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Title: *In Vivo* Generation of Both CAR T Cells and CAR NK Cells Using a CD7 Targeted Lentiviral Vector

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CAR-T cell therapy has shown remarkable clinical efficacy against various cancers, including hematologic malignancies. CAR-NK cells have shown promising results in cancer therapy, especially B-cell lymphoma, with a lower risk of GVHD, serious cytokine release syndrome (CRS) and immune effector-cell associated neurotoxic syndrome (ICANS) compared to allogenic CAR-T cells[1]. INT2104 utilizes a lentiviral vector platform that incorporates CD7-targeting to specifically transduce T cells and NK cells upon intravenous (i.v.) administration. Here we describe the ability of a single i.v. administration of INT2104 to transduce both T and NK cells *in vivo* generating functional CAR20+ cells resulting in the depletion of CD20+ B cells.

In vitro assessments of INT2104 confirmed transduction of both CD7+ NK cells and T cells, including CD4+ and CD8+ subsets. A humanized CD34-engrafted NSG-Tg (Hu-IL15) mouse model, which constitutively expresses human interleukin-15 to enhance NK-cell development in these mice, was utilized to demonstrate effective NK cell transduction following INT2104 administration. Mice receiving a single bolus INT2104 dose of 1×10^7 transducing units by tail vein injection were compared to control mice receiving no vector. Weekly analysis of blood confirmed CD20+ B cell depletion compared to untreated control mice. At select timepoints throughout the study, the distribution and persistence of transduced CAR+ NK cells and T cells was assessed examining select tissues from euthanized animals. Beginning on Day 7 post INT2104 administration, CAR T and NK cells were detected in bone marrow, spleen, and liver.

After demonstrating targeted gene delivery to T and NK cells using humanized mouse models, CAR20 vector was administered i.v. to cynomolgus macaques. Depletion of peripheral blood B cells in vector-treated NHPs was evident as early as Day 4 post administration. The complete absence of circulating B cells was maintained in one animal and analysis of secondary lymphoid organs in this animal confirmed systemic B cell depletion at necropsy 1-year post treatment. Separately, to demonstrate that both CAR+ T cells and NK cells were generated in NHPs following i.v. administration of a CAR20 vector, two NHPs were treated with CAR20 vector and sacrificed 4 days post treatment. Immunohistochemical analyses of spleen and liver were conducted with an anti-CAR antibody and markers for T cells (CD3), NK cells (NKG2A), as well as hepatocytes (HNF4α). CAR+ cells were detected in spleen with CAR+ signal colocalizing to both CD3+ T cells (Figure 1a) and NKG2A+ NK cells (Figure 1b). No CAR+ hepatocytes were detected among the 8685 hepatocytes present in the representative liver sections analyzed to

cells, with no off-target transduction observed in hepatocytes. A FIH study for INT2104 is scheduled to begin in 4Q2024.

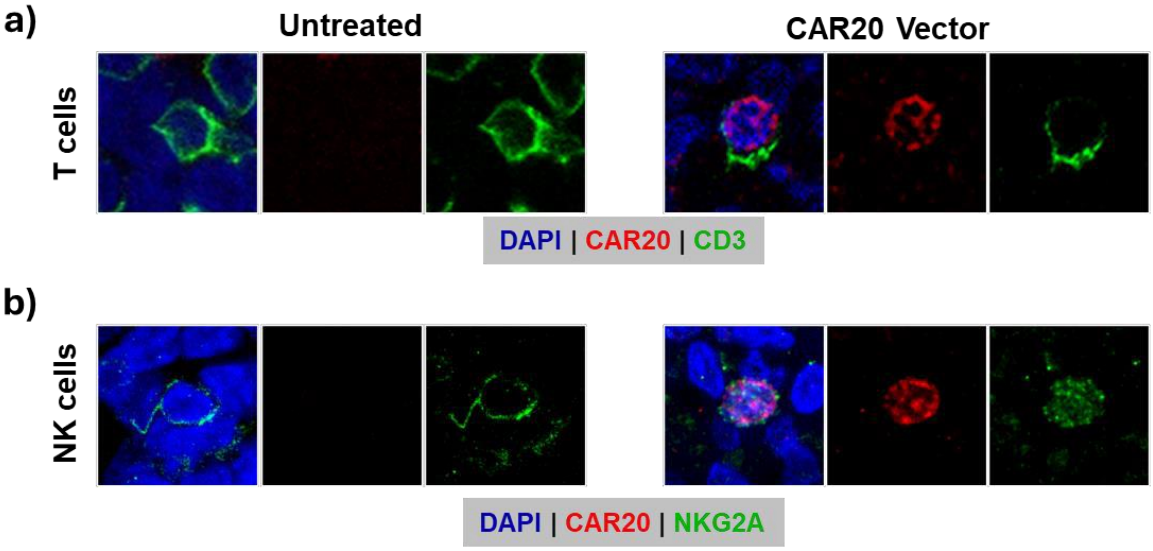

Figure 1: CD7 targeted CAR20 vector treatment in NHPs results in CAR+ T cells and NK cells. Representative CD3+ T cells (a) and NKG2a+ NK cells (b) colocalize with CAR+ signal in NHP spleen.

1. Liu, E., et al. “Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors.” *The New England Journal of Medicine.* 382(6) (2020): 545-553.