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Table 2. Patient characteristics and adverse events after TCR gene–transduced lymphocyte transfer

Cohort	Cell doses allocated	Patient ID	Age/ sex	MAGE-A4 expressions				Previous therapy			Tumor lesions	Number of IFNγ$^+$ CD8$^+$ T-cell infused	Adverse events (grade)
				PCR[a]	IHC[b]/ 57B Ab	IHC[b]/ MCV-1 Ab	IHC[b]/ MCV-4 Ab	Surgery	Radiotherapy	Chemotherapy			
1	2×10^8	TCR-MA-102	68/M	2,880		NA		−	+	CDDP/5-FU	Liver	1.46×10^7	None
	2×10^8	TCR-MA-104	56/M	4,847	20%	20%	20%	−	+	CDDP/5-FU	Esophagus	1.24×10^7	None
	2×10^8	TCR-MA-106	73/M	2,215	10%	0	5%	−	+	CDDP/5-FU, TS-1	Esophagus, lymph node	1.48×10^7	Skin reaction()[d]
2	1×10^9	TCR-MA-208	67/M	7,942	30%	90%	10%	−	+	CDDP/5-FU	Lymph node[c]	6.8×10^7	None
	1×10^9	TCR-MA-209	57/M	1,352	70%	10%	50%	+	+	CDDP/5-FU	Lymph node	1.3×10^8	None
	1×10^9	TCR-MA-210	54/M	312	30%	20%	20%	−	−	CDDP/5-FU, Docetaxel	Esophagus, lung, lymph node	9.6×10^7	Skin reaction()[d]
	1×10^9	TCR-MA-212	43/M	1,765	20%	10%	5%	+	+	CDDP/5-FU, Docetaxel	Lymph node[c]	2.6×10^8	Skin reaction()[d]
3	5×10^9	TCR-MA-213	68/M	749		NA		+	+	CDDP/5-FU	Lymph node[c]	5.3×10^8	None
	5×10^9	TCR-MA-314	64/M	82	Focal	Focal	Focal	+	+	CDDP/5-FU	Lymph node	6.6×10^8	None
	5×10^9	TCR-MA-315	57/F	NA	20%	20%	20%	+	+	CDDP/5-FU	Lung, lymph node	9.75×10^8	Skin reaction()[d]

Abbreviations: NA, not available; 5-FU, 5-fluorouracil; CDDP, cisplatin.

[a]Copy numbers amplified by real-time PCR.

[b]Positive percentage in tumor samples by IHC staining.

[c]Minimal lesions, unable to evaluate by RECIST.

[d]Skin reactions were related to peptide vaccinations.

tetramer$^+$ and tetramer$^-$ fractions. The tetramer$^+$ CTLs were all originated from the transferred TCR-transduced cells (Fig. 3C). In patients TCR-MA-106, -208, and -212, tetramer$^+$ CD8$^+$ T cells were detected until days 119, 287, and 91, respectively (Fig. 3D).

To determine whether the transferred TCR-transduced CD8$^+$ T cells could react to MAGE-A4–expressing tumor cells, lymphocytes collected from 3 patients (TCR-MA-106, -208, and -212) were assayed by ELISPOT targeting the 11–18 tumor cell line, as well as cells pulsed with MAGE-A4 peptide. In all of these patients, the PBMCs were able to react to MAGE-A4–expressing tumor cells (Fig. 4) in an HLA class I–restricted manner.

Clinical course after transfer of MAGE TCR–transduced T cells

None of the 10 patients experienced any adverse events for the first 14 days after T-cell transfer. In 4 patients, we observed skin reactions such as redness and induration, graded as 1, at the peptide vaccine sites (Table 2). During the 63-day study period, 7 patients developed PD (progressive disease) within 2 months (Supplementary Fig. S6). Among them, 6 patients with tumor progression received subsequent rounds of chemotherapy. Seven patients survived for a median of 9 months (range, 3–15 months).

In TCR-MA-208, -212, and -213, who had minimal lesions at baseline, no disease progression was observed at 21, 26+, and 24+ months, respectively (Supplementary Fig. S6). TCR-MA-208 had a lymph node tumor in the mediastinum, which was treated with chemotherapy and radiotherapy. At the time of cell transfer, the tumor size was minimal, and no tumor activity was visible on an FDG ([^{18}F]fluoro-2-deoxyglucose)-PET (positron emission tomography) scan. The tumor did not enlarge for 21 months, at which time CT and FDG-PET scans revealed tumor relapse at another lymph node. Before entering our study, TCR-MA-212 had experienced multiple tumor recurrences in the lymph nodes and bones, all of which developed soon after chemotherapy and radiotherapy. At the time of cell transfer, no measurable tumors were detectable by CT scan, and FDG-PET scan revealed no active tumor uptake. To date, the patient has been free from disease progression for 26 months. A small tumor was seen in TCR-MA-213 along the esophageal–gastric anastomotic site (Supplementary Fig. S7). The tumor has not enlarged at the time of this writing, 24 months after the transfer of TCR gene–transduced lymphocytes. The uptake of FDG was still active 3 months after the transfer.

Discussion

In this clinical study, we tested the hypothesis that TCR gene–engineered T cells would exhibit the capacity to persist for long periods in cancer patients not subjected to lymphodepleting pretreatment. Peripheral blood–derived T cells transduced with MAGE-A4–specific TCRs were safely transferred into patients with esophageal cancer and persisted for long periods *in vivo*. Although the transferred cells maintained tumor-specific reactivity in patients, objective tumor regression was not observed. Dose-dependent appearance of the transferred cells was found in the peripheral blood in the first 14 days followed by the immediate decline and the long-term persistence in the patients at the low but detectable level, although the number of the transgene evaluated should be practical rather than absolute.

In clinical trials with T cells genetically engineered to express tumor-reactive receptors, a high level of persistence of the infused cells seems to be necessary but not sufficient for tumor regression.

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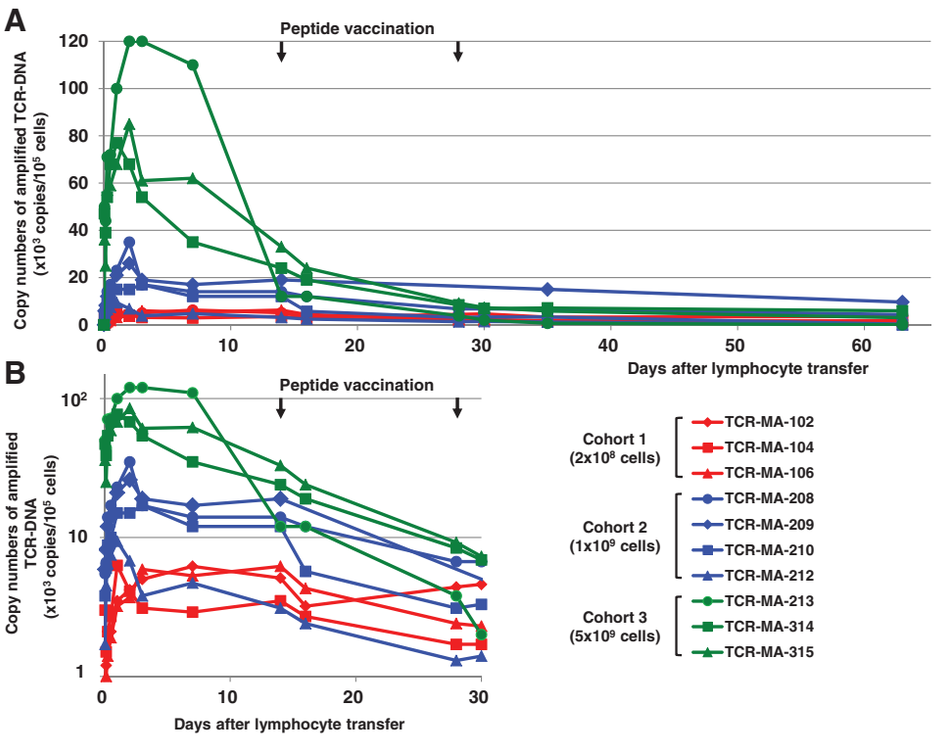

Figure 1.
Cell kinetics of MAGE-A4–specific TCR–transduced T cells after transfer into 10 patients. A, panels show kinetics of 3 patients who received 2×10^8 cells, 4 patients who received 1×10^9 cells, and 3 patients who received 5×10^9 cells. Peripheral blood was collected at baseline and at predetermined time points over a period of 63 days. DNA samples were extracted from the PBMCs, and TCR gene copy numbers were measured by qPCR. The detection limit of the transduced cells is 100 copies/10^5 cells. B, cell kinetics from day 1 to day 30. The kinetics are shown as logarithmic expressions. On days 14 and 28, MAGE-A4 peptides were given to all patients except TCR-MA-209. In cohort 3, the peak levels ranged from 80 to 120 ($\times 10^3$ copies/10^5 cells). The average number of the TCR-transgene per cell was 7 in the preinfusion state, which indicates that 11% to 17% of TCR gene–transduced T cells appeared in the peripheral blood.

In a study of MART-1–specific TCR–engineered T cells in patients with metastatic melanoma, 2 patients who experienced definite tumor regressions had persistent T cells in their peripheral blood for more than 1 year, as determined by measurements of DNA levels of the genetically marked cells (6). In that study, patients with clinical responses received T cells that had been subjected to shorter periods of *in vitro* culture than those administered to the patients that did not exhibit a response. Subsequent trials with high-affinity TCRs specific for the melanoma differentiation antigens MART-1 and gp100 (27), or the cancer-testis antigen NY-ESO-1 (7), utilized relatively briefly cultured cells for transfer, but reported either a mild correlation with some exceptions (27), or no correlation (7) between high-level persistence of transferred T cells and the clinical responses. A study of GD2-reactive CAR showed no correlation between tumor response and the dose of transferred cells or their detection level in peripheral blood (28). Recently, however, a successful trial of CD19-CAR therapy for hematopoietic malignancy reported sustained *in vivo* expansion and persistence of transferred cells in patients (9). However, the dose effects and threshold for durable clinical effects await further

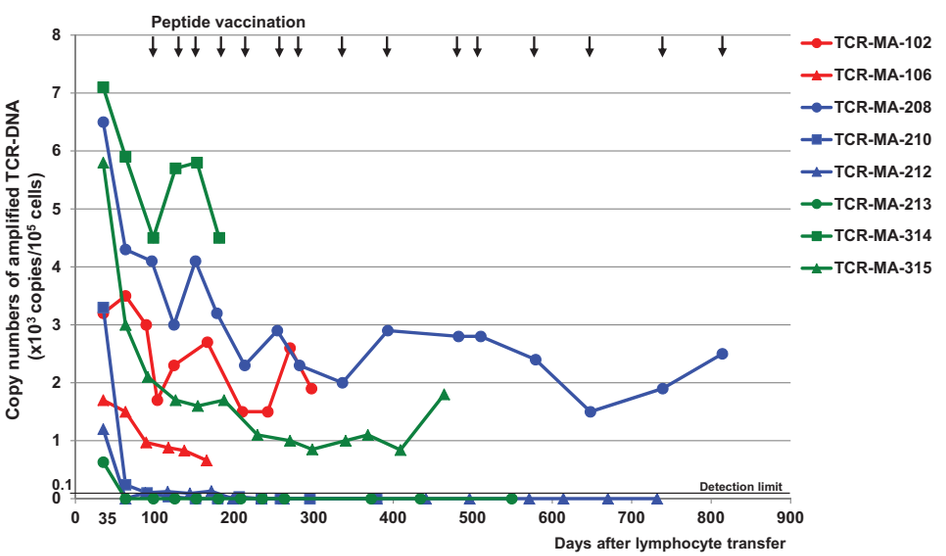

Figure 2.
Long-term cell kinetics of MAGE-A4–specific TCR–transduced T cells in 8 patients. Peripheral blood was collected on the day of patients' visits to the clinic after 35 days. DNA samples were extracted from the PBMCs, and TCR gene copy numbers were measured by quantitative PCR. The detection limit of the transduced cells is 100 copies/10^5 cells. MAGE-A4 peptides were also vaccinated on the same day, as indicated in the case of TCR-MA-208.

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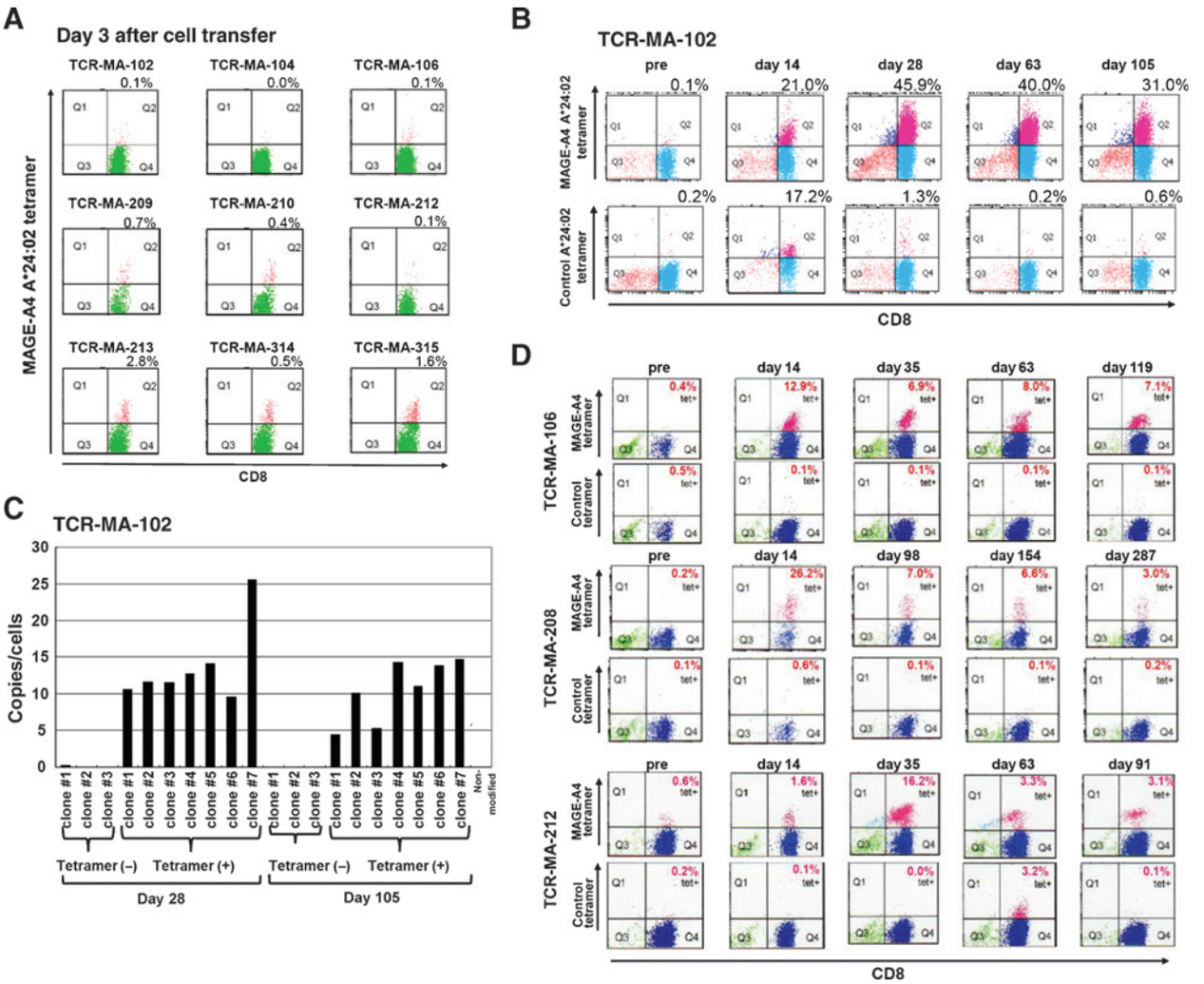

Figure 3.
Tetramer analysis of the TCR gene–transduced T cells at preinfusion and peripheral T cells after adoptive transfer of TCR-transduced T cells. A, PBMCs were collected 3 days after the adoptive transfer of TCR-transduced lymphocytes, and directly stained with MAGE-A4 peptide/HLA-A*24:02 tetramer in 9 patients. B, PBMCs collected from TCR-MA-102, before and after transfer of TCR-transduced lymphocytes. $CD8^+$ T cells were selected, stimulated *in vitro* with MAGE-A4 peptide, and assayed for tetramer of MAGE-A4 peptide/HLA-A*24:02. Irrelevant peptide/HLA-A*24:02 tetramers were used as a control. C, T-cell clones were established from PBMCs of TCR-MA-102 on days 28 and 105. The cloned cells were derived from the MAGE-A4 peptide/HLA-A*24:02 $tetramer^+$ and $tetramer^-$ fractions. D, PBMCs collected from TCR-MA-106, -208, and -212 before and after transfer of TCR-transduced lymphocytes. $CD8^+$ T cells were selected, stimulated *in vitro* with MAGE-A4 peptide, and assayed for MAGE-A4 peptide/HLA-A*24;02 tetramers.

analyses. In this study, we observed discordance between the persistence of transferred cells and tumor regression, consistent with the idea that prolonged persistence of the transferred cells is not a sufficient condition for efficient tumor control in adoptive therapy. Given that our protocol achieved a considerable degree of T-cell survival without lymphodepletive pretreatment, our data also support the idea that pretreatment enhances antitumor responses via multiple mechanisms, such as depletion of immunosuppressive cell populations, reduced competition for activating cytokines between endogenous and transferred cells, or increased functionality of APCs, as suggested in animal models (29, 30). Another possible explanation for the lack of tumor regression is that lymphodepletion was not required for persistence of infused cells, but in this case the cells simply did not reach the numerical threshold for tumor destruction. The transferred cells reached 2%–3% of PBMCs at most, declined after day 14, and persisted at much lower levels (e.g., 0.04%) after 1 month. Although some CAR trials reported clinical responses with similar levels of detectable infused cells in peripheral blood (8–12), other trials with TCR-engineered cells reported clinical responses in patients with higher levels of infused cells (6, 7, 27).

It has been demonstrated that the inhibitory tumor microenvironments in various tumor types impact their clinical prognoses. In esophageal tumors, PD-L1 or -L2 was expressed in approximately 40% in esophageal cancer (31), and M2 macrophages ($CD68^+CD163^+$ cells) infiltrated to more than half of esophageal tumor tissues (32). Although we did not analyze these inhibitory factors in patients' tumor samples, they might have played a role

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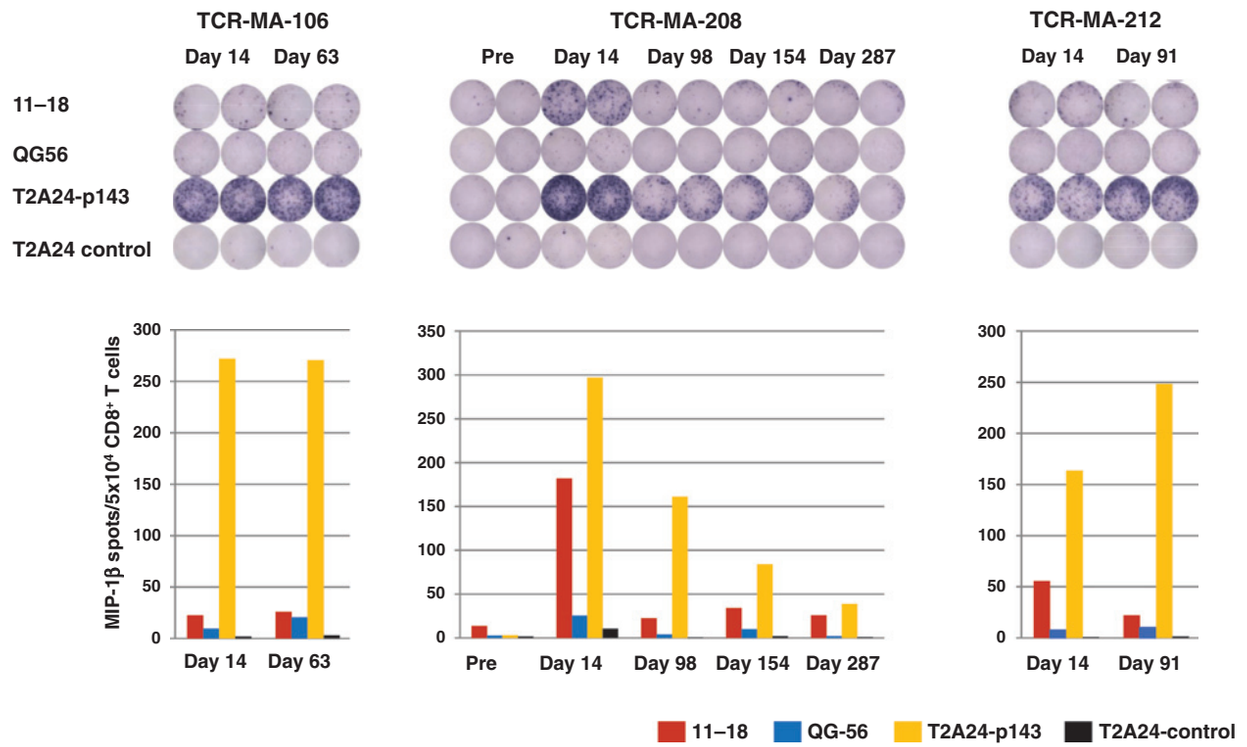

Figure 4.
Reactivity to MAGE-A4 peptide and MAGE-A4+ tumor cells in PBMCs after TCR-transduced T-cell transfer. PBMCs collected from TCR-MA-106, -208, and -212, before and after transfer of TCR-transduced lymphocytes. $CD8^+$ T cells were selected, stimulated *in vitro* with MAGE-A4 peptide, and subjected to ELISPOT assays. The target cells were 11–18 ($MAGE\text{-}A4^+$, $HLA\text{-}A^*24{:}02^+$), QG-56 ($MAGE\text{-}A4^+$, $HLA\text{-}A^*24{:}02^-$), and T2A24 cells pulsed with MAGE-A4 peptide. T2A24 cells pulsed with an irrelevant peptide were used as control targets.

in tumor responses in this study. The other issue we should consider is loss or decrease of tumor-antigen expression during tumor progression over the clinical course, although they were all positive at baseline in this study. In fact, in TCR-MA-104, the TCR-transferred T cells infiltrated a tumor site showing high expression of MAGE-A4 antigen, whereas in TCR-MA-210, whose tumor expressed lower antigen levels, no TCR transference was detected in the tumor.

We have initiated a clinical trial utilizing the same MAGE-A4 TCR–engineered cells accompanied by lymphodepletive pretreatment as a therapy for esophageal, head/neck, ovarian cancer, and melanoma patients. In the trial, we will evaluate whether the pretreatment regimen enhances the clinical response irrespective of whether the infused cells persist for increased periods of time.

In this study, 7 patients had measurable tumors, and none of these patients exhibited tumor shrinkage. Even after the tumors started to progress, the patients survived for a median of 9 months (range, 3–15 months) while receiving chemotherapy. Because the patients who entered this clinical trial had therapy-refractory disease, their survival times are considered to be quite long given their disease status (33, 34). Among the 10 patients, 3 have exhibited a long-term lack of disease progression. Although they had minimal disease at the time of cell transfer, they have all remained progression free for more than a year without any treatment other than T-cell transfer and MAGE-A4 peptide vaccine. This finding suggests that this sort of TCR-transduced T-cell therapy might be beneficial for patients bearing minimal tumors. In one patient, tumor activation was observed by FDG-PET uptake; this patient has exhibited stable disease for 24 months. On the basis of these observations, we conclude that MAGE-A4–targeting TCR-transduced T cells exert constant *in vivo* antitumor activity and may have clinical potential.

Nine of the patients received MAGE-A4 vaccines both on days 14 and 28. Although the patients' derived MAGE-A4-peptide–specific T cells were much expanded with *in vitro* peptide stimulation, these vaccinations did not increase the levels of infused cells in the peripheral blood; indeed, the levels declined in many cases. Because the MAGE-A4 peptide vaccine promoted inhibition of tumor growth when it was administered along with MAGE-A4 TCR–transduced T cells in our preclinical studies in NOD-SCID mice (35), we initially expected that the vaccine would stimulate TCR-transduced T cells, and that the levels of the infused cells would be elevated in peripheral blood. However, this was not the case. One possible explanation of this unexpected observation is that cognate peptide vaccine induced T-cell apoptosis at injection sites, consequently reducing the total number of TCR-transduced T cells (36, 37).

Although we did not observe any toxicity related to the TCR-transduced lymphocyte transfer we performed in this study, other studies have reported toxicities induced by TCR-transduced T-cell transfer (27, 38–41). In a study using high-affinity TCR against MART-1 and mouse-derived TCR against gp100, melanocyte-related toxicities (including skin, eye, and ear toxicities) occurred frequently (27). Moreover, an on-target effect on a normal organ

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was observed in a study in which CEA-targeting mouse-derived TCR was used to treat colorectal cancer patients (38). In addition, affinity-enhanced MAGE-A3-specific TCR-transferred T cells caused serious cardiac toxicity with unexpected recognition of titin, a cardiac muscle antigen, in patients treated for melanoma and myeloma (39, 40). It is theoretically possible that a TCR could induce toxicity by reacting to peptides from a different antigen that includes sequences similar to the target peptide. For example, in a previous study, T-cell transfer using mouse-derived TCR targeting MAGE-A3 induced severe toxicity in the central nervous system (41); this toxicity was caused by a reaction to a similar peptide of the MAGE-A12 antigen, which is expressed at low levels in normal brain tissues. In this study, we first investigated whether the MAGE-A4-TCR we used could induce a cross-reaction; however, we did not observe cross-reactions to any of 7 peptides similar to the MAGE-A4 peptide. Because it is technically challenging to predict the unexpected cross-reactivity of artificially affinity-enhanced TCRs that have not undergone *in vivo* negative selection, the use of wild-type TCRs with physiologically high avidity may represent a safe approach for selecting TCRs for clinical use.

Disclosure of Potential Conflicts of Interest

H. Ikeda, N. Imai, and J. Mineno report receiving commercial research grants from Takara Bio Inc. No potential conflicts of interest were disclosed by the other authors.

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