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Title: *In Vivo* Delivery of an Engineered Lentiviral CAR19 Vector for the Treatment of Autoimmune Diseases

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Chimeric antigen receptor (CAR) T cell therapy has revolutionized the treatment of B cell malignancies and multiple myeloma. More recently, CAR T therapy has been highlighted as a potential intervention for autoimmune diseases. Conventional autologous CAR T therapy includes a multifaceted, complicated process including patient pretreatment using lymphodepleting chemotherapy, complex manufacturing and logistics for the generation and delivery of the cell product which prolong vein-to-vein time. Alternative approaches meant to overcome these limitations, including allogenic CARs or faster manufacturing processes, are currently in clinical development. *In vivo* generation of CAR T cells is a fast-growing area of research, with the promise of reducing the complexity of autologous CAR generation, eliminating the need for lymphodepletion, and expanding patient access. We describe a lentiviral vector engineered to target CD7+ T cells and NK cells following a single intravenous administration to generate autologous CAR+ cells within the body, without lymphodepletion. This vector, INT2106, confers specific targeting of T cells and NK cells by pseudotyping the lentivirus with both a CD7 Binder and a detargeted VSV-G fusogen called "Gen 2.1 Fusogen". INT2106 delivers a fully human CAR19 transgene, resulting in the generation of functional CAR T and NK cells with specific activity against CD19+ B cells, the same cells targeted by autologous CAR T therapies being tested in clinical studies to combat autoimmune diseases.

In vitro evaluation of INT2106 demonstrates specific transduction of CD7+ cells. Biologic activity of the fully human CAR19 was evident through specific killing of CD19-expressing B cell lymphoma lines across a range of Effector to Target cell ratios. This biological activity was confirmed to be dependent on CD19-expression on the tumor cells by data showing reduced killing of B cells with CD19 expression knocked out. Incubation of INT2106 with various B cell lymphoma lines or primary B cells isolated from healthy human donors does not result in the transduction of B cells, an important safety consideration regardless of whether the CAR cells are generated *in vivo* or *ex vivo***.** An *in vivo* demonstration of INT2106 was performed using human CD34-engrafted NSG mice. Following treatment with INT2106 via a single intravenous tail vein injection, circulating B cells in vector-treated mice were depleted, with loss of CD19+ B cells across multiple tissues including the blood, spleen, and bone marrow. Coincident with B cell depletion, CAR19+ cells were detectable in the blood of treated mice, with peak concentrations measured approximately three weeks after administration. INT2106 treatment was well-tolerated with no vector associated safety signals observed.

Taken together, these data suggest that intravenous administration of INT2106 can effectively target CD7+ cells, leading to functional CAR19+ cell generation and CD19+ B cell depletion, the intended biological effect. INT2106 is in development

as an off-the-shelf treatment for autoimmune diseases without the need for patient preconditioning or cell manufacturing.