Immune monitoring of PNK-007, an allogeneic, off the shelf NK cell in a Phase I study of acute myeloid leukemia

#LB-070

William van der Touw[1], Lin Kang[1], Julie Curtsinger[2], Vanessa Voskinarian-Berse[1], Bhavani Stout[1], Mohamad A. Hussein[3], Sarah Cooley[2], Jeffery S. Miller[2], Robert Hariri[1], Xiaokui Zhang[1]

[1]Celularity, Inc, Warren, NJ; [2]University of Minnesota, Minneapolis, MN; [3]Celgene Corporation, Summit, NJ.

INTRODUCTION

Background

- Natural Killer (NK) cells are innate immune cells which play an important role in host immune surveillance against pathogenic infection and cell transformation. Multiple studies adoptively transferring NK cells in clinical settings have demonstrated the potential of NK cells to induce remissions for hematological indications with a consistent safety profile.
- Celularity has developed a GMP procedure for generating Placenta-derived intermediate Natural Killer cells (PNK-007) from placental/umbilical cord blood CD34+ cells. This technology platform enables the scalable commercial production of a uniform allogeneic NK cell therapy.
- PNK-007 shows cytotoxic activity against various cancer cell lines and is being evaluated for the treatment of relapsed/refractory AML patients in a Phase I study. Here, we provide translational data monitoring PNK-007 *in vivo* persistence and phenotypic characterization for patients enrolled in the multi-center CCT-PNK-001-AML study in the context of immune profiling and disease state.

PNK-007 manufacturing process overview

Donor Isolation

Cryopreserved CD34+ Bank

CD34 Donor cell Screen/Testing

Cryopreserved PNK-007 Drug Substance

Cytokine Cocktail-driven Expansion/Differentiation Process

Freeze for DS Release

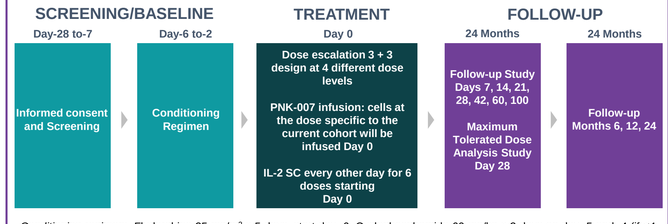
- **Donor Capacity**: 10,000 - 50,000 fold expansion
- **Viability**: >80% NK viability (7AAD)
- **Purity**: >85% CD56+/CD3-
- **Functionality**: >50% K562 cytotoxicity @ 10:1 E:T
- **Process Capacity**:12 billion cell yield (DS)
- **Quality**: Systems, testing, residuals, closure

PNK-007 Product

- Recovery Culture
- Washing
- Formulation
- Release Assays

- Shipping 2-8° C
- Administer IV

Overview of PNK-001-AML clinical protocol



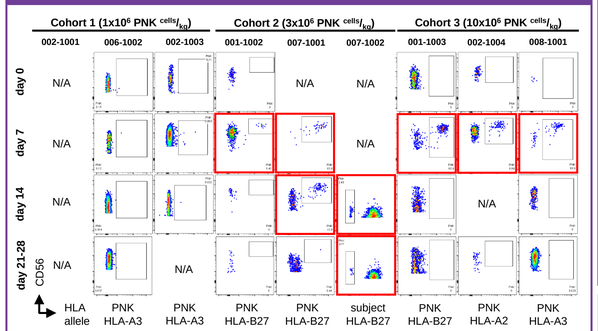
Conditioning regimen: Fludarabine 25 mg/m^2 x 5 days, start day -6, Cyclophosphamide 60 mg/kg x 2 days on day -5 and -4 (if <4 months since transplant, omit day -4 dose).

IL-2 to facilitate NK cell survival and expansion: IL-2 at 6 million units SC beginning Day 0, every other day for 6 total doses.

Acetaminophen and diphenhydramine: Acetaminophen 650 mg PO and diphenhydramine 25 mg PO prior to PNK-007 infusion and 4 hours after PNK-007 and IL-2 SC

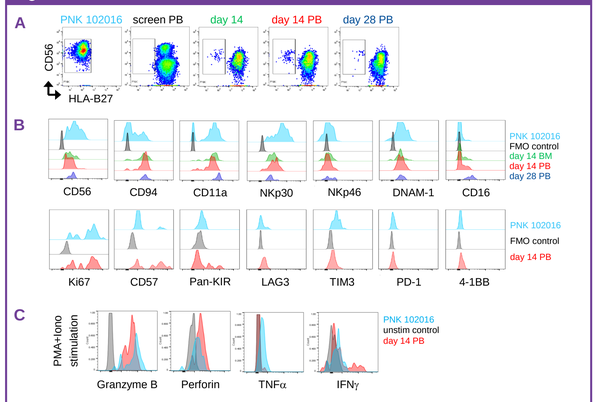
RESULTS

Figure 1. PNK-007 cell persistence in peripheral blood



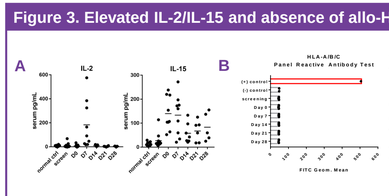
NK cells among patient peripheral blood mononuclear cells (PBMC) were identified based on the CD45+CD3-CD56+ population shown after excluding debris, dead cells, and doublets. The indicated HLA allele specific antibodies distinguished PNK-007 from patient endogenous NK cells. Plots highlighted in red indicate the presence of PNK-007 persistence detected based upon negative and positive staining controls.

Figure 2. PNK-007 cells further mature *in vivo*



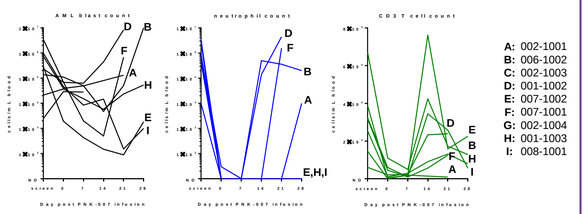
Subject 007-1002 PBMC (and bone marrow aspirate when available) was characterized for PNK-007 cell persistence and extended phenotype characterization. **A)** Persistent PNK-007 cells were identified by gating on HLA-B27-CD56+ cells. PNK-007 102016 drug substance prior to infusion is shown as a staining control. **B)** Gated PNK-007 cells from A) were analyzed for expression of NK lineage and immunomodulatory cell surface markers. **C)** To measure cytokine responses, total cells were stimulated 4 hours in the presence of PMA+ionomycin +brefeldin A followed by surface staining and intracellular staining following cell fixation and permeabilization.

Figure 3. Elevated IL-2/IL-15 and absence of allo-HLA antibodies in serum



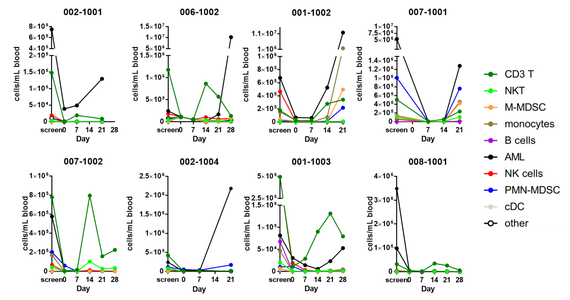
A) IL-2 and IL-15 were measured in AML serum samples by Luminex (Millipore) relative to normal serum. All patients are plotted versus post PNK-007 infusion day. Day 7 IL-2 spike corresponds to the rhIL-2 dosing window. **B)** Alloantibodies against HLA-A/B/C were tested using FlowPRA flow cytometry test (OneLambda). Alloantibody binding is shown as FITC signal geometric mean versus controls.

Figure 4. AML blast and immune reconstitution post PNK-007 infusion



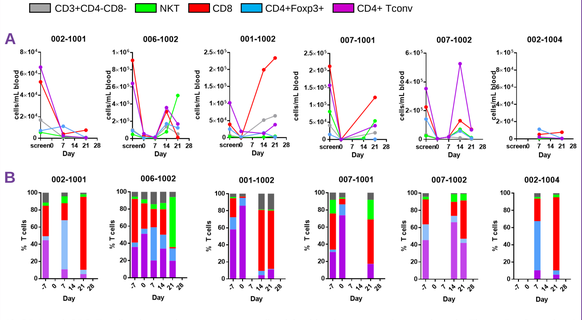
Peripheral blood characterized by hematology analyzer complete blood count and flow cytometry. **Left black)** AML blasts percentages were identified by FACS based upon light scatter, aberrant expression of CD34, CD33, CD45, and CD56. **Center blue)** Neutrophils were determined by CBC count. **Right green)** CD3 T cells were identified by FACS based upon CD45+CD3+ cells among lymphocytes.

Figure 5. PBMC cell profiling post PNK-007 infusion



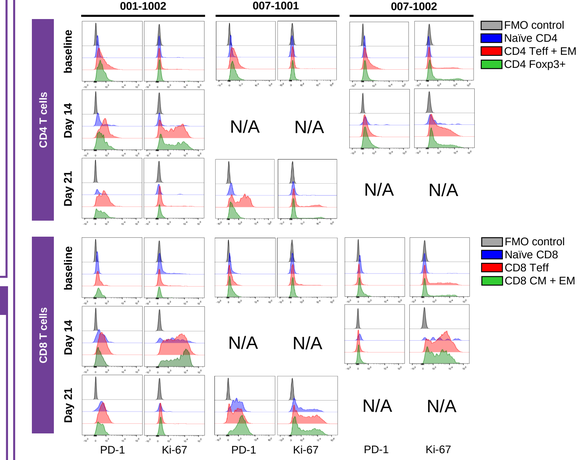
PBMC were characterized by multiparameter flow cytometry (CD45, CD38, CD11b, CD14, CD19, CD33, CD56, CD3, HLA-DR, CD34) to distinguish immune cell subsets. Cell percentages were normalized to leukocyte cell counts to determine immune cell subset blood concentration.

Figure 6. Blood T cell recovery and subset distribution post PNK-007 infusion



A) CD4 and CD8 T cell subsets shown were normalized for blood concentration as done in Figure 5. Over the 4 weeks post PNK-007, the T cell compartment expands from day 7 to 14. The day 14 sample was unavailable for subjects 002-1001, 007-1001 and 002-1004. T cells contract by day 21, except in 001-1002 and 007-1001 where CD8 T cells remain elevated. **B)** The percentage of each T cell subset among CD3+ T cells is summarized highlighting the higher proportion of Treg at day 7 and sustained proliferation of CD8 T cells in 001-1002 and 007-1001 beyond day 14.

Figure 6. Immune checkpoint expression on T cells post PNK-007 infusion



Sample permitting, T cell subsets were monitored for proliferation (Ki-67) and expression of PD-1 immune checkpoint. Total CD4 T cells of effector and regulatory subsets were proliferating at day 14 while all CD8 T cell subsets were proliferating. While T cell PD-1 expression was upregulated from baseline post PNK-007 injection on CD4 and CD8 T cell subsets, variability among patients was high with 007-1002 expressing negligible PD-1 at all time points.

CONCLUSIONS

Conclusions

- Circulating PNK-007 cells persist between 7 to 28 days (mean = 11 days) following IV infusion at dose level ≥3x10^6 cells/kg.
- Persistent PNK cells maintain NK lineage marker expression with subsets upregulating CD57 and KIR. PNK-007 stain for granzyme B, perforin, IFNγ, but not inhibitory checkpoint molecules PD-1, TIM3, and LAG3.
- Serum analysis demonstrated absence of allo-HLA antibodies in all subjects
- Cy/Flu + PNK-007 reduces AML blasts in the blood between days 0 and 7 post infusion, but increasing blast counts was observed by day 28.
- RR AML patients have poor recovery of neutrophils, lymphocytes, and myeloid-derived suppressor cell expansion associated with AML blasts.
- Treg are a significant proportion of T cells at day 7. T cell expansion occurs by day 14 followed by contraction by day 21. PD-1 expression can be high but is variable by patient.

Discussion

Our data demonstrate the feasibility of monitoring allogeneic PNK-007 and immune correlatives in patients for up to 28 days post infusion. Limited normal hematopoiesis was observed in conditioned RR AML patients and we observed presence of immune-suppressed subpopulations. The results of this study will be used to inform the design of future trials in AML.