Renewed momentum in ocular gene and cell therapy, broadening application to chronic diseases

BY ROD MCNEIL

Gene and cell therapies offer the prospect of ground-breaking new avenues for the treatment of diseases, reflected in a renewed explosion of interest and investment in retinal gene therapy. **Rod McNeil** reports recent clinical trial readouts across a diverse range of investigational ocular gene and cell therapy candidates.

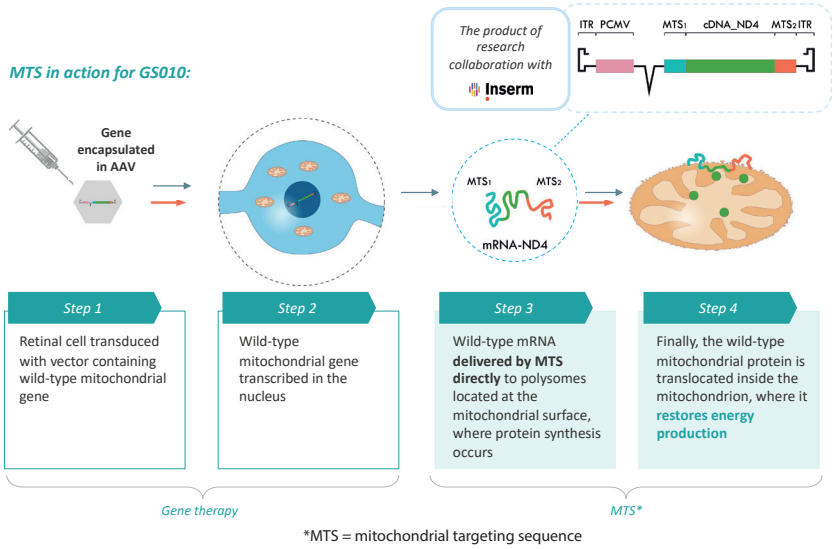
"Gene therapy is literally giving sight to children who would otherwise not see," said Dr Jean Bennett, delivering the Charles L Schepens MD Lecture jointly with Prof Albert Maguire at the American Academy of Ophthalmology 2019 Retina Subspecialty Day. Dr Bennett has developed gene transfer approaches to test treatment strategies for retinal degenerative and ocular neovascular diseases and her work led to the first approved gene therapy product targeting a retinal disease worldwide."

Gene therapy has definitely arrived. There are currently at least five different retinal gene therapy phase 3 trials active and many more planned, with more than 30 clinical sites with ~1167 patients enrolled in gene transfer clinical trials to date involving subretinal and intravitreal delivery. The majority of these studies use an adeno-associated virus (AAV) vector.

Gene therapy for choroideremia

Investigational gene therapy timrepigene emparvovec (BIIB111/AAV2-REP1, Biogen) is an AAV2 vector administered by subretinal injection being evaluated as a treatment for choroideremia (CHM). Biogen announced November 2019 completion of patient enrolment in the global phase 3 STAR clinical trial of 170 adult males with CHM evaluating treatment with a single subretinal injection of timrepigene emparvovec. Data from the proof-of-concept phase 1/2 studies demonstrate a slower rate of decline in visual acuity (VA) at 24 months in patients treated with timrepigene emparvovec compared with untreated patients in the natural history study. At two years over 90% of patients treated with timrepigene emparvovec maintained VA. In a subset of treated patients with moderate to severe VA loss, 21% experienced a VA improvement of at least 15 letters from baseline compared with 1.0% of untreated patients.

Figure 1: Gene therapy to produce working mRNA, with MTS* technology to shuttle mRNA directly to affected mitochondria.



*MTS = mitochondrial targeting sequence

Source: GenSight Biiologics.

GenSight Biologics targets novel gene therapies for LHON and retinitis pigmentosa patients

GenSight Biologics' lead gene therapy candidate is a recombinant adeno-associated viral vector serotype 2 containing the wild-type NADH Dehydrogenase 4 (ND4) gene (rAAV2/2-ND4, GS010) being investigated for the treatment Leber Hereditary Optic Neuropathy (LHON). GS010 targets LHON via a mitochondrial targeting sequence (MTS) proprietary technology platform, which, when associated with the gene of interest, allows the platform to specifically address defects inside the mitochondria using an AAV vector (Figure 1). Based on results from two pivotal phase 3 clinical trials, GenSight expects to submit an application for marketing approval in Europe in the third quarter of 2020.

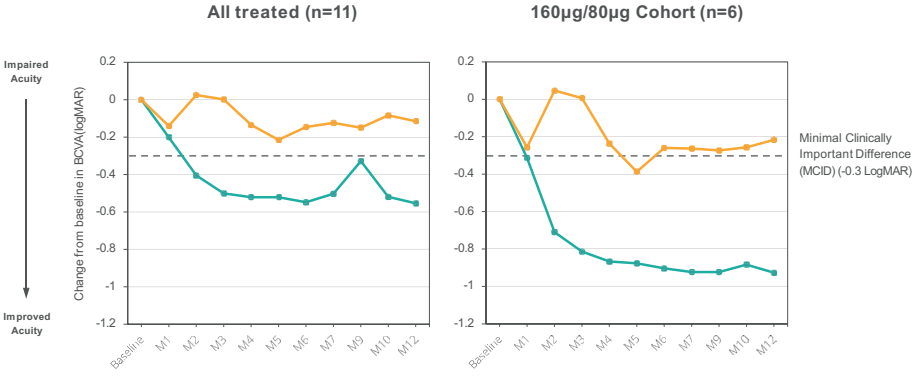
Results at 96 weeks (n-39) from the RESCUE phase 3 clinical trial in 39 subjects with LHON due to the ND4 mutation with an onset of vision loss up to six months prior to study treatment showed a durable bilateral improvement in VA of 25.0 ETDRS letters equivalent versus nadir (i.e. worst best corrected visual acuity (BCVA) after baseline, up to the week of interest) in GS010-treated eyes. Sixty-three percent

of subjects achieved a 'clinically relevant recovery' (CRR) from nadir in at least one eye at week 96, while in the REVERSE phase 3 clinical trial in LHON subjects, the rate of CRR from nadir was 78%, significantly better outcomes than the natural history (~28%) in any prior studies [1]. Clinically significant and sustained bilateral visual improvement with unilateral GS010 gene therapy was observed, with a non-clinical study demonstrating presence of GS010 DNA in the untreated contralateral eye.

Optogenetics is a neuromodulation technique that uses light to control cells that have been genetically modified to express light-sensitive ion channels. GenSight's GS030-DP is an optogenetic gene therapy targeting retinal ganglion cells, encoding an optimised form of channelrhodopsin, ChrimsonR, ChR-tdT, which is administered via a modified AAV2 vector (AAV2.7m8). A medical device GS030-MD (stimulating goggles) processes special 'event' images of the visual world, modulating a light source projected onto the retina in real time. An ongoing multicentre phase 1/2 study (PIONEER) will evaluate the safety and tolerability of escalating intravitreal doses of GS030-DP and repeated light stimulation in subjects with end-stage non-syndromic retinitis pigmentosa [2]. GenSight believes this technology offers the possibility of application to other retinal diseases where photoreceptors have degenerated, and may be transferable to the dry form of age-related macular degeneration (AMD).

Figure 2: Sepofarsen phase 1/2 trial top-line results: sustained improvement in BCVA for at least one year in patients with LCA.

All responses (7/7) were maintained for a minimum of six months after a maintenance dose.



LCA, Leber's congenital amaurosis.
Source: ProQR Therapeutics.

Figure 3: Sepofarsen phase 1/2 trial top-line efficacy data in patients with LCA.

Target registration dose level: 160µg/80µg (n=6)

Group mean	Treated eye	Contralateral eye
BCVA (LogMAR)	-0.93 P<0.01 vs. baseline	-0.22 P=N.S. vs. baseline
FST Red (Log)	-0.66 P<0.01 vs. baseline	0.05 P=N.S. vs. baseline
FST Blue (Log)	-0.63 P<0.01 vs. baseline	0.12 P=N.S. vs. baseline
Mobility (levels)	+4.0 P<0.01 vs. baseline	+2.7 P<0.05 vs. baseline

Significance assessed by mixed effects repeated measures model

LCA, Leber's congenital amaurosis.
Source: ProQR Therapeutics.

Sepofarsen for Leber congenital amaurosis

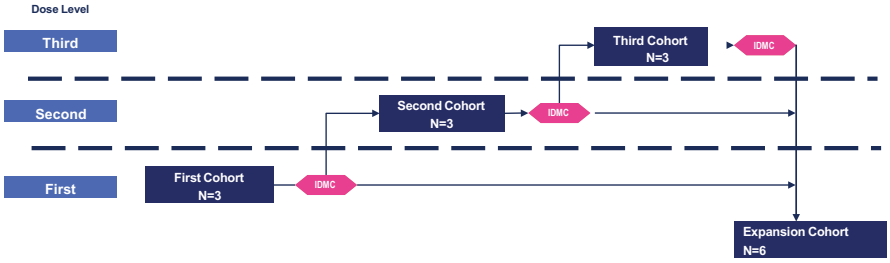
Intravitreal sepofarsen (QR-110, ProQR Therapeutics) is an investigational RNA-based oligonucleotide designed to address the underlying cause of Leber's congenital amaurosis 10 (LCA10) due to the p.Cys998X mutation in the CEP290 gene.

Top-line results from a phase 1/2 dose range finding trial of sepofarsen in patients with LCA10 due to the p.Cys998X mutation

Table 1: Clinical trials of gene therapy for LCA-RPE65.

NCT	Sponsor	Sites	Phase	N target	N recruited
NCT00643747 AAV2	UCL	Moorfields Eye Hospital	1/2	12	12
NCT00516477 AAV2	Spark Therapeutics	The Children's Hospital of Philadelphia (CHOP)	1	12	12
NCT00481546 AAV2	CHOP	CHOP, Univ Florida	1	15	15
NCT01208389 AAV2	Spark Therapeutics	CHOP	1	12	12
NCT00999609 AAV2	Spark Therapeutics	CHOP/Univ Iowa	3	30	31
NCT02781480 AAV2/5- OPTIRPE65	MeiraGTx	Moorfields Eye Hospital/Kellog Eye Center	1/2	15	15
NCT00821340 AAV2	Hadassah Med	Hadassah Med	1	10	3
NCT00749957 AAV2	Applied Genetic Technologies	Casey Eye Institute/Univ Massachusetts	1/2	12	12
NCT01496040 AAV2/4	Univ Nantes	Nantes University Hospital	1/2	9	9

Figure 4: Phase 1/2 trial of AAV-RPE65.



- **Dosing complete:** 15 patients treated (nine adults in three dose escalation cohorts and six pediatric patients in an expansion cohort)
- **Six-month topline data released May 2019:** AAV-RPE65 was generally well-tolerated, with a safety profile consistent with other approved and investigational ocular gene therapies; statistically significant improvement across several secondary endpoints that assessed clinical activity.
- **Long-term follow up ongoing**
- **Natural History study ongoing:** >30 RPE65-deficiency patients well characterized

Source: MeiraGTx.

in the CEP290 gene show that the target registration dose (80µg with a 160µg loading dose) was associated with a clinically meaningful and statistically significant improvement in vision and had a favourable benefit / risk profile (Figures 2 and 3). Further, a six-month dosing frequency was associated with durable improvements in vision, and the response observed at 12 months in the target registration dose was equal to or greater than the response at the three-month interim analysis.

Illuminate (PQ-110-003) is an ongoing randomised, sham-controlled 24-month phase 2/3 trial of sepofarsen that will initially enroll 30 adults and children (eight years of age and over) who have LCA10 due to one or two copies of the p.Cys998X mutation in the CEP290 gene and a baseline BCVA of 3.0 logMAR or better. This pivotal trial is designed as the sole registration trial for the clinical programme and is on track to deliver data in the first half of 2021. A study of RNA antisense oligonucleotide for intravitreal injection (QR-421a) for Usher syndrome is on schedule to deliver 12-patient interim data in quarter 1 2020.

AAV2/5-OPTIRPE65 gene therapy for RPE65-associated retinal dystrophy

RPE65-deficiency causes impaired rod photoreceptor function from birth and slowly progressive retinal degeneration, with outer retinal degeneration by the third decade (Table 1). AAV2/5-OPTIRPE65 (MeiraGTx) consists of an AAV vector serotype 5 carrying an optimised hRPE65 promoter and a codon-optimised hRPE65 gene [3]. MGT003 is a phase 1/2 trial of subretinal AAV2/5-OPTIRPE65 in participants aged three years or older, confirmed bi-allelic RPE-65-associated retinal dystrophy and structural evidence of photoreceptor preservation on spectral domain optical coherence tomography (SD-OCT) (Figure 4). Fifteen patients were enrolled (nine young adults and six children) at two participating sites, Moorfields Eye Hospital and University of Michigan Kellog Eye Center. Treatment was generally well tolerated with an expected safety profile. Inflammation was observed at intermediate and high dose (three out of six patients), effectively treated with extension of steroid cover.

Data suggest treatment with AAV2/5-OPTIRPE65 improves functional vision, retinal function and central vision. Through 24 weeks, statistically significant improvement in vision-guided mobility was observed in all treated subjects (study eye vs. fellow eye) [4]. Over the six-month period, retinal sensitivity in treated eyes improved and there were significant improvements in visual function among eyes treated at the 1x1011 dose: median improvement in BCVA of 4.3 letters across all 1x1011 patients (n=9), median improvement of 5.3 letters in children (n=6, p=0.031). The 1x1011vg/mL dose has been chosen as the optimal dose for continued clinical development.

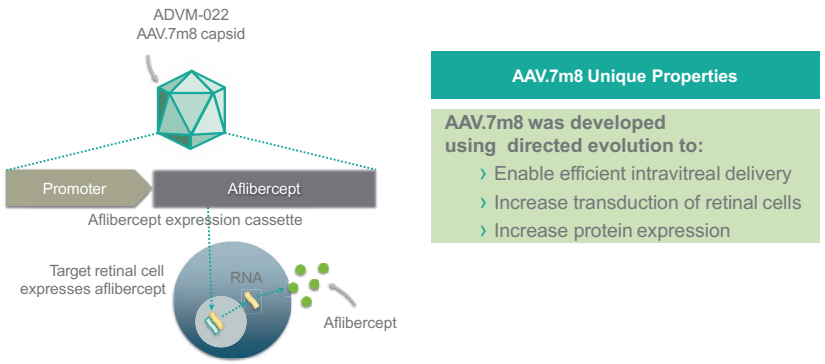
AAV-CNGB3 and AAV-CNGA3 (MeiraGTx) for the treatment of patients with achromatopsia are being evaluated in phase 1/2 clinical trials. AAV-RPGR (MeiraGTx) for the treatment of X-linked retinitis pigmentosa (RP) due to retinitis pigmentosa GTPase regulator (RPGR)-deficiency is also in phase 1/2 clinical development. Research continues into potential gene

Table 2: hRPC therapy for retinitis pigmentosa: phase 2a clinical trial efficacy results to date.*

Month(s) post-treatment	Mean improvement in VA in treated eye, letters	Mean improvement in VA in treated eye (excluding two patients with procedure-related vision loss), letters	Mean change in VA in untreated eye, letters
1	+8.3 (n=8)	+14.5 (n=6)	+1.6 (n=8)
2	+5.4 (n=8)	+13.0 (n=6)	+2.8 (n=8)
3	+6.1 (n=8)	+17.8 (n=6)	+6.8 (n=8)
6	+18.8 (n=4)	+28.7 (n=3)	+7.8 (n=4)
9	+12.0 (n=1)	+12.0 (n=1)	-1.0 (n=1)

Abbreviations: hRPC, human retinal progenitor cells; VA, visual acuity.
*Source: Dugel P. AAO 2019 Retina Subspecialty Day Meeting, October 2019.

Figure 5: ADVM-022 is specifically designed for long-term intraocular VEGF suppression with a single intravitreal injection.



ITR, inverted terminal repeat
Source: Grishanin, R. et al. Mol. Ther. 2019;27:118–29

therapy treatments for AMD as well as gene regulation using VEGF2 Ab eye drops.

Subretinal human retinal progenitor cells (hRPC) in RP

Human retinal progenitor cells (hRPC) represent an allogeneic cell-based therapeutic approach to retinal disease. The approach offers broad therapeutic potential across a range of retinal diseases, initially focused on inherited retinal degenerative diseases.

Investigator Dr Pravin Dugel recently provided an update from an ongoing phase 1/2a clinical trial of ReNeuron's hRPC cell therapy candidate for retinitis pigmentosa [5]. Potential benefits of subretinal hRPC therapy include direct delivery into the subretinal space, the cells are cryopreserved, enabling on-demand shipment to site of care, and therapeutic effect independent of genetic subtype of disease. A good safety profile was observed in an open-label trial (n=12), allowing progression to a phase 2a clinical trial. Participants will be followed for two years to evaluate the safety and tolerability of hRPC. The primary efficacy endpoint is change in ETDRS letters from baseline to 24 months in the treated eye.

Among 22 subjects, dose escalation was generally well tolerated and there was no evidence of inflammation or proliferative vitreoretinopathy. No immune-related adverse events have been observed. Two patients experienced procedure-related vision loss. Clinically meaningful efficacy signals were consistently seen, with rapid and profound improvement in VA in some patients. The phase 2a segment of the study, which uses a cryopreserved hRPC formulation, enrols subjects with some remaining retinal function. Table 2 shows efficacy results from a recent follow-up in the phase 2a segment of the study.

Early evidence of safety and efficacy of an AAV8 viral vector encoding RPGR (BIIB112, NSR-RPGR, Biogen) for subretinal treatment of X-linked retinitis pigmentosa (XLRP) shows improvements in microperimetry with favourable safety (n=18) [6]. An ongoing part II, phase 2/3 study named XIRIUS is evaluating AAV8-RPGR retinal gene therapy for patients with RPGR-associated XLRP at clinic centres in the USA and UK. Data readouts from both clinical trial programmes are expected in the second half of 2020.

Long-term outcomes of human embryonic stem cell-derived retinal pigment epithelium (hESC-RPE) cell transplantation

Transplantation of hESC-RPE cells (OpRegen) in advanced dry AMD patients (n=15) appears to be well tolerated, with interim data from an ongoing phase 1/2a clinical study providing preliminary evidence of improved structural changes [7]. Results persisting through the last time point examined (>3 years) include subretinal pigmentation, decreased drusen density, irregular reflectance above areas of atrophy and changes in the ellipsoid zone on OCT imaging.

Gregori et al. reported results from multicentre trials showing that hESC-RPE cell transplants (50,000 to 200,000) were safe at four years post-transplantation in patients with Stargardt disease and atrophic AMD (n-23; AMD =10, SD=13) with no evidence of graft rejection, inflammation, retinal detachment, tumour or ectopic tissue formation [8]. Vision improvement of three lines or more of vision after 12 and 48 months of follow-up was observed in 35% and 15% for all treated eyes, compared with 13% and 5% for untreated eyes.

Astellas Institute for Regenerative Medicine is sponsoring a two-stage phase 1b/2, multicentre clinical trial evaluating subretinal ASP7317 for dry AMD, an investigational treatment derived from pluripotent human stem cells.

Autologous cultivated limbal stem cell transplantation

Holoclar (Chiesi Farmaceutici) received conditional marketing authorisation in December 2014 by the European Union (EU) as a treatment for moderate to severe limbal stem cell deficiency (LSCD) due to ocular burn(s) in adults, the first stem cell therapy recommended for approval in the EU. In a multicentre retrospective case series of 106 patients treated for moderate to severe LSCD, a total of 75 (72.1%) treatments were reported with a successful outcome and 51 patients (49.0%) had an improvement of at least one line of VA (or one category for the severely impaired cases). An ongoing phase 4 clinical trial, due to complete toward end 2021, will further evaluate the efficacy and safety of autologous cultivated limbal stem cell transplantation for restoration of corneal epithelium in patients (n=70) with LSCD. The first NHS-funded patient with LSCD caused by chemical eye burns was treated with Holoclar at Newcastle upon Tyne Hospitals NHS Foundation Trust in October 2019.

One-time intravitreal gene therapy for neovascular AMD and DR: turning the eye into a 'biofactory'

Adverum Biotechnologies' ADVM-022 gene therapy candidate is designed to provide long-term vascular endothelial growth factor (VEGF) suppression with a single intravitreal injection (Figure 5). This novel gene therapy candidate utilises a proprietary next-generation vector capsid, AAV.7m8, carrying an aflibercept coding sequence under the control of a proprietary expression cassette. Intravitreal delivery provides potential for wide distribution of vector and the ability to transduce a broader tissue area. Robust protein levels and long-term safety have been demonstrated in non-human primates out to 30 months post injection.

OPTIC cohort 1 clinical trial data demonstrate intravitreal ADVM-022 is safe and well-tolerated, with robust sustained anatomical improvements and zero rescue injections through a median of 34 weeks in a tough-to-treat population of neovascular AMD patients with relatively good vision but recalcitrant fluid despite intensive prior anti-VEGF therapy [9]. Enrolment in further trial cohorts is ongoing and 52-week follow-up data from cohort 1 and 24-week data from cohort 2 is expected in H1 2020. Adverum plans to submit an Investigational New Drug application for diabetic retinopathy (DR) in the first half of 2020 and to then initiate a clinical trial of ADVM-022 for DR patients.

Dr Aaron Osborne, Chief Medical Officer of Adverum Biotechnologies, commented in an interview with *Eye News*: "Non-

surgical gene therapy for chronic eye diseases represents a new frontier, with one-time intravitreal gene therapy for nAMD and other retinal conditions offering transformational potential for patients requiring long-term anti-VEGF therapy. It offers the prospect of in-office or clean-room delivery and expresses an anti-VEGF protein which is known to be highly effective. We have not seen any evidence of a decline in protein expression or a stop-point in preclinical studies conducted thus far. We envisage a one-time treatment that provides continuous VEGF suppression for effective maintenance of vision in nAMD patients."

Vector development and optimisation technologies create additional opportunities to treat broader indications beyond rare, inherited genetic disorders, believe gene therapy companies. Progress also continues to be made with development of advanced precision subretinal delivery techniques for gene and cell therapy, including investigation of transvitreal subretinal delivery without vitrectomy and ab externo suprachoroidal delivery systems.

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