) deficiency [1]. It was demonstrated that the ex vivo gene transduction system could be safely and effectively used to transduce ADA cDNA into the patient's autologous peripheral lymphocytes. In 1991, gene therapy for advanced malignant lymphoma was performed using tumor necrosis factor (TNF)-α cDNA-transduced tumor-infiltrating lymphocytes by Rosenberg et al. in the USA [2]. Since those epoch-making trials, more than 2,000 cancer patients have been enrolled in various cancer gene therapy clinical trials (Table 1).

As current cancer gene therapy cannot target tumor cells, there are more ongoing immunogene therapy trials than trials of other cancer gene therapy protocols. Numerous preclinical studies have confirmed that engineering murine tumor cells to express various immunostimulatory molecules can lead to enhanced tumor immunogenicity. Among the immunogene therapy protocols, we have been particularly interested in cytokine gene therapy using human granulocyte-macrophage colony-stimulating factor (GM-CSF) cDNA. GM-CSF stimulates potent, specific, and long-lasting antitumor immunity in multiple murine tumor model systems, including melanoma and renal cell carcinoma (RCC). Immunization requires the participation of both $CD4^+$ and $CD8^+$ T lymphocytes and likely involves improved tumor antigen presentation by dendritic cells and macrophages recruited to the vaccination site [3–5].

In Japan RCC occurs most frequently in those in the age range of 50–70 years, men die of the disease more frequently than women, and approximately 2,800 RCC patients die annually. Almost 30% of patients have metastatic disease at the time of diagnosis. Although nephrectomy is curative for stage I–III patients, there is no effective treatment for patients with stage IV RCC. Nearly half of patients with stage IV RCC die within one year of diagnosis. Neither interferon-α nor interleukin-2 is effective in these patients. Because spontaneous tumor regression occurs in some patients, an immunological approach may be helpful in those with stage I–II disease. For more advanced-stage patients, however, new therapy is required [6–10].

Table 1 Clinical trials of cancer gene therapy underway in the USA as of May 18, 1999 (*HSV-TK* herpes simplex virus-thymidine kinase)

Trial using	No. in progress
Antisense oligonucleotides	5
Chemoprotection	9
Ex vivo immunogene therapy	60
In vivo immunogene therapy	59
Prodrug/HSV-TK/ganciclovir	30
Tumor suppressor genes	23
Single-chain antibody	2
Other	5
Total	193

A phase I study of autologous RCC vaccination was reported by Simons et al. in 1997 [4]. Eighteen patients were evaluated in their double-blind dose-escalation study, and eight received GM-CSF-transduced cells. No dose-limiting toxicity was observed. Delayed-type hypersensitivity (DTH) tests using irradiated autologous renal cancer cells showed that DTH skin reactions were dose dependent. Infiltration of eosinophils and lymphocytes occurred at the vaccine and DTH sites. Patients who received GM-CSF-transduced cells 4×10^7 monthly for a total of three times had significant DTH skin reactions, whereas those who received 4×10^6 cells monthly for a total of three times did not. Simons et al. were unable to produce sufficient GM-CSF-transduced cells to attempt monthly administration of 4×10^8 cells for a total of three times [4].

The antitumor effects of GM-CSF-transduced cells are thought to be induced by dendritic cells. Major histocompatibility complex (MHC) class II expression of dendritic cells is believed to be enhanced by locally produced GM-CSF and tumor antigen is presented to MHC class II molecules. This activates CD4+ cells followed by cytotoxic T lymphocyte activation to attack metastasized tumor cells.

Background to cancer gene therapy at the Institute of Medical Science, University of Tokyo

As the vaccination of autologous GM-CSF-transduced renal tumor cells was considered a promising therapy for RCC patients based on animal studies, clinical studies using GM-CSF-transduced autologous renal cell vaccine-based immunogene therapy for RCC were initially planned in Japan in collaboration with Dr. Richard Mulligan, Harvard Medical School, Boston, MA, USA, and CellGenesys, Inc. (formerly Somatix Therapy), Foster City, CA, USA, in 1992. In 1993, the Japan version of the US clinical protocol was evaluated at the pre-Institutional Review Board (IRB) level in a collaborative conference. Between 1995 and 1996, the Institute of Medical Science, University of Tokyo (IMSUT) Institutional Review Board discussed the protocol. The protocol was submitted to the Japanese Ministry of Health and Welfare and Ministry of Education, Science, Culture, and Sport in December 1996. When our former collaborative partner Somatix merged with CellGenesys in April 1997, it was decided to culture and transduce renal cells at the Clinical Cell Technology Facility, Research Hospital, IMSUT.

Trial runs were conducted between October 1997 and March 1998. Cell processing and GM-CSF gene transfer were performed exactly as reported by Simons et al. As shown in Table 2, we performed five preclinical qualification analyses of cultured retroviral human GM-CSF cDNA-transduced renal cancer cells from nephrectomized specimens of patients who had given informed written consent. Our results confirmed that preparation of RCC cells for autologous vaccine at our facility was

Table 2 Trial runs of cell processing and GM-CSF gene transfer

Sex and age	Stage	RCC pathology	GM CSF production ($ng/10^6/24$ h)	No. cells recovered	Tumor weight (g)* (weight resected)	HLA-DQα gene transfer	
						Pre	Post
F 74	II	Alveolar type, clear cell, granular cell subtype, G2 > G1, INFβ, pT2, pN0, pV0	66.5	2.0×10^7	0.11 (14)	DQα 1.1, 1.2	DQα 1.1, 1.2
M 55	I	Cystic type, alveolar type, common type, clear cell subtype, G1 > G2, INFα, pT2, pN0, pV0	13.6	8.0×10^6	0.84 (3)	DQα 1.3, 3	DQα 1.3, 3
F 43	IV	Expansive type, alveolar type, common type, mixed subtype, G2, INFα, pT2, pV0, pM1 (liver), pN0	4.20	5.0×10^6	0.12 (40)	DQα 1.2, 3	DQα 1.2, 3
M 60	IV	Alveolar type, common type, granular cell subtype, G2 > G3, INFα, pT2, pN0, pMx, pV1a, cM1(BM)	90.2	2.0×10^7	2.60 (20)	DQα 1.3, 3	DQα 1.3, 3
M 55	IV	Common type, clear cell subtype, G2, INFβ, pT3a, pV1b, pN0	89.1	1.8×10^8	14.0 (50)	DQα 1.3, 3	DQα 1.3, 3

* Tumor weight, processed weight; figures in parentheses refer to resected weight
Inf, infiltration [11]

feasible. In April 1998, we submitted our preclinical data on cell processing and gene transfer to the IMSUT IRB. In August 1998 government permission to initiate gene therapy was finally granted.

Case reports

In the Japanese version of the RCC gene therapy clinical protocol, we administered lethally irradiated autologous GM-CSF-transduced cells 4×10^7 cells three times at 4-week (more than dose level 2) intervals, for a total of 1.2×10^8 cells as reported by Simons et al. We first administered 4×10^7 cells, followed by 2×10^7 cells every 2 weeks. Our protocol requires a minimum dose of 1.4×10^8 cells for each patient. Two patients with stage IV renal cancer have been recruited to our study so far.

The first patient was a 60-year-old man with a large right renal tumor with multiple lung metastases and direct liver infiltration who satisfied all of the inclusion criteria and had an Eastern Cooperative Oncology Group performance status of 0–1. After giving written informed consent, on October 5, 1998, he underwent nephrectomy, and the cell processing and gene transduction began on the same day. GM-CSF 49 $ng/10^6/24$ h was produced. In late October, a fraction of the GM-CSF-transduced RCC cells was transported to BioReliance Corp. (Kockville, MD, USA) for safety checks including sterility, the presence of *Mycoplasma* sp. and endotoxins and replication-competent retrovirus (RCR). The results showed that the transduced cells were safe, and the intradermal administration of a total of GM-CSF-transduced RCC cells 1.4×10^8 every 2 weeks commenced from December 1998 to April 1999 (a total of 10 injections).

The second patient was a 70-year-old man who had a large right renal tumor with sacral bone metastasis (7×7 cm). He also met the inclusion criteria and had an Eastern Cooperative Oncology Group performance status of 1. Nephrectomy was performed on April 6, 1999. Cell processing and gene transduction were started on the same day, and GM-CSF 96 $ng/10^6/24$ h was obtained. In the beginning of May, a fraction of GM-CSF-transduced RCC cells was transported to BioReliance for safety checks as for the first patient. After the safety of the transduced cells had been confirmed, injections of a total GM-CSF-transduced RCC cells 2.8×10^8 were administered intradermally from June 3, 1999, to December 1999. We did not observe any severe generalized adverse reactions except for local erythema and itching at the vaccination sites. The serum GM-CSF level was not affected, but the eosinophil level increased 48 h after injection. A significant DTH reaction was observed. Detailed immunological and clinical data on these two patients will be reported elsewhere.

Conclusions

Our preclinical studies demonstrated that cell processing and gene transfer could be carried out safely and effectively at the IMSUT cell processing facility. GM-CSF-transduced autologous tumor cells were safely administered to two stage IV RCC patients and an immunologic reaction was induced. Some degree of clinical benefit is expected. Further clinical evaluation including three more patients is required before proceeding to the next step.

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