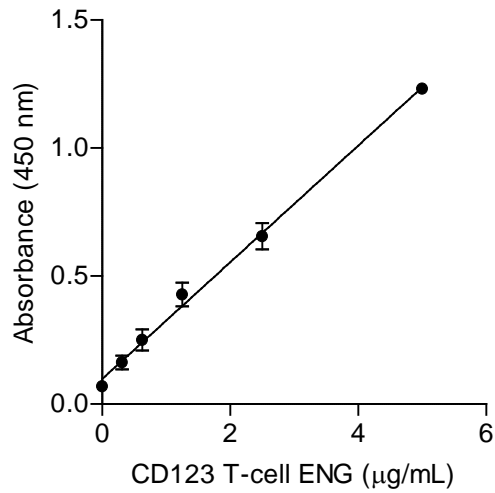
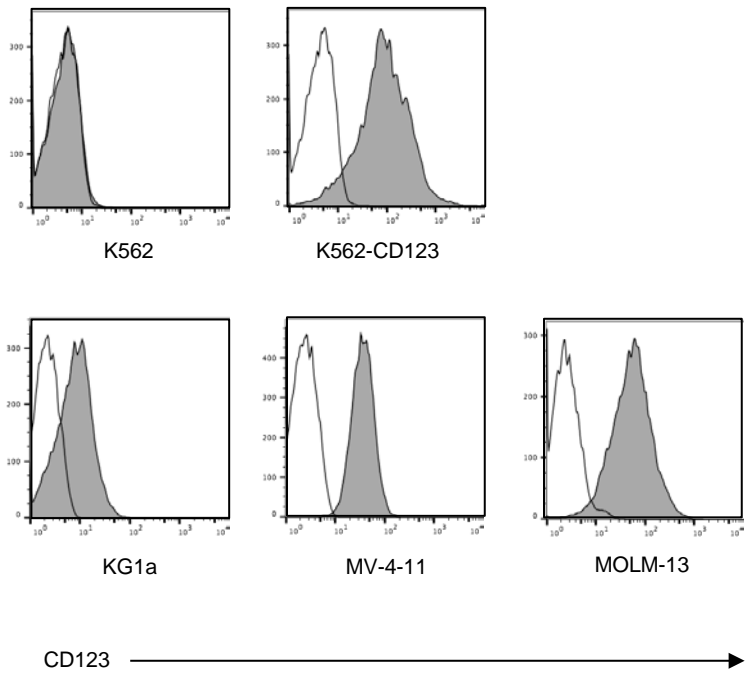


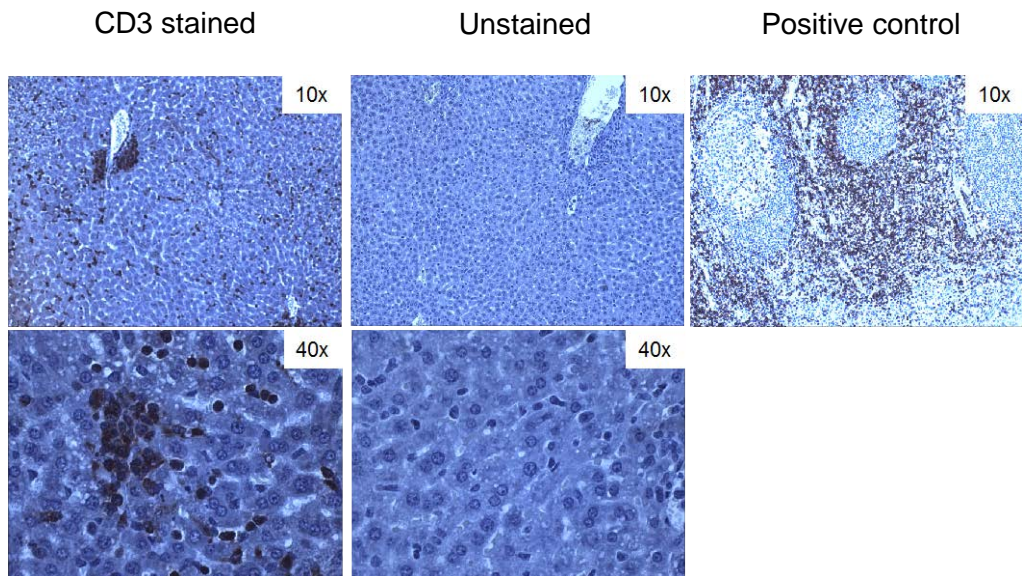
Supplemental Figure 1: CD123-ENG T cells exhibit similar T-cell phenotype to non-transduced, *ex-vivo* expanded T cells. T cells were stained with CD3, CD4, CD8, CD45RA, and CCR7 antibodies and analyzed by FACS; central memory (CM): CD45RA⁻, CCR7⁺; effector memory (EM): CD45RA⁻, CCR7⁻; naïve: CD45RA⁺, CCR7⁺; n=5; non-transduced vs CD123-ENG-T cells for all T-cell subsets: p=ns).



Supplemental Figure 2: Standard curve of developed ELISA to detect CD123 T-cell ENG protein. Recombinant CD123 T-cell ENG protein was added to non-tissue culture treated plate coated with recombinant human CD123. ELISA was performed as described in the materials and methods section. Linearity of assay was maintained at CD123 T-cell ENG protein concentrations from 0 to 5 µg/mL.



Supplemental Figure 3: Myeloid leukemia cell lines express CD123. Representative FACS histograms of CD123 expression of AML cell lines (KG1a, MV-4-11, MOLM-13). K562 and K562 expressing CD123 (K562-CD123) served as negative and positive controls.

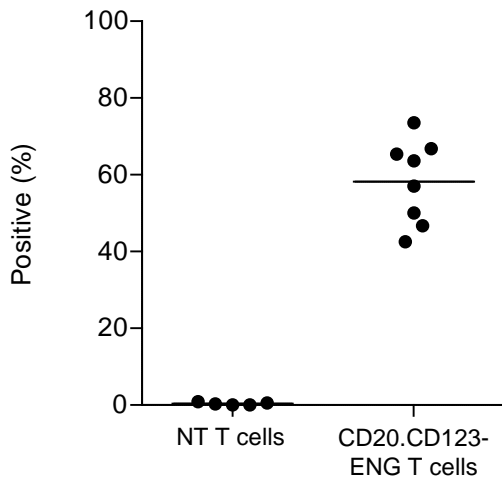


Supplemental Figure 4: Human T-cell infiltration into liver of NSG mice treated with CD123-ENG T cells. Immunohistochemical analysis for human CD3 of liver tissue obtained at necropsy on day 29 post leukemia cell injection from a mouse treated with 1×10^7 CD123-ENG T cells. Unstained murine liver tissue and human thymic tissue served as negative and positive control. Paraffin embedded tissue sections were deparaffinized in xylene and rehydrated in graded alcohol followed by water. Heat-induced epitope retrieval was performed and sample was blocked in 3% H_2O_2 . Polyclonal anti-human rabbit CD3 (Dako, Carpinteria, CA) was used for staining with hematoxylin counterstain.

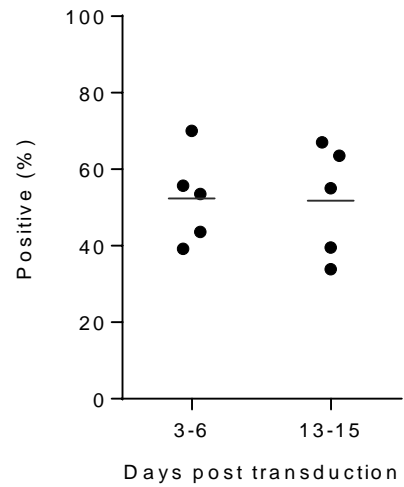
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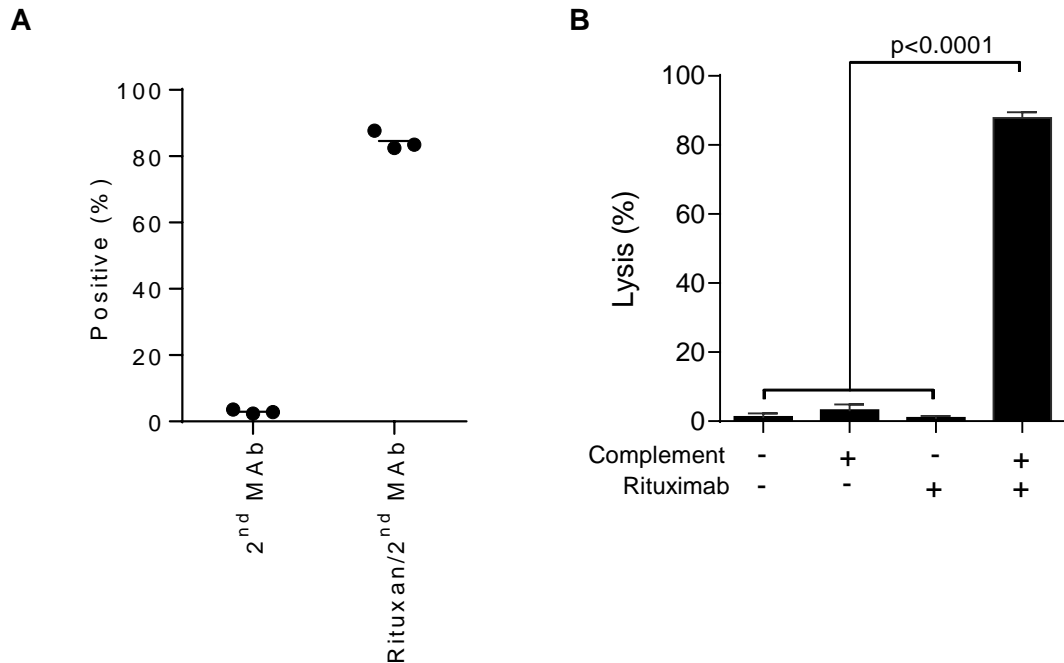
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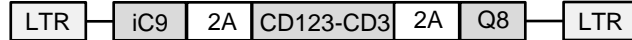
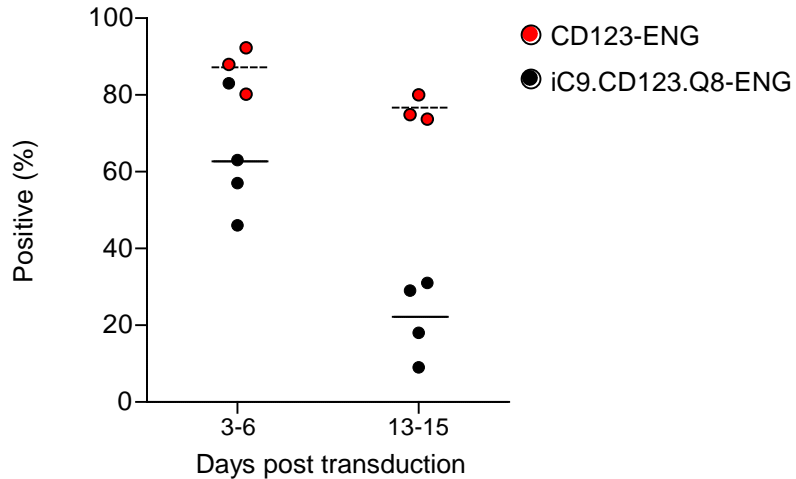
C



Supplemental Figure 5: Generation of CD20.CD123-ENG T cells. (A) Schematic of retroviral vector encoding CD20-2A-CD123-ENG. (B) FACS analysis for CD20 5-7 days post-transduction using a CD20-PE antibody (BD Biosciences). n=8; mean: 60.3%; range: 42.5-73.5%. (C) CD20 expression measured with FACS on indicated days post-transduction. n=5; mean D3-6: 52.4%; range: 39.2-70%; mean D13-15: 51.8%; range: 33.9-67%



Supplemental Figure 6: Selection and *in vitro* elimination of CD20.CD123-ENG T cells. T cells were double transduced with retroviruses encoding CD20.CD123 and GFP-ffLuc (n=3). On day 4 post transduction CD20.CD123-ENG/GFP-ffLuc T-cells were sorted with anti-CD20 Miltenyi beads. Post sorting T cells were expanded for 6 to 7 days before determining CD20 expression by FACS analysis, and conducting a 4 hour chromium release cytotoxicity assay in the presence of rituximab +/- complement. Untreated T cells served as controls. **(A)** On average, 84.6% (range: 83.6 - 88.8%) of T cells were positive for CD20 expression as judged by FACS analysis using rituximab as a primary MAb and an anti-FC MAb-FITC (2nd MAb). **(B)** In cytotoxicity assays, 88.8% (range: 86.0 – 91.2%) of T cells were killed in the presence of rituximab plus complement where as <4% of untreated T cells or T cells incubated with rituximab or complement were killed (n=3; assay performed in triplicates; rituximab plus complement treatment vs all other conditions: p<0.0001).

A**B**

Supplemental Figure 7: Expression of inducible caspase 9 (iC9) in CD-123 ENG T cells is toxic. (A) Scheme of retroviral vector. A minigene was synthesized (ThermoFisher Scientific) encoding iC9, a 2A sequence, the CD123-engager molecule, a 2A sequence, and a CD34 minitope (Q8) to allow FACS detection¹, and subcloned into a SFG retroviral vector (iC9.CD123-ENG.Q8). **(B)** T cells were either transduced with CD123-ENG or iC9.CD123-ENG.Q8 RD114-pseudotyped retroviral particles. Percent positive cells was determined early (3-6 days) and late (13-15 days) post transduction using FACS analysis for mOrange (CD123-ENG T cells) or Q8 (anti-CD34-PE; Abcam, Cambridge, MA). For iC9.CD123-ENG.Q8 T cells there was a significant decrease of transduced cells within 14 days of culture (n=4, day 3-6 mean: 62.25% (range 46-83%) vs day 13-15 mean: 21.75% (range 9-31%): p<0.005). In contrast, no decrease was observed for CD123-ENG T cells (n=3, day 3-6 mean: 86.8% (range 80-92%) vs day 13-15 mean: 76.2% (range 74-80%): p=ns).

1. Philip B, Kokalaki E, Mekkaoui L et al. A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy. *Blood* 2014;124:1277-1287.