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Preclinical Efficacy of a Novel CD8-Targeted Fusosome for In Vivo CAR T Cell Therapy

Vandana Chaturvedi, PhD[1], *Jesse Green, PhD*[1], *Garrett Zipp, BS*[1], *Aaron Lampano, BS*[1], *Ouwen Liang, BS*[1], *Allison O'Rourke, PhD*[1], *Brian Granger, BS*[1], *Kutlu G Elpek, PhD*[2], *Neal van Hoeven, PhD*[1], *Terry Fry, MD*[1]

[1] Sana Biotechnology, Seattle, WA
[2] Sana Biotechnology, Cambridge, MA

SG299 is a CD8-targeted fusosome; a self-inactivating (SIN) lentiviral vector (LVV) pseudotyped with a CD8-targeted fusogen, which carries a transgene encoding a CD19-directed chimeric antigen receptor (CAR). We evaluated the preclinical efficacy of a representative CD8/CD19CAR fusosome comparable to SG299 for its ability to control tumor growth *in vivo* in an NSG mouse model engrafted with human CD19 positive tumor cells and human peripheral blood mononuclear cells (PBMC). We compared direct intravenous (IV) administration and ex vivo exposure of fusosome to resting T cells followed by IV injection of vector-cell mixtures, as has been proposed for extracorporeal delivery (ECD) methods. We have also evaluated the performance of multiple lots of fusosomes to assess consistency in manufacturing and have evaluated donor-to-donor variability in five PBMC donors in this model.

To measure anti-tumor efficacy, immunodeficient NSG mice were challenged IV with NALM6 tumors expressing firefly luciferase (5E5 cells/mouse) on day 4. For direct IV, PBMC (1E7 cells/mouse) were injected IV on day -1 followed by CD8/CD19CAR fusosome (1E7 IU/mouse) on day 0. For ECD, PBMC were incubated with CD8/CD19CAR fusosome for 1 hr and the cell and fusosome mix were injected IV on day 1. Tumor growth was monitored by bioluminescence imaging over a 28-day period. In addition, we have investigated both pharmacokinetics of fusosome induced CAR T cells and NALM6 tumors by flow cytometry.

CD8/CD19CAR fusosome demonstrated statistically significant control of NALM6 tumors by both direct IV and ECD routes of delivery. Kinetic analysis of peripheral blood demonstrated tumor control and in vivo CART cell generation were dose dependent. The peak CAR T cell response was at D14. CARs detected at all timepoints were CD8+ specific; no CD4 $^{+}$ CAR T cells were observed. In addition, all mice that received fusosome demonstrated protection from morbidity and mortality as measured by reduced weight loss and improved survival compared to control or tumor only mice. Lot-to-lot performance variability of fusosomes was minimal; three lots of fusosome that had similar physical and functional attributes showed efficient and equivalent tumor control with multiple PBMC donors. Finally, an assessment of donor-to-donor variability across five PBMC donors using a single lot of fusosome showed donor-specific variations in tumor control as measured by BLI but demonstrated a consistent protection from morbidity and mortality. Collectively, these studies demonstrate preclinical efficacy of our CD8/CD19CAR candidate, lot consistency and verify our ability to generate CD19-directed CAR T cells *in vivo* using a specifically targeted platform.

Disclosures Chaturvedi: *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company. **Green:** *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company. **Zipp:** *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company. **Lampano:** *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company. **Liang:** *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company. **O'Rourke:** *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company. **Granger:** *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company. **Elpek:** *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company. **van Hoeven:** *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company. **Fry:** *Sana Biotech:* Current Employment, Current equity holder in publicly-traded company.

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