Supplementary materials and methods

Generation of autologous Vγ9Vδ2 T-cell products for infusion

For small-scale 10-day Vγ9Vδ2 T-cell culture tests, PBMCs were isolated from 7.5 ml whole blood in BD Vacutainer Cell Preparation Tubes with sodium heparin (Becton-Dickinson, Franklin Lakes, NJ). Patients judged appropriate for the study based on this small-scale culture test then underwent leukapheresis to isolate PBMC and harvest plasma using Fresenius AS.TEC204 with a C4Y white blood cell set (FRESENIUS KABI, Bad Homburg, Germany). Sodium citrate (ACD-A solution; TERUMO, Tokyo, Japan) was used as the anticoagulant. PBMC and plasma were isolated by density gradient centrifugation using Lymphoprep (AXIS-SHIELD Poc AS, Oslo, Norway) and at least 40 vials of plasma and 40 vials of PBMC were cryopreserved until use.

To prepare autologous Vγ9Vδ2 T-cells for infusion, PBMC were thawed and stimulated with 5 μmol/L zoledronate (Novartis Pharma KK Tokyo, Japan) in AlyS203 γδ medium (Cell Science and Technology Institute, Sendai, Japan) containing 1,000 IU/mL human recombinant IL-2 (Proleukin™; Chiron, Amsterdam, The Netherlands), and 10% autologous plasma. Fresh medium containing IL-2 (1,000 IU/mL) was added every 2–3 days and the cultures were transferred into new flasks or culture bags as necessitated by the degree of cell growth to maintain cell density below 1×10^6/mL. Fourteen days after in vitro stimulation, ex vivo expanded Vγ9Vδ2 T-cells were harvested and screened for their sterility (negative for endotoxin, bacteria, fungi, and mycoplasma contamination) and purity (>70%). Cell surface expression of the activation marker CD69 and NKG2D as a surrogate marker for cytotoxic activity were examined by flow cytometry. Following approval for use after these tests, the cultured cells were washed twice with RPMI-1640 (ThermoFisher Scientific, Waltham, MA) and resuspended in normal saline (FUSO Pharmaceutical Industries, Osaka, Japan) for administration to the patient.

Monoclonal antibodies (mAb) used in this study

The following fluorescent dye-conjugated mAbs were obtained from Beckman Coulter, BD Pharmingen (San Diego, CA), BioLegend (San Diego, CA) and eBiosciencee (San Diego, CA) : FITC-labeled anti-T-cell receptor (TCR)Vγ9 (IMMU360), anti-CD3 (UCHT1), PE-labeled anti-CD19 (J3-119), anti-NKG2D (ON72), anti-CD56 (N901), anti-TCRpanαβ (IP26A), PC5-labeled anti-CD3 (UCHT1), anti-CD27 (1A4CD27), ECD-labeled anti-CD45RA (2H4LDH11LDB9) (Beckman Coulter), PE-labeled anti-TCRVγ9 (B3), FITC-labeled CD103a (H4A3), CD103b (H4B4), PE-labeled anti-CD69

(FN50) (BD Pharmingen), Alexa Fluor 647-labeled anti-CD3 (HIT3a), FITC-labeled anti-CD4 (RPA-T4), Pacific Blue-labeled anti-CD8 (HIT8a), anti-CD14 (M5E2), anti-CD45 (HI30), APC-labeled anti-CD45 (HI30), Alexa Fluor 488-labeled anti-FOXP3 (259D), Mouse IgG1, κ isotype control (MOPC-21) (BioLegend), Fixable Viability Dye eFluor450 (eBiosciencee).

Repertoire analysis by Sequencing TRD CDR3 regions

Total RNA was extracted from PBMCs and cultured Vγ9Vδ2 T-cells of indicated patients using the AllPrep DNA/RNA Mini Kit (Qiagen). Total RNA was converted to complementary DNA (cDNA) with Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA); unbiased adaptor-ligation PCR was performed as described previously (Reference #1) with modification for TRD. Primers and adaptors used in this study were described in Supplementary Table S1. After PCR amplification, index (barcode) sequences were added by amplification with Nextera XT index kit v2 setA or setD (Illumina, San Diego, CA). The indexed amplicon products were mixed and sequenced with the Illumina Miseq paired-end platform (2 x 300bp). All the paired-end reads were classified by index sequences. Assignment of sequences was performed by determining sequences with the highest identity in a data set of reference sequences from the international ImMunoGeneTics information system® (IMGT) database (http://www.imgt.org). Nucleotide sequences of CDR3 regions ranged from conserved Cysteine at position 104 (Cys104) of IMGT nomenclature to conserved Phenylalanine at position 118 (Phe118) was translated to deduced amino acid sequences. A unique sequence read (USR) was defined as a sequence read having no identity in the assignment of gene segments and deduced amino acid sequence of CDR3 with the other sequence reads. The copy number of identical USR were counted in each sample and then ranked in order of the copy number. Percentage occurrence frequencies of sequence read with V and J genes in total sequence reads were calculated.

Reference #1: Kitaura K, Shini T, Matsutani T*, et al.* A new high-throughput sequencing method for determining diversity and similarity of T cell receptor (TCR) α and β repertoires and identifying potential new invariant TCR α chains. *BMC Immunology* 2016;17:38. doi: 10.1186/s12865-016-0177-5