Intravenous Delivery of a Bi-functional AAV Gene Therapy to Reduce Endogenous ApoE4 and Express ApoE2 in ApoE4 Humanized Mice

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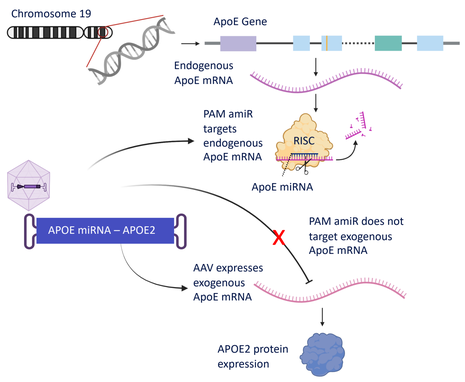
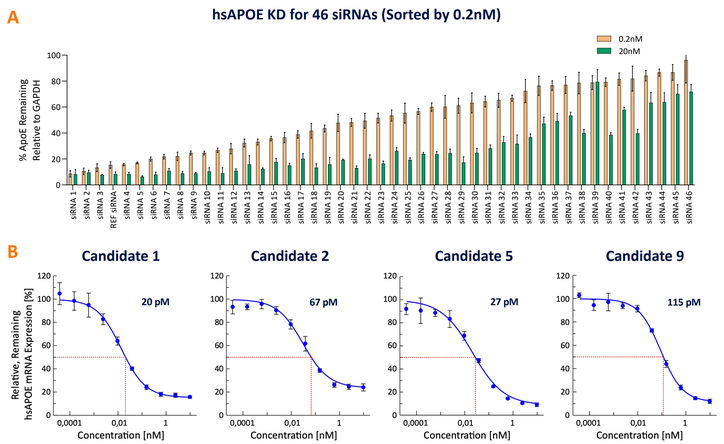
INTRODUCTION

Apolipoprotein E (ApoE) genotype represents the most significant genetic risk factor for Alzheimer's disease (AD), with distinct allelic variants exerting differential effects on disease susceptibility and progression. The ApoE2 allele is associated with a reduced risk and offers a protective effect, whereas the ApoE4 allele markedly elevates the risk of developing AD and is linked to an earlier onset of symptoms. Consequently, therapeutic strategies aimed at modulating ApoE expression, such as adeno-associated virus (AAV)-mediated delivery of the protective ApoE2 isoform and/or knockdown of ApoE4 expression, are emerging as promising avenues for intervention in AD pathogenesis. Here, we describe the identification of siRNAs targeting APOE in silico, confirmation of potent siRNAs *in vitro*, and subsequent embedding of top performing siRNA sequences into proprietary amiRNA cassettes for *in vitro* and *in vivo* evaluation. We then demonstrate IV delivery of investigational AAV therapeutic candidates that combine expression of human APOE-targeting artificial microRNAs with concomitant ApoE2 expression via single dose IV-delivery using Voyager's proprietary, VCAP-Gen2 BBB-penetrant capsid. We observed significant reduction of endogenous ApoE4 in key AD-relevant brain regions of ApoE4 knock in mice, while significantly increasing expression of the ApoE2 isoform. We conclude that utilization of a novel BBB-penetrant capsid delivering a bi-functional payload effectively modulates the level of ApoE2 and 4, and is a viable approach that will be further evaluated in pre-clinical studies.



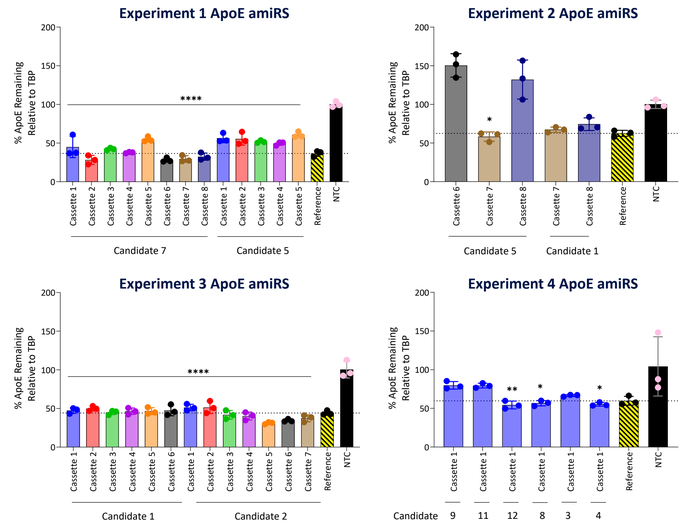
- Pan-APOE miRNA for maximal efficacy
- Intronic artificial miR scaffold for single promoter co-regulation and maximum miRNA-cDNA co-expression
- Targeted codon optimization of APOE2 gene for complete miRNA evasion
- Optimization of short regulatory elements for self-complementary AAV vectorization

Figure 1. *In vitro* siRNA Screening Identifies Multiple Potent APOE-targeting siRNAs



(**A**) APOE mRNA silencing by potent siRNAs *in vitro*. 46 sequences selectively targeting APOE were designed, synthesized and evaluated in HepG2 cells. siRNAs were evaluated at two different concentrations, 0.2 nM and 20 nM. Following transfection, cells were harvested, and APOE and GAPDH mRNA were quantified by RT-qPCR. (**B**) Dose response curves of the top performing siRNA candidates in HepG2 cells. 24 hr after transfection APOE siRNA, cells were harvested, and APOE and GAPDH mRNA were quantified by RT-qPCR.

Figure 2. Projected Therapeutic Level of hAPOE Silencing Achieved with amiRNAs *in vitro*



Best performing siRNA candidates were embedded in Voyager's proprietary amiRNA cassettes and evaluated in HEK293T cells in multiple experiments. 48 hr after transfection of 0.5 µg of APOE amiR expressing plasmids, cells were harvested, and APOE and TBP mRNA were quantified by RT-qPCR. A reference amiR was included as a positive control for each set tested. Several candidates exhibited knockdown as good, or better, than the knockdown achieved by the reference amiR. The best performing amiRs were selected for evaluation *in vivo*. *p<0.05, **0.01, ***0.001, ****0.0001 1-way ANOVA with Tukey's Multiple Comparisons.

Figure 3. Single IV-dose Delivery of APOE miRNA via VCAP Gen. 2 Capsids Results in Robust APOE mRNA Reduction in Key AD Brain Regions

hAPOE4KI Mouse amiRNA Screen

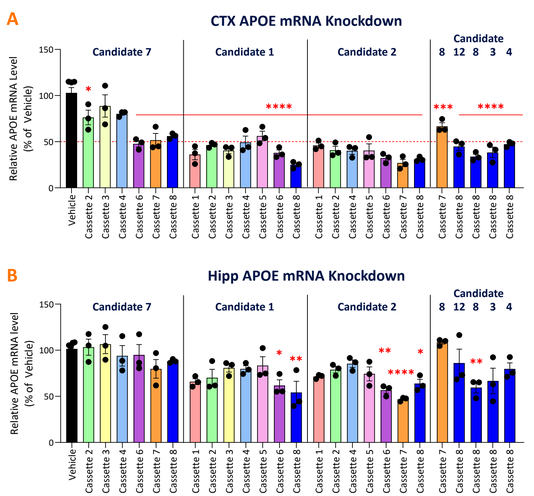
Objective:
Evaluate target engagement of ApoE amiRs *in vivo*

Mice:
7–8-week-old B6(SJL)-*Apoe*$^{tm1.1(APOE*4)Adiuj}$/J (male and female)

Vector:
VCAP-2nd Generation.APOE amiRNA

Dose:
IV administration 1e13 vg/kg

Endpoints:
APOE knockdown in cortex and hippocampus, biodistribution



Substantial knockdown of hAPOE observed in cortex (**A**) and hippocampus (**B**) of APOE4KI mice 28 days after intravenous administration of AAV. hAPOE expression measured using multiplexed RT-qPCR with hAPOE expression level normalized to a host reference gene transcript with vehicle control group as the comparator. *p<0.05, **0.01, ***0.001, ****0.0001 1-way ANOVA with Tukey's Multiple Comparisons.

Figure 4. A Single IV Injection of Bi-functional Vector Leads to a Dose Dependent hAPOE4 Protein Reduction and hAPOE2 Delivery in Mouse Cortex and Hippocampus

hAPOE4KI Mouse Bi-unctional Payload Dose Response

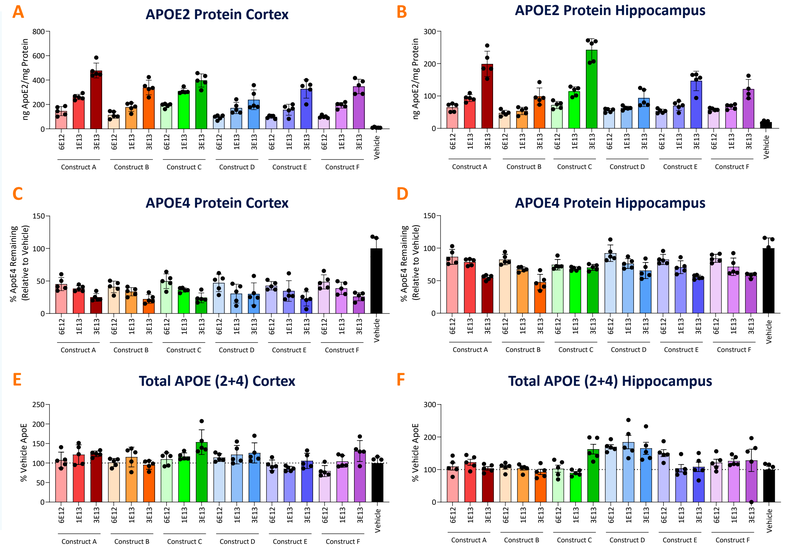
Objective:
Investigate pharmacology and safety of different doses of lead bi-functional APOE constructs in APOE4 KI mice

Mice:
7–8-week-old B6(SJL)-*Apoe*$^{tm1.1(APOE*4)Adiuj}$/J (male and female)

Vector:
VCAP-2nd Generation.APOE miRNA.APOE2 cDNA

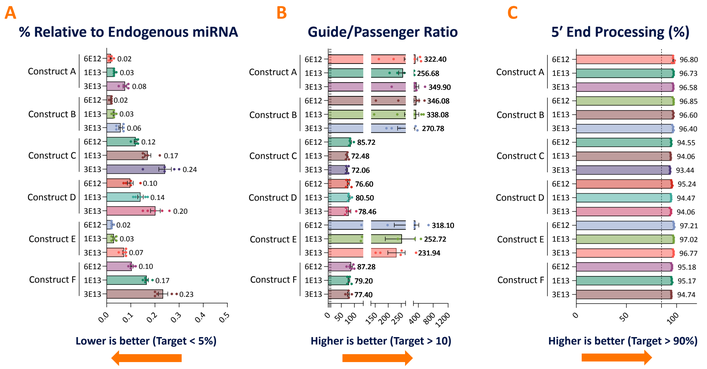
Dose:
IV administration 6e12, 1e13, 3e13 vg/kg

Endpoints:
APOE2, APOE4, pan APOE ELISAs; APOE knockdown, biodistribution



Delivery of APOE2 and reduction of APOE4 in APOE4KI mice resulting in physiologically normal APOE levels with single IV administered bifunctional vector. Dose respondent APOE2 delivery in cortex (**A**) and hippocampus (**B**) measured with APOE2 specific ELISA. Recombinant APOE2 was used as a standard to quantify APOE2. Dose respondent APOE4 reduction in cortex (**C**) and hippocampus (**D**) measured with APOE4 specific ELISA, with the vehicle control group serving as a comparator. Total hAPOE (2+4) in cortex (**E**) and hippocampus (**F**) measured with pan hAPOE ELISA, with the vehicle control group serving as a comparator. Total APOE levels in treated groups are consistent with levels observed in vehicle group, indicating near physiological levels.

Figure 5. Global miRNA Profiling Identifies Several Candidates with Favorable amiR Safety Profile



Small RNAseq profile of mouse cortex 28 days following IV administration of bifunctional vector. (**A**) Dose respondent percent of artificial miRNA delivered relative to endogenous miRNA. Target profile is <5% to avoid potential toxicity associated with saturation of RNAi pathway. (**B**) Ratio of guide strand to passenger strand counts. Target profile is >10 to avoid potential off-target effects from the passenger strand. (**C**) Percent of expressed amiRs with perfect processing of the 5' end of the guide strand. Target profile is >90% to limit potential off-target effects from incorrectly processed amiRs.

CONCLUSIONS

- Several siRNA candidates demonstrate robust potency and selectivity for APOE *in vitro*
- Vectorized amiRNA candidates produced significant APOE reduction in the cortex and hippocampus of APOE4KI mice
- IV injection of bifunctional APOE amiRNA-codon optimized APOE2 vectors produced dose dependent APOE4 protein reduction and APOE2 protein expression in the cortex and hippocampus of APOE4KI mice. Total APOE levels were modulated to physiological levels
- Global miRNA profiling identifies several candidates with favorable amiR safety profile
- These data support the continued development of IV delivered AAV therapeutics that combine expression of human APOE-targeting artificial microRNAs with concomitant APOE2 expression