Development of a WT1-TCR for clinical trials: engineered patient T cells can eliminate autologous leukemia blasts in NOD/SCID mice

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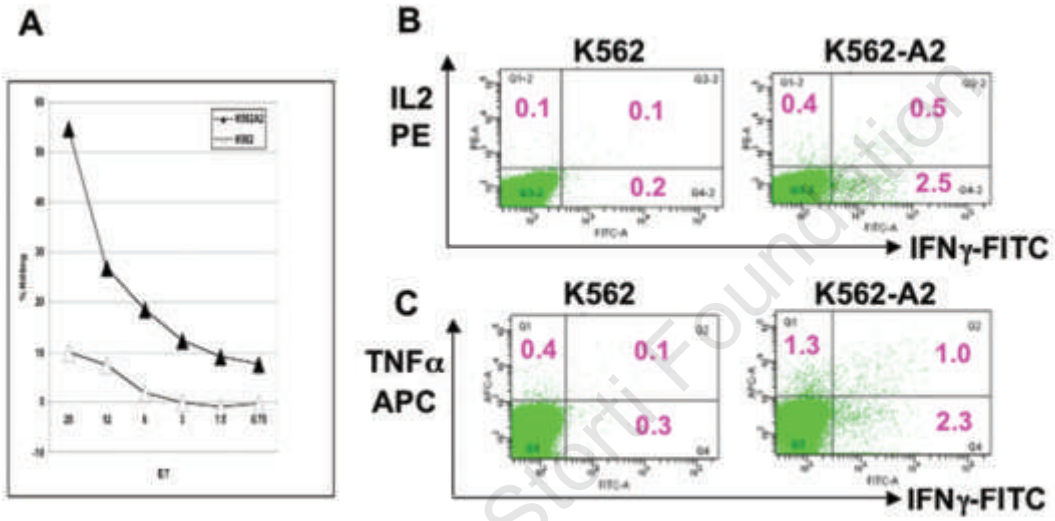
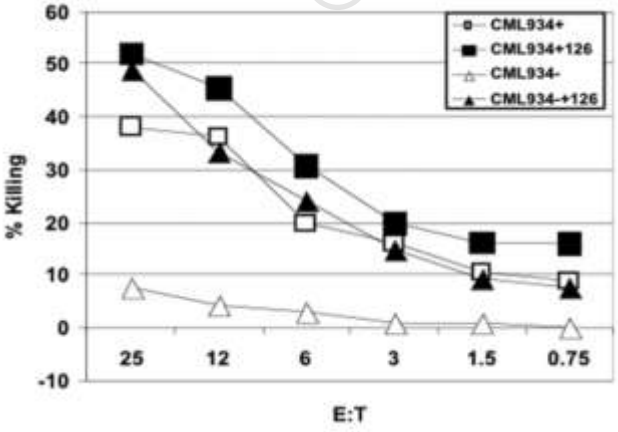
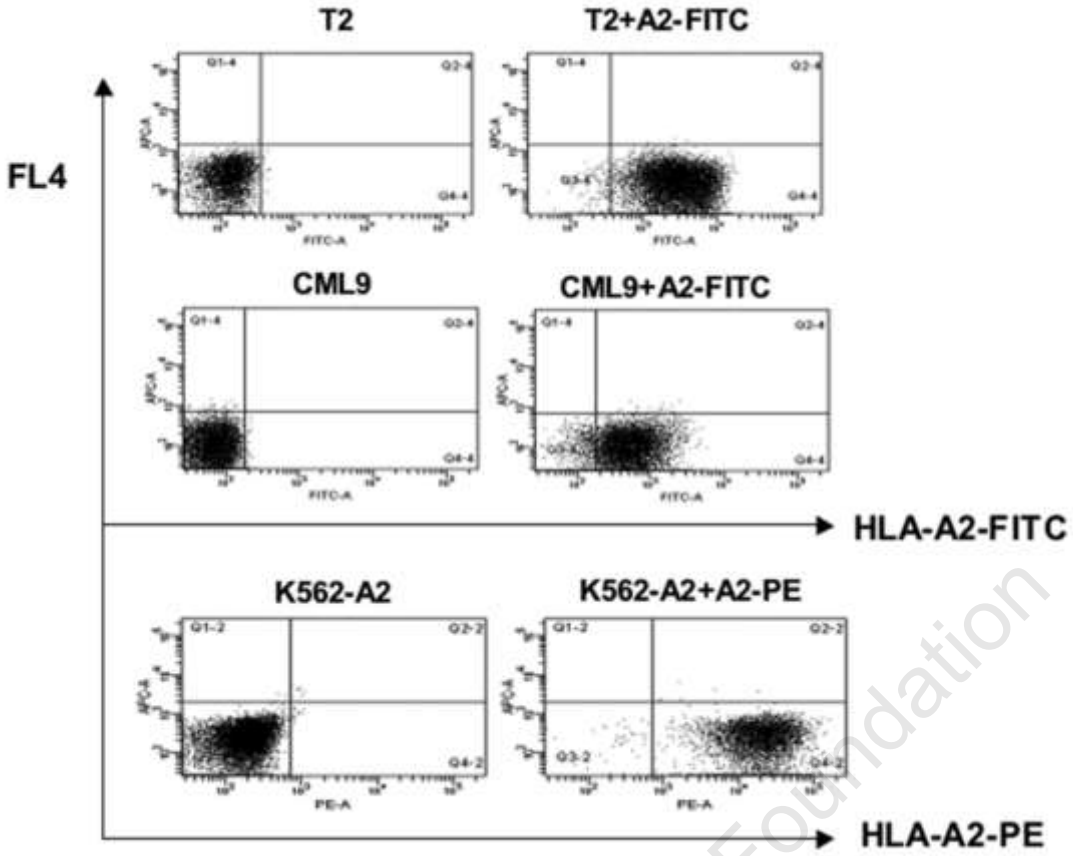


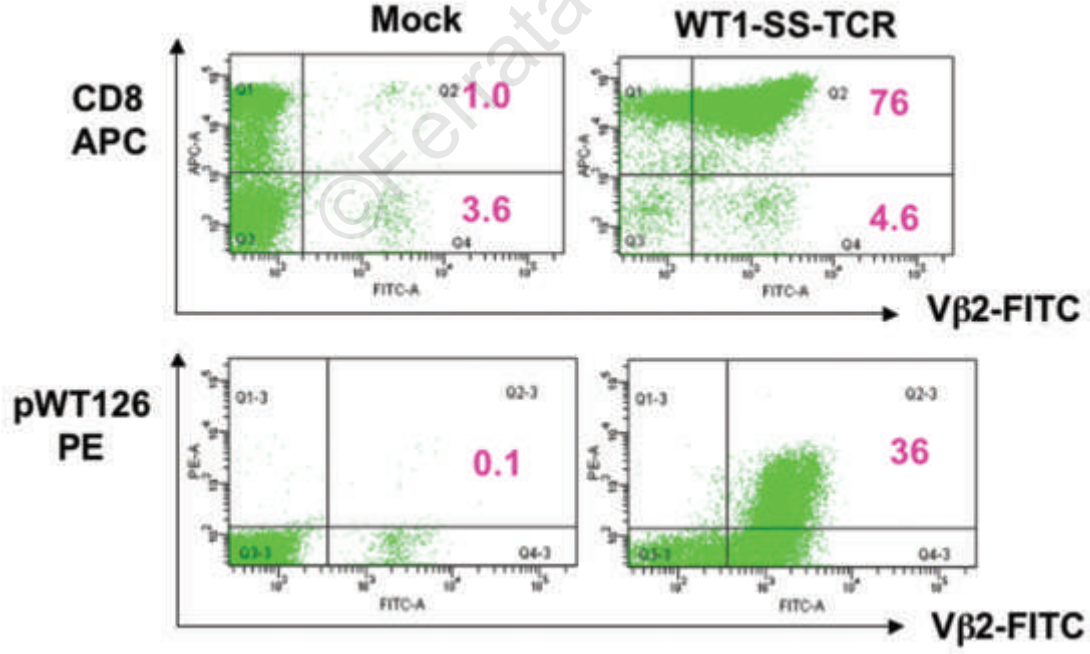
Supplementary Figure 1. Recognition of target cells endogenously expressing WT1 by WT1-SS-TCR transduced T cells. Leukemia cell line K562 endogenously expresses WT1 antigen. In order to provide a target for the WT1-SS-TCR transduced T cells, K562 cells were transdduced with HLA-A2 molecule, then used as target cells in cytotoxicity (A) and cytokine production (B-C) assays. The A2 expression of K562-A2 is shown in Supplementary Figure 3.



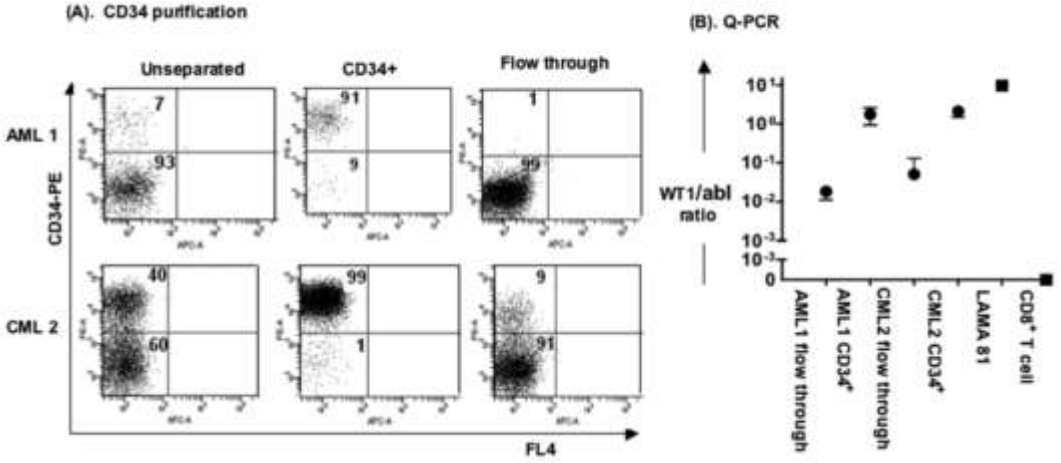
Supplementary Figure 2. Killing of CD34+ leukemia progenitor cells by WT1-SS-TCR transduced T cells. CD34+ and CD34- cells were isolated from a CML donor, then used as target cells with or without loading of pWT126 peptide in a cytotoxicity assay. As shown in the figure, without loading of pWT126 peptide, the WT1-SS-TCR transduced T cells only selectively killed the CD34+ cells, not the CD34- cells. But when loaded with pWT126 peptide, they were all killed by the WT1-SS-TCR transduced T cells.



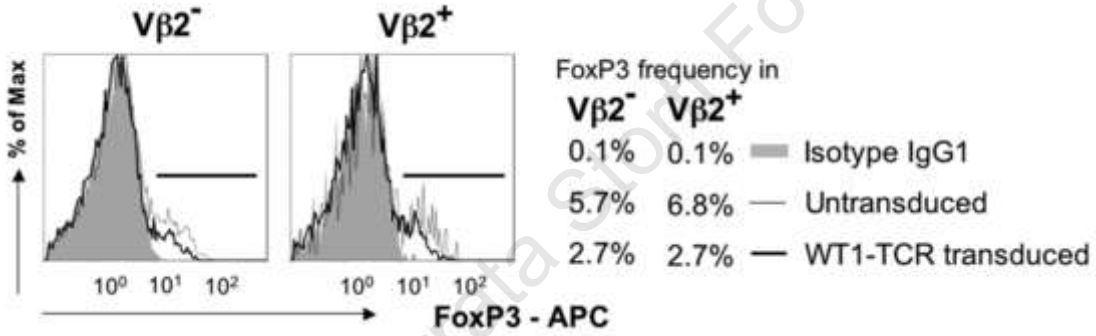
Supplementary Figure 3. HLA-A2 staining of target cells K562-A2 leukemia line, leukemia patient sample CML9 and stimulator T2 cell line.



Supplementary Figure 4. Expression of WT1-SS-TCR in two-round stimulated T cells. WT1-SS-TCR freshly transduced T cells were peptide stimulated in vitro for two rounds, then stained for TCR and tetramer expression. As shown in the figure, using mock transduced T cells (left panel) as a control, after two-rounds stimulation, majority of WT1-SS-TCR transduced T cells (right panel) express the WT1-specific TCR (Vb2), and nearly half of these T cells can bind to their specific tetramer.



Supplementary Figure 5. CD34 Purification from patient samples and Q-PCR analysis of WT1 gene expression. (A). CD34$^+$ cells were purified from leukemia patient samples by using anti-CD34 PE Ab staining and followed by anti-PE microbeads purification according to the manufacturer's instructions. Left panels show CD34-PE staining of unseperated leukemia samples from one AML (upper panel) and one CML (lower panel). Middle column shows the purified CD34$^+$ cells from these two patient samples. Right column shows the flow through fractions of the purification. (B). Total RNA was extracted from different fractions of the CD34 purification. After reverse transcription, cDNAs from different fractions were subjected to quantitative PCR to analyse the WT1 gene expression. Abl gene was used as an internal standard, the level of WT1 gene expression is expressed as WT1/abl ratio of copy numbers. The leukaemia cell line LAMA81 was used as a positive control, and the CD8$^+$ T cells were used as negative control.



Supplementary Figure 6. FoxP3 expression before and after WT1-TCR transduction. Bulk human T cells were retrovirally transduced with the WT1-SS-TCR. Three days after transduction, T cells were surface stained for CD4-APC-Cy7, CD25-PE, Vb2-FITC and permeabilized then stained for intracellular FoxP3-APC or an isotype IgG-APC control, respectively. Cells were gated on CD4$^+$CD25^{hi} T cells and separated into Vβ2- and Vβ2$^+$ (representing the introduced TCR and endogenous Vβ2+ T cells) populations. As shown in the figure, following transduction with the WT1-SS-TCR, the total frequency of FoxP3$^+$ T cells was reduced, suggesting that non-Treg expanded preferentially during the transduction period. There was no evidence of preferential TCR gene transfer into Treg cells, as the frequency of FoxP3$^+$ cells was similar in the Vβ2- and Vβ2+ population. The graphic and statistic evaluation was performed with Expo32 software (Beckman-Coulter).