INTRODUCTION

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults (Gittelman, 2018). With intensive research efforts and a multimodal management that consists of surgery, radiotherapy and chemotherapy with temozolomide, the prognosis remains very poor with 5-year survival rate of less than 10% (Kamya-Matsuoka, 2015). Therefore, there is an unmet medical need to develop novel treatments for GBM.

It has been demonstrated that ex vivo expanded human peripheral blood NK (PiNK) cells from healthy donors exhibited in vitro and in vivo anti-tumor activities against patient derived GBM cells (Lee, 2015). It was also reported that both CD133+ medulloblastoma cells and CD133+ cancer stem cells were susceptible to NK cells-mediated killing in vitro (Castriconi, 2007). Ishikawa et al (2004) reported Phase I data for an intracranial and intravenous autologous NK cell injection that showed tumor regression in a small number of patients. Several ongoing clinical trials (ClinicalTrial.gov: NCT03360708, NCT01588769, NCT00331526, NCT03383978, NCT00003067) are investigating the potential of adoptive NK cell immunotherapy for GBM.

Celularity has established an ex vivo process to generate Placential derived intermediate Nature Killer (PiNK) cells from placental CD34+ cells with an average of 1.0 x 10^6 fold expansion and ~90% purity of CD56+CD3+ cells. Here, we have demonstrated that PiNK cells exhibited in vitro cytotoxic activity against tumor cell lines and cytokine secretion activity following exposure to tumor cells. In vivo efficacy studies further demonstrated the activity of PiNK against tumor growth in a U-87MG orthotopic NSG mouse model. Taken together, our data support the application of PiNK cells for the development of an allogeneic adoptive immunotherapeutic for patients with GBM.

METHODS

Ex vivo expansion of PiNK cells: Placental CD34+ cells were cultivated in the presence of cytokines including thrombopoietin, SCF, Flt3 ligand, IL-7, IL-15 and IL-2 for 35 days to generate PiNK cells.

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

In vitro cytotoxicity of PiNK cells against GBM cells: The cytotoxicity of PiNK cells against tumor cell lines was assessed by a PIKI6d/TO-PRO-3 FACS based assay (4-hour assay). A range of effector to target (E:T) ratios were assessed as indicated. Tumor cell lines including U-251, LN-18, U-87 MG, U-118 MG and K562 were obtained from ATCC. K562 was used as assay control. To dissect the mechanism of PiNK cells against GBM cells, the PiNK cells were pretreated with 10ng/mL blocking antibodies as indicated or corresponding isotype control; or 500M perforin inhibitor (Concanamycin A, CMA), or corresponding control at 5% CO2, 37°C for 30min, followed by 4h cytotoxicity assay against U-87 MG cells. The percentage of cytotoxicity change was reported as: (Cytotoxicity by blocking antibodies/perforin inhibitor (CMA) – Cytotoxicity by control) / Cytotoxicity by control * 100%.

In vitro cytokine secretion of PiNK in the presence of GBM cells: PiNK cells (1x10^5 cells) were incubated with GBM cells (1x10^5 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). The data were analyzed using Milliplex Xponent and Analyst software.

In vivo U-87 MG orthotopic NSG mouse model: Luciferase-expressing U-87 MG cells (1x10^5) were stereotactically injected into the cranium of NSG mice at Day0. Single dosing of 0.5x10^5 PiNK at Day14 or repeated dosing of 0.5x10^5 PiNK at Day14 and Day21 by intracranial injection (IC) were administered. Live bioluminescence imaging (BLI) were taken twice per week until study termination. In addition, clinical symptoms were monitored and body weight were measured weekly. The data were analyzed by GraphPad Prism.

RESULTS

Ex Vivo Cultivation Procedure Established to Produce PiNK Cells

Celularity has established an ex vivo process to generate PiNK cells from placental CD34+ cells with an average of 1.0 x 10^6 fold expansion and 88.3% ± 6.3% purity of CD56+CD3+ cells (n=20 donors) (Figure 1A, 1B). PiNK cells expressed similar percentages 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).

In Vivo Anti-tumor Activity of PiNK Cells Against Orthotopic U-87 MG NSG Mouse Model

The U-87 MG orthotopic mouse model was used to assess in vivo anti-GBM activity of PiNK cells. IC with single dosing of 0.5x10^5 PiNK at Day14 or repeated dosing of 0.5x10^5 PiNK at Day14 and Day21 were evaluated. No abnormal clinical symptoms were observed in the animals with either single or two repeated IC injections of 0.5x10^5 PiNK cells. PiNK cells with single (n=6 mice per group) or repeated dosing (n=3 mice per group), significantly reduced BLI signal on Day 25, 28 and 35 compared with the PBS control (n=6 mice per group) (P<0.05). Furthermore, PiNK cells with repeated IC dosing significantly reduced BLI signal on Day25, 28 and 35 compared with PiNK single IC dosing (P<0.05) (Figure 5A, 5B).

CONCLUSION

Celularity has established an ex vivo process to generate PiNK cells from placental CD34+ cells with an average of 1.0 x 10^6 fold expansion and ~90% purity of CD56+CD3+ cells. PiNK cells exhibited in vitro cytotoxic and cytokine secretory activities against several GBM tumor cell lines, whereas PiNK cells showed no cytotoxicity against unrelated healthy PBMC. In addition, our data demonstrated that Perforin, TRAIL, NKG2D and DNAM-1 played important roles in PiNK-mediated cytotoxicity against U-87 MG. Furthermore, in vivo anti-tumor activity of PiNK was demonstrated in U-87 MG orthotopic NSG mouse model. Taken together, our data support the development of PiNK for the treatment of GBM.

REFERENCES