iPSC-derived neoantigen-specific cytotoxic T-lymphocyte therapy

for Ewing sarcoma

Midori Ishii, Jun Ando, Satoshi Yamazaki, Tokuko Toyota, Kazuo Ohara,

Yoshiki Furukawa, Yoshiyuki Suehara, Mahito Nakanishi, Kazutaka Nakashima,

Koichi Ohshima, Hiromitsu Nakauchi, Miki Ando

**Supplementary Figure S1**: Gating strategies for flow cytometry.

Gating strategy for flow cytometry of EWS/FLI1-CTL clones, EWS/FLI1-bulk CTL, and EWS/FLI1-rejTs. Lymphocytes were gated based on FSC-A and SSC-A, followed by doublet removal. Dead cells (marked with propidium iodide; PI+) were then removed. (A) To confirm the antigen specificity of CTLs, they were gated as CD3+CD4- cells. (B) To define the memory phenotype subsets of EWS/FLI1-CTL clones and EWS/FLI1-rejTs, they were gated as CD3+CD8+ cells. (C) To examine expression of LAG-3, PD-1. and TIM-3 by EWS/FLI1-CTL clones and EWS/FLI1-rejTs, lymphocytes were gated based on FSC-A and SSC-A, followed by doublet removal. Dead cells (PI+) were then removed. (D) To examine expression of HLA-A2, PD-L1, and PD-L2 by TC-71 and A673 cells, cells were gated based on FSC-A and SSC-A, followed by doublet removal. Dead cells (PI +) were then removed.

**Supplementary Table S1**: The break/fusion sequences in TC-71, A673, and RD-ES cells.

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| TC-71 FWD | CCTTCTTATGACTCAGTCAGAAGAGGAGCATGGGGCAATAACATGAATTCTGGC |
| TC-71 REV | CCAAGTCAATATAGCCAACAGAGCAGCAGCTACGGGCAGCAGAACCCT |
| A673 FWD | CCTTCTTATGACTCAGTCAGAAGAGGAGCTTGGGGCAATAACATGAATTCTG |
| A673 REV | CCAAGTCAATATAGCCAACAGAGCAGCAGCTACGGGCAGCAGAACCCT |
| RD-ES FWD | GCTGGCCTATAATACAACCTCCCACACCGACCAATCCTCACGATTGAGTGTCAAAGAAGACCCTTCTTATGACTCAGTCAGAAGAGGAGCTTGGGGCAATAACATGAATTCTGGCC |
| RD-ES REV | CCAAGTCAATATAGCCAACAGAGCAGCAGCTACGGGCAGCAGAGTTCACTGCTGGCCTATAATACAACCTCCCACACCGACCAATCCTCACGATTGAGTGTCAAAGAAGACCCT |