Supplemental Information

Adeno-Associated Virus Delivery of Anti-HIV Monoclonal Antibodies Can Drive Long-Term Virologic Suppression

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Fig. S1. AAV-mAb vector design. Related to Figures 1, 2 and 4. The AAV expression vector contains: a CMV enhancer/promoter; a SV40 intron; a SV40 polyA signal; constant regions of IgG1 heavy and light chains from rhesus (rh) monkey, a rhesus signal peptide (SP), and the human (hu) variable domains (VH and VL) of an anti-HIV monoclonal antibody (mAb). The expression cassette is flanked by AAV serotype 2 inverted terminal repeats (ITR). The CH3 domain of the IgG constant region contains two amino acid modifications (M428L and N434S) for extended half-life of the mAb (Ko et al., 2014; Zalevsky et al., 2010). In this bicistronic single-stranded AAV (ssAAV) vector, both heavy and light chains of IgG are expressed from one open reading frame using a F2A "self-processing" peptide from foot-and-mouth disease virus. The furin cleavage sequence "RKRR" for the cellular protease furin is added for removal of amino acids that were left on the heavy chain C-terminus following F2A self-processing. The peptide linker "SGSG" is added for improved furin enzyme-mediated cleavage (Fuchs et al., 2016). The 3' untranslated region contains multiple binding sites for conserved endogenous miRNAs that are specifically expressed in antigen presenting cells (miRNAbs). This is designed to render the mAb transcripts sensitive to translational inhibition by the miRNAbs expressed in antigen presenting cells (Agudo et al., 2014; Boisgerault et al., 2013; Majowicz et al., 2013). Abbreviations: CMV, cytomegalovirus; SV40, simian virus 40; SP, signal peptide; VH, variable heavy domain; CH, constant heavy domain; H, hinge; VL, variable light domain; CL, constant light domain; pA, polyadenylation signal; rh, of rhesus monkey origin; hu, of human origin.
Fig. S2

A

SHIV-AD8 env

1st recovery
2nd recovery
3rd recovery

MKVGIKKNYQLWLRGKIMLQLMMLCSAVENLWTVTVGGPVWKEATTLFTCASDAKAYDTEHTVNHVATACBPTDPNQPQEVENVETNFNMWKN

SHIV-AD8 env

1st recovery
2nd recovery
3rd recovery

EQMHEDISLWDQSLKPCVLTPCBLTCDTGWGVNTIINSSSMEEMRGIKNCFSNITTISIRDKVEDAYLFYPLDVPI1NDNTSYRLISCTSTYITQA

SHIV-AD8 env

1st recovery
2nd recovery
3rd recovery

CPKVSFEPIPIHYCTPAGFIALKCDKKFNSTGCNPSTQCTCHI1RPVSSTQLLNSLAEVEEVRSNFSNTDKNI1QVILKESVNEICTRPNNTR

SHIV-AD8 env

1st recovery
2nd recovery
3rd recovery

KS1H1GPGRATYTGDI1GD1RQAHCNI1RTKWNLTNQI1ATLKEQFGNNTI1VFQGSGGDPEI1YMHSNCGGEFYFCNSQTLASNFWNGTWNLTQ

SHIV-AD8 env

1st recovery
2nd recovery
3rd recovery

SNQDTITLPKCR1KQ1INMWSVEVGKAMYAPP1RQGI1RC5SN1TGL1ILTDBGNINN1DTEFPRGGDMRDNWRELSLYKVVKEPLGVAPTKVRRVFQ

SHIV-AD8 env

1st recovery
2nd recovery
3rd recovery

RERKRAVGAGMFLGFGLAGSTMGAA11TLVQARLLL1G1VQQNL1K1AE1AQH1L1L1V1Y6WQ1KQ1ARL1AVERYL1QDQ1LGWCSK1L1CTT

SHIV-AD8 env

1st recovery
2nd recovery
3rd recovery

AVPWNASHSNSNK1MD1INWNMTMWEERE1INDN1TGY11YTL11EESNQ1QNE1K1EQELE1L1DKWAS1WNWF1ITNW1LY1K1FI1M11VQ1GL1GL1IR1FGTLV1SMV

SHIV-AD8 env

1st recovery
2nd recovery
3rd recovery

BYVRO1Q1SPLSFQ1HT1L1P1TPRI1DPR1P1EG111E1GG1DRD1D1R1S1VR1LVGG1F1L1F1P1D1D1L1R1S1C1L1F1S1Y1H1R1L1D1L1L1Y1A1RT1V111L1R1G1W1O1E1A1K1Y1W1N1L1Q1Y1WN1Q1E1

B

Neutralization assay

Neutralization assay

Fig. S2. Env sequence comparisons of SHIVs recovered from rh2438 and neutralization sensitivity of viruses with variant envelope sequences. Related to Figure 1. (A) PBMCs from rh2438 were collected on weeks 84, 88 and 93 post AAV administration. Each collection was co-cultured with naive PBMCs or 221 cells. On days 16, 18 and 23, respectively, cell culture supernatant was harvested, and isolated viral RNA was subjected to RT-PCR. A total of 12, 12, and 5 individual clones from the respective recovery event were sequenced, and the translated env consensus sequence of each set was aligned against that of the SHIV-AD8 parental clone. Identical amino acids are depicted as dots, mismatches show the changed amino acids, amino acid positions not covered by sequencing are depicted as dashes. Abbreviations: envelope glycoprotein 120 (gp120), envelope glycoprotein 41 (gp41), variable loops (V1 to V5), CD4 binding site (CD4bs). The regions “N160” and “N332” are involved in the binding of "apex" and "high-mannose-patch" antibodies, respectively. (B) TZM-bl neutralization assays of SHIV-AD8 and viruses recovered from rh2438 using the broadly-neutralizing mAbs 3BNC117 (left panel) or 10-1074 (right panel) starting at 10 μg/ml. Monoclonal antibody VRC34.01 was used as a negative control. The dashed line indicates 50% relative light units (RLU) and represents 50% neutralization activity against the tested virus strains. A lower RLU indicates higher neutralization.
Fig. S3. mAb levels in serum following rAAV administrations (12-monkey study). Related to Figure 4. Levels of anti-HIV broadly neutralizing antibodies (A) N6, (B) PGT128, (C) PGT145, (D) 35O22 from the quad-group, and (E) 3BNC117 and (F) 10-1074 from the bi-group were quantified in serum by ELISA. AAV1 booster inoculations were conducted 24 weeks following the AAV8 administrations. Note: macaque r13029 had to be euthanized at week 76 post-infection due to SHIV related complications.
Fig. S4. Anti-antibodies (ADA) levels in serum following rAAV administrations (12-monkey study). Related to Figure 4. Levels of host anti-antibodies generated to each of the antibodies delivered in Fig. 7: (A) N6, (B) PGT128, (C) PGT145, (D) 35O22, (E) 3BNC117 and (F) 10-1074 were quantified in serum by ELISA. AAV1 booster inoculations were conducted 24 weeks following the AAV8 administrations. Note: macaque r13029 had to be euthanized at week 76 post-infection due to SHIV related complications.
Table S1

IC50 for SHIV-AD8 using broadly-neutralizing monoclonal antibodies. Related to Figures. 1, 2 and 4.

<table>
<thead>
<tr>
<th>mAb</th>
<th>Class</th>
<th>IC50 against SHIV-AD8 (µg/ml)</th>
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<tbody>
<tr>
<td>10-1074</td>
<td>V3 (mannose patch)</td>
<td>0.02</td>
</tr>
<tr>
<td>3BNC117</td>
<td>CD4bs</td>
<td>0.04</td>
</tr>
<tr>
<td>10E8</td>
<td>MPER</td>
<td>0.40</td>
</tr>
<tr>
<td>35O22</td>
<td>gp120-gp41 interface</td>
<td>0.01</td>
</tr>
<tr>
<td>PGT145</td>
<td>V1-V2 (apex)</td>
<td>0.15</td>
</tr>
<tr>
<td>N6</td>
<td>CD4bs</td>
<td>0.40</td>
</tr>
<tr>
<td>PGT128</td>
<td>V3 (mannose patch)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

IC50 was determined by performing a luciferase-based neutralization assay with broadly-neutralizing monoclonal assays using TZM-bl cells.