Prospective Evaluation of Safety of Immune-cell Therapy for Patients with Various Types of Advanced Cancer

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Abstract. *Several types of immune-cell therapies, such as αβ T-cell, γδ T-cell, and dendritic cell (DC) vaccine therapies, are clinically employed for cancer treatment. The safety of immune-cell therapy for the treatment of patients with malignancies should be maintained by continuous assessment of adverse events. In the present study, we surveyed the adverse events associated with immune-cell therapy using large-scale prospective data and analyzed the side-effect profiles. For the assessment of adverse events associated with immune-cell therapy, we evaluated 771 treatment profiles (484 for αβ T-cell therapy, 58 for γδ T-cell therapy, 206 for DC vaccine therapy, and 23 for concurrent therapy with αβ T-cells and DC vaccines) from 144 patients with various malignancies. For the assessment of fever, fatigue, and itching, each of these adverse events was found to be grade 1 or 2 in most of the treated patients, except for one patient who had grade 3 itching. It was suggested that αβ T-cell therapy could elicit a more rapid and direct immune reaction in patients than DC vaccine therapy, as shown by the earlier development of fever and higher incidence rate of fatigue. It was found that grade 1 or 2 reaction at the injection site developed in 10.2% of the patients injected with DC vaccines. Most of the grade 3 non-hematological and hematological adverse events were associated with disease progression or side-effects of chemotherapy, and were not considered to be related to immune-cell therapy. In conclusion, immune-cell therapy, such as αβ T-cell, γδ T-cell, or DC vaccine therapy, was well-tolerated for cancer treatment.*

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Immune-cell therapy is one of the cell therapies in which autologous or allogeneic cells are cultured and processed *ex vivo* and administered to patients for the treatment of various diseases (1). Recently, immune-cell therapy has been utilized as a novel option for the treatment of various malignancies, such as the clinical use of sipuleucel-T for prostate cancer (Provenge®; Dendreon Corp., Seattle WA, USA), although surgery, radiotherapy, and the use of cytotoxic or hormonal drugs are considered established conventional cancer therapies (2). Sipuleucel-T was approved for autologous immune-cell therapy for the treatment of metastatic hormone-refractory prostate cancer by the U.S. Food and Drug Administration in 2010. Therefore, given that the circumstances surrounding immune-cell therapy, such as the technology and legal systems, are now in place, immune-cell therapy against cancer may be in the transition phase from therapeutic research to a more generalized option for patients with various malignancies (1).

There is still little evidence on the efficacy and safety of immune-cell therapy in cancer treatment; however, without additional side-effects, it could have the potency to yield favorable effects as monotherapy or in combination with other conventional therapies, such as chemotherapy or radiotherapy. We previously reported that the autologous αβ T-cellular therapy essentially does not have undesirable side-effects because (i) it does not cause harmful immunological reactions such as graft *versus* host reaction, (ii) it does not cause transmission of infectious diseases derived from allogeneic materials, and (iii) it does not have strong toxic side-effects caused by the administration of a large amount of cytokines (3). We also reported that no severe side-effects were found in patients injected with dendritic cell (DC) vaccines, which were loaded with tumor lysate using closed-flow electroporation systems (4).

The safety and anticipated side-effects of immune-cell therapy should be evaluated, although it is considered essentially safe for the treatment of patients with malignancies. In the present study, we surveyed the adverse events associated with immune-cell therapy using large-scale

prospective data and analyzed the side-effect profiles of patients subjected to various immune-cell therapies. Additionally, in this study, the adverse events were evaluated for each injection of effector cells or a DC vaccine, because the quality of cell products was not always equivalent in terms of cell number or population, unlike therapeutic chemical drugs, although the harvested immune cells always meet our quality control standards.

Materials and Methods

Patients and protocols. We subjected patients with various types of cancer to effector cell therapy or DC vaccine therapy once every two weeks for three months or longer and evaluated the antitumor effect after the sixth injection. We evaluated the type, frequency, and onset time of adverse events after each injection in patients who were subjected to immune-cell therapy for various types of cancer at Seta Clinic Osaka from January 2011 to September 2013 prospectively. The criteria for the selection of patients in the present study were as follows: (i) age 20 years or older, (ii) Eastern Cooperative Oncology Group performance status (PS) was 0 or 1, and (iii) adequate bone marrow, liver and renal functions. This study was approved by the Research Ethics Committee of the Seta Clinic Group in October 2010 (approval number: SCG10051) and all the patients provided written informed consent.

Immune-cell therapy. For effector cell therapy, we prepared αβ T- or γδ T-cells cultured *ex vivo* with interleukin-2 (IL2) and an immobilized antibody to CD3 or IL2 and bisphosphonate, respectively (5, 6) For DC vaccine therapy, peripheral blood mononuclear cells were collected from the patients by leukapheresis and the adherent cell fraction was used for the DC culture using a medium supplemented with IL4 and granulocyte/macrophage colony-stimulating factor (GM-CSF). The DCs pulsed with tumor-specific peptides or the autologous tumor lysate were injected subcutaneously into the patients with various types of cancer (4,7). The combination of immune-cell therapy with chemotherapy or radiotherapy was not prohibited, although immune-cell therapy was carried out on a different day to avoid cytotoxic damage of the αβ T-, γδ T-cells, or DCs when the patients underwent conventional standard therapy. Immune-cell therapy was discontinued when disease progression was determined by diagnostic imaging or on the basis of clinical symptoms during the course of the immune-cell therapy consisting of six injections.

Toxicity assessment. We investigated the adverse events associated with non-hematological and hematological toxicities after every treatment using questionnaires and by interviews with the doctors or nurses. In the questionnaires, there were questions on major adverse events, such as fever, fatigue, itching and injection site reaction, which were previously proven to be the common side-effects of immune-cell therapy. In the questionnaires, a column was also provided for comments on any side-effect or symptom. We questioned the patients regarding the time of onset for each adverse event and we also received their body temperature on the day of immune-cell therapy and the following day. When the patients had fever of more than 38˚C, they were allowed use an antipyretic. We prescribed an anti-histamine as needed for the patients injected with αβ T- or γδ T-cells or a DC vaccine when subsequent itching or urticaria occurred owing to allergic reaction. If an allergic reaction occurred after the initial injection of the αβ T- or γδ T-cells, we washed the effector cells twice for the second and following treatment immediately before injecting them into the patients. We performed blood tests just before each treatment and evaluated the hematological toxicity. As for the adverse events, we extracted all the adverse events that were possibly related to immune-cell therapy and their grades were evaluated after every treatment on the basis of National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) ver.4.0.

Table I. *Characteristics of 144 patients subjected to immune-cell therapy.*

Characteristic	No. of patients (%)
Gender	
Male	76 (52.8)
Female	68 (47.2)
Age, years (Median: 63)	35-87
Stage	
Advanced or recurrent	116 (80.6)
Adjuvant	28 (19.4)
Combination therapy	
Yes	77 (53.5)
Chemotherapy	67 (46.5)
Radiotherapy	3 (2.1)
Both	7 (4.9)
None	67 (46.5)
Primary site	
Lung	22 (15.3)
Head & neck	17 (11.8)
Pancreas	16 (11.1)
Colorectum	16 (11.1)
Breast	15 (10.4)
Stomach	10 (6.9)
Biliary tract	9 (6.3)
Ovary	9 (6.3)
Prostate	7 (4.9)
Liver	5 (3.5)
Other	18 (12.5)

Statistical analyses. A Chi-square or Fisher's exact test was performed to evaluate the significance of results. Additionally, statistical examination was carried out using McNemar's test to assess the onset time of adverse events. We used R ver. 3.0.0 (R Foundation, Vienna, Austria) for the statistical analyses and considered $p<0.05$ as statistically significant.

Results

Patient background characteristics and treatments. For the three-year period, 144 patients with different types of cancers were registered in the present study. Patient background characteristics are shown in Table I. We included 144 participants: 22 cases of lung cancer, 17 head and neck, 16

Table II. *Incidence rate of fever for various immune-cell therapies.*

Type of cell	CTCAE grade	Treatment no.						Total (%)
		1	2	3	4	5	6	
αβT (n=484)	1	3	3	3	1	1	11	13 (2.7)
	2		1			1	2	
γδT (n=58)	1							0 (0)
DC (n=206)	1		1				2	3 (1.5)
αβT+DC (n=23)	1						1	1 (4.3)

CTCAE: Common Terminology Criteria for Adverse Events; DC: dendritic cell.

Table III. *Onset time of fever for various immune-cell therapies.*

Type of cell	CTCAE grade	Day 0		Day 1		Total (%)
		Evening	Morning	Noon	Evening	
αβT (n=484)	1	7	1	1	2	11	13 (2.7)
	2	1	1			2	
γδT (n=58)	1						0 (0)
DC (n=206)	1		1		2		3 (1.5)
αβT+DC (n=23)	1	1					1 (4.3)

CTCAE: Common Terminology Criteria for Adverse Events; DC: dendritic cell.

Table IV. *Incidence rate of fatigue for various immune-cell therapies.*

Type of cell	CTCAE grade	Treatment no.						Total (%)
		1	2	3	4	5	6	
αβT (n=484)	1	22	16	7	5	8	4	62	67 (13.8)
	2	2	2	1				5	
γδT (n=58)	1			3		2		5	6 (10.3)
	2	1						1	
DC (n=206)	1	3	3	3	3	2	3	17	19 (9.2)
	2				2			2	
αβT+DC (n=23)	1				1	1			2 (8.7)

CTCAE: Common Terminology Criteria for Adverse Events; DC: dendritic cell.

Table V. *Incidence rate of itching for various immune-cell therapies.*

Type of cell	CTCAE grade	Treatment no.						Total (%)
		1	2	3	4	5	6	
αβT (n=484)	1	13	3	2	2	3	3	26	
	2			1	1	1		3	30 (6.2)
	3						1	1	
γδT (n=58)	1	1	1						2 (3.4)
DC (n=206)	1	2	2	2	3	8	5	22	
	2				1			1	23 (11.2)
αβT+DC (n=23)	1						1		1 (4.3)

CTCAE: Common Terminology Criteria for Adverse Events; DC: dendritic cell.

colorectal, 16 pancreatic, 15 breast, 10 stomach, 9 ovary, 9 bile duct, 7 prostate, 5 liver, 4 malignant melanoma, 2 esophageal, and 2 of skin cancer; the remaining patients had other types of cancers, such as brain and kidney and other malignancies. Seventy-seven patients were subjected concurrently to immune-cell therapy and a standard therapy, such as chemotherapy or radiotherapy, although 67 patients underwent immune-cell therapy only. In addition, 116 patients with advanced or recurrent cancer and 28 subjected to postoperative adjuvant therapy were included in this study. One-hundred and eight (71.5%) out of the 144 enrolled patients completed the six injections of immune-cell therapy and 80 patients completed seven or more by immune-cell therapy. However, at the time of the sixth injection of immune-cell therapy, 36 patients withdrew from this study owing to progressive disease (PD) according to Response Evaluation Criteria in Solid Tumours (RECIST) (8) by imaging diagnosis or as requested by the patients.

For the evaluation of adverse events associated with immune-cell therapy, we finally collected 771 questionnaires from these 144 patients. The 771 treatments included 484 regarding αβ T-cell therapy (62.7%), 58 for γδ T-cell therapy (7.5%), 206 for DC vaccine therapy (26.7%), and 23 for concurrent therapy with αβ T-cells and DC vaccine (2.9%) in the 144 patients.

Adverse event assessment.

Fever. Table II shows the incidence rate of fever that developed in the course of the various immune-cell therapies. For the patients subjected to αβ T-cell therapy, γδ T-cell therapy, DC vaccine therapy, and αβ T- and DC vaccine concurrent therapy, fever developed in 13 (grade 1 in 11 patients and grade 2 in two) (2.7%: 13/484), none (0%: 0/58), three (grade 1) (1.5%: 3/206) and one patient (grade 1) (4.3%: 1/23), respectively. There were no significant differences in the incidence rates of fever among patients subjected to these treatments. For the patients subjected to αβ T-cell therapy, fever appeared on the day of the treatment in more than half of the patients; on the other hand, fever developed on the next day of treatment in all the patients subjected to DC vaccine therapy (Table III).

Fatigue. Fatigue developed in 67 (grades 1 and 2 in 62 and 5 patients, respectively) (13.8%: 67/484), 6 (grades 1 and 2 in 5

Table VI. *Incidence rate of injection site reaction for various immune-cell therapies.*

Type of cell	CTCAE grade	Treatment no.						Total (%)
		1	2	3	4	5	6	
αβT (n=484)	1	2	1	1		1		5 (1.0)
γδT (n=58)	1							0 (0)
DC (n=206)	1	3	2	2	4	4	3	18 20 (9.7)
	2					1	1	2
αβT+DC (n=23)	1					1		1 (4.3)

CTCAE: Common Terminology Criteria for Adverse Events; DC: dendritic cell.

Table VII. *Incidences of other toxicities.*

CTCAE grade	1	2	3	Total
Pain	18			18
Dyspnea	1	2		3
Diarrhea	3			3
Constipation	2			2
Urticaria	1		1	2
Biliary tract infection			1	1
Urinary tract infection			1	1
Hypothermia		1		1
Chills	1			1
Limb edema	1			1
Nausea	1			1
Dysgeusia	1			1
Total	29	3	3	35

CTCAE: Common Terminology Criteria for Adverse Events.

and 1 patient, respectively) (10.3%: 6/58), 19 (grades 1 and 2 in 17 and 2 patients, respectively) (9.2%: 19/206) and 2 patients (grade 1) (8.7%: 2/23) of those subjected to αβ T-cell therapy, γδ T-cell therapy, DC vaccine therapy, and concurrent αβ T-cell and DC vaccine therapy, respectively (Table IV). There were no significant differences in the incidence rates of fatigue among patients subjected to these treatments.

Itching. Itching developed in 30 (grades 1, 2 and 3 in 26, 3 and 1 patient, respectively) (6.2%: 30/484), 2 (grade 1) (3.4%: 2/58), 23 (grades 1 and 2 in 22 and 1 patient, respectively) (11.2%: 23/206) and 1 patient (grade 1) (4.3%: 1/23) for those subjected to αβ T-cell therapy, γδ T-cell therapy, DC vaccine therapy, and concurrent αβ T- cell and DC vaccine therapy, respectively (Table V). The incidence rate of itching was significantly lower in patients subjected to αβ T-cell therapy than in those injected with DC vaccines ($p<0.05$), although there was no notable difference between patients subjected to αβ T- and γδ T-cell therapies. The incidence rate of itching was significantly lower after the second and following treatment than after the first treatment for the patients subjected to αβ T-cell therapy because the cells were washed twice immediately before injection when an allergic reaction occurred after the initial injection of the effector cells (data not shown).

Reactions at injection sites. For the evaluation of skin reactions at injection sites, the incidence rates were 9.7% (20/206) in patients injected with DC vaccines (grades 1 and 2 in 18 and two patients, respectively) and 4.3% (1/23) in patients subjected to concurrent αβ T-cell and DC vaccine therapy (grade 1). On the other hand, injection site reactions were very rare in the patients subjected to *i.v.* injection with effector cells, such as αβ T- or γδ T-cells (Table VI).

Other non-hematological toxicities. Of the 771 immune-cell therapies, grade 1 pain developed in 18 patients (10 with αβ T-cell therapy, 2 with γδ T-cell therapy, and 6 with DC vaccine therapy), and neither of the pain incidences were associated with immune-cell therapy. Grade 3 urticaria was found in 1 patient subjected to DC vaccine therapy and grade 1 urticaria developed in 1 patient subjected to γδ T-cell therapy. Allergic reaction is one of known side-effects of immune-cell therapy in the Seta Clinic Group as well as fever, fatigue, itching and injection site reaction. Grade 2 dyspnea appeared with disease progression in the patients subjected to DC vaccine therapy, and grade 3 biliary and urinary tract infection, which were not associated with immune-cell therapy, were observed in a patient subjected to αβ T-cell therapy. Grade 1 constipation, diarrhea, chill, edema and dysgeusia were found in patients treated with αβ T-cell therapy, and nausea was found in those treated with DC vaccine therapy (Table VII). In addition, no autoimmune reaction was observed in any of the patients. All these non-hematological toxicity events were related to disease progression or side-effects of chemotherapy, and no unknown adverse event related to immune-cell therapy was observed.

Hematological toxicity. In the present study, more than half of the enrolled patients were subjected to concurrent immune-cell therapy and standard cancer therapy. Out of the 771 immune-cell therapies, grade 3 hematological adverse events were observed in 46 patients (34 with αβ T-cell therapy, two with γδ T-cell therapy, and 10 with DC vaccine therapy), and most of the adverse events were not associated with immune-cell therapy (Table VIII). Most of the hematological toxicity events, such as anemia or liver dysfunction, were related to disease progression or side-effects of chemotherapy, and no adverse event related to immune-cell therapy was observed. For one patient with gastric cancer, grade 3 neutropenia was observed after the initial injection of αβ T-cells as monotherapy. The grade 3 neutropenia might have been associated with some oral

Table VIII. *Grade 3 hematological toxicities for various immune-cell therapies with or without chemotherapy.*

		Type of cell				Total (n=771) (%)
		αβT (n=484) (%)	γδT (n=58) (%)	DC (n=206) (%)	αβT+DC (n=23) (%)	
Marrow	Neutrophil	2 (0.4)	0	2 (1.0)	0	4 (0.5)
	Leukocyte	1 (0.2)	0	1 (0.5)	0	2 (0.3)
	Hemoglobin	7 (1.4)	0	3 (1.5)	0	10 (1.3)
	Platelet	0	0	0	0	0
Liver	ALP	5 (1.0)	1 (1.7)	0	0	6 (0.8)
	-GTP	11* (2.3)	1 (1.7)	3 (1.5)	0	15 (1.9)
	AST (GOT)	2 (0.4)	0	0	0	2 (0.3)
	ALT (GPT)	0	0	0	0	0
	T-Bil	5 (1.0)	0	0	0	5 (0.6)
	Albumin	1 (0.2)	0	0	0	1 (0.1)
Kidney	Cr	0	0	1 (0.5)	0	1 (0.1)
Total		34 (7.0)	2 (3.4)	10 (4.9)	0 (0)	46 (6.0)

ALP: alkaline phosphatase; γ-GTP: γ-glutamyltranspeptidase; AST (GOT): aspartate aminotransferase (glutamic-oxaloacetic transaminase); ALT (GPT): alanine aminotransferase (gutamic-pyruvic transaminase); T-Bil: total bilirubin. *Two patients had grade 4 γ-GTP level elevation among the 11 patients.

medicines, because the neutrophil number recovered after discontinuing the drugs, although the patients continued the αβ T-cell monotherapy.

Discussion

Several methods of immune-cell therapy have become available, such as adoptive T-cell immunotherapy using *ex vivo*-expanded cytotoxic T-lymphocytes (9-11). Various DC-based vaccines have also been employed in clinical trials or used in clinical practice of cancer immunotherapy (12, 13). The Seta Clinic Group is one of the pioneers that introduced immune-cell therapy against cancer in Japan, which was established in 1999 as a private clinic specializing in immune-cell therapy (14). Today, the Seta Clinic Group provides several types of immune-cell therapy, such as αβ T-cell, γδ T-cell, natural killer cell or DC vaccine therapy. The greatest advantage of this therapy may potentially be the absence of harmful side-effects, whereas standard therapies including chemotherapy have profound side-effects and suffer from limitations in efficacy (15). We also previously reported that the efficacy of immunotherapy against advanced lung cancer is limited but may extend the lifespan of the patient under certain conditions. Patients whose PS is 2 or more enjoyed the benefits of these immune-cell therapies and maintain their quality of life, although they were unable to receive chemotherapy in clinical practice (5). Koretz *et al*. reported that adverse events such as chills, anorexia and nausea were observed during their randomized trial for IL2 *versus* IL2 plus lymphokine-activated killer cells in 38 patients with either melanoma or renal cell cancer (16). Ardon *et al*. also reported 38 serious adverse events in their clinical trial for DC-based tumor vaccination in 77 patients with glioblastoma (17). In the present study, surveillance of adverse events was re-evaluated, using large-scale prospective data from over 100 cancer patients undergoing immune-cell therapy at the Seta Clinic.

For the assessment of fever, fatigue and itching, each adverse event was found to be grade 1 or 2 in most of the treated patients, except 1 patient who had grade 3 itching after the infusion of αβ T-cells. The incidence rate of fatigue was slightly higher in the patients subjected to αβ T-cell therapy than in those who were injected with DC vaccines. Additionally, it was observed that low-grade fever appeared earlier in the patients infused with αβ T-cells than in those injected with DC vaccines. For immune-cell therapy, the cause of the adverse events may be related to the immune reaction elicited by the encounter between the injected cells and the host immune system. The present data suggest that αβ T-cell therapy could elicit a more rapid and direct immune reaction in the patients than DC vaccine therapy. For the patients infused with γδ T-cells, the incidence rates of fatigue and itching were essentially equivalent to those for the patients infused with αβ T-cells, although there were no patients who developed a fever after γδ T-cell therapy.

Some investigators found that injection site reactions were the most common events, similar to the present results that showed that injection site reactions developed in 10.2% of the patients injected with DC vaccines (18). Fatigue is one of the most common side-effects caused by not only immune-cell therapy but also by chemotherapeutic drugs. In

the patients subjected to immune-cell therapy, the incidence rate of fatigue could be more accelerated than that for fever or itching induced by an immunogenic reaction, because more than half of the patients underwent concurrent immune-cell therapy and chemotherapy.

The side-effects of immune-cell therapy were reported to be mainly due to IL2 administration (16,19). For αβ T- or γδ T-cell therapy, IL2 is used for the *ex vivo* expansion of these effector cells at 2- to 3-week culture intervals. Infinitesimal IL2 remaining in the harvested cells is administered to the patients, even after most of the IL2 is removed by washing. Itching could be caused by residual IL2, because additional washing of the harvested cells reduced the incidence rate of itching in the patients infused with αβ T-cells. One potential disadvantage of immune-cell therapy, particularly DC vaccines, is that it could lead to the generation of an autoimmune response. In most of the trials of DC-based cancer vaccines, no notable organ toxicity or autoimmune response was identified, although autoimmune toxicity associated with DC vaccines was reported in a melanoma vaccine trial (20, 21). In the present study, no autoimmune response was observed in any of the treatments. For the assessment of adverse events except fever, fatigue, itching and injection site reactions, most of the adverse events were not associated with immune-cell therapy. Additionally, for the hematological adverse events, most of the toxicity events were related to disease progression or side-effects of chemotherapy, and no adverse event related to immune-cell therapy was found.

In conclusion, the present study shows that immune-cell therapy, such as αβ T-cell, γδ T-cell, or DC vaccine therapy was well-tolerated, and the administration of these cells did not enhance toxicity when the patients were subjected to concurrent immune-cell therapy and chemotherapy.

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