Immune Monitoring of CD34+ Placental Cell Derived Natural Killer Cell Therapy (PNK-007) in Phase I Study of Multiple Myeloma

#4457

William Van Der Touw[1], Lin Kang[1], Joseph Dennis Tario[2], Bhavani Stout[1], Vanessa Voskinarian-Berse[1], Valentina Rousseva[1], Paul K. Wallace[2], Robert Hariri[1], and Xiaokui Zhang[1]

[1]Celularity, Inc, Warren, NJ; [2]Roswell Park Comprehensive Cancer Center, Buffalo, NY

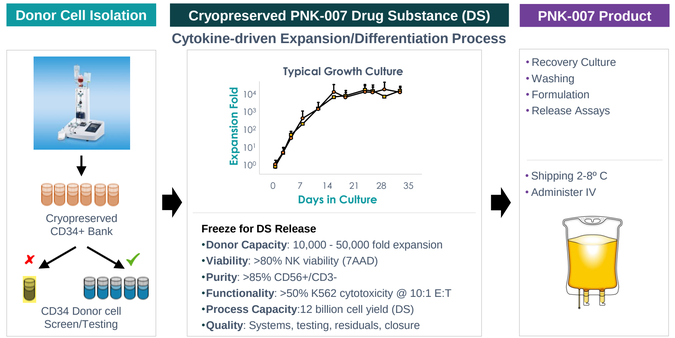
INTRODUCTION

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 remission for hematological indications with a consistent safety profile.

Celularity has developed a novel proprietary GMP procedure that enables the scalable production of an off-the-shelf, allogeneic NK cell therapy. Using this technology platform, Celularity developed PNK-007, a Placental Hematopoietic Stem Cells Derived Natural Killer cell therapy.

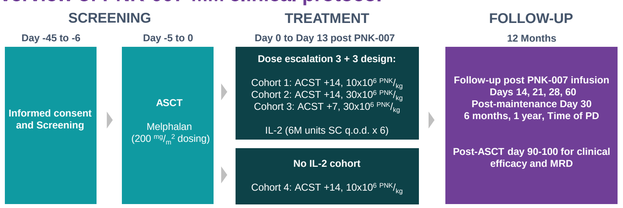
PNK-007 shows cytotoxic activity against various cancer cell lines and has been evaluated in a Phase I study for the treatment of relapsed/refractory acute myeloid leukemia and in multiple myeloma patients undergoing autologous stem cell transplant (ASCT). Here, we present translational data from multiple myeloma monitoring minimal residual disease (MRD) using EuroFlow validated assay for 1 year. We characterize immune reconstitution and immune correlates associated with the clinical protocol and PNK-007 administration.

PNK-007 manufacturing process overview



CD34 donor cells are screened and tested for use in PNK-007 manufacturing. Cells are harvested following a 35 day expansion and differentiation process, then frozen as Drug Substance. Qualified Drug Substance undergoes a final formulation and release process and is distributed as a fresh formulated product.

Overview of PNK-007-MM clinical protocol



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.

EuroFlow MRD summary of enrolled patients

		Cohort 1						Cohort 2			Cohort 3			Cohort 4		
Prior lines		0	1	0	0	0	0	2	0	0	0	0	5	0	0	0
Subject		002-1001	002-1002	002-1003	002-1008	002-1005	003-1001	005-1001	008-1001	008-1002	001-1001	009-1001	009-1002	002-1006	008-1003	002-1007
Baseline	MRD	+	+	+	+	+	+	NE	+	+	-	+	+	-	-	-
Day 90	MRD	-	-	+	-	+	+	+	-	-	-	-	+	-	-	-
1 Year	MRD	NE	-	-	-	DD	+	+	-	-	ND	ND	WD	NE	-	-

MRD assay performed on fresh bone marrow aspirate at LOD < 10^{-5} according to EuroFlow guidance. Subjects in green indicate conversion from MRD(+) to MRD(-) by 1 year, orange indicates unchanged MRD(+) status, gray indicates MRD(-) status at time of enrollment. Abbreviations legend: ND: Not Done; NE: Not Evaluable due to insufficient sample, DD: Discontinued due to Physician Decision, WD: Patient withdrawal

RESULTS

Figure 1. EuroFlow MRD assessment of patient bone marrow post-ASCT

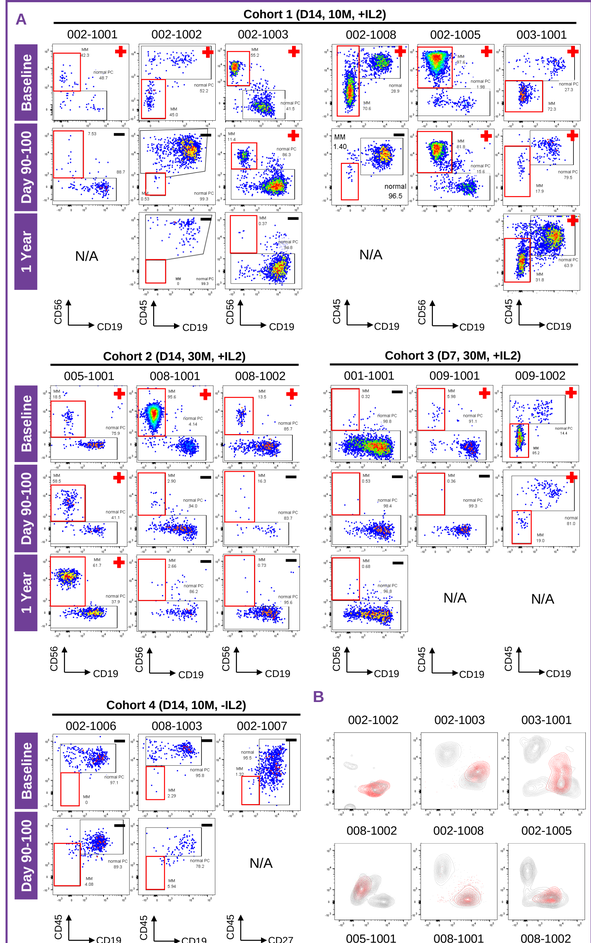


Figure 1. Patient bone marrow aspirate was collected at sites and processed by central lab for MRD testing. **A)** Samples were stained with EuroFlow MRD Tube 1 detecting CD138, CD27, CD38, CD56, CD45, CD19, CD117, and CD81. Total CD38+138+ plasma cells are shown using the indicated markers to differentiate normal plasma cells (black gates) from abnormal plasma cells (red gates). Baseline sample shown for subject 005-1001 is MRD(+) peripheral blood due to unavailability of bone marrow aspirate (BMA). **B)** BMA were stained with EuroFlow MRD Tube 2 detecting CD138, CD27, CD38, CD56, CD45, CD19, Ig-λ, and Ig-κ. Normal (gray) and abnormal (red) plasma cells as determined in A) are shown for usage of the Ig-λ or Ig-κ light chain by intracellular staining to confirm myeloma clonality. MRD(-) samples with insufficient numbers of abnormal plasma cells are not shown.

Figure 2. PNK-007 dosing does not interfere with ASCT

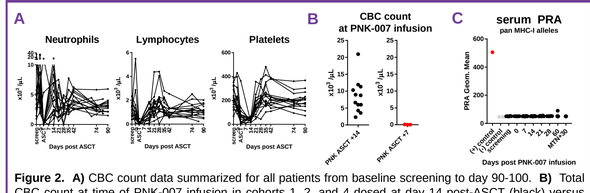


Figure 2. A) CBC count data summarized for all patients from baseline screening to day 90-100. **B)** Total CBC count at time of PNK-007 infusion in cohorts 1, 2, and 4 dosed at day 14 post-ASCT (black) versus cohort 3 dosed at day 7 post-ASCT (red). **C)** Panel Reactive Assay (PRA) test was performed to detect anti-HLA antibodies in the serum. Among 15 patients, 8 patients had 6/6 mismatches, 4 patients had 5/6 mismatches, 2 patients had 4/6 mismatches, and 1 patient had 3/6 mismatches for alleles within HLA-A/B/C.

Figure 3. rhIL-2 does not enhance post-ASCT NK cell recovery

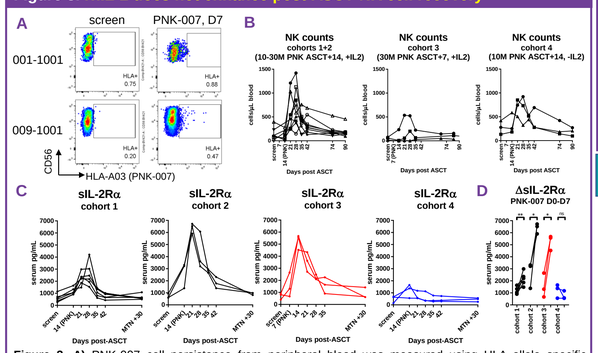


Figure 3. A) PNK-007 cell persistence from peripheral blood was measured using HLA allele specific antibodies to distinguish PNK-007 from patients' own NK cells. Total CD56+CD3- NK cells are shown from cohort 3 using anti-HLA-A03 to distinguish PNK-007 at day 7, the earliest timepoint collected post-infusion. PNK-007 persistence was not observed in any other cohorts (data not shown) at post infusion day 7. **B)** Total NK cell frequency determined by flow cytometry comparing infusion day 14 + IL-2 (left), infusion day 7 + IL-2 (center) and infusion day 14 – IL-2 (right). **C)** Soluble levels of IL-2 receptor alpha in the serum measured using the Milliplex human soluble cytokine receptor panel (EMD Millipore). Cohorts 1 and 2 are shown in black, cohort 3 shown in red, and cohort 4 shown in blue. **D)** Paired T test comparing soluble IL-2 receptor levels in serum pre-infusion to 7 days post-infusion. (*p<0.05, **p<0.005, ns: not significant)

Figure 4. rhIL-2 increases circulating T_{reg} post-ASCT

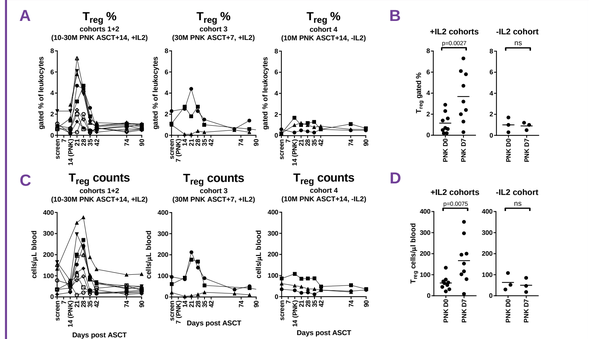


Figure 4. Patient PBMC were stained with CD4, CD25, and CD127 to identify regulatory T cells (T_{reg}) **A)** T_{reg} percentage were monitored up to day 90 post ASCT separated by cohorts receiving rhIL-2, not receiving rhIL-2, and PNK-007 dosing at ACST Day +7. **B)** Paired T test analysis of T_{reg} change at PNK-007 pre-infusion versus Day +7. **C** and **D)** Same analysis as part A) and B) but based on T_{reg} blood concentration.

Figure 5. T cell activation and cytokine profile monitoring post PNK-007

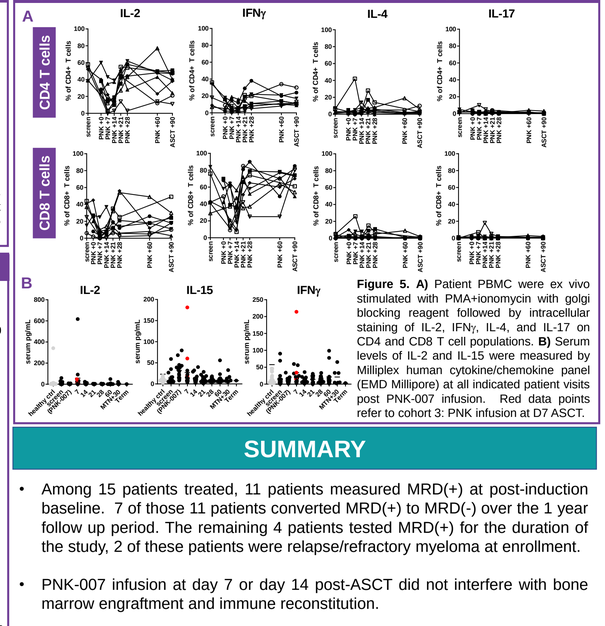


Figure 5. A) Patient PBMC were ex vivo stimulated with PMA+ionomycin with golgi blocking reagent followed by intracellular staining of IL-2, IFNγ, IL-4, and IL-17 on CD4 and CD8 T cell populations. **B)** Serum levels of IL-2 and IL-15 were measured by Milliplex human cytokine/chemokine panel (EMD Millipore) at all indicated patient visits post PNK-007 infusion. Red data points refer to cohort 3: PNK infusion at D7 ASCT.

SUMMARY

- Among 15 patients treated, 11 patients measured MRD(+) at post-induction baseline. 7 of those 11 patients converted MRD(+) to MRD(-) over the 1 year follow up period. The remaining 4 patients tested MRD(+) for the duration of the study, 2 of these patients were relapse/refractory myeloma at enrollment.
- PNK-007 infusion at day 7 or day 14 post-ASCT did not interfere with bone marrow engraftment and immune reconstitution.
- Serum analysis demonstrated absence of allo-HLA antibodies in all subjects.
- PNK-007 cell persistence was not detectable by flow cytometry in patients at day +7 post PNK-007, the earliest post-infusion timepoint measured.
- rhIL-2 administration did not significantly affect reconstitution of patients' NK cells. However, rhIL-2 stimulated shedding of soluble IL-2 receptor in the serum, indicative of a negative feedback loop. rhIL-2 also significantly enhanced transient levels of T_{reg} relative to patients not receiving rhIL-2.
- Patients' CD4 and CD8 T cell populations showed inhibited effector response 7-14 days post PNK-007 dosing, potentially in response to rhIL-2 or increased numbers of T_{reg}. At all measured timepoints up to day 90 post ASCT, T cells primarily secreted IL-2 and IFNγ in response to PMA/ionomycin stimulation with minimal levels of IL-4 and IL-17.

Discussion

Translational data from this Phase I study of PNK-007 established that dosing up to $3x10^7$ cells/kg at 14 or $1x10^7$ cells/kg at 7 days post-transplant did not impair engraftment or immune reconstitution. EuroFlow MRD assessment of the bone marrow showed conversion of 7 of 11 patients from MRD(+) to MRD(-) over the course of 1 year.

The administration of IL-2 in this clinical study did not appear to benefit NK cell reconstitution but instead stimulated soluble IL-2Rα antagonism and increased systemic levels of T_{reg}, highlighting potential disadvantages of rhIL-2 administration in this setting. Our results support the feasibility of PNK-007 in the MM + ASCT setting and will help inform the design of further clinical studies.

For a clinical summary associated with this clinical trial, please visit **Poster #4451** titled "Results of a Phase I Study of PNK-007, Allogeneic, off-the-shelf NK cell, post autologous transplant, in Multiple Myeloma (NCT02955550)".