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Potent *ex vivo* Expanded, Human CD34+ Cord Blood-derived Natural Killer Cells for Cancer Immunotherapy

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INTRODUCTION

Multiple myeloma (MM) is a malignancy of antibody-secreting plasma cells characterized by complex genetic and epigenetic abnormalities that accumulate over time as the disease progresses from asymptomatic precursor conditions to symptomatic MM (Bruno, 2005). Despite significant improvements in patient outcomes following the introduction of immunomodulatory drugs (IMiDs) , proteasome inhibitors (PIs), histone deacetylase inhibitors and monoclonal antibodies, MM remains an incurable disease and the prognosis of patients with relapsed/refractory MM remains very poor (Cook, 2018). As such, there is an unmet medical need to develop novel treatments for relapsed/refractory MM patients.

It has been postulated that during disease progression, the escape of MM cells from immune surveillance is associated with the impairment of NK cell function (Dosani, 2015). NK cells from MM patients often display reduced expression of activating receptors NKG2D, NKp30, 2B4, and DNAX accessory molecule-1 (DNAM1), and decreased capacity for antibody-dependent cell-mediated cytotoxicity (ADCC) (Dosani, 2015.

Shah et al (2013) demonstrated that ex vivo expanded umbilical cord blood (UCB) NK cells showed anti-MM activity in vitro and in vivo. The infusion of activated and expanded peripheral blood (PB) NK cells inhibits tumor growth and prolongs survival in a murine model of MM (Garg, 2012). These observations together with the reported safety, *in vivo* persistence and efficacy results from adoptive NK cell immunotherapy in MM patients (Pittari, 2017; Shah, 2017) provide a rationale for the use of NK cell-based therapies for the treatment of MM.

Celularity has established an *ex vivo* process to generate PNK-007 cells from UCB CD34$^+$ cells with average of 1.0 x 10^5 fold expansion and ~90% purity of CD56$^+$CD3$^-$ cells. Despite their less mature phenotype as evidenced by the low expression of CD16 and KIRs in comparison to NK cells derived from PB, PNK-007 cells displayed substantial anti-tumor activity *in vitro* against MM tumor cell lines including primary MM cells, cytokine secretion activity following exposure to tumor cells, together with *in vivo* efficacy against RPMI8226 xenograft murine model. Celularity is currently conducting a Phase I study to assess the safety, maximum tolerated dose and potential clinical efficacy of PNK-007 in MM patients undergoing autologous stem cell transplant (NCT02955550).

METHODS

***Ex vivo* expansion of PNK-007 cells**: UCB CD34$^+$ cells were cultivated in the presence of cytokines including thromobopoietin, SCF, Flt3 ligand, IL-7, IL-15 and IL-2 for 35 days to generate PNK-007 cells.

Phenotype characterizations of PNK-007 cells: PNK-007 cells were subjected to phenotypic characterization via multi-color flow cytometry using a FACSCanto II (BD Biosciences). Data analysis was performed using FlowJo software (TreeStar).

***In vitro* cytotoxicity of PNK-007 against MM cells:** 4h cytotoxicity of PNK-007 against tumor cell lines was assessed by a PKH26/TO-PRO-3 FACS based assay (Lee-MacAry et al, 2001). A range of effector to target (E:T) ratios were assessed as indicated. MM tumor cell lines: RPMI8226, IM-9 and NCI-H929 were obtained from ATCC, OPM2 was obtained from DSMZ. Primary BM MM cells were obtained from Tissue solution. Counting beads (Invitrogen) were introduced to 24h cytotoxicity to identify viable MM cells after 24h coculture.

***In vitro* cytokine secretion of PNK-007 in the presence of MM cells:** PNK-007 cells were incubated with MM cells in 96-well U-bottom tissue culture plates at an E:T ratio of 1:1 for 24 hours. After incubation, the supernatant was collected and cytokine concentrations were determined by Luminex analysis using MILLIPLEX MAP magnetic bead kits according to the protocol provided by the manufacturer (EMD Millipore). Data were analyzed using Milliplex Xponent and Analyst software.

***In vivo* RPMI8226 xenograft NSG murine model:** NOD/SCID IL-2Rγ^{null} (NSG) mice were preconditioned with busulfan (30mg/kg, intraperitoneal injection) at Day-2 and inoculated with 5x10^6 RPMI8226 cells (subcutaneously) or 2x10^5 RPMI8226 (intravenously) at Day0. 1x10^7 PNK-007 cells were given intravenously at single dose or repeated doses at the time points indicated with/without IL-15 (0.5μg) intraperitoneally (IP) every other day for two weeks. Tumor size was measured twice per week until study termination. Survival was calculated using Kaplan-Meier method (GraphPad Prism).

RESULTS

Ex vivo Cultivation Procedure Established to Produce PNK-007 Cells

Celularity has established an *ex vivo* process to generate PNK-007 cells from UCB CD34$^+$ cells with an average of 1.0 x 10^5 fold expansion and 88.3% ± 6.3% purity of CD56$^+$CD3$^-$ cells (n=20 donors) (Figure 1A, 1B). PNK-007 express comparable percentage of NKG2D, NKp46, NKp30, CD94, CD226 and 2B4, higher percentage of NKp44, and lower percentage of CD16 and KIRs in comparison to NK cells derived from PB (Figure 1C).

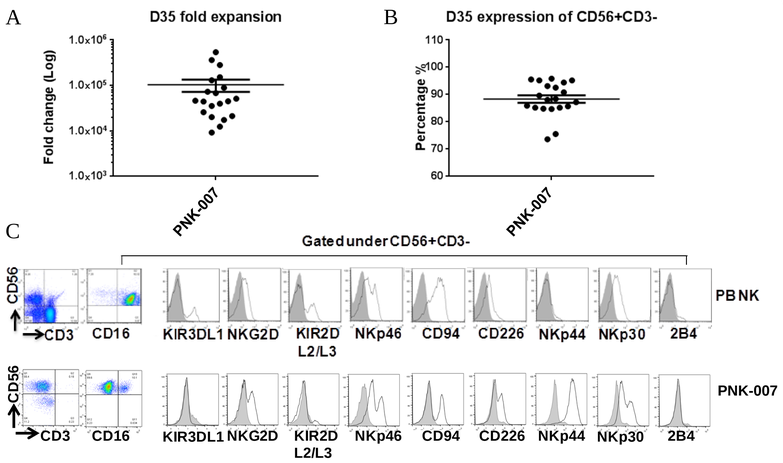

Figure 1. Fold expansion, purity and phenotype characterization of UCB CD34$^+$ derived PNK-007 cells. A) Fold expansion. B) Purity by % CD56$^+$CD3$^-$ of *ex vivo* expanded PNK-007 cells (n=20 donors). C) Expression of NK surface markers of PNK-007 cells in comparison with PB NK cells under the gate of CD56$^+$CD3$^-$.

In Vitro Anti-tumor Activity of PNK-007 Against MM Cells

PNK-007 cells exhibited 36.0%, 12.3%, 16.4%, and 10.0% cytotoxicity against RPMI 8226, IM-9, NCI-H929, and OPM-2 MM cell lines, respectively, in a 4-h cytotoxicity assay at E:T ratio of 3:1 (Figure 2A). PNK-007 cells did not show any cytotoxic activity against PBMCs from unrelated healthy donors at any of the E:T ratios tested up to 100:1 (Figure 2B), indicating that PNK-007 cells were capable not only of lysing tumor cells but also of discriminating between healthy and tumor targets. In a 24-hour cytotoxicity assay at an E:T ratio of 3:1, the cytotoxicity of PNK-007 was 77.8%, 14.9%, 55.8%, and 49.3% for RPMI 8226, IM-9, NCI-H929, and OPM-2, respectively (Figure 2C). PNK-007 exhibited up to 68% cytotoxic activity against primary bone marrow samples from MM patients (Figure 2D). PNK-007 cells secreted immunomodulatory cytokines, namely IFN-γ, GM-CSF, and TNF-α, in the presence of all four MM tumor cell lines tested (Figure 3). In summary, PNK-007 cells were not only capable of directly lysing tumor cells, but could also indirectly stimulate anti-tumor responses through their ability to secrete immunomodulatory cytokines.

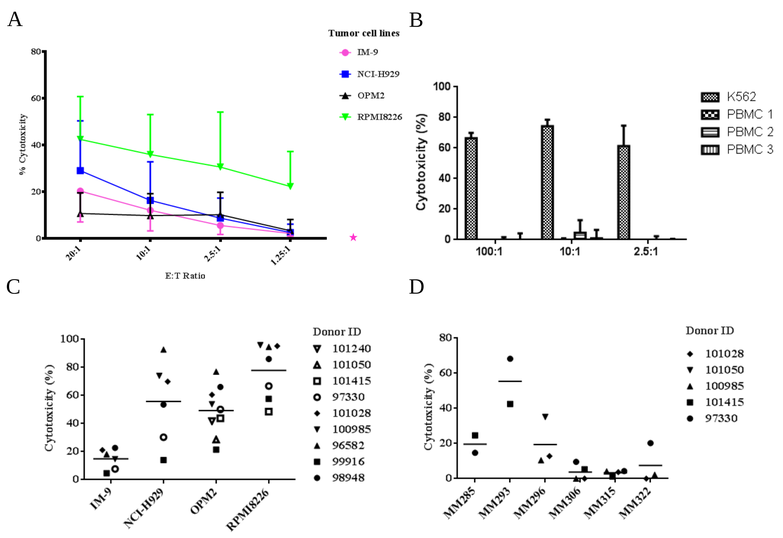

Figure 2. *In vitro* cytotoxicity of PNK-007 against MM cells. A) PNK-007 (n=10 donors) exhibited cytotoxicity against MM tumor cell lines at different E:T ratios as indicated by 4h cytotoxicity assay. B) PNK-007 (n=3 donors) displayed no cytotoxicity against unrelated healthy PBMC donors (n=3 donors). C) PNK-007 (n=9 donors) exhibited cytotoxicity against MM tumor cell lines at E:T ratio of 3:1 by 24h cytotoxicity assay. D) PNK-007 (n=6 donors) exhibited cytotoxicity against primary MM cells at E:T ratio of 3:1 by 24h cytotoxicity assay.

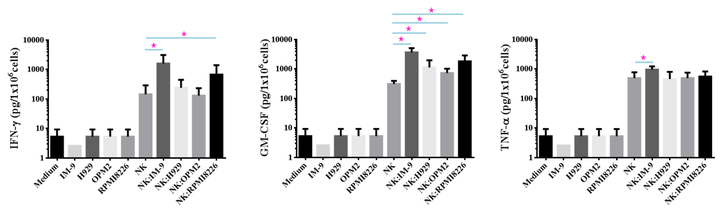

Figure 3. PNK-007 secreted IFN-γ, GM-CSF and TNF-α in the presence of MM cells at an E:T ratio of 1:1 for 24h. The error bars represent the standard deviation calculated from the mean of 8 PNK-007 donors. (P<0.05)

In Vivo Anti-tumor Activity of PNK-007 Cells Against MM Xenograft NSG Murine Model

Previously, Celularity has developed a specific animal model that allows for the evaluation of safety, biodistribution, persistence, and maturation of PNK-007 *in vivo*. The model utilized busulfan (a myeloblative conditioning agent) along with recombinant human IL-15 to facilitate PNK-007 cell survival and expansion in NSG mouse strain. Utilizing this *in vivo* model, PNK-007 was shown to be well tolerated with the doses ranging from 5 x 10^6 to 50 x 10^6 cells/mouse. PNK-007 persistence was up to 28 days post infusion in the PB, bone marrow, spleen, liver, and lung by quantitative polymerase chain reaction (qPCR) analysis.

In RPMI8226 subcutaneously xenograft NSG model, PNK-007 cells with repeat dose at Day 5 and Day 7 showed significantly reduced tumor size in comparison with vehicle control (Figure 4A, 4B). In RPMI8226 disseminated NSG model, PNK-007 with repeat dose at Day6, Day8, Day12 and Day15 improved survival rate in comparison to vehicle control (P<0.05) (Figure 4C, 4D).

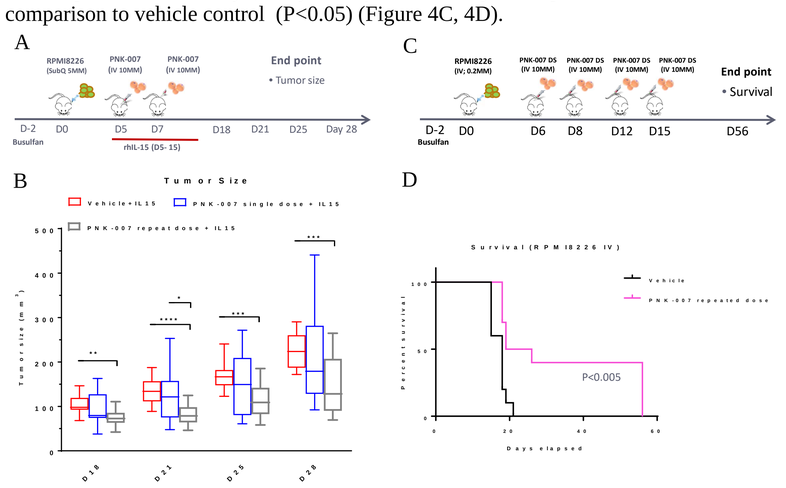

Figure 4. *In vivo* anti-tumor activity of PNK-007 cells against MM xenografted NSG mice. A) NSG mice were preconditioned with busulfan on Day-2 and inoculated with 5x10^6 subcutaneously RPMI8226 cells on Day0. On Day5, vehicle or PNK-007 (1x10^7 per mouse) were intravenously administered. For repeated dose groups, PNK-007 (1x10^7 per mouse) were administered again on Day7. Recombinant human IL-15 (0.5μg) was supplemented via intraperitoneal injection on Days 5, 7, 9, 11, 13, 15 for all groups. Tumor size was measured twice per week until study termination. B) PNK-007 with repeated dose significantly reduced tumor growth compared with vehicle control (n=10 mice per group). C) RPMI8226 cells (2x10^5 per mice) were intravenously inoculated in NSG mice on Day0 after two days of busulfan preconditioning, repeated dose of 1x10^7 PNK-007 cells were infused at Days as indicated. D) By Kaplan-Meier method, PNK-007 with repeated dose showed significant increased survival rate in RPMI8226 IV model in comparison with vehicle control (P<0.05).

CONCLUSION

Celularity has established an *ex vivo* process to generate PNK-007 cells from UCB CD34$^+$ cells with an average of 1.0 x 10^5 fold expansion and ~90% purity of CD56$^+$CD3$^-$ cells. PNK-007 displayed immature phenotype as evidence by low expression of CD16 and KIRs in comparison with PB NK. We are currently investigating the impact of the low CD16 expression on ADCC activity against various tumor cell lines. PNK-007 exhibited *in vitro* cytotoxic and cytokine secreting activities against several MM tumor cell lines, as well as cytotoxic activity against primary MM cells. PNK-007 showed no cytotoxicity against unrelated healthy PBMC, implying the property of allogeneic NK product attacking tumor cells in presence of normal mismatched PBMCs. Furthermore, PNK-007 demonstrated the *in vivo* tumor reduction and survival benefit in MM xenograft NSG model. Taken together, our data support the development of PNK-007 for the treatment of MM. Celularity is currently conducting a Phase I study to assess the safety, maximum tolerated dose and potential clinical efficacy of PNK-007 in MM patients undergoing autologous stem cell transplant (NCT02955550).

REFERENCE

1. *Bruno B et al, Leukemia 2005;19(10):1729-38.*
2. *Cook G et al, Crit Rev Oncol Hematol, 2018, 121:74-89.*
3. *Dosani T et al, Blood Cancer J 2015;5:e321.*
4. *Garg TK et al, Haematologica 2012;97(9):1348-56.*
5. *Lee-MacAry AE, et al, J Immunol Methods 2001;252(1-2):83-92.*
6. *Pittari G et al, Front Immunol, 2017, 8:1444.*
7. *Shah N et al, Plos One, 2013, 8(10):e76781.*
8. *Shah N et al, Br J Haematol. 2017 May;177(3):457-466.*