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POSTER ABSTRACTS

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Feasibility of Extracorporeal Delivery of Fusosomes to Generate CAR T Cells In Vivo

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Fusosomes are viral vectors pseudotyped with modified paramyxovirus envelopes targeting specific cell types. A CD8-targeted fusosome delivering a CD19CAR transgene has the potential to provide an off-the-shelf therapeutic approach to generate *in vivo* CD19-directed chimeric antigen receptor (CAR) T cells in patients. For an *in vivo* gene delivery platform there are several approaches for administration that could be considered including direct intravenous (IV) administration or extra-corporeal delivery (ECD). With ECD, patients would first undergo apheresis, then fusosomes would be retained with patient peripheral blood mononuclear cells (PBMCs) in the apheresis bag for a brief period of time before the entire contents of the bag is IV delivered into the same patient. Here, we demonstrate the feasibility of this approach to generate *in vivo* CAR T cells in a clinical setting with a mock infusion and discuss the translation of these findings to direct IV administration.

A healthy donor was apheresed to collect PBMCs using standard apheresis equipment. No further cell enrichment, separation, or washing was performed. CD8-targeted fusosome formulated in an isotonic cryoprotective buffer was administered into the apheresis bag by gravity via an infusion set at a dose of ~0.5 IU/cell, gently mixed and retained for 30 min. The contents of the bag were mock infused into sample collection bags at a controlled flow rate using an infusion pump. Samples were collected before and after fusosome addition, after 30 min retention, and every 30 min during the mock infusion. Cell counts and viability were measured by complete blood count (CBC) and flow cytometry analyses. Fusosome binding to CD8 $^{+}$ T cells was measured by flow cytometry. Cytokines and complement components in supernatants were measured by ELISA or MSD. Residual fusosome in the supernatant was measured by ddPCR. Transduction of CD8 $^{+}$ T cells was measured by flow cytometry and vector copy number (VCN) analysis after PBMCs were washed, activated with CD3/CD28 beads, and cultured for 8 days. Early transcription events were measured by ddPCR using specific amplicons targeting different DNA species generated during reverse transcription.

The CD8-targeted fusosome was successfully deliveredusing aseptic techniques to a freshly collected apheresis bag.PBMC viability stayed high (>%95) throughout the process. There were no major changes in white blood cell, platelet, or red blood cell levels as measured by CBC analysis. There were no changes in CD8 $^{+}$ T cell phenotype throughout the time course. Fusosome binding to CD8 $^{+}$ T cells was detected as early as fusosome addition to the apheresis bag (~5 min) and increased over the course of the process. Analysis of vector genomes in the supernatants indicated presence of unbound residual fusosome. Exposure of PBMC to fusosome did not induce cytokine secretion or complement activation throughout the process. Early events of transduction of cells could be detected by analysis of reverse transcription intermediates. Analysis of cultured cells via both flow cytometry and VCN analysis indicated generation of CD8 $^{+}$ CAR T cells upon short-term exposure to fusosome. *In vivo* delivery ofCD8-targeted fusosome encoding a CD19CAR transgene enables a novel CAR T cell approach. This study demonstrated that administration of fusosomes via ECD is operationally feasible in a clinical setting and scale as a novel method of administration to generate CD8 $^{+}$ CD19CAR T cells *in vivo*. ECD setup also served as an *in vitro* pharmacokinetic model for direct IV infusion. In scenarios where *in vivo* exposure is limited due to very rapid clearance (e.g. few minutes), ECD has the benefit of reducing the volume of distribution and bringing T cells and fusosomes in close proximity for efficient gene delivery. The findings of study suggest that with rapid binding of fusosomes to CD8 $^{+}$ T cell leading to early transcription events, and an *in vivo* half-life of more than 30 min, fusosome may generate CAR T cells successfully via direct IV administration.

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