It's a mountain not a hill: Progress made in realizing AAV-delivered inhibitors for an HIV cure

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Disclosure

MRG is an inventor on patents related to eCD4-Ig and AAV-expressed eCD4-Ig and a co-founder/shareholder of Emmune, Inc., a company licensing eCD4-Ig from UF Scripps.

Community Summary

 <u>Key Question</u>: Can HIV inhibitors (antibodies or eCD4) delivered by adeno-associated virus (AAV) vectors and expressed by muscle cells suppress an ongoing infection in monkeys in the absence of daily cART?



- <u>Key Result</u>: AAV9 vectors expressed concentrations of an SIV antibody at therapeutic concentrations in monkeys.
- <u>Next Steps</u>: Apply key findings from AAV-expressed SIV antibody study for HIV bNAbs and assess in monkey model.

Project Goal: Can AAV-expressed HIV inhibitors suppress viremia after ART lift?



Key examples that have moved the field forward

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Miami Monkey



Star Performer A (5L7 antibody) Antibody / ADA levels 0 weeks 40 weeks 350 Monkey 84-05



Martinez-Navio et al., Front Immunol, 2020

Martinez-Navio et al., Immunity, 2019

eCD4 is an antibody-like HIV entry inhibitor

Peptide origin

Sequence

- CCR5 N-term MDYQVSSPIYDINYYTSEPSQK..
- E51 CDR-H3 ...NSIAGVAAAGDYADYDGGYYYDMD.....

CCR5mim6GDYYDYDGGYYYDGD



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Gardner MR and Kattenhorn LM et al, Nature 2015

AAV-expressed eCD4 for SHIV suppression



10

0

10

10 20 30 40 50 60 70

10

0

0

10

10 20 30 40 50 60 70

Observing viral suppression after ART lift

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 7 of 10 AAV.eCD4 macaques with partial control

AAV-expressed eCD4 for SIV suppression



AAV-expressed eCD4 for SIV suppression



Engineering new eCD4 expression cassettes



AAV-expressed HIV bNAbs in clinical trials

Adeno-associated virus vectored immunoprophylaxis to prevent HIV in healthy adults: a phase 1 randomised controlled trial

Oa OPEN ACCESS

Frances H Priddy, David J M Lewis, Huub C Gelderblom, Hana Hassanin, Claire Streatfield, Celia LaBranche, Jonathan Hare, Josephine H Cox, Len Dally, Daryl Bendel, David Montefiori, Eddy Sayeed, Jim Ackland, Jill Gilmour, Bruce C Schnepp, J Fraser Wright, Philip Johnson

- 2019: IM inoculation (up to 3) AAV1.PG9
 - Could not quantify PG9 by ELISA (LOD = 2.5 ug/mL)
 - ADA against PG9 observed

medicine

ARTICLES https://doi.org/10.1038/s41591-022-01762-x

Check for updates

Safety and tolerability of AAV8 delivery of a broadly neutralizing antibody in adults living with HIV: a phase 1, dose-escalation trial

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- 2022: IM inoculation (up to 8) AAV8.VRC07
 - Max dose: 2.5×10¹² vg/kg
 - Max VRC07 concentration ~1-3 ug/mL
 - ADA against VRC07 in 3 of 8 participants

Can improvements be made?





• Antibody?



AD Lewis et al. JVI 2002;76:8769-8775



AB Balazs et al. Nature (2011) doi:10.1038/nature10660

AAV-expressed ITS01 transgene cassette design



- ITSO1 is an anti-SIV antibody isolated from an SIV-infected rhesus macaque (Mason et al., PLOS Path, 2016)
 - M428L/N434S mutations in Fc to increase half-life
- Transfer plasmid design: 2A peptide splitting antibody heavy and light chain
- Construct includes CASI promoter, shortened WPRE, SV40 polyA signal sequence
- Size: ~4.28 kb

ITS01 with P2A peptide expresses best in mice

• Each mouse received 25 uL of 10¹¹ vg total in a single IM injection (gastrocnemius muscle)



	F2A	P2A	T2A
Average conc. (µg/mL)	153	312	160
Range (µg/mL)	110 - 207	168 - 402	113 - 212

NHP study design



n = 3 or 5

- Capsids evaluated: AAV1, AAV8, AAV9, AAV-NP22, AAV-KP1
 - n = 5 for AAV8 and AAV9 groups; n = 3 for AAV1, AAV-NP22, and AAV-KP1 groups
- Inoculation dose: 2.5×10¹² vg/kg
- Inoculation sites (x8): upper and lower left and right quads, left and right deltoids, left and right biceps

Selecting the AAV9 capsid for IM inoculation



	AAV1	AAV8	AAV9	AAV-NP22	AAV-KP1
Average conc. (ug/mL)	216	47	224	73	35
Range (ug/mL)	172 - 272	19 – 109	102 - 307	26 - 160	<1 - 62

Wider selection of AAV9-negative macaques to choose from?



- 360 animals screened from 6 different primate centers
- AAV-negative %: 8% AAV1, 16% AAV8, 42% AAV9

Applying what we learned to AAV-expressed bNAbs



- AAV1.10-1074 dose: 1E+11 vg
- AAV9.10-1074 dose: 5E+10 vg

Applying what we learned to AAV-expressed bNAbs



- AAV1.10-1074 dose: 1E+11 vg
- AAV9.10-1074 dose: 5E+10 vg

Applying what we learned to AAV-expressed bNAbs



- AAV1.10-1074 dose: 1E+11 vg
- AAV9.10-1074 dose: 5E+10 vg

Take home message

- AAV9 is a suitable replacement for AAV1 for IM inoculations in rhesus macaques
 - AAV9-negative macaques easier to find?
- Codon optimization is ok for IM inoculations
- P2A peptide might be the 2A of choice
 - At least 2A shop with other antibodies
- ADA not as big of an issue when the cargo is not overly immunogenic/welltolerated
- Spread out the injections maybe 1-2E+12 vg per injection site
 - One injection site per kg?

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