Oligonucleotide Therapeutics – Restoring Sight to the Blind

Vision is one of our most priceless senses. Without it, we miss out on precious visuals, such as witnessing a child's face light up with joy or seeing a glorious sunset, and even the important relational activity of reading the expressions on someone's face during a conversation. Some people are born without the gift of sight and others endure losing it later in life. Imagine facing the knowledge that you are losing your sight with small children to care for, or a career that you may lose, then experiencing every single day becoming more difficult and independence slowly slipping out of your control. Any other time in history blindness would be a final sentence. Now, oligonucleotide therapeutics are beginning to restore sight and prevent the progression of diseases that cause blindness.

Oligonucleotide-based therapeutics are displaying astonishing results in treating many different diseases of the eye.

The eye is a model target organ for oligonucleotide therapeutics because it is easily accessible, immune privileged, and isolated (1). Additionally, one eye can be used as an experimental target while the other is used as a control (2).

In fact, the eye is such an ideal target that the first FDA approval of an oligonucleotide-based drug was given to Vitravene (fomivirsen), which treats cytomegalovirus (CMV) retinitis. Fomivirsen, a first generation ASO, was delivered to the eye by intravitreal (IVT) administration and did not require a delivery agent. Developed by Isis Pharmaceuticals (now Ionis Pharmaceuticals) then licensed to Novartis, fomivirsen had great success in arresting the development in CMV retinitis. However, it was withdrawn because the number of cases of CMV

retinitis was dramatically reduced due to the development of highly effective antiretroviral drugs (1).

Despite that early success, it has taken a while for oligonucleotide drugs to be developed enough to achieve clinical usefulness. After decades of persistence, the investment is producing results, and oligonucleotide-based drugs are safely and effectively treating a broad range of conditions, including ocular diseases. Already, several different oligonucleotide medications are showing promising results in clinical trials for the treatment of ophthalmic conditions.

Although the human eye is a relatively small organ, there are a surprisingly large number of diseases that can affect it. Therefore, it should be expected that it requires many different techniques to treat ocular diseases. Sometimes, a single disease could be treated in multiple ways. Here are a few common ophthalmic diseases, and the solutions that are being developed to treat them.

Leber congenital amaurosis (LCA) is one of the most common causes of blindness in children. It is an inherited retinal disorder that is associated with at least 25 genes. 15-20% of cases are caused by mutations in *CEP290*. One form of the disease, LCA10 is caused by an intronic mutation in *CEP290* that causes a defective splice site and inserts an exon that does not belong in the mRNA, resulting in a premature termination codon (3). This defect results in a non-functional CEP290 protein, which ultimately causes severe visual impairment, with many patients merely able to detect the difference between light and dark, and a full one-third are completely unable to perceive light at all.

ProQR Therapeutics has developed Sepofarsen to restore vision loss in people with this mutation. Sepofarsen is an ASO that binds to the defective splice site, excluding exon X, and restoring correct splicing. (*To learn more about targeting RNA splicing, see this article*.) This allows the cells in the retina to produce fully functional CEP290 protein. In a phase 1/2 trial, eleven patients received intravitreal injections of sepofarsen in one eye. It was well tolerated, with no serious adverse events, ***and most patients experienced a rapid and durable improvement in vision***. Astonishingly, one exceptional responder improved from only being able to perceive light to gaining 20/400 vision (4)!

Perhaps even more significant, are the results in one patient who only received a single course of sepofarsen then declined additional doses. This patient showed remarkable improvements that peaked around three months after the injection, with sustained efficacy over the course of 15 months that was similar to those who received multiple, regular injections (5).

A phase 2/3 clinical trial is ongoing and includes adults and children eight years and older. After the first twelve months of treatment in one eye, and a benefit/risk analysis, it was decided that treatment may begin in the second eye and those that were in the sham group may switch to

treatment with sepofarsen. An additional trial has begun in which children under the age of eight are receiving treatment.

Autosomal dominant optic atrophy (ADOA) is the most common inherited optic nerve disorder. It causes progressive vision loss that begins in a child's first year as well as hearing loss and muscle pain and weakness. ADOA is caused by mutations in the *OPA1* gene, which produces the OPA1 protein that maintains the mitochondria so they can produce energy, regulate cellular metabolism, and carry on their other normal functions. When there is not enough OPA1 protein, retinal ganglion cells die because they have high energy needs, and the mitochondria are not able to meet the demand.

Stoke Therapeutics has completed preclinical studies using TANGO ASOs to target the OPA1 gene. Doing so prevented non-productive splicing, which reduced the production of naturally occurring non-productive mRNA and increased productive mRNA. This resulted in increased OPA1 protein levels. The increased levels of OPA1 protein partially restored mitochondrial function, and increased ATP production. As a result, Stoke Therapeutics announced that lead optimization is underway to identify a clinical candidate in 2021.

Glaucoma is the second leading cause of blindness (second to cataracts) and is the leading cause of irreversible blindness. It is actually a group of diseases associated with increased intraocular pressure (IOP) which damages the optic nerve and causes the retinal ganglion cells and retinal nerve fiber layers to degenerate (2, 3, 6). The result is irreversible eye damage.

Sylentis developed Bamosiran (SYL040012), a siRNA, to treat primary open-angle glaucoma (POAG) and ocular hypertension. It works by inhibiting synthesis of ADRβ2 which reduces IOP. Results showed a significant reduction in IOP. This medication is particularly interesting because it is the first siRNA therapeutic for an ocular disease that is administered topically, rather than by intravitreal injection. This non-invasive method of administration contains clear advantages, as it greatly reduces the risk of adverse events and is far easier for a patient to feel comfortable with accepting this method of administration.

Quark Pharmaceuticals is developing QPI-1007, a siRNA that preserves retinal ganglion cells by silencing the synthesis of caspase 2, an enzyme that plays a central role in cell apoptosis. It is being studied as a treatment for non-arteritic ischemic optic neuropathy (NAION) and acute, primary angle-closure glaucoma.

Isarna Therapeutics developed ISTH0036, which is an ASO that selectively targets the *TGF-β2* mRNA, in order to decrease TGF-β2 levels, as elevated levels are associated with increased IOP. ISTH0036 was studied in patients with primary open angle glaucoma who would undergo trabeculectomy (a surgery for glaucoma that allows fluid to be drained). The study showed that ISTH0036 was effective in controlling IOP postoperatively and did not display toxicity (7). Isarna

is about to initiate Phase 2a development in neovascular age-related macular degeneration (nAMD) and diabetic macular edema (DME).

Retinal disorders:

Diabetic retinopathy and diabetic macular oedema (DME) are retinal disorders that are complications of diabetes. In diabetic retinopathy blood vessels in the retina are damaged. As it progresses, fluid and blood can leak into the retina, causing swelling, at which point it has become diabetic macular oedema.

Age-related macular degeneration (AMD) is a retinal disorder that is a major cause of blindness (2). AMD causes damage to a part of the retina called the macula, blurring your central vision. There are two types of AMD. Dry AMD (atrophic AMD) results from the macula becoming thinner as you age, and tiny clumps of a protein called drusen grow. Wet AMD (neovascular AMD) occurs when abnormal leaky blood vessels grow under the retina (this is called choroidal neovascularization (CNV)), causing damage and scarring of the macula. Vascular endothelial growth factor (VEGF) is a key component in the development of choroidal neovascularization (CNV) and wet AMD.

Age-related macular degeneration (AMD), diabetic macular oedema (DME), and diabetic retinopathy are all treatable with anti-VEGF medications which help prevent abnormal blood vessel growth and reduce swelling, so oligonucleotide medications are sometimes tested as treatments for more than one of these conditions.

- Bevasiranib (Cand5) was the first siRNA to advance to Phase III trials (8). It is a chemically modified naked siRNA that was intended to treat DME and wet AMD by down-regulating vascular endothelial growth factor (VEGF) (2). While it was safe and showed good preliminary results, on the recommendation of the Independent Data Monitoring Committee, OPKO Health decided to terminate its Phase III clinical study as it was unlikely to meet its primary end point.
- Another of the earliest trials utilizing siRNA was conducted by Allergan and Sirna Therapeutics Inc. In this trial, patients with choroidal neovascularization (CNV) that resulted from neovascular AMD received one intravitreal injection of Sirna-027 (also called AGN211745). Sirna-027 is a siRNA designed to reduce the levels of VEGFR-1, significantly inhibiting CNV (2, 3). It was well tolerated, and foveal thickness decreased within 2 weeks of treatment (9). However, it did not meet the efficacy endpoint of improving visual acuity in a phase II study and displayed an off-target effect which could have undesirable impacts on the vascular and immune systems, so development was halted (3).

- PF-655 is a siRNA that was designed by Quark Pharmaceuticals to silence expression of *RTP801* which is rapidly upregulated in response to ischemia, hypoxia, and/or oxidative stress, resulting in cell apoptosis. A phase I trial evaluated PF-655 for treatment of AMD, diabetic retinopathy, and macular oedema, and PF-655 was advanced to phase II trials to treat patients with visual loss due to DME, and neovascular AMD. Currently, it is in Phase IIB for DME.

Dry Eye Syndrome occurs when the tears are unable to lubricate the eyes well enough. It can lead to inflammation and even damage to the surface of the eye. It can have many different causes and a wide range of signs and symptoms.

Tivanisiran (SYL1001) was developed by Sylentis. It is an siRNA in the form of eye drops that inhibits *TRPV1,* the gene that encodes the receptor that senses, transmits, and regulates pain, in addition to acting as a mediator of the innate inflammatory response in corneal tissues. By reducing *TRPV1* expression, tivanisiran decreased ocular pain and improved conjunctival hyperaemia (10).

Retinitis pigmentosa causes progressive vision loss as the light-sensing cells of the retina gradually deteriorate. In one form, mutations in the rhodopsin gene (*RHO*) cause the production of misfolded rhodopsin which results in the death of rod cells, leading to the eventual death of cone cells. *RHO* mutations are a cause of autosomal dominant retinitis pigmentosa (adRP) (3).

Ionis Pharmaceuticals discovered an ASO (ION357, also known as QR-1123) that binds to the mutated RNA, preventing production of the misfolded protein while allowing the normal protein to perform its function, which slows the rate of photoreceptor degeneration. Ionis has a partnership with ProQR, which is conducting a Phase 1/2 clinical trial of QR-1123. Preclinical studies in mouse models showed a measurable reduction in the levels of mutant *RHO,* which slowed the rate of photoreceptor degeneration and preserved their function (11).

Usher Syndrome is a genetic disease that causes people to be both blind and deaf. It is another form of retinitis pigmentosa in which abnormal accumulation of pigmented material on the retina eventually causes retinal degeneration. It is associated with mutations in at least ten genes and one of them, USH2A encodes two alternatively spliced usherin transcripts. Hundreds of pathogenic variants of the gene have been recorded, with the most common defect being a frameshifting mutation in exon 13 that results in a lack of functional usherin protein (3)

ProQR has developed QR-421a, an ASO that causes exon 13 to be skipped during pre-mRNA splicing. This allows production of a shorter but functional usherin protein. Interim findings of a

phase 1/2 trial show that a single intravitreal injection was safe and well tolerated, and two of the eight participants who received QR-421a displayed benefit in visual function and retinal structure. Based on these early results, ProQR plans to expand the mid dose group with additional individuals and begin a high dose group.

Oligos are producing results that would have been unimaginable just one century ago.

We have long been able to provide better vision for people with nearsightedness or farsightedness through the use of glasses and the more recent use of contact lenses, but a cure to vision problems, eye diseases, and blindness has been an unreachable dream. Surgery and laser surgery are now able to correct some problems but are not without the risk of complications. Cures for eye diseases and blindness would be an incredible achievement.

As effective oligonucleotide drugs are developed for ocular diseases, they will provide new methods of treatment while streamlining the process of developing future oligonucleotide therapies, allowing a broader range of treatments for ocular diseases to be created at a rapid pace.

After reading through the current progress, it is easy to see the oligonucleotide-based therapeutics are halting the progression of ocular diseases, reversing visual impairment, and improving pain – quite the triumph for the oligo community!

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