Immunological response and durability of expression following sequential intravitreal administration of AAV2.7m8 gene therapy to the contralateral eye in non-human primates

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Purpose

The adeno-associated viral (AAV) capsid AAV2.7m8 can transduce the retina following intravitreal (IVT) injection, which offers improved safety and convenience over subretinal injection, but introduces the vector into a less immune-privileged compartment.

Adverum is developing ADVM-022, an intravitreally-delivered gene therapy candidate consisting of AAV2.7m8 encoding aflibercept, an approved standard of care to treat wet age-related macular degeneration (wAMD).

Many patients develop wAMD bilaterally with variable time of onset.

To assess this risk, we investigated the effect of prior exposure of AAV2.7m8-aflibercept (AAV2.7m8-afli) vector in one eye on the transduction efficacy and ocular tolerability of the same AAV vector in the contralateral eye of non-human primates (NHPs).

Study Design and Methods

- Research-grade AAV2.7m8-aflibercept vector was produced in the baculovirus/SF9 cell system and purified by iodixanol gradient centrifugation.
- Male African green monkeys enrolled in the study were prescreened for the absence of pre-existing AAV2.7m8 neutralizing antibodies in serum using an in vitro transduction inhibition assay in HEK293T cells.
- NHPs were dosed IVT in one eye with 2x10¹² vg AAV2.7m8-afli, and 2 months later the contralateral eye received an equal IVT dose of the same AAV vector. Vehicle controls were injected with formulation buffer in both eyes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>N</th>
<th>Dose/eye (vg)</th>
<th>Dosing schedule</th>
<th>Eye Treated</th>
<th>Follow up post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AAV2.7m8-afli</td>
<td>3</td>
<td>2x10¹²</td>
<td>Day 0</td>
<td>OD</td>
<td>9 months</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>1</td>
<td>0</td>
<td>Day 0</td>
<td>OD</td>
<td>9 months</td>
</tr>
</tbody>
</table>

- Aflibercept expression was monitored by ELISA in vitreous and aqueous fluids throughout the study duration up to 9 months, and in ocular tissue at study termination.
- Serum and vitreous nAbs and IgGs to AAV2.7m8 were assessed at various time points post dosing.
- Ocular tolerability was evaluated by ophthalmic examinations (ocular coherence tomography [OCT], fundus imaging, slit lamp, tonometry) throughout the study, and by histopathology of eye tissues at termination.

Results

- Sustained aflibercept expression observed in ocular compartments following bilateral dosing with AAV2.7m8-afli, with meaningful levels detected in both eyes over subretinal injection, but introduces the vector into a less immune-privileged compartment.
- Adverum is developing ADVM-022, an intravitreally-delivered gene therapy candidate consisting of AAV2.7m8 encoding aflibercept, an approved standard of care to treat wet age-related macular degeneration (wAMD).
- Many patients develop wAMD bilaterally with variable time of onset.
- To assess this risk, we investigated the effect of prior exposure of AAV2.7m8-aflibercept (AAV2.7m8-afli) vector in one eye on the transduction efficacy and ocular tolerability of the same AAV vector in the contralateral eye of non-human primates (NHPs).

Conclusions

- Our data demonstrate that development of immunity following IVT administration of AAV2.7m8 capsid in one eye does not completely block transduction following sequential dosing in the contralateral eye in NHPs.
- Even in the presence of neutralizing antibodies, staggered IVT dosing to both eyes with AAV2.7m8-aflibercept does not exacerbate inflammatory response with histological observations limited to minimal perivascular infiltrates and mild inflammation.
- These data suggest that patients with pre-existing neutralizing antibodies can be safely enrolled in clinical studies and that it is possible to use the same vector to treat bilateral disease with variable time of onset.