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POSTER ABSTRACTS

801.GENE THERAPIES

In Vitro and In Vivo Specificity and Biodistribution of a Novel CD8-Targeted Fusosome

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Autologouschimeric antigen receptor (CAR) T cells represent a groundbreaking therapy for treating B-cell malignancies. However, the complexity, cost, time and toxicity associated with manufacturing and administering current CAR T cell therapies hinder their broader applications. To overcome CAR T limitations, Sana has developed a novel fusosome platform that can deliver a T cell targeted CAR gene therapy 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, transduces CD8+ cells in lymphoid tissues, showing preclinical safety and high selectivity, laying the groundwork for clinical evaluation of an *in vivo* CAR gene therapy.

In vitro tissue cross reactivity (TCR) study was assessed to evaluate binding of the CD8 fusogen binder, a CD8-binding scFv, on tissues from Nemestrina macaques and compared these with tissues from human 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 vector integration 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 35 days for vector biodistribution in CD8 enriched PBMCs and at terminal timepoint in panel of 17 tissues using ddPCR. In addition, we also demonstrated SG299 specificity with help of flow cytometry, in 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.

The TCR study demonstrated that the CD8 fusogen binder is specific for the target tissues containing T cells and similar binding across multiple human and Nemestrina tissues. SG299 fusosome demonstrated CD8-specificity evaluated by transduction of cell types with CD8 receptor (SupT1 and PanT cells). There was no detectable off-target transduction in sets of primary as well as immortalized cell lines evaluated including pulmonary cells, renal epithelial cells, lymphoblast, endothelial cells and CD8KO cells.

VCN analysis of nemestrina macaques PBMC and tissue samples following treatment with SG299 demonstrated dose dependent integrations per diploid genome through Day 35. All animals dosed 4 x 10^9 IU/kg showed quantifiable VCN signal through Day 34 in PBMC population enriched for CD8+ cells (0.0032 - 0.6544 vc/dg, vector copy per diploid genome). One out of 4 animals dosed at 1 x 10^9 IU/kg showed quantifiable VCN signal at Day 34 (0.0276 vc/dg). Tissues collected at necropsy on Day 35 demonstrated quantifiable VCN signal in spleen, lymph node, CD8+ splenocyte and lymphocyte, draining lymph node, heart ventricle, and injection site, for animals dosed at 4 x 10^9 IU/kg. One of 4 animals dosed 1 x 10^9 IU/kg showed quantifiable VCN in spleen, CD8+ splenocyte, and injection site.

Flow cytometric analysis of peripheral blood of mice treated with SG299 showed a dose dependent increase in CAR T cells, and the CAR T cells were CD8+ T cells specific with no CAR T cell detection amongst the CD4+ T cells.

Collectively, these studies demonstrate high on target specificity of SG299 fusosome to transduce CD8+ T cells *in vitro* and *in vivo*. Therefore, SG299 represents a novel therapeutic opportunity to generate CAR T cells *in vivo* and potentially overcome challenges associated with *ex vivo* CAR T therapies.

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