A recent announcement from Adverum Biotechnology reports that a clinical trial (NCT04418427) patient lost sight in the treated eye after recombinant adeno-associated virus (rAAV)-mediated gene transfer of the anti-vascular endothelial growth factor (VEGF) drug aflibercept (Eylea). The patient was treated for diabetic macular edema (DME) with the company’s lead candidate, ADVN-022, which is the vectored form of aflibercept packaged in the artificial rAAV capsid AAV2.7m8. The incident occurred in the cohort treated at the higher dose ($6 \times 10^{11}$ viral genomes [vg]/per eye) causing hypotony with panuveitis and vision loss. No adverse event has been reported from the cohort treated at the lower dose ($2 \times 10^{11}$ vg/eye). Similarly, none of the patients treated with ADVN-022 for neovascular pathologies in age-related macular degeneration (AMD) developed any complications at either doses.

The company is conducting a thorough review of their data from the ADVM-022 program to determine the underlying cause of this incident. An immune reaction to the following: an artificial vector capsid used, an artificial chimeric protein being expressed, and/or a foreign DNA sequence being introduced, are all factors that can contribute to such adverse events.\(^1\) The delivery route and delivery technique also can cause complications.\(^1\) In addition, the cell types transduced, the biological function of the transgene expressed and the disease stage of the patient can also influence the risk of such an incident.\(^1\) Likely, it is a combination of several of these factors, with the disease stage of the patient and biological function of the transgene expressed being the most important considerations.

Gene therapy in the eye with rAAV vectors has proven to be safe. To date there are >50 clinical trials registered for eye gene therapies that use a variety of rAAV serotypes and delivery routes. AAV2, the most widely used serotype thus far, has been employed in subretinal and intravitreal deliveries for different disease conditions, including Leber congenital amaurosis 2, which became the first Food and Drug Administration (FDA)-approved gene therapy in the eye. Other eye disorders that used AAV2 for gene transfer are choroideremia, wet AMD, Leber hereditary optic neuropathy, as well as X-linked retinitis pigmentosa.\(^2,3\)

AAV8 has been used subretinally to deliver anti-VEGF antibodies and intravitreally to treat achromatopsia and retinoschisis.\(^2,3\) Suprachoroidal injections of AAV8 are planned to deliver an anti-VEGF antibody.\(^3\) AAV5 is being used to treat photoreceptor-specific mutations in retinitis pigmentosa by subretinal injections. Artificial capsid variants of AAV2 (rAAV2tYF) that contain three tyrosine to phenylalanine mutations are being used to treat retinoschisis by intravitreal delivery.\(^2\) None of these trails resulted in a severe adverse event that caused vision loss in the treated eye.\(^1–3\) Although transient inflammation has been often observed in a vector-dose-dependent manner immediately after viral delivery, these inflammatory episodes appear to be manageable with immunosuppressive drugs.\(^1\) They are more commonly associated with intravitreal injections, as this delivery route is not behind the retinal blood barrier.

Surprisingly, the incident reported by Adverum occurred 30 weeks after the initial intravitreal injection. This suggests that an immune response to the artificial capsid, the chimeric protein being expressed or the foreign DNA sequence are unlikely to have been a major contributing factor to this serious adverse event. Similarly, the delivery route and delivery technique are not likely to have been the cause for this adverse outcome, although both do affect the number of cells transduced.

In 2006, the FDA-approved ranibizumab (Lucentis), a monoclonal antibody derived from bevacizumab (Avastin) that binds VEGF, for the treatment of wet AMD. Since then, a few more anti-VEGF drugs have been approved, including aflibercept (Eylea) to treat neovascular pathologies in the eye. Anti-VEGF therapies have proven to be overall safe; however, inflammatory events, which are generally
manageable, are a common reoccurring theme of anti-VEGF treatments, suggesting a possible link between VEGF inhibition and inflammation. There also is an increasing body of literature indicating that VEGF plays an important neuroprotective role in the retina. Consequently, alternative avenues are being pursued to treat neovascular pathologies without affecting VEGF function.

In a companion article to this editorial, we show that inhibition of VEGF by rAAV2.7m8-mediated gene transfer of conbercept, a recently developed anti-VEGF protein, can cause a vascular sheathing pathology, which is reminiscent of vasculitis in humans at a high dose. Both conbercept and aflibercept are similarly designed drugs in that they both are composed of different domains of VEGFR1 and VEGFR2 fused to the constant Fc domain of human IgG1. Beovu, which is a single chain anti-VEGF antibody, has also been reported to cause vasculitis in one study. We show that the vascular sheathing pathology is due to immune cells infiltrates. Importantly, the pathology is preceded by increased expression in vascular cell adhesion molecule 1 (VCAM1) and intercellular cell adhesion molecule 1 (ICAM1). Both proteins promote extravasation of immune cells from the vasculature.

To test if an immune response to the vector capsid or the protein being expressed caused increased VCAM1 and ICAM1 expression, we injected mice deficient in B and T cells. As expected, these mice did not develop a vascular sheathing pathology, as they lack associated immune infiltrates. However, they still upregulated VCAM1 and ICAM1 expression. This indicates that changes in gene expression that promote extravasation of immune cells are a direct consequence of suppressed VEGF function. Thus, inflammatory episodes associated with VEGF inhibition may be promoted by gene expression changes that enhance extravasation of immune cells over time. Compounded with a disease-associated inflammation that is common in patients with edemas, inhibition of VEGF could result in more severe inflammatory events.

To test if disease-associated inflammation can exacerbate extravasation of immune cells, we injected the lower dose of AAV2.7m8-Conbercept, found to be safe in wild-type mice, in a mouse model of AMD. Approximately 50% of eyes still developed a uniform vascular sheathing pathology. Thus, the severe adverse event in the patient treated with AAV2.7m8-Aflibercept could have been caused by a combination of excessive inflammation due to the disease condition and inhibition of VEGF function, which over time would have further exacerbated the disease-associated inflammation. Since DMES are caused primarily by retinal vascular pathologies, including a breakdown of the retinal blood barrier, expression of aflibercept in endothelial cells may have increased the risk of this event by further increasing the immune cell permeability of the retinal vasculature. This contrasts the situation in AMD patients, where neovascular pathologies are primarily caused by the choroidal vasculature. Owing to its distance from the vitreous, it is less amenable to rAAV-mediated transduction through intravitreal injection. This may explain why no adverse event has been reported in AMD patients treated at the same dose.

In summary, this event reported by Adverum highlights the need to use animal with advanced disease in preclinical safety studies to unravel the rare incidents that may unfold at different vector doses. Determining the minimum dose required to resolve edemas and prevent them from re-developing will also improve long-term safety of this kind of therapy.

REFERENCES


10. Kroger N, Matonti F, Roubeix C, et al. VEGF is an autocrine/paracrine neuroprotective factor for in-


