Supplemental methods

Retroviral vector and TCR-T cell preparation

PBMC were isolated using Ficoll-Paque^{TM}PLUS (Cytiva, Tokyo, Japan) from healthy volunteers and cultured with IL-2 (600 IU/mL), anti-CD3 antibody (30 ng/mL), and RetroNectin® (Takara Bio Inc., Shiga, Japan). Proliferating lymphocytes were infected with the siTCR^{TM} retroviral vector (MS3-NY-ESO-1-siTCR) twice, at 24 hours and 48 hours after the start of culture. After 10–12 days, the T cells were harvested. The vector includes DNA encoding TCR-α and TCR-β chains specific for NY-ESO-$1_{157\text{-}165}$ and HLA-A*02:01. Gly 50 and Ala 51 in the CDR2 region of the TCR-β chain were replaced with Ala and Glu, respectively, resulting in a reduction in K_d from 21.4 μM for the wild-type to 1.9 μM. The retroviral vector also contains interfering RNA constructs that specifically down-regulate endogenous TCR. The coding sequences of the TCR-α and -β transgenes are codon-optimized and also resistant to siRNA against endogenous TCR.

RNA analysis for endogenous TCR expression

We evaluated effects of endogenous TCR knock-down in the T cells that were

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transduced with the retrovirus vector. At the same time, non-transgenic PBMCs were cultured and harvested under the same conditions as the genetically modified cells. Cells were homogenized by QIAshredder (QIAGEN, MD, USA), and total RNA was isolated using RNeasy Mini Kit (QIAGEN), then RT-PCR was performed using PrimeScript RT reagent Kit (Takara Bio Inc, Shiga, Japan) and SYBER Premix EX Taq (Takara Bio Inc.), with wt-TCRA-F primer (TakaraBio Inc, Shiga, Japan) and wt-TCRA-R primer (Takara Bio Inc.) for TCR-α RNA, or wt-TCRB-F primer (Takara Bio Inc.) and wt-TCRB-R primer (Takara Bio Inc.) for TCR-β RNA, respectively. The primers were wt-TCRA-F primer (5'-gtgcaaacgccttcaacaaca-3') and wt-TCRA-R primer (5'-gaccagcttgacatcacaggaac-3') for TCR-α (Takara Bio Inc.) and wt-TCRB-F primer (5'-cgccctcaatgactccagatac-3') and wt-TCRB-R primer (5'-cctgggtccactcgtcattc-3') for TCR-β. Suppression effects of endogenous TCR were calculated as TCR-α RNA in siTCR gene-modified cells/TCR-α RNA in non-gene modified cells, or TCR-β RNA in siTCR gene-modified cells/TCR-β RNA in non-gene modified cells. We used 3 samples from different healthy volunteers.

DNA microarray in the TCR-T cells

Two blood cell samples from four healthy individuals were infected with siTCR™ retroviral vector or TCR gene retrovirus without siRNA, respectively. After 9 days in culture, the cells were harvested, and total RNA was isolated with Fast Pure RNA kit (Takara Bio Inc.). Targets for DNA microarray analysis were prepared from each sample using the Quick Amp Labeling Kit one-color (Agilent Technologies, Santa Clara, CA, USA). The target samples were used for Whole Human Genome oligo DNA microarray using Gene Expression Hybridization Kit (Agilent Technologies, Santa Clara, CA, USA). Numerical data was obtained using Agilent Feature Extraction (Agilent Technologies, Santa Clara, CA, USA).

TBI-1301 cell kinetics after infusion

Heparinized peripheral blood was collected at baseline and at pre-determined time points during the 56-day period. PBMCs were isolated and cryopreserved. The PBMCs were thawed prior to the qPCR assay, and DNA was isolated from them using a DNA extraction kit. Primers for proviral DNA (retroviral packaging signal region, found in TCR-transduced cells) and human IFN-γ DNA (genes from whole T cells) from the Provirus Copy Number Detection Primer Set,

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Human (Product code 6167, Takara Bio Inc.) were used for quantitative PCR assay using the Cycleave PCR Core Kit (Product code CY501, Takara Bio Inc.). Ten microliters of a 10 ng/μL solution of the isolated DNA samples were amplified by 50 cycles of three-step PCR reactions. For standard curve generation, serially diluted (2,000 to 0.02 mg/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 IFN-γ, were amplified at the same time. The DNA concentration of IFN-γ or proviral NY-ESO-1-TCR was calculated from the standard curve. The copy number of NY-ESO-1-TCR DNA in the PBMC was represented by the ratio of proviral DNA to IFN-γ DNA values.

Major inclusion and exclusion criteria for the trial enrollment

Major inclusion criteria for trial entry were as follows: patients had histologically or cytologically confirmed solid tumors, had a solid tumor which is unresectable and refractory to standard therapy (chemotherapy, radiotherapy, etc.), were HLA-A*02:01 or HLA-A*02:06 positive, had NY-ESO-1 expression verified by PCR or immunohistochemistry, an ECOG Performance Status of either 0 or 1,

were aged 20 years or more at the time of consent, were not undergoing treatment (surgery, chemotherapy, radiotherapy, etc.) and expected sufficient recovery from prior treatment at the time of lymphocyte collection for gene transfer, had a life expectancy of 16 weeks or longer after consent, and had no severe damage to major organs (bone marrow, heart, lung, liver, kidney, etc.). Major exclusion criteria were: unstable angina, cardiac infarction, or heart failure; uncontrolled diabetes mellitus or hypertension; active infection; obvious interstitial pneumonia or lung fibrosis by chest X-ray; active autoimmune disease requiring steroids or immunosuppressive therapy; serious hypersensitivity; tumor cell invasion into the CNS; and active multiple cancers.