Enhancing efficacy and specificity of AAV5 capsid via small, intermediate, and large peptide insertions

Bas Bosma*, **Sebastian Kieper***, Tom van der Zon, Pablo de Miguel Herraiz, Morgane Wartel, Arifa Ewaz, Macha de Dreu, Giso Brasser, Lukas Schwarz, Nikoleta Efthymiopoulou, Mercedes Valls, Romain Galmes, Jolanda M.P. Liefhebber, Elena de Miguel, Tycho Hoogland, Paula Miranda and Anggakusuma

[1]uniQure biopharma B.V., Amsterdam, The Netherlands; *Equal contributed

uniQure

INTRODUCTION

Rational capsid design is a potential method for enhancing the targeting profile of AAV. AAV5 capsids have several advantages relative to other AAV serotypes including its relative safety and reduced immunogenicity which allow administration even with pre-existing neutralizing antibodies. One limitation of AAV5 capsids are their limited tropism which restricts delivery of cargoes to the liver when administered IV or to the brain with intraparenchymal administration. Here, we present several effective approaches for enhancing transduction of target tissues and for re-directing AAV5's tropism towards cells that are usually not transduced. We demonstrate that AAV5 capsids tolerate the insertion of peptides of varying sizes into surface-exposed loops in a way that neither affects capsid assembly nor genome packaging. Engineered capsids remain infectious across different cells lines and display altered transduction profiles compared to the wild-type capsids. Remarkably, insertion of high-density lipoprotein (HDL) binding peptides (6-30 amino acids) into surface-exposed loops influenced binding of the AAV5 capsid to liver cells by exploiting the HDL particle as a carrier to improve liver biodistribution. Similarly, introducing larger peptides (~160 amino acids) with epitope binding properties (such as single-domain antibodies) into the capsid surface enabled targeting of cell types that express the target epitope. Taken together, these approaches allow for the creation of AAV5 capsids with custom targeting profiles that significantly extend the range of applications of AAV5 capsid in gene therapy

METHODS

Next-generation (NG) AAV5 capsids were generated by:

- Site-directed mutagenesis
- Small (8 aa) or intermediate (30 aa) peptide insertion into VR-loops of VP3
- Large (160 aa) peptide insertion into VR-loops of VP1 only
- Production of research-grade AAV for proof-of-concept testing via HEK293T triple, quadruple pDNA transfection or BEVs
- *In vivo* testing by intrastriatum or intravenous injection into wild-type C57BL/6 mice or intravenous injection into C57BL/6-Tg(ApoA1)1Rub/J human ApoA1 transgenic mice followed by histological and molecular analysis of target tissues

RESULTS

Capsid design approaches and AAV production

VP protein stoichiometry of AAV5 NG capsid proteins was compared by SDS-Page **(Figure 1)**. NG AAV5 vector yields were determined in the crude cell lysates by Q-PCR **(Table 1)**.

- Mutagenesis or Peptide insertions did not interfere with capsid assembly and genome packaging

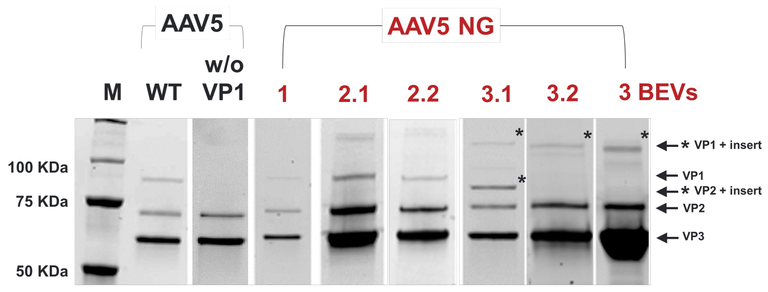

Figure 1 - SDS PAGE of purified NG AAV5 produced in HEK293T or Sf+ insect cells (BEVs)

Table 1: Capsid design approaches and productivity

AAV5 Generation	Approach	Production Platform	Productivity in CLB (GC/ml)
WT AAV5	Natural serotype	HEK triple pDNA transfection	$3.6x10^{11}$
AAV5 w/o VP1	no VP1	HEK triple pDNA transfection	$4.5x10^{10}$
AAV5 NG1	Site directed Mutagenesis (@VP3, 60 x 1 aa)	HEK triple pDNA transfection	$1.3x10^{11}$
AAV5 NG2.1	Small Peptide Insertion (@VP3, 60 x 8 aa)	HEK triple pDNA transfection	$2.1x10^{11}$
AAV5 NG2.2	Small Peptide Insertion (@VP3, 60 x 30 aa)	HEK triple pDNA transfection	$1.8x10^{11}$
AAV5 NG3.1	Large Peptide Insertion (@VP1, ~5 x 160 aa)	HEK quadruple pDNA transfection	$1.6x10^{11}$
AAV5 NG3.2	Large Peptide Insertion (@VP1, ~5 x 160 aa)	HEK triple pDNA transfection	$1.5x10^{11}$
AAV5 NG3 BEVs	Large Peptide Insertion (@VP1, ~5 x 160 aa)	Insect triple BEVs	$2.8x10^{11}$

AAV5 NG1 - mutagenesis

AAV5 NG1 capsid was generated by site-directed mutagenesis. Upon bilateral intrastriatal injection, the spread of AAV5 NG1 - eGFP in the CNS was enhanced while the liver was de-targeted (~200-fold reduction compared to wild-type) **(Figure 2)**.

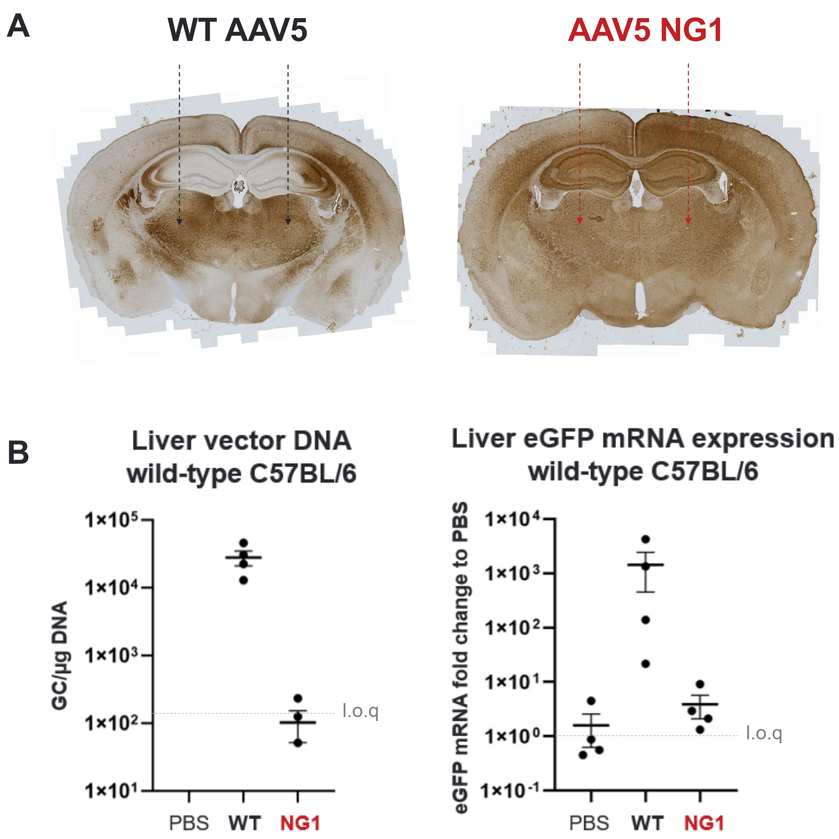

Figure 2 - A Histological analysis of the brain (chromogenic **eGFP** detection) and **B** molecular analysis of the liver (vector and eGFP mRNA copies).

AAV5 NG2 - small peptide insertions

AAV5 NG2.1 was generated by insertion of small HDL binding peptides (8 aa) into VP3. Upon intravenous injection, enhanced liver transduction and transgene activity of AAV5 NG2.1 - SEAP was observed (**Figure 3**).

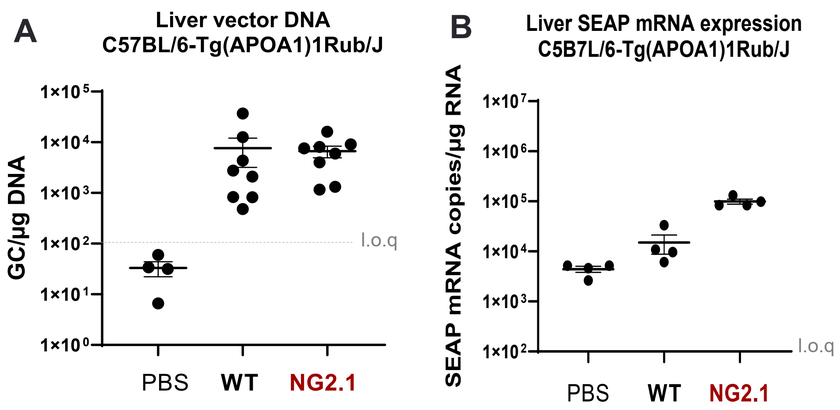

Figure 3 - A Vector genome copies and **B** transgene mRNA copies in the liver of C57BL/6-Tg(ApoA1)1Rub/J mice.

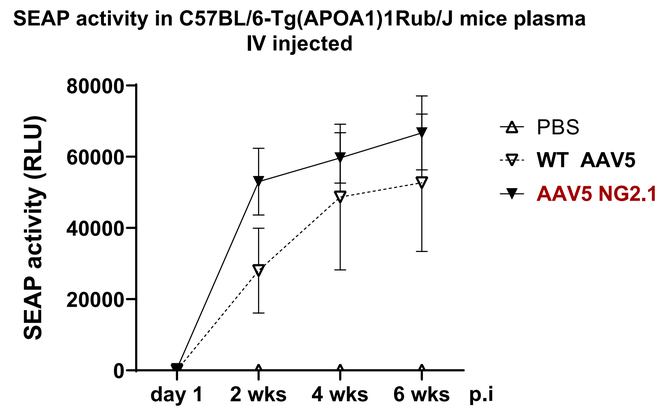

Figure 3 - C SEAP reporter activity in the plasma of C57BL/6-Tg(ApoA1)1Rub/J mice intravenously injected with respective AAV.

AAV5 NG3 - large peptide insertions

AAV5 NG3.1 and 3.2 were generated by insertion of single domain antibodies into VP1. Intrastriatal injection demonstrated localized vector spread of AAV5 NG3.1 - eGFP and more global spread of AAV5 NG3.2 - eGFP which is in accordance with cognate surface antigen expression patterns **(Figure 4)**.

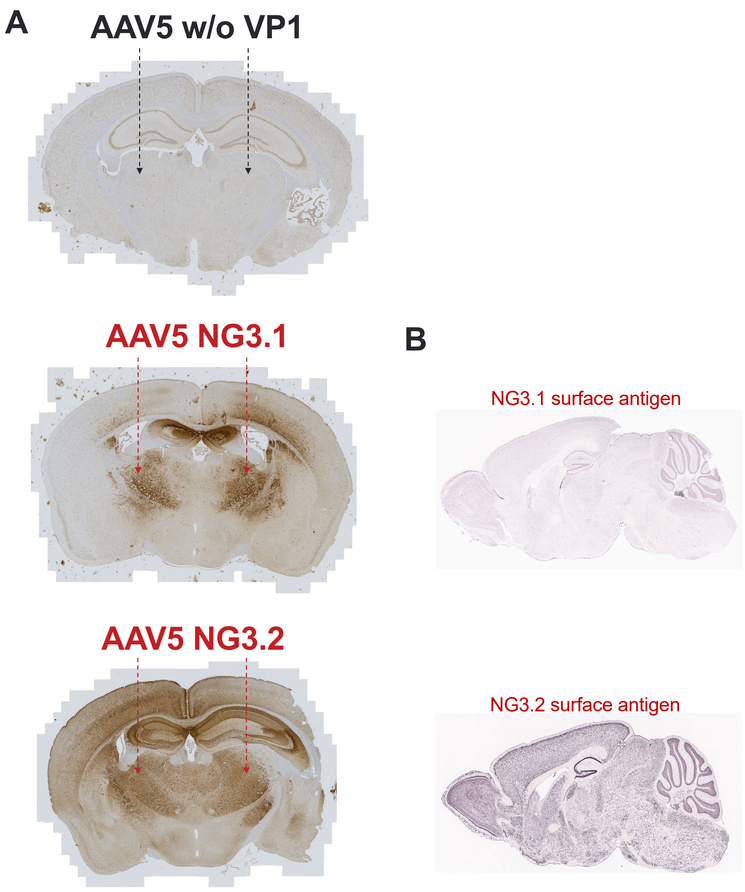

Figure 4 - A Chromogenic detection of **eGFP** transgene in intrastriatal injected C57BL/6 mice brain. **B Cognate surface antigen** expression profile via ISH

CONCLUSION

Site-directed mutagenesis, small, intermediate and large peptide insertions allow for the generation of AAV5 capsids with custom targeting profiles. Taken together, these approaches can significantly enhance the range of applications of the AAV5 serotype.

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