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Safety evaluation of immune-cell therapy for malignant tumor in the Cancer Immune-cell Therapy Evaluation Group (CITEG)

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ABSTRACT

Background aims: With the aim of strengthening the scientific evidence of immune-cell therapy for cancer and further examining its safety, in October 2015, our hospital jointly established the Cancer Immune-Cell Therapy Evaluation Group (CITEG) with 39 medical facilities nationwide.
Methods: Medical information, such as patients' background characteristics, clinical efficacy and therapeutic cell types obtained from each facility, has been accumulated, analyzed and evaluated by CITEG. In this prospective study, we analyzed the adverse events associated with immune-cell therapy until the end of September 2022, and we presented our interim safety evaluation.
Results: A total of 3839 patients with malignant tumor were treated with immune-cell therapy, with a median age of 64 years (range, 13–97 years) and a male-to-female ratio of 1:1.08 (1846:1993). Most patients' performance status was 0 or 1 (86.8%) at the first visit, and 3234 cases (84.2%) were advanced or recurrent cases, which accounted for the majority. The total number of administrations reported in CITEG was 31890, of which 960 (3.0%) showed adverse events. The numbers of adverse events caused by treatment were 363 (1.8%) of 19661 administrations of αβT cell therapy, 9 of 845 administrations of γδT-cell therapy (1.1%) and 10 of 626 administrations of natural killer cell therapy (1.6%). The number of adverse events caused by dendritic cell (DC) vaccine therapy was 578 of 10748 administrations (5.4%), which was significantly larger than those for other treatments. Multivariate analysis revealed that αβT cell therapy had a significantly greater risk of adverse events at performance status 1 or higher, and patients younger than 64 years, women or adjuvant immune-cell therapy had a greater risk of adverse events in DC vaccine therapy. Injection-site reactions were the most frequently reported adverse events, with 449 events, the majority of which were associated with DC vaccine therapy. Among all other adverse events, fever (228 events), fatigue (141 events) and itching

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(131 events) were frequently reported. In contrast, three patients had adverse events (fever, abdominal pain and interstitial pneumonia) that required hospitalization, although they were weakly related to this therapy; rather, it was considered to be the effect of treatment for the primary disease.
Conclusions: Immune-cell therapy for cancer was considered to be a safe treatment without serious adverse events.

Introduction

Immunotherapies against cancer are undergoing drastic changes with the advent of immune checkpoint inhibitors (ICIs) [1]. The biomarkers related to the efficacy of ICIs have been investigated, and it has been found that the expression of programmed cell death-ligand 1 in tumor, tumor mutation burden and host immune status might affect the clinical response [2–4]. In particular, the difference in the immune function of the host might be one of the major factors affecting the clinical efficacy of ICIs [4,5].

Adoptive immune-cell therapy is a type of treatment in which the patient's immune cells are amplified *ex vivo* and returned to the body to activate the immune function and antitumor effect [6,7]. Meta-analysis of studies of immune-cell therapies, such as activated T lymphocyte, cytokine-induced killer cell and dendritic cell (DC) vaccine therapies, has shown their effectiveness in gastric cancer and lung cancer [8,9]. Furthermore, double-blind studies also have demonstrated their effectiveness against hepatocellular carcinoma and prostate cancer [10–12]. We and others have reported that the combination of adoptive immune-cell therapy and a standard therapy for cancer was beneficial and brought about a better prognosis than those with a standard therapy alone in terms of overall survival [7,13-16]. Moreover, it has been demonstrated that immune-cell therapies also were useful as postoperative adjuvant therapies for various cancers [17–21]. Thus, it is conceivable that the therapeutic effect of ICIs can be enhanced while improving the patient's immune status.

In fact, we experienced treating a case of esophageal cancer in which complete remission was observed when a low dose of an ICI was combined with an immune-cell therapy using $\alpha\beta$T cells [5]. Furthermore, it also has been demonstrated that even a low dose of an ICI may be effective if the immune status of the host is maintained [22]. Therefore, adoptive immune-cell therapy may increase the therapeutic effect in the future when combined with ICIs. However, there are several concerns about the frequency and severity of adverse events due to the combination of ICIs and immune-cell therapies; therefore, sufficient verification of adverse events associated with immune-cell therapies is essential.

In October 2015, we collaborated with 39 medical facilities nationwide and established the "Cancer Immune-cell Therapy Evaluation Group (CITEG) in order to collect, analyze and evaluate the clinical efficacy and safety of immune-cell therapies on the basis of medical information such as patients' background characteristics, diagnosis, age, sex, performance status (PS) and type of immune-cell therapy. In this prospective study, we analyzed the adverse events associated with immune-cell therapies in 31 890 administrations in 3839 cases collected by the end of September 2022 and conducted an interim evaluation of its safety. Such a study with large-scale, real-world data is unprecedented and is considered to provide extremely important information.

Patients and Methods

Patients

In October 2015, we launched CITEG jointly with medical facilities nationwide (39 facilities) to collect and analyze medical information (e.g., background characteristics, clinical efficacy and the type of immune-cell therapy) (jRCTc030190249, jRCTc030190251, jRCTc030190254, jRCTc030190256). The criteria for the selection of patients in the present study were as follows: (i) diagnosed as having malignancy; (ii) adequate bone marrow, liver and renal functions; (iii) no uncontrolled heart disease, interstitial pneumonia or autoimmune disease and (iv) negative serological tests for HIV. This study is being conducted as paid clinical trial and the patient is responsible for the cost of immune-cell therapy. This study was approved by the Research Ethics Committee of the Seta Clinic Group on September 30, 2015, and all the patients provided written informed consent.

In this prospective study, from October 2015 to the end of September 2022, the content of treatment, the type of immune-cell therapy, the number of administrations and adverse events in malignant tumor cases (3839 cases) with immune-cell therapies at facilities belonging to CITEG were tabulated and safety was evaluated.

Ethics approval and consent to participate

Each center's institutional review board or ethics committee approved the study. The trial followed the principles of the Declaration of Helsinki and the Japanese Ethical Guidelines for Clinical Research. All patients provided their written informed consent including the publication.

Immune-cell therapy

Autologous cells were used as the cell source for all immune-cell therapies administered in this clinical trial. For effector cell therapy, we prepared $\alpha\beta$T-cells cultured *ex vivo* with interleukin-2 (IL-2) and an immobilized antibody to CD3 or $\gamma\delta$T-cells cultured *ex vivo* with IL-2 and bisphosphonate [7,23]. Natural killer (NK) cells were prepared according to the method of NKBIO Co. [24]. 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 IL-4 and granulocyte/macrophage colony-stimulating factor. The DCs pulsed with tumor-specific peptides or the autologous tumor lysate were injected subcutaneously into the patients with various types of cancer [25]. The combination of an immune-cell therapy with surgical operation, chemotherapy, radiotherapy, molecular targeting therapy or endocrine therapy was not prohibited, although the immune-cell therapy was carried out on a different day to avoid cytotoxic damage of $\alpha\beta$T-cells, $\gamma\delta$T cells, NK cells or DCs when the patients underwent a standard conventional therapy.

Assessment of toxicity

As reported previously, we investigated the adverse events associated with nonhematological toxicities after every treatment using a questionnaire and by interviews with doctors or medical staff members [26]. In the questionnaire, 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 therapies. In the questionnaire, a column also was provided for comments on any side effects or symptoms. As for the adverse events, we extracted all the adverse events that were possibly related to immune-cell therapies, and their grades were

evaluated after every treatment based on National Cancer Institute Common Terminology Criteria for Adverse Events, ver.4.0.

Statistical analyses

To determine the odds ratio (OR) for adverse events caused by immune-cell therapies, univariate and multivariate logistic regression analyses were performed. The χ^2 test or Welch test were used to analyze the statistically significant difference between multiple groups. All statistical analyses were either one-sided or two-sided and performed using JMP, version 15.0.0 for Microsoft Windows 10 (SAS, Cary, NC, USA). Results were considered statistically significant when $P < 0.05$.

Results

The patients' background characteristics are represented in Table 1. A total of 3839 patients were enrolled in this study, with a median age of 64 years, 2005 (52.2%) aged ≥64 years and 1834 (47.8%) aged <64 years. There were 1846 male patients (48.1%) and 1993 female patients (51.9%). Regarding their general condition, 2552 of the 3839 patients (66.5%) had an Eastern Cooperative Oncology Group PS score of 0 and 781 patients had a score of 1 (20.3%). Most of the patients who received immune-cell therapies (3234 patients, 84.2%) had advanced or recurrent diseases. In addition, 484 patients (12.6%) received immune-cell therapies to prevent disease recurrence. In total, 507 cases (21.3%) were treated with immune-cell therapy alone without any combination therapy, whereas 873 cases (36.7%) were treated with immune-cell therapy in combination with chemotherapy. As shown in Table 2, pancreatic, colorectal, lung, gastric and breast cancers accounted for the majority, and uterine, ovarian, liver and esophageal cancers also were included. There was no significant difference in the occurrence of adverse events by tumor type.

Table 1
Patients' background characteristics.

Characteristic	Number (%)
Total, N	3839
Median age 64 y (range 13-97 y)	
≥64 y	2005 (52.2)
<64	1834 (47.8)
Sex	
Male	1846 (48.1)
Female	1993 (51.9)
PS	
0	2552 (66.5)
1	781 (20.3)
2	242 (6.3)
3	142 (3.7)
4	45 (1.2)
Unknown	77 (2.0)
Clinical status	
Advanced/recurrent	3234 (84.2)
Adjuvant	484 (12.6)
Others	121 (3.2)
Combination therapy (n = 2381)	
None	507 (21.3)
Surgical operation	37 (1.6)
Chemotherapy	873 (36.7)
Radiotherapy	47 (2.0)
CT + RT	80 (3.4)
Molecular targeting therapy	53 (2.2)
Endocrine therapy	66 (2.8)
SO + CT	126 (5.3)
CT + MT	208 (8.7)
SO + CT + RT	31 (1.3)
SO + CT + MT	30 (1.3)
ICI	25 (1.0)
Others[a]	298 (12.5)

CT, chemotherapy; ICI, immune checkpoint inhibitors; MT, molecular targeting therapy; N, number of patients; PS, performance status; RT, radiotherapy; SO, surgical operation.
[a] Others indicate hyperthermia, transcatheter arterial chemoembolization and bisphosphonate.

A total of 31890 doses of immune-cell therapy were administered to 3839 patients over 7 years until September 30, 2022. Of these, 960 (3.0%) were reported to have adverse events (Table 3). Adverse events occurred within 2 days after administration in more than 95% of the patients, as shown in Figure 1A. Adverse events occurring later were considered to be associated with the existing disease or concomitant treatments such as chemotherapy, radiotherapy and surgical operation. Table 3 shows the number of adverse events of each treatment. $\alpha\beta$T-cell therapy was administered 19661 times to 3470 patients, with 363 adverse events (1.8%). $\gamma\delta$T cell therapy was administered to 175 patients 845 times with nine adverse events (1.1%), and NK cell therapy was administered to 164 patients (626 times) with 10 adverse events (1.6%). DC vaccine therapy was performed 10748 times in 1416 patients, and there were 578 adverse events (5.4%). The incidence of adverse events in DC vaccine therapy was significantly greater than that of other immune-cell therapies, and $\alpha\beta$T cell therapy had significantly more adverse events than $\gamma\delta$T cell therapy (Table 3). When the frequency of occurrence of each adverse event was tabulated for each administration frequency, $\alpha\beta$T, $\gamma\delta$T and NK cells of effector cells often occurred within 3–4 times, as shown in Figure 1B. In contrast, there was a risk of adverse events occurring after the fifth infusion with DC vaccine therapy. Because the incidence of these adverse events is attributable to an aggregation of factors that include the concomitant use of other immune-cell therapies, we investigated and analyzed the adverse events in $\alpha\beta$T cell therapy or DC vaccine therapy alone, which had a significantly higher frequency of adverse events than others.

As shown in Table 4, we analyzed the clinical background factors that are likely to cause adverse events due to $\alpha\beta$T-cell therapy. As a result, multivariate analysis showed that $\alpha\beta$T-cell therapy significantly increased the risk of adverse events in PS1 or higher (PS1 ≤: $P = 0.0015$, OR 1.478, 95% confidence interval [CI] 1.161–1.881). In comparison, the risk of adverse events was conversely low when used for adjuvant therapy or in combination with molecular targeting therapy, endocrine therapy and others (adjuvant: $P = 0.0182$, OR 0.632, 95% CI 0.432–0.925; molecular targeting therapy: $P = 0.0425$, OR 0.231, 95% CI 0.056–0.952; endocrine therapy: $P = 0.0194$, OR 0.186, 95% CI 0.045–0.762; other treatment: $P = 0.0250$, OR 0.579, 95% CI 0.359–0.934). Similarly, when the risk of adverse events associated with DC was analyzed by clinical background factors, multivariate analysis revealed that younger than 64 years of age, female or adjuvant therapy were significantly more likely to develop adverse events (<64; $P = 0.0205$, OR 1.258, 95% CI 1.036–1.528: female; $P < 0.0001$, OR 1.717, 95% CI 1.409–2.093: Adjuvant; $P = 0.0034$, OR 1.372, 95% CI 1.110–1.695). In contrast, the risk of adverse events was conversely low in combination therapy cases such as surgical operation alone or surgical operation with chemotherapy (surgical operation: $P = 0.0252$, OR 0.104, 95% CI 0.014–0.755; surgical operation with chemotherapy: $P = 0.0376$, OR 0.524, 95% CI 0.285–0.964).

Specific details of adverse events are shown in Table 5. Injection-site reactions were the most frequent, accounting for 94.2% in DC vaccine therapy. Fever and fatigue have been reported frequently as adverse events associated with $\alpha\beta$T-cell therapy, whereas itching has also been frequently reported as a local symptom associated with DC vaccine therapy. The frequency of these adverse events was almost the same as those previously reported [26].

Here, we describe three cases of patients in whom adverse events were serious and required hospitalization (Table 5). Grade III fever was observed in a 70-year-old woman with cervical cancer who underwent chemotherapy (paclitaxel and carboplatin). On the 12th day of the chemotherapy, when $\gamma\delta$T-cell therapy was performed, a

Table 2
Primary diagnosis.

Primary site	Number of patients, n (%)	CTCAE grade		Incidence rate[a]
		Grade 0	Grade ≤1	
Pancreas	726 (18.9)	650	76	10.5%
Colorectum	555 (14.5)	498	57	10.3%
Lung	343 (8.9)	319	24	7.0%
Stomach	312 (8.1)	281	31	9.9%
Breast	279 (7.3)	240	39	14.0%
Uterine cervix	249 (6.5)	219	30	12.0%
Liver	205 (5.3)	179	26	12.7%
Ovary	195 (5.1)	163	32	16.4%
Biliary tract	125 (3.3)	113	12	9.6%
Esophagus	124 (3.2)	114	10	8.1%
Prostate	115 (3.0)	102	13	11.3%
Multiple	58 (1.5)	54	4	6.9%
Gallbladder	57 (1.5)	51	6	10.5%
Urinary bladder	52 (1.4)	49	3	5.8%
Kidney	35 (0.9)	32	3	8.6%
Brain	33 (0.9)	29	4	12.1%
Non-Hodgkin lymphoma	27 (0.7)	27	0	0.0%
Others	349 (9.0)	298	51	14.6%

AE, adverse events; CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events.
[a] Not significant.

high fever appeared. Febrile neutropenia was determined to be myelosuppression due to anticancer drug therapy, but it was difficult to distinguish fever associated with immune-cell therapy. The next case in which abdominal pain occurred corresponding to a grade 3 adverse event was a 40-year-old woman with liver, lung and lymph node metastases of uterine cancer. The patient was in palliative care because she was refractory to chemotherapy, and $\alpha\beta$T therapy was performed when the primary disease was exacerbating. On the second day after administration of $\alpha\beta$T-cell therapy, the abdominal pain worsened, and computed tomography revealed that it was accompanied by swelling of the liver with metastasis. In another case of a 70-year-old woman with lung and bone metastases after surgery for rectal cancer, $\alpha\beta$T-cell therapy was started about 2 months after radiotherapy for bone metastasis. About 1 and a half months after the start of $\alpha\beta$T-cell therapy, cough and shortness of breath developed, and interstitial pneumonia was diagnosed. Interstitial pneumonia was considered to be highly associated with radiotherapy for bone metastasis, but since it occurred after $\alpha\beta$T-cell therapy, it seemed that a causal relationship could not be denied, and it was judged that there was a weak relationship.

As mentioned previously, immune-cell therapies for cancer can be safely performed without serious adverse events. However, in patients after radiotherapy, patients undergoing anticancer drug treatment and patients with worsening symptoms, immune-cell therapies should be carried out with caution.

Discussion

We previously examined the adverse events of immune-cell therapies and demonstrated their safety, but additional data are required owing to the small number of patients in that study [26]. In this present prospective study conducted in collaboration with CITEG, we further accumulated a larger number of cases and examined adverse events associated with immune-cell therapies by large-scale real-world evidence based on the treatment records of more than 30 000 infusions in nearly 4000 cases.

Immune-cell therapies were performed for highly prevalent malignant tumors, including pancreatic, colorectal, lung, stomach and breast cancers. The results of this study showed no significant difference in the incidence of adverse events among tumors (Table 2). This was

Table 3
Overall incidence rates of AEs associated with immune-cell therapy.

All patients	Cumulative research period (September 30, 2015, to September 29, 2022)			
Number of patients				3839
Number of administrations				31890
Number of AEs				960
Incidence rate of AE (%)				3.0
Type of immune-cell therapy				
Type of immune-cell therapy	Number of patients	Number of administrations	AEs	
			Number of AEs	Incidence rate (%)
$\alpha\beta$T	3470	19661	363	1.8[a]
$\gamma\delta$T	175	845	9	1.1
NK	164	626	10	1.6
DC	1416	10748	578	5.4[b]

AE, adverse event; DC, dendritic cell vaccine therapy; NK, natural killer cell therapy.
[a] $P = 0.0331$ ($\alpha\beta$T vs $\gamma\delta$T).
[b] $P < 0.0001$ (DC vs $\alpha\beta$T, $\gamma\delta$T and NK).

Table 4
Risk factors for adverse events in relation to patients' background characteristics.

Characteristic	Number of administrations	CTCAE grade		Univnterariate analysis			Multivariate analysis		
		Grade 0	Grade ≤1	*P* value	OR	95% CI	*P* value	OR	95% CI
Univariate and multivariate analyses of adverse events in $\alpha\beta$T-cell therapy									
Total, N	17478	17158 (98.2%)	320 (1.8%)						
Median age, y (range) 64 (13-97)									
≥64, n (%)	9594 (54.9%)	9419 (98.2%)	175 (1.8%)	–	1				
<64, n (%)	7884 (45.1%)	7739 (98.2%)	145 (1.8%)	0.9409	1.008	0.808–1.259			
Sex, n (%)									
Male	8176 (46.8%)	8043 (98.4%)	133 (1.6%)		1				
Female	9302 (53.2%)	9115 (98.0%)	187 (2.0%)	0.0596	1.241	0.991–1.553			
PS, n (%)									
0	12995 (74.4%)	12786 (98.4%)	209 (1.6%)		1			1	
≤1	4483 (25.6%)	4372 (97.5%)	111 (2.5%)	**0.0002**	**1.553**	**1.231–1.960**	**0.0015**	**1.478**	**1.161–1.881**
Clinical status, n (%)									
Advanced/recurrent	14347 (82.1%)	14067 (98.0%)	280 (2.0%)		1			1	
Adjuvant	2599 (14.9%)	2567 (98.8%)	32 (1.2%)	**0.0127**	**0.626**	**0.433–0.905**	**0.0182**	**0.632**	**0.432–0.925**
Others	532 (3.0%)	524 (98.5%)	8 (1.5%)	0.4629	0.767	0.378–1.557	0.4563	0.763	0.374–1.555
Combination (n = 2381)									
None	2615 (15.0%)	2552 (97.6%)	63 (2.4%)		1			1	
Surgical operation	191 (1.1%)	189 (99.0%)	2 (1.0%)	0.2408	0.429	0.104–1.765	0.3090	0.479	0.116–1.977
Chemotherapy	5076 (29.0%)	4973 (98.0%)	103 (2.0%)	0.2779	0.839	0.611–1.152	0.1561	0.793	0.575–1.093
Radiotherapy	200 (1.1%)	191 (95.5%)	9 (4.5%)	0.0759	1.909	0.935–3.897	0.1050	1.809	0.883–3.705
CT + RT	498 (2.8%)	489 (98.2%)	9 (1.8%)	0.4144	0.746	0.368–1.509	0.3081	0.692	0.340–1.405
Molecular targeting therapy	332 (1.9%)	330 (99.4%)	2 (0.6%)	0.0513	0.246	0.060–1.008	**0.0425**	**0.231**	**0.056–0.952**
Endocrine therapy	431 (2.5%)	429 (99.5%)	2 (0.5%)	**0.0206**	**0.189**	**0.046–0.775**	**0.0194**	**0.186**	**0.045–0.762**
SO + CT	725 (4.1%)	713 (98.3%)	12 (1.7%)	0.2281	0.682	0.366–1.271	0.2044	0.668	0.358–1.246
CT + MT	1139 (6.5%)	1120 (98.3%)	19 (1.7%)	0.1556	0.687	0.409–1.153	0.1236	0.661	0.391–1.120
SO + CT + RT	144 (0.8%)	143 (99.3%)	1 (0.7%)	0.2124	0.283	0.039–2.057	0.1583	0.240	0.033–1.744
SO + CT + MT	120 (0.7%)	119 (99.2%)	1 (0.8%)	0.2871	0.340	0.047–2.475	0.2360	0.301	0.041–2.192
ICI	120 (0.7%)	119 (99.2%)	1 (0.8%)	0.2871	0.340	0.047–2.475	0.2623	0.321	0.044–2.340
Others	1660 (9.5%)	1636 (98.6%)	24 (1.4%)	**0.0315**	**0.594**	**0.370–0.955**	**0.0250**	**0.579**	**0.359–0.934**
Univariate and multivariate analyses of adverse events in DC vaccine therapy									
Total, N	8406	7920 (94.2%)	486 (5.8%)						
Median age, y (range) 64 (13-97)									
≥64, n (%)	3670 (43.7%)	3489 (95.1%)	181 (4.9%)	–	1			1	
<64, n (%)	4736 (56.3%)	4431 (93.6%)	305 (6.4%)	**0.0034**	**1.327**	**1.098–1.603**	**0.0205**	**1.258**	**1.036–1.528**
Sex, n (%)									
Male	3845 (45.7%)	3685 (95.8%)	160 (4.2%)		1			1	
Female	4561 (54.3%)	4235 (92.9%)	326 (7.1%)	**<0.0001**	**1.773**	**1.460–2.153**	**<0.0001**	**1.717**	**1.409–2.093**
PS, n (%)									
0	7092 (84.4%)	6677 (94.1%)	415 (5.9%)		1				
≤1	1314 (15.6%)	1243 (94.6%)	71 (5.4%)	0.5226	0.919	0.709–1.191			
Clinical status, n (%)									
Advanced/recurrent	6064 (72.1%)	5748 (94.8%)	316 (5.2%)		1			1	
Adjuvant	2052 (24.4%)	1898 (92.5%)	154 (7.5%)	**0.0001**	**1.476**	**1.209–1.802**	**0.0034**	**1.372**	**1.110–1.695**
Others	290 (3.4%)	274 (94.5%)	16 (5.5%)	0.8190	1.062	0.634–1.781	0.7243	1.099	0.650–1.858
Combination (n = 2381)									
None	1773 (21.1%)	1650 (93.1%)	123 (6.9%)		1			1	
Surgical operation	112 (1.3%)	111 (99.1%)	1 (0.9%)	**0.0362**	**0.121**	**0.017–0.873**	**0.0252**	**0.104**	**0.014–0.755**
Chemotherapy	2656 (31.6%)	2473 (93.1%)	183 (6.9%)	0.9515	0.993	0.783–1.258	0.6943	1.050	0.823–1.339
Radiotherapy	156 (1.9%)	152 (97.4%)	4 (2.6%)	**0.0432**	**0.353**	**0.129–0.969**	0.0625	0.382	0.139–1.052
CT + RT	121 (1.4%)	119 (98.3%)	2 (1.7%)	**0.0383**	**0.225**	**0.055–0.923**	0.1028	0.308	0.075–1.268
Molecular targeting therapy	165 (2.0%)	159 (96.4%)	6 (3.6%)	0.1102	0.506	0.220–1.167	0.1968	0.574	0.247–1.334
Endocrine therapy	256 (3.0%)	242 (94.5%)	14 (5.5%)	0.3825	0.776	0.439–1.371	0.3243	0.750	0.423–1.330
SO + CT	294 (3.5%)	282 (95.9%)	12 (4.1%)	0.0698	0.571	0.311–1.046	**0.0376**	**0.524**	**0.285–0.964**
CT + MT	691 (8.2%)	652 (94.4%)	39 (5.6%)	0.2454	0.802	0.553–1.163	0.5367	0.886	0.603–1.302
SO + CT + RT	77 (0.9%)	75 (97.4%)	2 (2.6%)	0.1548	0.358	0.087–1.474	0.1984	0.393	0.095–1.632
SO + CT + MT	103 (1.2%)	100 (97.1%)	3 (2.9%)	0.1250	0.402	0.126–1.288	0.1008	0.376	0.117–1.209
ICI	116 (1.4%)	112 (96.6%)	4 (3.4%)	0.1549	0.479	0.174–1.321	0.3433	0.610	0.219–1.696
Others[a]	684 (8.1%)	649 (94.9%)	35 (5.1%)	0.1005	0.723	0.492–1.065	0.1279	0.738	0.498–1.091

CI, confidence interval; CT, chemotherapy; CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; ICI, immune checkpoint inhibitors; MT, molecular targeting therapy; N, number of patients; OR, odds ratio; PS, performance status; RT, radiotherapy; SO, surgical operation.
Significant differece in bold.
[a] Others indicate hyperthermia, transcatheter arterial chemoembolization and bisphosphonate.

consistent with the risk of adverse events in ICI therapy being tumor-independent and likely affected primarily by the immune status of the host [1]. The overall incidence of adverse events was very low at 3% (Table 3). According to the type of immune-cell therapy, the frequency of adverse events was significantly greater in DC vaccine therapy than in other immune-cell therapies. In addition, it was found that patients younger than 64 years or female showed a significantly greater frequency of adverse events in DC vaccine therapy (Table 4). This may be related to the fact that young patients and female patient are more susceptible to immune responses such as allergic reactions [27,28]. Adverse events were more common in the group that used DC vaccine therapy as adjuvant therapy. This was thought to be due to the fact that many patients younger than the age of 64 or women were included in the adjuvant therapy group.

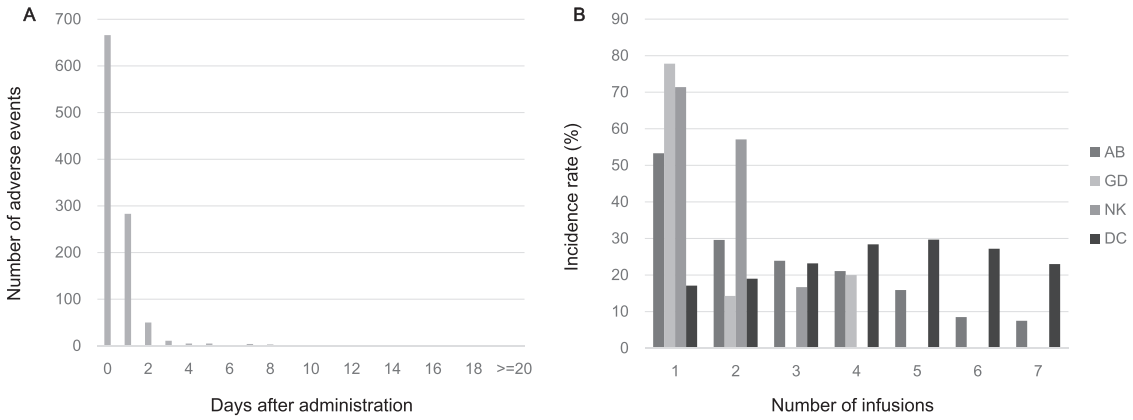

Figure 1. Numbers and frequency of adverse events associated with immune-cell therapies. (A) Number of adverse events days after administration. (B) Proportion of adverse events per dose in each immune-cell therapy. AB, $\alpha\beta$T-cell therapy; DC, dendritic cell vaccine therapy; GD, $\gamma\delta$T-cell therapy; NK, natural killer cell therapy.

Table 5
Details and number (rate) of adverse events for each immune-cell therapy.

	CTCAE grade		$\alpha\beta$T (%)	$\gamma\delta$T (%)	NK (%)	DC (%)
	Grade 1/2	Grade 3				
Injection-site reaction	449	0	26 (5.8%)	0 (0%)	0 (0%)	**423 (94.2%)**[a]
Fever	227	1	**175 (76.8%)**[a]	6 (2.6%)	1 (0.4%)	46 (20.2%)
Fatigue	141	0	**107 (75.9%)**[b]	3 (2.1%)	1 (0.7%)	30 (21.3%)
Itching	131	0	30 (22.6%)	1 (0.8%)	7 (5.3%)	**93 (71.0%)**[a]
Maculopapular rash	7	0	3 (42.9%)	0 (0%)	0 (0%)	4 (57.1%)
Headache	6	0	5 (83.3%)	0 (0%)	0 (0%)	1 (16.7%)
Diarrhea	5	0	3 (60.0%)	0 (0%)	1 (20.0%)	1 (20.0%)
Nausea	5	0	5 (100%)	0 (0%)	0 (0%)	0 (0%)
Abdominal pain	4	1	4 (80.0%)	0 (0%)	0 (0%)	1 (20.0%)
Allergic reaction	4	0	2 (50.0%)	0 (0%)	0 (0%)	2 (50.0%)
Chills	4	0	2 (50.0%)	2 (50.0%)	0 (0%)	0 (0%)
Vomiting	4	0	3 (75.0%)	0 (0%)	1 (25.0%)	0 (0%)
Urticaria	4	0	3 (75.0%)	0 (0%)	0 (0%)	1 (25.0%)
Back pain	2	0	1 (50.0%)	0 (0%)	0 (0%)	1 (50.0%)
Dyspnea	0	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)

CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; DC, dendritic cell vaccine therapy; NK, natural killer cell therapy.
Significant differece in bold.
[a] $P < 0.0001$.
[b] $P = 0.0006$.

In contrast, $\alpha\beta$T-cell therapy resulted in a significantly greater incidence of adverse events in patients with poor general conditions (PS1 or greater). This trend was similar to those in other reports [26,29,30]. It was speculated that $\alpha\beta$T-cell therapy has greater adverse events than other cell therapies because it has the potential to induce more specific reactions and be more powerful antitumor effects than other cell therapies. When the risk of adverse events in $\alpha\beta$T-cell therapy was examined for each concomitant therapy, it was found that the risk of adverse events was low in patients with concomitant therapies such as surgery, molecular targeting therapy, endocrine therapy and other therapies (Table 4). This may be due to factors such as the inclusion of many patients in a good general condition in each group. Finally, although the number of patients was small, it should be noted that the combination of DC vaccine therapy or $\alpha\beta$T-cell therapy with ICI does not increase the risk of adverse events.

Regarding NK cell therapy, minor adverse events such as chills have been reported [31], and we also observed 10 minor adverse events of 626 administrations, with a low frequency of 1.6% (Tables 3 and 5). As for the $\gamma\delta$T-cell therapy, we have already confirmed its safety [23], and we were able to reconfirm in this study that this therapy has no serious adverse events. Factors such as contamination with a small amount of IL-2 or the effect of dimethyl sulfoxide, a commonly used cryoprotectant, have been pointed out as factors for these adverse events, but these adverse events could be controlled by the administration of antihistamine or anti-inflammatory drugs.

As described previously, adverse events associated with immune-cell therapies were limited and not serious, and it was concluded that they can be safely implemented. ICIs, which are currently used as immunotherapeutic drugs, have insufficient therapeutic effects; therefore, their use in combination with anticancer drugs, molecular targeting drugs and other drugs may become mainstream. However, in order to enhance the effect of ICIs, it is essential to improve the immune status of the host [4]. Therefore, the safety of immune-cell therapy is extremely important to maintain the immune status of the host, and their use in combination with ICI should be promoted. Sufficient safety was confirmed in this study, and we are currently conducting a trial to test the safety and efficacy of immune-cell therapies in combination with low doses of ICIs.

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Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Author Contributions

Conception and design of the study: RT, TK and SG. Acquisition of data: Investigators of CITEG, SO, HI, EO, SO HI and EO. Analysis and interpretation of data: RT, SO, TK, SG and investigators of CITEG. Drafting or revising the manuscript: RT, TK, HI, MS, KT, KS, HY, KO, HT, KT, MM, EM, TM, YN, KH, SM, MK, AT, HK, TM, HS, KS, TY, ST, KM, KN, KY, YY, HI, KK, KO, SN, MO, YY, SO, HI, EO and SG. All authors have approved the final article.

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Data Availability

All data are available through the corresponding author.

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