Adoptive Transfer of MAGE-A4 T-cell Receptor Gene-Transduced Lymphocytes in Patients with Recurrent Esophageal Cancer

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Abstract

Purpose: Preparative lymphodepletion, the temporal ablation of the immune system, has been reported to promote persistence of transferred cells along with increased rates of tumor regression in patients treated with adoptive T-cell therapy. However, it remains unclear whether lymphodepletion is indispensable for immunotherapy with T-cell receptor (TCR) gene–engineered T cells.

Experimental Design: We conducted a first-in-man clinical trial of TCR gene-transduced T-cell transfer in patients with recurrent MAGE-A4–expressing esophageal cancer. The patients were given sequential MAGE-A4 peptide vaccinations. The regimen included neither lymphocyte-depleting conditioning nor administration of IL-2. Ten patients, divided into 3 dose cohorts, received T-cell transfer.

Results: TCR-transduced cells were detected in the peripheral blood for 1 month at levels proportional to the dose administered, and in 5 patients they persisted for more than 5 months. The persisting cells maintained ex vivo antigen-specific tumor reactivity. Despite the long persistence of the transferred T cells, 7 patients exhibited tumor progression within 2 months after the treatment. Three patients who had minimal tumor lesions at baseline survived for more than 27 months.

Conclusions: These results suggest that TCR-engineered T cells created by relatively short-duration in vitro culture of polyclonal lymphocytes in peripheral blood retained the capacity to survive in a host. The discordance between T-cell survival and tumor regression suggests that multiple mechanisms underlie the benefits of preparative lymphodepletion in adoptive T-cell therapy. Clin Cancer Res; 21(10): 2268–77.

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Introduction

Initial studies of adoptive T-cell therapy for patients with malignancy reported a lack of prolonged persistence of the transferred cells and limited clinical responses (1, 2). In later studies, lymphodepletive preparative regimens using chemotherapy alone or in combination with total body irradiation were reported to enhance persistence of the transferred cells, accompanied by an increased clinical response, for example, in adoptive therapy of melanoma patients utilizing tumor-infiltrating lymphocytes (TIL; refs. 3, 4). In these trials, persistence of transferred T cells was correlated with tumor regression (5). Technology for the engineering of antigen receptor genes presents opportunities for novel T-cell–based therapies. Such approaches could potentially expand the application of adoptive therapy with tumor-reactive T cells to patients with tumor types that are difficult to isolate, and for which it is therefore challenging to expand tumor-reactive T cells. Lymphodepleting pretreatments have also been incorporated into adoptive therapy with T cells genetically engineered to express tumor-specific T-cell receptors (TCR) or tumor-reactive chimeric antigen receptors (CAR); these therapies have resulted in durable tumor regression in patients with metastatic melanoma, synovial cell sarcoma, and hematopoietic malignancy (6–12).

In contrast to TIL-based cells used for transfer, TCR gene–engineered T cells are created from polyclonal T cells in peripheral blood that have undergone minimal in vivo exposure to chronic antigen stimulation and the tumor microenvironment. Such gene-engineered T cells are cultured in vitro for relatively short periods, for example, 7 to 14 days before transfer. These differences between TILs and gene-engineered T cells may influence the degree to which these cells are prone to clonal exhaustion.
Translational Relevance

This article describes the first TCR gene T-cell therapy for esophageal cancer. The regimen did not include preparative lymphodepletion. Monitoring the in vivo kinetics of transferred cells by sensitive quantitative PCR and specific tetramers, it demonstrated that the transferred T cells persisted for more than 5 months, trafficked to tumor sites, and maintained tumor-specific reactivity in patients. None of the patients exhibited tumor shrinkage in the short term. However, 3 patients who had minimal disease at the time of cell transfer remained free from disease progression for more than a year without any treatment. These findings suggest that this sort of TCR-transduced T-cell therapy might be beneficial for patients bearing minimal tumor burdens. Also, the discordance between T-cell survival and short-time tumor response suggests that multiple mechanisms underlie the benefits of lymphodepletive preconditioning in adoptive T-cell therapy.

following transfer, and may therefore result in different requirements for lymphodepletive pretreatment to achieve in vivo persistence. However, the necessity of preparative lymphodepletion, both to ensure survival of gene-engineered T cells and to achieve durable tumor regression in cancer patients, has not yet been carefully investigated.

We previously reported establishment of a CTL clone that recognizes MAGE-A4, which in an HLA-A*24:02-restricted fashion (13). We constructed a retrovirus vector, MS-bPa, for transduction of T cells with TCR-α and -β chains derived from the MAGE-A4, specific T-cell clone. Translational studies of MAGE-A4-specific TCR genes confer MAGE-A4 specificity on 28%-52% of CD8+ T cells (14). These T cells exhibited HLA-A24:02-restricted cytotoxicity against MAGE-A4–expressing tumor cells; moreover, they stably exerted antigen-specific functions for over 6 months in vitro (14).

In this study, we investigated whether the unique nature of TCR gene–engineered T cells preserved the capacity to survive in hosts. Given that 38%-52% of tumor tissues from esophageal squamous cell carcinoma express the MAGE-A antigen (15, 16), we performed a first-in-man clinical trial of TCR gene therapy for recurrent esophageal cancer patients, targeting MAGE-A4, without any preparative lymphodepleting regimen or administration of IL2. In this study, we monitored the in vivo kinetics of transferred cells by sensitive qPCR of inserted vectors as well as the specific tetramer. T cells engineered to express MAGE-A4–specific TCR were safe, persisted for long periods, trafficked to tumor sites, and maintained tumor-specific reactivity. Unexpectedly, we observed discordance between T-cell persistence and tumor response, suggesting that lymphodepleting pretreatment in humans contributes not only to T-cell survival but also other mechanisms beneficial to the clinical response.

Materials and Methods

Preparation of TCR gene–transduced lymphocytes

Lymphocytes were collected from each patient by processing 5 L of peripheral blood by apheresis. In the cell-processing facility, the lymphocytes were cultured with IL2, anti-CD3 antibody, and RetroNectin (Takara Bio Inc.) under institutional GMP control. Proliferating lymphocytes were infected with the retroviral vector, MS-bPa, which was constructed from DNA encoding MAGE-A4, HLA-A*24:02–specific TCR-α and -β chains (14). After 7–10 days in culture, the lymphocytes were harvested and then frozen until use. The IFNγ responding cells were assayed as described previously, with some modification (17). Briefly, the cells were stimulated with MAGE-A4 peptide. Brefeldin A was added, and they were then incubated with anti-CD8 monoclonal antibody (Becton Dickinson). After permeabilization and fixation, cells were stained intracellularly with anti-IFNγ monoclonal antibody.

Study design

This study was a phase I, cell dose–escalating clinical trial of MAGE-A4–specific TCR gene-transduced lymphocyte transfer for treatment of patients with recurrent esophageal tumors expressing the MAGE-A4 antigen. The primary objective was to determine clinical safety, and the secondary objective was to assess cell kinetics in peripheral blood and infiltration of the TCR-transduced lymphocytes into the tumor tissue. The other aims were to determine MAGE-A4–specific immune responses and clinical responses.

Patients were eligible for study entry if they met each of the following criteria: had recurrent or metastatic esophageal tumors that expressed the MAGE-A4 antigen, were positive for HLA-A*24:02, had a performance status (PS) of 0, 1, or 2; were between 20 and 75 years old, had a life expectancy of 4 months or more, and did not have impaired organ function.

The patients were divided into 3 cohorts of 3 patients each: Cohort 1, 2 × 10^8 cells (whole cells including TCR-transduced lymphocytes) per dose; cohort 2, 1 × 10^7 cells per dose; and cohort 3, 5 × 10^6 cells per dose. In case of impaired PS due to disease progression, patients discontinued the clinical study. When a patient was withdrawn from the trial before safety evaluation before day 35, they were replaced with another patient. After withdrawing, they were followed up to assess clinical events and cell kinetics in the peripheral blood.

Clinical safety was evaluated according to the NCI Common Terminology Criteria for Adverse Events ver.3.0 (NCI-CTCAE ver.3.0; ref. 18). Tumor responses were assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST ver1.0; ref. 19).

The study was conducted in accordance with the current version of the Declaration of Helsinki. Written informed consent was obtained from all patients participating in this study. The protocol was approved by the institutional review board of Mie University Hospital (Mie, Japan) and the Ministry of Health, Labor, and Welfare of Japan. This clinical trial was registered in the UMIN Clinical Trials Registry as ID: UMIN000002395.

Expression of MAGE-A4 antigen

MAGE-A4 expression was assessed by quantitative real-time PCR (qRT-PCR) using specific primers (20), or by immunohistochemistry (IHC) using the monoclonal antibodies 57B (21). MCV-1 and MCV-4. MCV-1 and -4 were produced from hybridomas generated in our laboratory by cell fusion of the mouse myeloma cell line SP2/0 and splenocytes harvested from C/B F1 mice (CLEA Japan, Inc.) immunized with recombinant MAGE-A4 protein. MCV-1 and -4 recognize amino acids 255–277 and 71–95 of MAGE-A4, respectively. MCV-1 reacted...
to MAGE-A2, -A4, and -A12, and MCV-4 covered MAGE-A1 and -A4, respectively. MAGE-A4 expression in tumors was judged primarily by qPCR. Immunohistochemical staining was alternatively used if the tumor sample was unavailable for PCR. The cut-off value of PCR-amplified copies for MAGE-A4 was 12.2, which was determined as the mean copy number +2 SD of the amplified products in normal human tissues. Tumor samples expressing 12.2 or more PCR-amplified copies were judged as MAGE-A4 positive (22). The sensitivity was qualified by the GAPDH gene amplification. For IHC, 57B monoclonal antibody was used first, as 57B covers the MAGE-A antigen family (23). Samples that were also positive for MCV-1 and MCV-4 were judged as MAGE-A4 positive. Tissue samples with 5% positive or more stained area were judged as MAGE-A4 positive. Focally stained samples were also positive.

Treatment protocol

After preparation of TCR-transduced lymphocytes, patients were given the lymphocytes intravenously without preconditioning treatment. On days 14 and 28, patients were subcutaneously given 300 μg of MAGE-A4 peptide (NYKRCpep1; PolyPeptide Laboratories) emulsified with incomplete Freund adjuvant (IFA; Montanide ISA-51VG; SEPPIC). On days 35 and 63, safety and clinical responses were assessed. After completing the study, patients who wished to do so received continuous MAGE-A4 peptide vaccination on a monthly basis.

Cell kinetics and tumor infiltration of TCR-transduced lymphocytes

Heparinized peripheral blood was collected at baseline and at predetermined time points during the 63-day period. On day 35, if the patients had tumors at esophageal lesions, esophageal tumors were endoscopically biopsied. After day 63, peripheral blood mononuclear cells (PBMC) were collected on the days of patients’ visits to the clinic. PBMCs were isolated and cryopreserved. The PBMCs were thawed before qPCR assay, from which DNAs were isolated using the DNA extraction kit. Primers for proviral DNA sequence (retroviral packaging signal region, existing in TCR-transduced cells) and human IFNγ DNA (genes of whole T cells) from the Provirus Copy Number Detection Primer Set, Human (Product code 6167, Takara Bio Inc.) were used for qPCR assay using the Cycler PCR Core Kit (Product code CY501, Takara Bio Inc.). Ten microliters of 10 ng/μl solution of the isolated DNA samples were amplified by 50 cycles of 3-step PCR reactions. For standard curve generation, serially diluted (2,000 to 0.02 fg/μL) DNA Control Template for Provirus, Human (a component of the Provirus Copy Number Detection Primer Set, Human), which is the plasmid with target sequences for proviral DNA and human IFNγ were amplified at the same time. We generated the standard curve by the use of a plasmid that encodes one copy of the target proviral sequence and one copy of IFNγ gene sequence. The amplification efficiencies of the retrovirus transgene and human IFNγ were within twofold across the standard curves (Supplementary Fig. S1). Each DNA concentration of IFNγ or proviral vector for MAGE-A4 TCR expression was calculated from the standard curve. The copy number of the MAGE-A4-TCR DNA in the PBMCs was represented by the ratio of proviral DNA and IFNγ DNA values.

Tetramer analysis and ELISPOT assays

CD8+ T cells were sorted from patients’ PBMCs collected at each time point after transfer, and they then were cultured with MAGE-A4 peptide–pulsed non-CD8+ T cells with peptide concentration of 10 nmol/L. The cells were cultured in the presence of IL2 (10 U/mL), IL7 (20 μg/mL), and 10% human AB serum. After 8 days in culture, the stimulated CD8+ T cells were used as effector cells in the tetramer analysis and ELISPOT assay.

Using MAGE-A4 peptide/HLA-A*24:02 tetramer, the frequencies of MAGE-A4 TCR–positive T cells were analyzed by flow cytometry. MAGE-A4-tetramer+/CD8+ T cells were subjected to phenotypic analysis using monoclonal antibodies, CD45RO, CD45RA, CCR7, and CD62L. In parallel with the tetramer analysis, ELISPOT assays were performed by targeting T2A24 cells pulsed with MAGE-A4 peptide and the tumor cell lines 11–18 (MAGE-A4 positive), HL-A*24:02 positive and QG-56 (MAGE-A4+, HLA-A*24:02−). T2A24 cells are T2 cells transduced with the HLA-A*24:02 gene.

The ELISPOT assay was done as described previously with some modifications (13, 24). Briefly, ELISPOT plates (MAHA S4510; Millipore) were coated with anti-human CCL4 (MIP-1β) monoclonal antibody (R&D Systems). A total of 5 × 105 sensitized CD8+ T cells and 1 × 105 peptide-pulsed T2A24 cells, nonpulsed T2A24 cells, 11–18, or QG56 cells were placed in each well of the ELISPOT plate. After incubation for 22 hours at 37°C, the plate was washed, and then supplemented with biotinylated capture antibody and incubated overnight at 4°C. After washing, the cells were reacted with streptavidin-alkaline phosphatase conjugate, and then stained with an alkaline phosphatase conjugate substrate kit (Bio-Rad). The spots were counted using an ELISPOT Plate Reader (ImmunoSpot, CTL-Europe GmbH).

In addition, we prepared 7 peptides with amino acid sequences similar to that of the MAGE-A4 peptide, by screening the BLAST database program, bastp (http://blast.ncbi.nlm.nih.gov/Blast.cgi; Supplementary Table S1). Cells of the MAGE-A4–specific CTL clone #2-28, from which the TCR-α and β genes were cloned for this study, were tested as effector cells. ELISPOT assays were performed using T2A24 cells pulsed with each of these 7 peptides. T2A24 cell were pulsed with the EBNA-3A peptide as a negative control target.

Results

Preparation of TCR-transduced T cells

The specificity of engineered TCR has become a matter of utmost concern in clinical trials. To determine whether the MAGE-A4–targeting TCR could cross-react with other peptides with amino acid sequences similar to that of the MAGE-A4 peptide, we performed ELISPOT assays using MAGE-A4 CTL clone #2–28, using target cells pulsed with analogous peptides derived from known human proteins (Supplementary Table S1). As shown in Supplementary Fig. S2, we observed no cross-reactivity of clone #2–28 with any of the 7 analogous peptides.

After preparing cells, we analyzed their cell-surface phenotypes (Table 1). More than 89% were CD3+ T cells. Two patients had CD4+ T-cell dominance, 2 patients had CD8+ T-cell dominance, and the remaining 6 had equal distributions of CD4+ and CD8+ T cells. Among the CD8+ T cells, 9.7%–43.1% of lymphocytes responded to MAGE-A4 peptide, approximating the percentage of T cells transduced with the MAGE-A4–specific TCR gene.
MAGE-A4 tetracer-positive CD8⁺ T cells ranged from 1.8% to 12.6% (Table 1; Supplementary Fig. S3).

Esophageal cancer patients receiving transfer of MAGE-TCR–transduced T cells

From May 2010 to November 2012, 15 patients were enrolled in the clinical trial. They underwent aptamers. This, from which TCR-transduced lymphocytes were prepared. Five of these patients were withdrawn before lymphocyte transfer, because their general condition deteriorated due to rapid disease progression. The remaining patients were treated with TCR-transduced T cells at one of 3 doses: 2 x 10⁶ cells/dose (cohort 1, 3 patients), 1 x 10⁷ cells/dose (cohort 2, 4 patients), or 5 x 10⁷ cells (cohort 3, 3 patients). One patient in cohort 2, TCR-MA-209, experienced rapid disease progression complicated by worsened performance status; he was removed from the clinical trial without peptide vaccinations, and was assessed for clinical events and cell kinetics in the peripheral blood. Another patient, TCR-MA-212, was entered to cohort 2 (Table 2).

As shown in Table 2, all patients had esophageal carcinoma that was recurrent or metastatic after standard treatment, including chemotherapy, radiotherapy, and/or surgery. In all 10 patients, the tumors were pathologically diagnosed as squamous cell carcinomas. MAGE-A4 expression was examined by PCR in 9 patients. For TCR-MA-315, the antigen expression was assessed by IHC. MAGE-A4 expression levels varied from low (in patient TCR-MA-104 and -212; Table 2). Seven patients (TCR-MA-102, -104, -106, -209, -210, -314, and -315) had definite tumor lesions that could be assessed according to the RECIST criteria. Three patients (TCR-MA-208, -212, and -213) had minimal lesions, which were assessed by progression-free periods.

Cell kinetics and phenotypes of MAGE TCR–transduced T cells after transfer

By analyzing the TCR transgene copies, the infused cells were detected in peripheral blood in all patients (Fig. 1A), appearing soon after transfer, whereas MAGE-A4 tetramer-positive T cells were detected in peripheral blood in 5 patients who were given 1 x 10⁷ or 5 x 10⁷ cells (Fig. 3A). The number of cells was dependent on the initial dose during the first 14 days, reaching peak and plateau levels on days 3–7, and then decreasing over 14 days (Fig. 1B). Considering that the average retroviral vector copy number in infused cells was approximately 7 copies per cell, the transferred cells reached over 11%–17% of the PBMCs in patients of cohort 3 during the first 14 days. The frequency was calculated at the actual TCR transgene numbers divided by 7 copies per cell. This means that in TCR-MA-315, who received cells with 45.3% CD8⁺, including 43.1% of the responding population (Table 1), TCR-engineered cells may have constituted 2%–3% of CD8⁺ T cells in total PBMCs. After the MAGE-A4 peptide vaccinations on days 14 and 28, the levels of TCR-transduced lymphocytes decreased. In 7 of the patients, the transferred cells persisted in vivo at stable levels in peripheral mononuclear cells for 63 days.

Ex vivo phenotypic analysis of MAGE-A4-tetramer⁺/CD8⁺ T cells was performed at the timing of 12 hours and 14 days after the cell transfer. Although effector/effector memory T cells (CD8⁺/CD45RO⁺/CCR7⁻) were dominant in PBMCs at the timing of 12 hours, terminally differentiated effector memory CD8⁺ T cells (CD8⁺/CD45RA⁻/CCR7⁻) became a dominant population in patients' PBMCs at 14 days in both TCR-MA-314 and 315 (Supplementary Fig. S4; refs. 25, 26).

Long-term in vivo persistence of the MAGE TCR–transduced T cells and immune reactivity against MAGE-A4–expressing tumor cells

Over more than 800 days of observation, 5 patients exhibited long-term persistence of the transduced T cells, as determined by detection of TCR genes by quantitative PCR (Fig. 2). According to the average vector copy number and antigen-specific response in cells used for transfer (Table 1), these cells represented around 0.01%–0.04% of the peripheral CD8⁺ T cells in patients TCR-MA-102 and -208.

We collected T cells from these patients, stimulated them in vitro with the MAGE-A4 peptide, and performed tetramer and ELISPOT assays. The data from 4 patients, TCR-MA-102, -106, -208, and -212, are presented in Fig. 3, in which the T cells were collected for more than 63 days of study period for tetramer analyses. In TCR-MA-102, the lymphocytes were detected as late as day 105, at which time large numbers of tetramer⁺ CD8⁺ T cells were detected following in vitro stimulation with MAGE-A4 peptide (Fig. 3B). Concurrently, T-cell clones were established from PBMCs on days 28 and 105. These T cells were derived from the MAGE-A4

Table 1. Phenotypes of manufactured lymphocytes after TCR gene transduction

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>CD3 (%)</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>MAGE-A4 tetracer⁺ cells (%)</th>
<th>IFNγ responded cells*</th>
<th>Copies of TCR transgene/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCR-MA-102</td>
<td>89.4</td>
<td>59.9</td>
<td>30.3</td>
<td>4.5</td>
<td>6.3</td>
<td>24.3</td>
</tr>
<tr>
<td>TCR-MA-104</td>
<td>91.8</td>
<td>71.7</td>
<td>27.1</td>
<td>0.5</td>
<td>1.8</td>
<td>5.5</td>
</tr>
<tr>
<td>TCR-MA-106</td>
<td>96.7</td>
<td>29.3</td>
<td>67.6</td>
<td>0.3</td>
<td>8.6</td>
<td>9.7</td>
</tr>
<tr>
<td>TCR-MA-208</td>
<td>95.5</td>
<td>81.1</td>
<td>17.6</td>
<td>0.5</td>
<td>4.1</td>
<td>22.4</td>
</tr>
<tr>
<td>TCR-MA-209</td>
<td>97.9</td>
<td>57.5</td>
<td>45.4</td>
<td>2.6</td>
<td>7.9</td>
<td>21.8</td>
</tr>
<tr>
<td>TCR-MA-210</td>
<td>94.7</td>
<td>40.9</td>
<td>60.5</td>
<td>3.3</td>
<td>11.1</td>
<td>15.9</td>
</tr>
<tr>
<td>TCR-MA-212</td>
<td>99.7</td>
<td>14.1</td>
<td>88.9</td>
<td>0.6</td>
<td>7.5</td>
<td>29.6</td>
</tr>
<tr>
<td>TCR-MA-213</td>
<td>95.6</td>
<td>42.7</td>
<td>54.1</td>
<td>2.8</td>
<td>9.1</td>
<td>19.6</td>
</tr>
<tr>
<td>TCR-MA-214</td>
<td>97.6</td>
<td>41.3</td>
<td>59.9</td>
<td>1.0</td>
<td>1.5</td>
<td>22.1</td>
</tr>
<tr>
<td>TCR-MA-314</td>
<td>97.6</td>
<td>41.3</td>
<td>59.9</td>
<td>1.0</td>
<td>1.5</td>
<td>22.1</td>
</tr>
</tbody>
</table>

*IFNγ releasing cells responded by peptide-pulsed target cells.
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.
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 1.](https://example.com/figure1.png)

**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 $2 \times 10^8$ cells, 4 patients who received $1 \times 10^9$ cells, and 3 patients who received $5 \times 10^9$ 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.

![Figure 2.](https://example.com/figure2.png)

**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.
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.
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.
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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