Molecular Therapy
Commentary

Breaking and Sealing Barriers in Retinal Gene Therapy

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The approval of Luxturna for the treatment of a rare form of autosomal recessive blindness validated decades of research that retinal disorders make for a promising target for gene therapy.[1] Many of the follow-up approaches emerging from the lab or in the clinic now rely, like Luxturna, on subretinal injection for delivering the gene therapy vector.[2] In this issue of *Molecular Therapy*, Cukras et al.[3] report on results from a phase I/IIa single-center, open-label clinical gene therapy trial targeting retinoschisis using, like Luxturna, an adeno-associated viral vector (AAV), yet injected intravitreally. Their results highlight the opportunities and limitations of this approach for this indication and beyond.

X-linked retinoschisis, or XLRS, is single gene disorder that leads to decreased visual acuity early in life that can impact day-to-day activities such as reading and the ability to drive. XLRS is caused by mutations in the retinoschisin gene, called *RS1*, which encodes for a secreted homo-octameric complex with pleiotropic functions. Functional measures of vision, such as the electro-retinogram waveform, are reduced, implicating dysfunction at the photoreceptor synapse, which is illustrative of a breakdown of the processing of the visual signal in the posterior segment. The name retinoschisis refers to the separations that arise within the neural retina as the disease progresses. These schisis cavities, and the retinal fragility that arises from it, complicates—if not prohibits—any drug delivery that requires invasive retinal surgery.

Subretinal injections, an essential component of Luxturna's efficient gene transfer and therapeutic effect, do involve an invasive surgical intervention to the retina. Not only is the vector formulation deposited below the retina by iatrogenically producing a retinotomy and self-resolving retinal detachment, but it is preceded by the partial removal of the vitreous jelly to facilitate the retinal maneuver. Since these interventions have the potential to harm the frail XLRS retina, an intravitreal injection would be highly preferred, as Cukras and colleagues[3] pursued here. Unlike subretinal injection, the intravitreal injection is routinely performed as an office procedure, for example, to administer one of several commercial anti-vascular endothelial growth factor (VEGF) protein drug products for the treatment of exudative age-related macular degeneration (AMD) and other conditions.[4] While these anti-VEGF drugs are administered into the vitreous gel in the center or anterior portion of the vitreous cavity, intravitreal injection of a gene therapy agent might require administration closer to the retina surface to adequately access the retinal cells. Intravitreal injection has the potential advantage of reaching a broad area of retina, whereas the effect of a subretinal injection is largely retained within the bleb, i.e., the subretinal space generated by the injection. Therefore, intravitreal injection of gene therapy agents is *theoretically* highly preferable over a subretinal approach, not just for XLRS, but arguably for most retinal gene therapy disease targets given its limited invasiveness, its ability for widespread retinal targeting, and the clinical experience and comfort practitioners have garnered with the procedure over the past decades.

In the work by the Cukras group leading up to this clinical study, the frailty of the retina in an XLRS mouse model was actually shown to promote vector penetration as compared to a healthy retina in a *wild-type* animal.[5] Several reports illustrate the challenges of achieving robust expression in neural retina following intravitreal administration, and some have implicated the inner limiting membrane at the vitreoretinal interface to be a physical and biochemical barrier for viral particles to cross efficiently.[6,7] The XLRS disease state—at least in part—seems to break down these barriers and allow for broad and efficient transduction of a large proportion of retinal cells and cell types, sufficient to achieve a clear treatment effect in the *Rs1*-KO mouse. Furthermore, RS1 is a secreted protein product, and it is anticipated that both transduced cells and, at some measure, non-transduced cells that take up extracellular RS1 protein, are functionally rescued. These aspects lower the delivery hurdle for this intravitreal approach and make retinoschisis arguably an ideal target for gene therapy via intravitreal injection. Indeed, in a mouse model, higher order function, including the rescue of molecular pathology at the photoreceptor-depolarizing bipolar cell synapse, can be restored by intravitreal AAV8-RS1 gene transfer.[8]

The safety studies conducted prior to this clinical trial, however, echo the limitations of the intravitreal approach observed in other preclinical and, more recently, clinical studies. In a dose-escalation safety study in rabbits, a self-resolving, dose-dependent vitreal inflammation was observed.[9] The issue of inflammation following AAV gene transfer via the intravitreal route was first described by Genzyme in the lead-up to their AAV2-sFlt program for AMD.[10] The inflammation, which is not obvious in rodents, is characterized by a delayed onset uveitis, which can lead to vitritis. Limited molecular studies have implicated T cell activation toward AAV capsid antigens.[10] While

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immunosuppressive regimens around the time of injection may allow control of these inflammatory events at some measure, these events force many programs to limit the dose to avoid any dose-limiting toxicity.

The clinical trial design by Cukras et al.[3] reflects this concern with a cautious dose-escalation in 3 groups (10^9, 10^{10}, and 10^{11} vector genomes [vg] per eye). The efficacious vector dose in mice was noted at 10^8 vg, and the first signs of inflammation in the rabbit study were seen in the 10^{10} range. It is worth noting that none of these preclinical doses are adjusted for the 100–1,000-fold larger volume of the ocular globe between mouse or rabbit and the human. Indeed, the authors describe that investigational product was overall well-tolerated, except for one individual at the highest dose. The inflammation that arose in the subject appeared analogous to that observed in larger animal models and was controlled by topical and oral corticosteroids.

In terms of efficacy, unfortunately, no significant gain of visual function was observed in the treated subjects. The patient at the highest dose with the inflammatory sequelae, however, did have an interesting yet complicated presentation: notwithstanding some initial and transient decline in visual acuity and function (likely due to the inflammatory events), some retinal cavity closure was observed in the treated but not opposing eye at the 2-week time point after injection. This effect did not last but may provide a hint of efficacy in man of intravitreally administered AAV.*RS1*.

This careful study, led by Dr. Sieving and born out of an extensive body of work on the molecular pathophysiology and treatment paradigms of retinoschisis, illustrates both the impact intravitreally administered retinal gene therapy can make on patients' lives and the areas where further development is needed. It is generally recognized that intravitreal administration would open up therapeutic opportunities for gene therapy in ophthalmology tremendously. However, the observed host responses remain poorly understood and warrant extensive study of the antigenic nature, the inflammatory mechanisms at play, and clinically relevant approaches to mitigate them. Second, gene transfer from the vitreous space into the retina in large eyes is clearly inefficient, in need of high dosing, and broader therapeutic success likely will hinge on development of more potent and better tolerated vector systems.

CONFLICTS OF INTEREST

J.W.M. is a consultant and advisor for Genentech/Roche, Bausch + Lomb, Kalvista Pharmaceuticals, and ONL Therapeutics; has equity in ONL Therapeutics; and has patents and/or royalties from ONL Therapeutics and Massachussettes Eye and Ear and Valeant Pharmaceuticals and Massachusettes Eye and Ear. L.H.V. is a founder and scientific advisor of Akouos and GenSight Biologics and a consultant to various biopharmaceutical companies in the gene therapy space, including Nightstar Therapeutics. Lonza, Solid, and Selecta Biosciences sponsored research for L.H.V.

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REFERENCES

1. U.S. Food and Drug Administration. (2018). Luxturna. https://www.fda.gov/biologicsbloodvaccines/cellulargenetherapyproducts/approvedproducts/ucm589507.htm.
2. Russell, S., Bennett, J., Wellman, J.A., Chung, D.C., Yu, Z.F., Tillman, A., Wittes, J., Pappas, J., Elci, O., McCague, S., et al. (2017). Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet *390*, 849–860.
3. Cukras, C., Wiley, H.E., Jeffrey, B.G., Sen, H.N., Turriff, A., Zeng, Y., Vijayasarathy, C., Marangoni, D., Ziccardi, L., Kjellstrom, S., et al. (2018). Retinal AAV8-RS1 Gene Therapy for X-Linked Retinoschisis: Initial Findings from a Phase I/IIa Trial by Intravitreal Delivery. Mol Ther. *26*, this issue, 2282–2294.
4. Miller, J.W. (2013). Age-related macular degeneration revisited–piecing the puzzle: the LXIX Edward Jackson memorial lecture. Am J Ophthalmol *155*, 1–35.e13.
5. Park, T.K., Wu, Z., Kjellstrom, S., Zeng, Y., Bush, R.A., Sieving, P.A., and Colosi, P. (2009). Intravitreal delivery of AAV8 retinoschisin results in cell type-specific gene expression and retinal rescue in the Rs1-KO mouse. Gene Ther. *16*, 916–926.
6. Auricchio, A., Kobinger, G., Anand, V., Hildinger, M., O'Connor, E., Maguire, A.M., Wilson, J.M., and Bennett, J. (2001). Exchange of surface proteins impacts on viral vector cellular specificity and transduction characteristics: the retina as a model. Hum. Mol. Genet. *10*, 3075–3081.
7. Dalkara, D., Kolstad, K.D., Caporale, N., Visel, M., Klimczak, R.R., Schaffer, D.V., and Flannery, J.G. (2009). Inner limiting membrane barriers to AAV-mediated retinal transduction from the vitreous. Mol. Ther. *17*, 2096–2102.
8. Ou, J., Vijayasarathy, C., Ziccardi, L., Chen, S., Zeng, Y., Marangoni, D., Pope, J.G., Bush, R.A., Wu, Z., Li, W., and Sieving, P.A. (2015). Synaptic pathology and therapeutic repair in adult retinoschisis mouse by AAV-RS1 transfer. J. Clin. Invest. *125*, 2891–2903.
9. Marangoni, D., Bush, R.A., Zeng, Y., Wei, L.L., Ziccardi, L., Vijayasarathy, C., Bartoe, J.T., Palyada, K., Santos, M., Hiriyanna, S., et al. (2016). Ocular and systemic safety of a recombinant AAV8 vector for X-linked retinoschisis gene therapy: GLP studies in rabbits and Rs1-KO mice. Mol. Ther. Methods Clin. Dev. *5*, 16011.
10. Maclachlan, T.K., Lukason, M., Collins, M., Munger, R., Isenberger, E., Rogers, C., Malatos, S., Dufresne, E., Morris, J., Calcedo, R., et al. (2011). Preclinical safety evaluation of AAV2-sFLT01- a gene therapy for age-related macular degeneration. Mol. Ther. *19*, 326–334.