In vitro and In vivo Specificity and Biodistribution of a Novel CD8-Targeted Fusosome

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Introduction

Autologous chimeric antigen receptor (CAR) T cells represent a ground-breaking therapy for treating B-cell malignancies. However, the complexity, cost, time and toxicity associated with manufacturing and administering these autologous CAR T cell therapies have hindered their broader applications. To address certain CAR T limitations, Sana has developed a novel in vivo targeted gene therapy approach, a fusosome, that can deliver a CAR transgene specifically to CD8+ T cells via systemic administration without ablative pre-conditioning. The fusosome is a lentiviral vector pseudotyped with a targeted fusogen that specifically directs gene transfer into CD8+ cells. Our CD8-targeted CD19-directed CAR fusosome, SG299, upon systemic administration, specifically transduces CD8+ cells in lymphoid tissues, showing preclinical safety and high selectivity, laying the groundwork for clinical evaluation of an in vivo CD19-directed CAR gene therapy.

Pre-clinical efficacy in mouse highlights specificity and tumor control

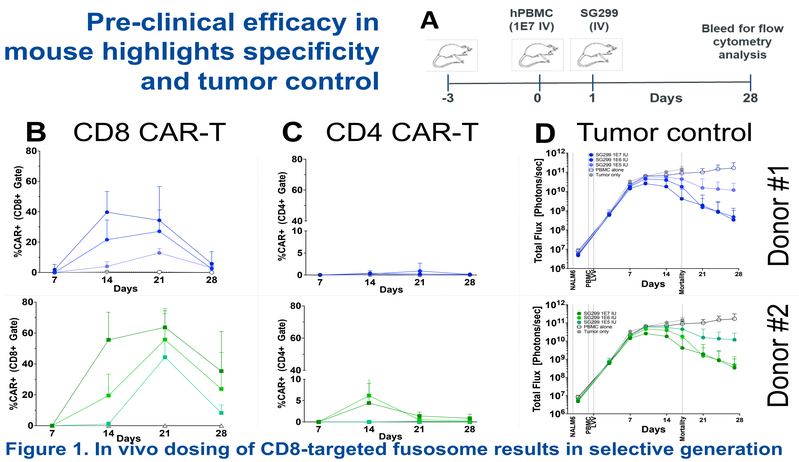

Figure 1. In vivo dosing of CD8-targeted fusosome results in selective generation CD8+ CAR-T cells leading to tumor control in NALM6 xenograft mouse model

A. Study design to evaluate gene transfer in PBMC and NALM6 tumor engrafted NSG model. B. Frequency of CD8 T cells expressing CD19CAR in peripheral blood. C. Frequency of CD4 T cells expressing CD19CAR in peripheral blood. D NALM-6 dynamics by BLI (Top, donor 1; Bottom, donor 2)

Plasma clearance of fusosome supports gene transfer to peripheral blood CD8+ cells

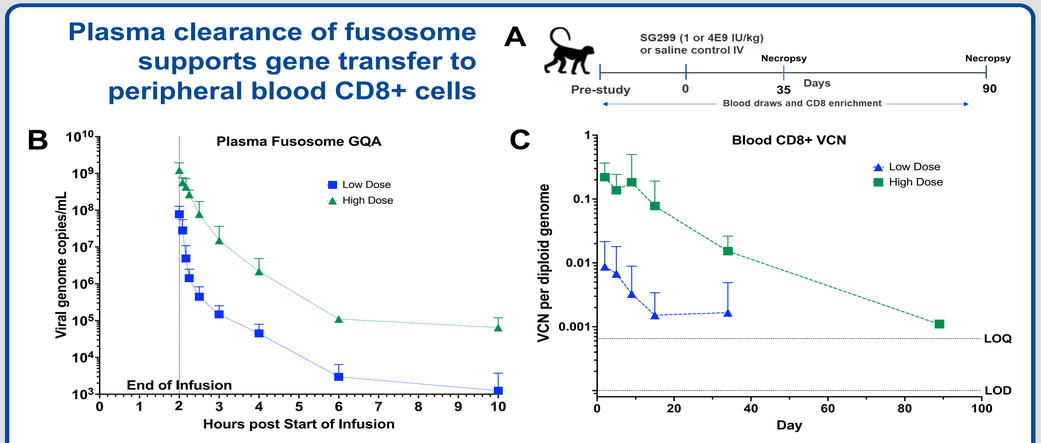

Figure 3. Pharmacokinetics and peripheral blood gene transfer in NHP model

A. Study design to evaluate SG299 gene transfer in Nemestrina macaques. Nemestrina macaques were dosed with saline (N=2), low dose (1E9 IU/kg, N=4) or high dose (4E9 IU/kg, N=4). Necropsy was done to one saline animal and two animals in each dose group on day 35 and remainder at day 90. B. Genome quantitation assay (GQA) to measure fusosome plasma levels performed by ddPCR. C. Gene transfer in CD8+ blood cells measured by vector copy number over time by ddPCR.

Methods

We demonstrated SG299 specificity and efficacy using a NALM6 tumor model in NSG mice. Briefly, NSG mice engrafted with human PBMCs were challenged with NALM6 tumors and treated with either SG299 or saline. Flow cytometry was used to evaluate CAR positivity. Bioluminescence was used to measure tumor growth.

In vitro tissue cross reactivity study was assessed to evaluate binding of the CD8 fusogen binder, a CD8-binding scFv, on frozen sections representing ~35 different tissues from human (4 donors) and Nemestrina macaques (2 donors).

SG299 specificity was also evaluated in vitro using panel of cell lines representing a breadth of receptor types. Cells were exposed to 8 IU/cell SG299 and gene transfer was evaluated using ddPCR. Reverse transcriptase inhibitor nevirapine was used as control for the in vitro studies.

To evaluate in vivo specificity and biodistribution, healthy Nemestrina macaques were treated with a single dose of SG299 at 1 or 4 x 10^9 IU/kg (n=2 or 4) or saline (n=1 or 2) via intravenous infusion and evaluated for up to 90 days for vector biodistribution in CD8 enriched PBMCs, lymphoid tissues and at terminal timepoint in panel of 17 tissues using ddPCR.

Off-target transduction shows specificity for CD8+ cells

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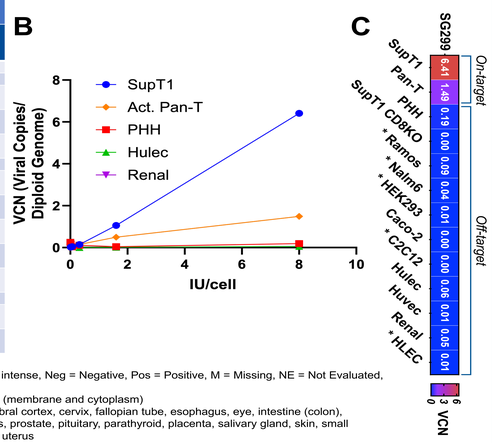
Tissue¶	Human tissue reactivity score* Mononuclear cells**	Human tissue reactivity score* Other elements of the tissue	Nemestrina tissue reactivity score* Mononuclear cells**	Nemestrina tissue reactivity score* Other elements of the tissue
Adrenal	2-3+ (rare)	Neg	1-3+ (rare)	Neg
Bladder	2-3+ (rare)	Neg	1-3+ (rare)	Neg
Blood cell	1-3+ (occas to freq)	Neg	2-3+ (freq)	Neg
Bone marrow	1-3+ (occas to freq)	Neg	1-3+ (rare to occas)	Neg
Brain	2-3+ (very rare)	Neg	1-3+ (rare)	Neg
Heart	2-3+ (rate)	Neg	1-3+ (rare)	Neg
Intestine (small)	2-3+ (occas to freq)	Neg	1-3+ (occas)	Neg
Kidney	2-3+ (very rare)	Neg	1-3+ (very rare)	Neg
Liver	1-3+ (rare to occas)	Neg	1-3+ (rare to occas)	Neg
Lung	1-3+ (rare to occas)	Neg	1-3+ (rare to occas)	Neg
Lymph node	2-3+ (occas to freq)	Neg	2-3+ (occas to freq)	Neg
Ovary	2-3+ (rare)	Neg	1-3+ (rare)	Neg
Spleen	2-3+ (occas to freq)	Neg	1-3+ (occas to freq)	Neg
Testis	2-3+ (very rare)	Neg	1-3+ (very rare)	Neg



* ± = equivocal, 1+ = weak, 2+ = moderate, 3+ = strong, 4+ = intense, Neg = Negative, Pos = Positive, M = Missing, NE = Not Evaluated, NS = Not Stained, occas = occasional, freq = frequent.
**Includes resident, migrating, infiltrating, and/or intravascular (membrane and cytoplasm)
¶ Other tested tissues with negative binding: cerebellum, cerebral cortex, cervix, fallopian tube, esophagus, eye, intestine (colon), mammary glands, skeletal muscle, peripheral nerve, pancreas, prostate, pituitary, parathyroid, placenta, salivary gland, skin, small intestine, spinal cord, stomach, thyroid, thymus, tonsil, ureter, uterus

Figure 2. CD8-targeted fusosome is specific to target tissues containing T cells in human and Nemestrina tissues

A. Tissue reactivity score based on binding of recombinant anti-human CD8 binder protein in tissue arrays. B. SG299 fusosome-mediated gene delivery is highly specific to CD8+ cell types. C. Heatmap showing vector copy number (VCN) in a panel of cell lines and primary cells transduced with SG299 at 8 IU/cell. CD8 is expressed only in "on-target" SupT1 and primary pan-T cells. Subsets of cell types (marked with * in A) evaluated for gene transfer in a dose range.

Day 90 NHP Necropsy shows persistent lymphoid tissue VCN and lymphoid-tissue specific gene transfer

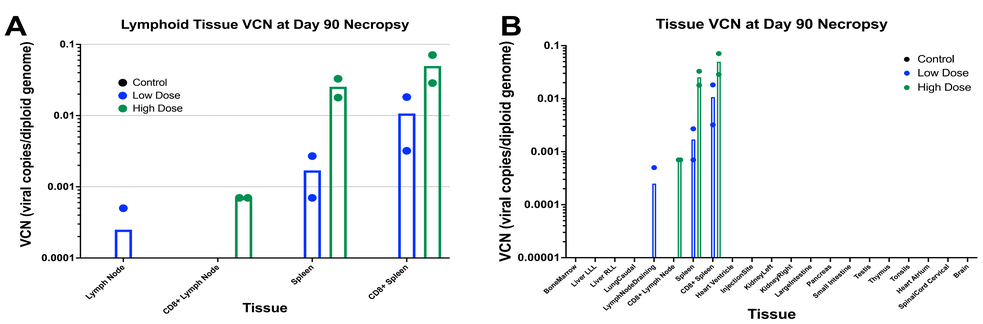


Figure 4. Measurements of gene transfer in lymphoid organs, CD8 enriched-tissue and organs of interest in Nemestrina macaques at 3 months (Day 90) post fusosome dosing

A. VCN in Lymphoid and CD8-enriched lymphoid tissues (N=2/dose) B. VCN in tissues of interest (N=2/dose)

Conclusion

- These studies demonstrate high on-target specificity of SG299 fusosome to transduce CD8+ T cells and express a CD19-directed CAR *in vitro* and *in vivo*.
- SG299 represents a novel therapeutic opportunity to generate CD19-directed CAR T cells *in vivo* and potentially overcome challenges associated with *ex vivo* CAR T therapies.

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