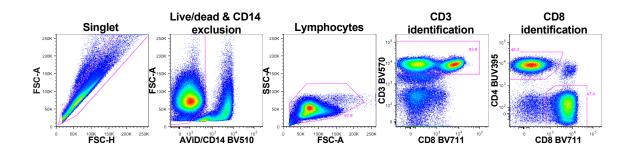
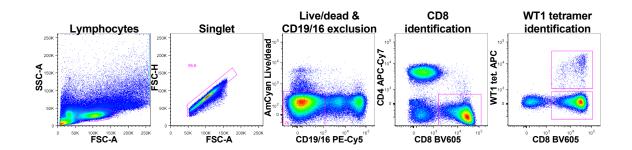
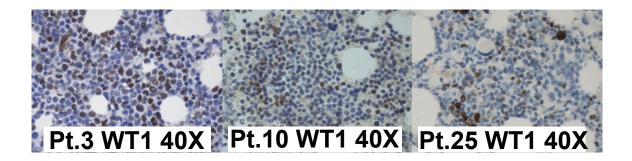
## **Supplementary Materials:**



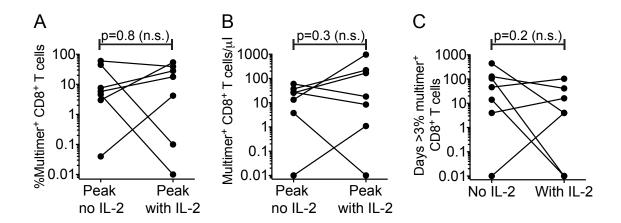
**Supplementary Figure 1: Gating strategy for intracellular assays**. Panels from left to right: Initial gating was on singlet (FSC-A vs. FSC-H), followed by exclusion of dead cells and CD14-expressing antigen presenting cells (FSC-A vs. AViD/CD14 BV510), followed by lymphocyte identification (FSC-A vs. SSC-A), followed by CD3 identification (CD3 BV570 vs CD8 BV711), followed by CD8 gating (CD4 BUV395 vs CD8 BV711). A representative example is shown.



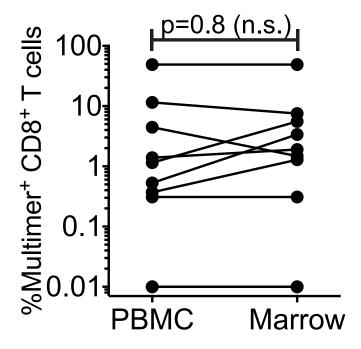
**Supplementary Figure 2: Gating strategy for surface stains.** Panels from left to right: Initial gating was on lymphocytes (SSC-A vs. FSC-A), followed by singlet exclusion (FSC-H vs FSC-A), followed by exclusion of dead cells and CD19/16-expressing cells (AmCyan Live/dead vs CD19/16 PE-Cy5), followed by CD8 identification (CD4 APC-Cy7 vs CD8 BV605), followed by WT1/HLA-A2 tet $^+$  cells identifying T<sub>TCR-C4</sub> (WT1 tet. APC vs CD8 BV605). A representative example is shown.



Supplementary Figure 3: WT1 expression in leukemic cells. Representative WT1 nuclear stain assessed by immunohistochemistry on the pre-HCT bone marrow biopsy performed for Pt. 3 (left), Pt. 10 (middle) and Pt (25 right panel). Magnification: 40X. The diameter of field is 0.4mm and each picture is representative of a larger (~0.3x 1cm) bone marrow biopsy core. On slide positive and batch negative controls marked appropriately.



**Supplementary Figure 4: Effect of low-dose s.c IL-2 on post-infusion T**<sub>TCR-C4</sub> **frequencies. (A)** Percent (% tetramer<sup>+</sup> CD8<sup>+</sup> T cells), **(B)** absolute numbers (tetramer<sup>+</sup> cells/ml) of peak frequencies and **(C)** days tetramer<sup>+</sup> remained >3% of CD8<sup>+</sup> T cells detected in peripheral blood of pts who received 10<sup>10</sup> cells/m<sup>2</sup> alone or the same cell dose followed by 14 days of low-dose s.c. IL-2 (n=7). A two-sided paired T-test was used for statistical analysis. n.s.: not significant (p>0.05). Peak frequencies and duration of T<sub>TCR-C4</sub> >3% of CD8<sup>+</sup> T cells was not increased, compared to infusions of the same dose without IL-2, suggesting that shorter persistence observed in some patients reflected the nature of the infused substrate cells rather than a requirement for exogenous factors. Two-sided paired t-tests were used for statistical analysis.



Supplementary Figure 5:  $T_{TCR-C4}$  do not preferentially localize to bone marrow in patients with no detectable AML. Percent tetramer<sup>+</sup> CD8<sup>+</sup> cells in blood (left) and bone marrow (right) at time points from patients (n=9) in whom blood and BM could be analyzed concurrently at any timepoint after  $T_{TCR-C4}$  infusions. A two-tailed paired t-test was used for statistical analysis. n.s.: non-significant (p<0.05). Two-sided paired t-tests were used for statistical analysis.

## **Supplementary Table 1: Clonotype composition of infusion products.**

Patients*	All Clonotypes** composing T <sub>TCR-C4</sub>	Of all clonotypes composing T <sub>TCR-C4</sub> , clonotypes detected at any timepoint after infusions	Of clonotypes detected at any timepoint after infusions, clonotypes that were additionally expanded in product
Pt 3	76	17	17
Pt 10	213	39	38
Pt 11	71	47	41
Pt 12	369	366	197
Pt 13	74	39	38
Pt 17	139	28	23
Pt 18	213	102	98
Pt 20	203	34	33
Pt 21	2080	290	245
Pt 24	181	79	77
Pt 25	312	83	81

<sup>\*</sup>Pt 16 did not have sufficient infusion product to perform clonotype composition analysis.

<sup>\*\*</sup>Only clonotypes with frequencies >0.001% are included.

## **Supplementary Table 2: Pt/donor EBV and CMV status.**

Pt	Donor EBV status*	Patient/donor CMV status	infusions received	CMV viremias obtained**
3	+	+/+	4	NO
10	+	-/-	1	NO
11	+	-/-	2	NO
12	+	-/+	1	YES
13	+	+/-	2	YES
16	+	+/-	2	YES
17	+	+/-	2	YES
18	+	+/-	1	YES
20	+	-/-	1	NO
21	+	+/-	1	YES
24	+	+/-	2	YES
25 *D4 FD) ( -4-4	+	+/+	2	YES

<sup>\*</sup>Pt EBV status was not directly determined, as per standard clinical practice at the FHCRC, since HCT recipients from EBV<sup>+</sup> donors sero-convert post-HCT. \*\*CMV serologies were not performed for CMV<sup>-/-</sup> pt/donor pairs.