Anti-CD33 chimeric antigen receptor targeting of acute myeloid leukemia

Carol O'Hear,[1,*] Joshua F. Heiber,[2,*] Ingo Schubert,[3] Georg Fey,[3] and Terrence L. Geiger[2,5]

[1]St. Jude Children's Research Hospital, Department of Oncology, Memphis, TN, USA; [2]St. Jude Children's Research Hospital, Department of Pathology, Memphis, TN, USA; and [3]University of Erlangen, Department of Biology, Germany

**CO and JFH contributed equally to this work.*

 doi:10.3324/haematol.2014.112748
Manuscript received on June 24, 2014. Manuscript accepted on December 2, 2014.
Correspondence: terrence.geiger@stjude.org

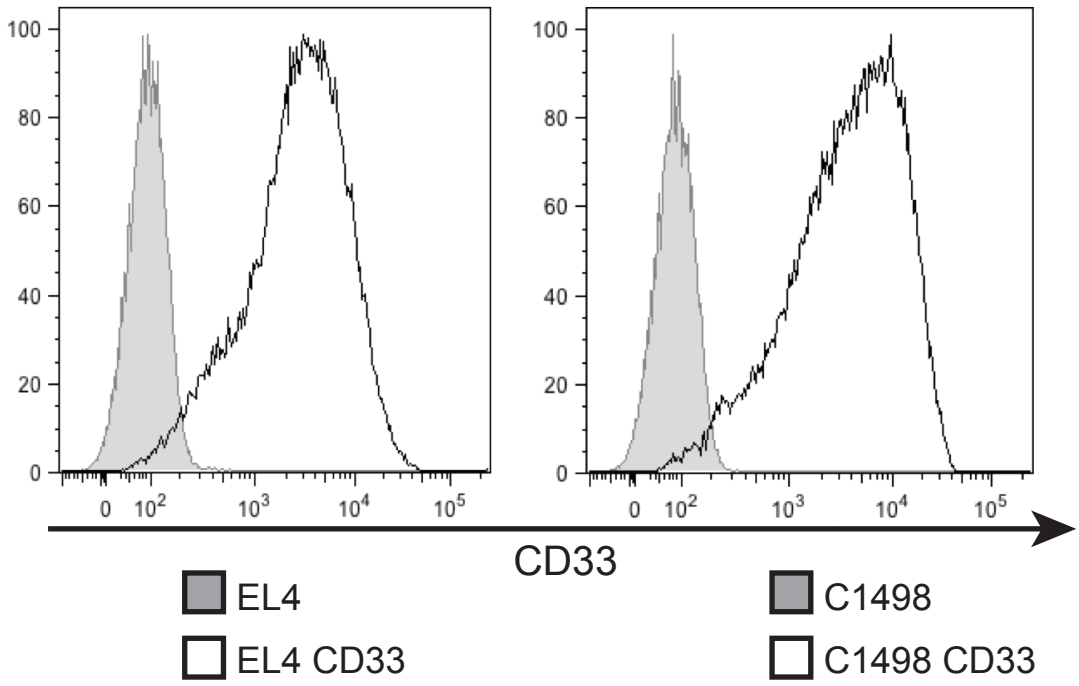
Supplemental Figure Legends

Supplemental Figure 1. Transduction of EL4 and C1498 cells with human CD33. Parent cell lines EL4 (murine T cell thymoma) and C1498 (murine AML) were transduced with human CD33 and stable lines flow cytometrically sorted. Histograms show CD33 expression on the transduced (EL4 CD33 and C1498 CD33) and parental cell lines.

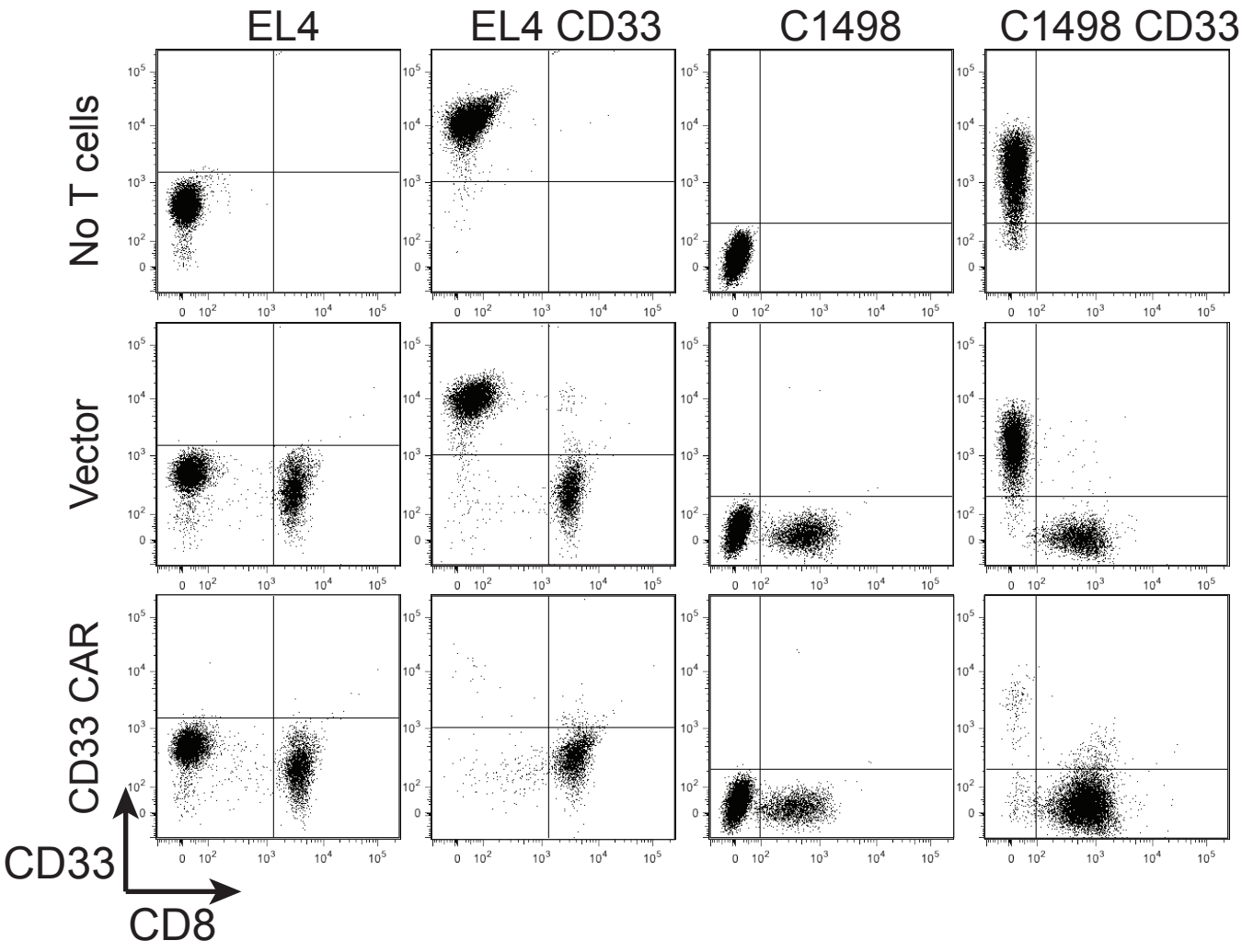
Supplemental Figure 2. Anti-CD33 CAR-modified T cells specifically target CD33-positive cells *in vitro*. Representative flow cytometry plots demonstrating the specific cytotoxicity of anti-CD33 CAR or vector-transduced T cells against C1498-CD33 and EL4-CD33 tumor cell lines or their parental CD33-negative controls. Quantitative flow cytometry was performed after 24 hour culture at an E:T ratio of 1:2.

Supplemental Figure 3. Anti-CD33 CAR-modified T cells release IL-2 and TNFα in the presence of CD33$^+$ targets. The indicated AML cell lines were co-cultured with anti-CD33 CAR or vector-transduced T cells at an E:T ratio of 1:2 for 24 hours. Supernatants were analyzed by ELISA for IL-2 (A) or TNFα (B). Means + 1 S.D. are plotted. *** $p<0.001$.

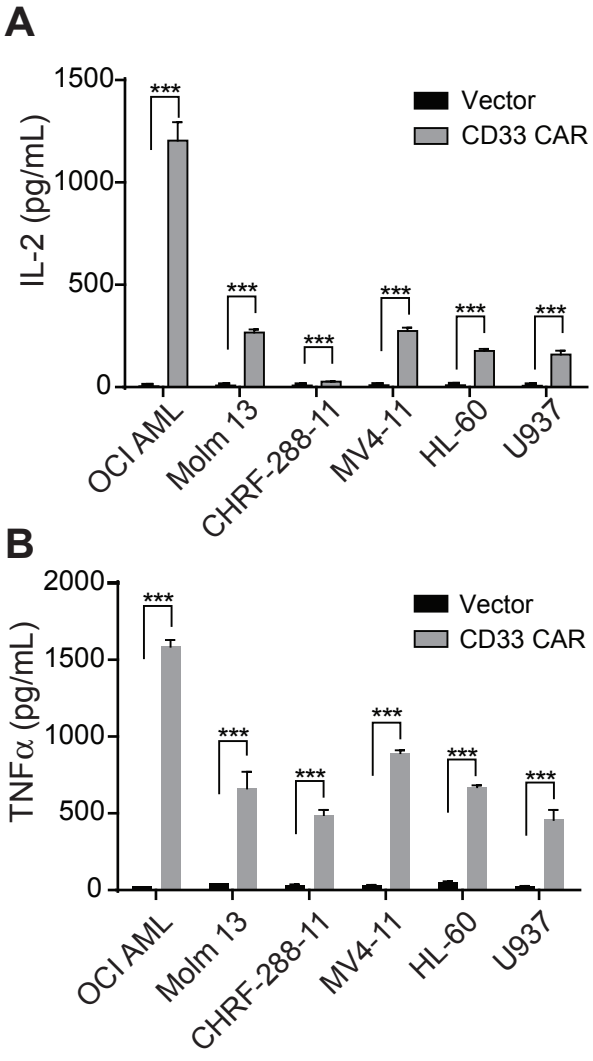
Supplemental Figure 4. Anti-CD33 CAR-modified T cells specifically target CD33$^+$ cells from normal bone marrow. Normal bone marrow samples were cultured alone or with anti-CD33 CAR or vector-transduced T cells at a 1:2 E:T ratio for 24 hours. Residual viable CD33$^+$ and CD33$^-$ cells were quantified by flow cytometry. Results represent the mean of triplicates + 1 S.D. *** $p<0.001$.



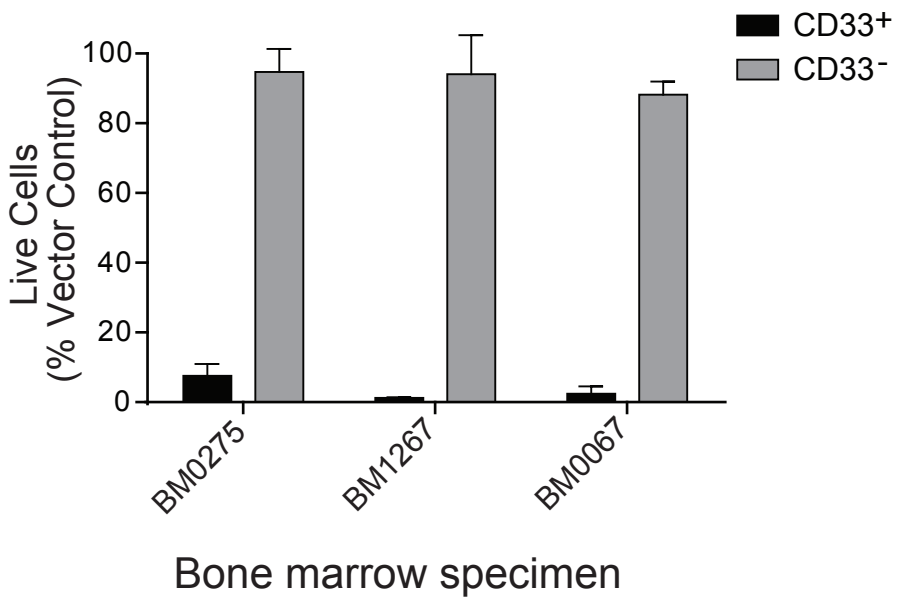
O'Hear et. al. Supplemental Figure 1



O'Hear et. al. Supplemental Figure 2



O'Hear et. al. Supplemental Figure 3



O'Hear et. al. Supplemental Figure 4