Gene Therapy: The Future of Ophthalmology?

AN INTERVIEW WITH THOMAS W. CHALBERG, PHD, AND SAMUEL B. BARONE, MD

The idea of injecting a virus into the human body capable of altering its genetic code seems like something straight from a science fiction novel. It seems almost far-fetched to conceive that the intersection of advances in virology, nanotechnogy, biochemistry, molecular biology, and genetics would set the stage for gene therapy: a forced but beneficial transduction of local genetic code to produce proteins with a therapeutic effect.

These ideas would seem far-fetched if not for the very real research emanating from laboratories such as the one at Avalanche Biotechnologies Inc., whose lead candidate, AVA-101—an adeno-associated virus serotype 2 (AAV 2) that transduces retinal pigment epithelium (RPE) cells in the outer retina, leading to continuous expression of the protein sFlt-1, which blocks local VEGF signaling—is already being studied in humans.

Retina Today recently interviewed the cofounder and CEO of Avalanche, Thomas W. Chalberg, PhD, as well as its chief medical officer, Samuel B. Barone, MD, to learn more about its technology and what it may mean for the future of ophthalmology, specifically for patients with VEGF-mediated diseases such as age-related macular degeneration (AMD). Although Dr. Chalberg conveys the strong intellectual appeal of the approach, in that "gene therapy really captures people's imaginations; scientifically it fulfills the dreams of scientists and physicians who continue to work in the field of genetics," he is also ready to point out the growing body of literature to support the concept.

And so, while it may still be a few years before such therapies are available for everyday use in clinics, a growing body of data underscores that, far from being a notion of fiction writers, gene therapy has the very real potential to change how retina specialists treat patients with diseases such as AMD in the next few years.

BACKGROUND

Retina Today: Avalanche Biotechnologies Inc. is predicated on finding solutions to ophthalmic diseases using gene therapies. This is a hot topic in modern medicine, but especially so in ophthalmology. Why is that? What is the promise of gene therapy and how might it transform the future of ophthalmology?

Thomas W. Chalberg, PhD: It is an interesting time in the field of gene therapy where there is a lot of interest and innovation.

I think gene therapy is a hot topic in ophthalmic research because it offers the potential for a one-time transformative therapy that fills unmet medical needs for patients suffering for wet AMD. In addition, it could apply to a number of target diseases that have never had any kind of available treatment. In much the same way that monoclonal antibodies represented a new area of discovery 15 or 20 years ago, gene therapy fills a relatively new space—a relatively open space, for that matter—with the promise of being the next generation of medical therapy and a new class of biologics.

More generally, I think gene therapy really captures people's imaginations; scientifically it fulfills the dreams of scientists and physicians who continue to work in the field of genetics. The idea of using DNA as a pharmaceutical is a sort of marvelous and wondrous proposition. But why now? Because there are positive clinical data emerging that demonstrates that gene therapy is working. For the first time, we are getting a sense that this is a real technology that is here now.

RT: Gene therapy is a nascent field, but it is by no means new. There is some history to build on, notably the Leber congenital amaurosis (LCA) trials headed by Jean Bennett, MD, PhD, and the National Eye Institute.[1]

What have you learned from those experiences, if anything, and have they shaped your approach to researching gene therapies?

Dr. Chalberg: The pioneering work in LCA has been informative and has catapulted the field forward, and, again, that was based on positive clinical data. The LCA program employed an adeno-associated virus serotype 2 (AAV 2), which is the viral vector used in our current lead candidate, AVA-101. It was also successfully administered in the same way, via subretinal injection. So there are a lot of things we learned from the LCA trial that we can directly translate into our program for AVA-101.

But the LCA trials were also significant in another way. The LCA work taught us a lot about how ophthalmology is leading the way in gene therapy and how it is a great fit for gene therapy. The eye is small and easily accessible, so it is possible to deliver the therapy right where you need it in a very small dose that minimizes systemic side effects. You can also directly visualize the retina externally, and there are proven diagnostic techniques for monitoring the treatment's effect and for potential adverse events. The eye is also somewhat immune-privileged, so the concern over a systemic immune response is reduced. That has been demonstrated both in our work and the LCA work.

When we take all of these factors and put them in combination with a disease model like wet AMD—where VEGF is a validated clinical target but there is a major limitation in the need for frequent readministration of anti-VEGF therapy—it is easy to see the promise of how gene therapy has the possibility to transform the future of ophthalmology.

RT: Most of the prior research with gene therapy took place in relation to heritable diseases. Is there any difference in approach to researching gene therapy for heritable disease states, such as LCA or juvenile X-linked retinoschisis (XLRS), versus acquired disease states, such as AMD?

Dr. Chalberg: One of the important elements we look for in indications we are developing is a strong understanding of the biological basis of the disease, whether that is based on genetics, for example in the case of XLRS, which is one of our programs for AVA-311; or whether it has a very well validated and known clinical target, VEGF, which is the case for AVA-101. Having that solid foundation in understanding the biologic rationale for the therapy is important when we think about designing programs that have a high probability for success.

OCULAR BIOFACTORY

RT: At the core of the Avalanche portfolio is a technology you refer to as Ocular BioFactory. Can you describe this concept and how it works?

Dr. Chalberg: The Ocular BioFactory is the name that we have given our platform technology, and it is a way to discover, manufacture, and develop new gene therapy vectors for the eye. Traditionally, gene therapy has used naturally occurring viral vectors, like AAV2, which, again, was used in the LCA studies. It is also what we use as the molecular basis of AVA-101. But there are some limitations of AAV2 and other naturally occurring AAV vectors. In order to overcome these limitations, we use a technique called *directed evolution* to derive new viral vectors.

The way this works is that we start with naturally occurring viral vectors that we can mutagenize into a large library of non-naturally occurring variants of AAV. We can screen the resulting library for advantageous properties and select useful variants from that library for further development. Then, through multiple rounds of diversity generation and selection, we can identify novel AAV variants that have improved properties compared with the first-generation technologies. This vector discovery work is something that we are actively working on in our research group, and it is also part of our work with Regeneron Pharmaceuticals.

RT: What is the biologic rationale for considering multiple AAV vectors in potentially directing therapy to various retinal diseases?

Dr. Chalberg: The retina is multiple layers thick, and each layer of the retina represents a distinct cell type. In turn, each of those cell types is implicated in different retinal diseases. For example, retinal ganglion cells are implicated in glaucoma; retinal pigment epithelium cells are implicated in wet AMD and dry AMD; and photoreceptors are implicated in certain kinds of retinal dystrophies. When we think about treating a disease, we need to be able to transduce the correct cell type with the vector, so it is necessary to design a construct that can efficiently penetrate the retinal layers and then transduce the appropriate cell type.

RT: Has this concept been validated in the literature, and has it met the rigors of scientific validation?

Dr. Chalberg: The idea of using directed evolution to identify AAV vectors that target different retinal cell types was pioneered at the University of California,

Berkeley, from which we licensed the technology. One publication in particular had a very powerful proof of concept that a vector produced through directed evolution could efficiently penetrate the retina following intravitreal injection and was effective in mouse models—and in that case, two different mouse models of diseases.[2]

AVA-101

RT: The lead candidate in the Avalanche portfolio is AVA-101, which secretes a therapeutic protein that blocks VEGF signaling. Is this route of VEGF blockade different in concept or function from intravitreal injection using currently available anti-VEGF agents?

Samuel B. Barone, MD: We are really excited about the promise that AVA-101 holds as a potential one-time transformative therapy. This mechanism of VEGF inhibition or blockade in AVA-101 is a secreted anti-VEGF protein called sFlt-1 that is produced by the AVA-101 vector. The mechanism is very similar to that of aflibercept (Eylea, Regeneron) because both therapies use the same domain for VEGF binding. What is very different is that with AVA-101, sFlt-1 is produced in the retina on a continuous basis to inhibit VEGF locally at the site of the disease. This would be a significant advantage over anti-VEGF intravitreal injections, which require frequent, chronic readministration.

RT: Is there an advantage to having the therapy locally available at the level of the retina?

Dr. Barone: Absolutely; this is right at the site of wet AMD activity. Additionally, there is built-in patient compliance and convenience with AVA-101 that is not available with repeated intravitreal injections. Further, AVA-101 offers a continuous supply of the sFlt-1 protein, which may be safer than repeated bolus intravitreal injections that provide very high peak protein levels in the vitreous, and it may be more effective because there are not the trough protein levels in the days preceding the next intravitreal injection.

RT: Those trough levels of VEGF inhibition are potentially when pathology can return and cause damage?

Dr. Barone: Exactly.

RT: Where is this technology in the development timeline?

Dr. Barone: We have human data at this point. AVA-101 is currently being evaluated in a phase 2a single-center, investigator-sponsored trial outside the United States in which the primary endpoint is safety. The results of our phase 1 study (NCT01494805) are being submitted for publication.[3] The data for the phase 2a portion will be coming out in mid-year 2015. We are in the process of planning a phase 2b, which we expect to begin enrolling in the second half of this year.

RT: What have you learned about the safety profile of AVA-101 at this point?

Dr. Barone: AVA-101 has been well tolerated, with no drug-related adverse events in the phase 1 study. In addition, there were encouraging signs of biologic activity. Subjects who were treated with AVA-101 showed decreased wet AMD activity as evidenced by sustained improvement of central retinal thickness on optical coherence tomography, a decreased need for anti-VEGF injections, and stable or improved visual acuity during the 1-year follow-up period.

OTHER PRODUCT DEVELOPMENTS

RT: The Avalanche portfolio includes AVA-201, which is also an sFlt-1 expressing gene–based therapeutic modality. How is this different from AVA-101?

Dr. Barone: The advantage of AVA-201 is that it may be able to be used for the prevention of wet AMD. It is similar to AVA-101 because it expresses the same naturally occurring anti-VEGF protein, sFlt-1. However, whereas AVA-101 uses a wild type AAV2 vector, AVA-201 employs a novel vector discovered from our directed evolution technology. We have the ability to deliver the transgene with an AAV vector that has been optimized so that it can be administered by a single intravitreal injection. Again, because of the potential advantage of being a one-time treatment, now with a less invasive route of administration, AVA-201 can play a role in patients who are at high risk, such as patients who have strong clinical or genetic markers predisposing them to wet AMD.

RT: What other products are in the Avalanche pipeline, and where are they in development?

Dr. Chalberg: AVA-101 is really just the tip of the iceberg. We are equally excited about other products in our pipeline. We think the Ocular BioFactory platform and the directed evolution capabilities, along with our ophthalmology experience and our expertise in gene therapy, puts us in a great position to continuously deliver a

pipeline of new potentially transformative treatments for different eye diseases. An example of that is AVA-311, which is a product for XLRS that we are developing in partnership with Regeneron. That is currently in preclinical studies, and Regeneron will be leading the clinical development. In addition, we are in the planning stages to investigate AVA-101 in other VEGF-mediated vascular diseases, like diabetic macular edema and retinal vein occlusion.

In addition to the programs we have announced, we are working on a couple of things that we have not yet announced but are really excited about, and we are looking forward to sharing them at an appropriate point in the future.

RT: Avalanche announced a partnership with Regeneron Pharmaceuticals Inc. in May 2014. What does this collaboration mean for Avalanche's development efforts and capabilities?

Dr. Chalberg: Regeneron is a terrific partner for their scientific leadership as well as their product development capabilities and commercialization track record. We are proud to have Regeneron as our partner on an R&D collaboration that includes, among other things, vector discovery and optimization using our Ocular BioFactory platform. Through this collaboration, we will be working on the development of certain targets; Regeneron has the right to nominate up to eight targets to be part of the collaboration. The first one, as I mentioned, is AVA-311, which is for the treatment of XLRS. Overall, we think the partnership with Regeneron is a great opportunity to expand our reach into additional indications to help patients suffering from blinding disease. ■

Thomas W. Chalberg, PhD, is a cofounder of Avalanche Biotechnologies Inc., a member of the board of directors since July 2006, and has served as president and chief executive officer since October 2010.

Samuel B. Barone, MD, is chief medical officer of Avalanche Biotechnologies Inc.

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CONTACT US

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