Supplementary Materials

Study Results

Eighteen patients received ML NK infusion but 3 were unevaluable due to insufficient cell dose (n=1) or early death (n=2). Among the 15 evaluable patients, 14 had AML and 1 MDS (clinical summaries in Table S1). Median age was 72 years (range, 43-83), and median number of prior therapies was 2 (range, 1-7). Among the toxicities attributed to ML NK cells, none occurred in more than one patient, and all were grade 1-2 (Tables S2-3). There were no dose limiting toxicities, and no deaths were attributed to ML NK cells. No GVHD or CRS was observed.

Among the 15 evaluable patients, 7 achieved CR (n=3) or CRi (n=4), and 3 had a best response of morphologic leukemia free state at day 14 by the IWG response criteria (15), for an overall response rate of 67% and a CR/CRi rate of 47%. Median leukemia-free survival among responding patients was 84 days, with one patient in ongoing remission after allogeneic stem cell transplant.

Two AML patients were retreated with lymphodepleting chemotherapy and infusion of ML-NK cells prepared from their original donors. The first was a 77 year-old man who achieved a CR lasting 126 days after his first course of study treatment. He then received a second infusion of ML-NK cells 57 days after disease progression. He had no evidence of leukemia on day 14 but died of sepsis on day 19. The second was a 71 year-old female who achieved a CR lasting 73 days after the first infusion of ML-NK cells. She received a second infusion of ML-NK cells 84 days after disease progression and achieved a second CR lasting 121 days, until her death from pneumonia.

Materials and Methods

Flow Cytometry Antibodies

Cells were stained for viability using zombie NIR (Biolegend), according to manufacturer's instructions and then surface antibody staining was performed for 15 minutes at 4°C in FACS buffer (PBS, 2% FBS, 1mM EDTA). Cells were washed twice and fixed using eBiosciences Fix/Perm, according to manufacturer's instructions. Permeabilized cells were stained for intracellular markers for 30 minutes at 4°C in 1X permeabilization buffer. Cells were washed and assessed. The following antibodies BD antibodies were used: CD8 (SK1), CD16 (3G8), Ki67 (B56), phospho (p)-STAT4 (38/p-Stat4), p-STAT5 (47/Stat5, pY694), p-ERK (pT202/pY204), p38 (pT290/pY182), p65 (pS529), and HLA-A2 (BB7.2), TCR-ab (WT131), Blimp-1 (6D3). The following Biolegend antibodies were used: T-bet (4B10), CD107a (H4A3), IFN-γ (B27), GATA3 (16E10A23), HLA-A2 (BB7.2) and HLA-E (3D12), TCR Va24-Ja18 (6B11), TCR gd (B1), TCF7 (7F11A10). The following eBiosciences antibodies were used: E4BP4 (MABA223), EOMES (WD1928), and HLA-A3 (GAP.A3). The following Beckman Coulter antibodies were used: CD45 (A96416), CD3 (UCHT1), and NKG2A (Z199). Runx3 (CBFA3) was purchased from R&D. The following Miltenyi antibodies were used: HLA-Bw6 (REA143), HLA-A9 (REA127), HLA-A2 (REA517), HLA-A2/A28 (REA142), HLA-Bw4 (REA274). Runx2 (D1L7F) was purchased from Cell Signaling.

CRISPR/Cas9 Gene Editing Efficiency

DNA was isolated from NK cells electroporated with Cas9 mRNA (Trilink) and respective sgRNA (Trilink, IDT, and Synthego) from 5-6 donors using Qiagen gentra puregene kit, according to manufacturer's instructions. CRISPR editing efficiency was determined by Next Generation Sequencing of the region around the respective sgRNA targeting site. Amplicons

were prepared using the primers F_5'AGAAGCTCATTGTTGGGATCCTG3' and R_5'ACAATGAGAACTCTATTCCCTGAAA3' for NKG2A (KLRC1); and F_5'AGCTAAGAGACATCCCTCCG 3', R_5' CTCTGTCACTCTACCTGGGTGR3' for Eomes. Sequencing data were analyzed with CRISPResso2 (48). CRISPR editing efficiency was calculated as 100 - Percent of WT allele reads.