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RNA Therapeutics in Ophthalmology - translation to clinical trials

**Aanchal Gupta^{1,2}, Konstantinos N. Kafetzis³, Aristides D. Tagalakis³,
Cynthia Yu-Wai-Man^{1,2}**

1. King's College London, London, SE1 7EH, United Kingdom.
2. Department of Ophthalmology, St Thomas' Hospital, London, SE1 7EH, United Kingdom.
3. Department of Biology, Edge Hill University, Ormskirk, L39 4QP, United Kingdom.

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Corresponding Author:

Dr Cynthia Yu-Wai-Man, MBBS FRCOphth PhD

King's College London

St Thomas' Hospital

Westminster Bridge Road

London SE1 7EH, UK

cynthia.yu-wai-man@kcl.ac.uk

Abstract

The use of RNA interference technology has proven to inhibit the expression of many target genes involved in the underlying pathogenesis of several diseases affecting various systems. First established in *in vitro* and later in animal studies, small interfering RNA (siRNA) and antisense oligonucleotide (ASO) therapeutics are now entering clinical trials with the potential of clinical translation to patients. Gene-silencing therapies have demonstrated promising responses in ocular disorders, predominantly due to the structure of the eye being a closed and compartmentalised organ. However, although the efficacy of such treatments has been observed in both preclinical studies and clinical trials, there are issues pertaining to the use of these drugs which require more extensive research with regards to the delivery and stability of siRNAs and ASOs. This would improve their use for long-term treatment regimens and alleviate the difficulties experienced by patients with ocular diseases. This review provides a detailed insight into the recent developments and clinical trials that have been conducted for several gene-silencing therapies, including ISTH0036, SYL040012, SYL1001, PF-04523655, Sirna-027, QR-110, QR-1123, QR-421a and IONIS-FB-L_{RX} in glaucoma, dry eye disease, age-related macular degeneration, diabetic macular oedema and various inherited retinal diseases. Our aim is to explore the potential of these drugs whilst evaluating their associated advantages and disadvantages, and to discuss the future translation of RNA therapeutics in ophthalmology.

1 Introduction

Gene therapy is a facet of biomedicine that focuses on the modulation, modification or correction of genes to treat or improve the clinical aspects of a disease with increasing promise for the treatment of previously incurable diseases, providing relief to patients suffering from such conditions by targeting the genetic root cause of a disorder rather than treating its symptoms. One such promising area of gene therapy is the silencing of genes by means of small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs), which demonstrate potential avenues for the integration of RNA therapeutics into clinical practice (1). Oligonucleotides inhibit gene expression through a sequence-dependent approach via degradation of the complementary messenger RNA (mRNA). Such post-transcriptional gene silencing was first observed in plants (2), then replicated by Fire *et al* in nematode models in 1998 (3), and this has led to the award of the Nobel Prize to Fire and Mello in 2006. Consequently, it became possible to target and downregulate specific genes relevant to particular disease pathologies (4).

Recent advances in the field of genomics have led to the sequencing of the genomes of several organisms, resulting in the rise of nucleic acid-based technologies for post-transcriptional silencing of gene expression and the investigation of gene function. One such example is the use of siRNAs, which was first demonstrated in the alteration of mammalian cell gene expression in 2001 via mediation of gene knockdown using sequence-dependent cleavage of specific complementary mRNA molecules (5). Early trials of nucleic acid gene silencing technology involved the use of long double-stranded RNA (dsRNA), however this was not effective in many mammalian cells due to the stimulation of the antiviral interferon response (6), which resulted in global post-transcription gene silencing and eventually cell death.

Further investigation of the mechanisms of siRNA revealed that evolutionarily-conserved machinery cleaves long dsRNA into short duplexes of 21-28 nucleotides in length (**Figure 1**). These molecules consist of two strands: a sense (passenger) and antisense (guide) structure. The duplex is integrated into an RNA-induced silencing complex (RISC) of proteins, which acts to remove the sense (passenger) strand. This leaves the guide strand to bind to complementary nucleotides in the mRNA sequence via hydrogen bonds (7–9). The RISC then cleaves the mRNA, inducing sequence-specific mRNA degradation (10–15). Further investigation into siRNA structure revealed that these molecules can downregulate mammalian gene expression without triggering the interferon response observed when dsDNA is used (5,16,17). The newer generations of siRNAs have also utilised the rapidly evolving field of nucleic acid chemical modifications to achieve improved performance, nuclease resistance and increased stability. As a result, alterations of the 2'-position of the ribose, such as the 2'-O-methyl (2'OMe) and 2'-fluoro (2'-F) modifications, are now widely used (18,19).

In 1978, Stephenson *et al* showed that an ASO could effectively inhibit the translation of Rous sarcoma virus proteins, and as a result inhibit viral replication *in vitro* (20). Twenty years later, the FDA approved the first ASO therapeutic to treat cytomegalovirus (CMV)-induced chorioretinitis (21). These first-generation ASOs were short (8-50 nucleotides long), synthetic, single-stranded oligodeoxynucleotides which were able to bind to mRNAs by complementary base pairing, forming RNA-DNA duplexes, which in turn were enzymatically cleaved, leading to their degradation and a subsequent decrease in protein levels. However, they had limited clinical potential due to the rapid degradation by endonucleases and exonucleases and low efficacy (22,23). Since the early 1990s, a variety of chemical modifications led to the development of second-generation ASOs. These modifications included the use of a phosphorothioate (PTO) backbone (replacement of one

oxygen atom in the ASO backbone with sulphur) (24) as well as alterations of the 2' ribose sugar, such as the 2'-O-methyl (2'OMe) and 2'-O-methoxy-ethyl (2'-MOE) modifications, resulting in higher target affinity and improved resistance to degradation (25). Nevertheless, these 2' substitutions interfered with the activation of RNase H, an integral part of the ASO mechanism, decreasing the efficacy of the ASOs (26). Therefore, a chimeric ASO was developed containing a central unmodified DNA region, "gap," flanked by 2'-modified nucleotides on both sides (gapmer), which could effectively activate RNase H (27). Finally, third-generation ASOs were developed to further improve nuclease resistance and increase binding affinity and biostability. These more advanced ASOs include the use of locked nucleic acids (28) as well as the phosphorodiamidate Morpholino oligomers (PMOs), in which the phosphate backbone of the nucleotides has been replaced by morpholine rings linked through phosphorodiamidate groups (27,29).

ASOs, depending on their chemistry and target, can both control gene expression and modify pre-mRNA splicing using several mechanisms. Once inside the cells, ASOs find and bind to their target mRNAs without the assistance of protein complexes, unlike siRNAs, forming RNA-DNA duplexes. These hybrids subsequently become the target of RNase H, a non-sequence-specific endonuclease enzyme, that catalyses the cleavage of RNA in RNA-DNA duplexes and degrades them. Alternatively, by binding to the mRNA, ASOs can block ribosomal access (steric block), which results in decreased protein expression. Furthermore, by targeting intron-exon junctions with ASOs, it is possible to destabilise the splicing sites and thus include or exclude particular exons in the mature mRNA product (**Figure 2**). This approach has already been approved for applications in disorders with known splicing defects, such as Duchenne muscular dystrophy (22,30–32).

Even though the field of gene therapy has grown to include a variety of technologies and therapeutic approaches, from ribozymes (33) and viral gene therapy (34) to the 2020 Nobel

prize winning CRISPR gene editing technique (35), siRNAs and ASOs are prominent in clinical research, utilising oligonucleotides for targeting, modulating and downregulating specific genes associated with particular pathologies (4,36). However, it should be noted that although these techniques share the same underlying principle of oligonucleotide-binding to RNA targets using complementary Watson-Crick base-pairing, a distinct difference is apparent between them. siRNAs consist of small RNA duplexes comprising of 'passenger' and 'guide' strands, whereas ASOs are single-stranded oligonucleotide molecules that must remain stable and identify targets without the aid of a 'passenger' strand (37). Moreover, ASOs can locate and bind to their target sequence without the assistance of protein complexes, unlike siRNAs.

The integration of this technology into therapeutic interventions has created a new era of pharmaceutical approaches that have already reached clinical trials. These novel approaches, though, are not without their own challenges, including target specificity, safety and efficiency (7). Luckily, since the eye is a closed compartment, ocular delivery presents fewer challenges for siRNA and ASO administration, and compounds have been successfully administered using topical eye drops, subconjunctival, intracameral or intravitreal injections (38–42). Local RNAi delivery to the target tissue confers several benefits, such as increased bioavailability, less adverse side effects, simpler formulation which makes production and administration easier, and greater localised efficacy with minimal systemic effects. Therefore, this reduces the incidence of off-target effects and overall improves the cost-effectiveness of such compounds (43). This has resulted in greater advancement in the use of RNAi technology for the treatment of ocular diseases, with siRNA and ASO-based approaches already in clinical trials.

This review aims to discuss clinical trials that have utilised RNA-targeting technologies for the treatment of glaucoma, dry eye disease and retinal disorders, such as age-related macular

degeneration, diabetic macular oedema and various inherited retinal diseases. The overall objective is to evaluate these clinical trials according to their potential as new therapies, and to discuss not only the benefits and challenges but also the extent to which they could contribute to the prevention of blinding eye diseases worldwide.

2 Glaucoma

Glaucoma is the leading cause of irreversible blindness worldwide, currently affecting 76 million people, and more than 11 million people with glaucoma are blind in both eyes (44). The disease is characterised by progressive optic neuropathy and damage to the optic nerve head, death of retinal ganglion cells and consequent irreversible loss of vision. Current standard of care is directed at reducing intraocular pressure (IOP) to prevent progressive optic nerve damage, either by using medical therapy or surgical intervention. Success of glaucoma filtration surgery is limited due to the excessive postoperative scarring causing closure of the drainage channel and subsequent increase in IOP (45,46). Antimetabolites, such as mitomycin-C (MMC) and 5-fluorouracil (5-FU), have been used as preventative measures against scarring. Although MMC has been shown to be effective in reducing fibrosis post-trabeculectomy compared to the use of placebo (47) and 5-FU (48), several adverse effects are associated with its use, such as hypotony, shallow anterior chamber, hyphaema, cataract progression, choroidal detachment and corneal endothelial defects (49). Although current standards of care represent effective interventions for many, the development of novel interventions targeting the underlying pathological mechanisms of glaucoma may present opportunities for further improvement and may address the shortcomings associated with current treatments.

2.1 Antisense oligonucleotide ISTH0036 targeting TGF- β 2 expression (2017) –

Phase 1 trial

Elevated levels of transforming growth factor beta 2 (*TGF- β 2*) have been associated with increased IOP and identified as one of the main pathophysiological mechanisms underpinning glaucoma development. Studies show substantially elevated *TGF- β 2* levels in

glaucoma patients (50–53), whilst the optic nerve head has 70 to 100-fold elevated concentrations (54).

A recent Phase 1 clinical trial (55) evaluated the safety and tolerability of single intravitreal injections of the ASO ISTH0036, which selectively targets the *TGF- β 2* mRNA, in patients with primary open angle glaucoma (POAG) undergoing trabeculectomy due to intolerance to medical therapy or disease progression despite maximal medical therapy. Preliminary clinical efficacy was also observed postoperatively in accordance with measurements of the secondary endpoints mentioned below (**Table 1**). Prior to the start of the trial, patients were weaned from previous medication-use by undergoing a washout period of 4 weeks for topical beta-blockers and prostaglandins, 7 days for topical alpha-2-agonists and carbonic anhydrase inhibitors, and over 14 days for any ophthalmic medications that had not been taken at a stable dose.

All patients underwent trabeculectomy with topical mitomycin-C (MMC, 100 μ l of 0.2 mg/ml solution) and a single postoperative intravitreal ISTH0036 injection of varying 50 μ l dose levels (6.75, 22.5, 67.5 and 225 μ g). Primary endpoints for this study were the type and frequency of adverse events (AEs). Vital signs, laboratory, slit-lamp and fundus examinations were carried out throughout the study, with an electroretinogram measuring retinal function performed at screening and at the end of the dose limiting toxicity period. Secondary endpoints were IOP at the end of the study, the number of interventions after trabeculectomy, bleb morphology, visual field, slit lamp biomicroscopy, best corrected visual acuity (BCVA) and optic disc assessment.

Of the 12 patients treated across the 4 dosage groups, none of the ocular adverse events that occurred (corneal erosion, corneal epithelial defect, extremes of IOP) were linked to ISTH0036 or the intravitreal injection procedure (**Table 2**); these were attributed either to the primary surgery or to other factors deemed unrelated to the investigational drug, injection

procedure or primary surgery. Additionally, the results showed that ISTH0036 demonstrated no toxicity and indicated an effectiveness in controlling IOP postoperatively, particularly at the two highest dose levels. Standard administration of MMC combined with a single intravitreal injection of ISTH0036 reduced the average IOP values from 27.3 ± 12.6 mmHg preoperatively to 9.8 ± 1.0 mmHg, 11.3 ± 6.7 mmHg, 5.5 ± 3.0 mmHg and 7.5 ± 2.3 mmHg at 6 weeks postoperatively for dose levels of 6.75, 22.5, 67.5 and 225 μ g, respectively. At 12 weeks postoperatively, the average IOP values were maintained at 9.7 mmHg ± 3.3 mmHg, 14.2 ± 6.5 mmHg, 5.8 ± 1.8 mmHg and 7.8 ± 0.6 mmHg, respectively. Individuals treated with 67.5 and 225 μ g doses had a postoperative IOP consistently below 10 mmHg, thus indicating a potential dose-response relationship. The maintenance of postoperative IOP under 10 mmHg using ISTH0036 is promising as previous studies have shown more favourable surgical outcomes if postoperative IOP was maintained below 10 mmHg (56). This approach has the potential to be more effective than existing treatments, especially given the lack of side effects compared to the cytotoxic antimetabolite MMC. Its extended *in vivo* half-life and pharmacodynamic activity for up to 8 weeks, which were seen in preclinical rabbit models following single intravitreal injections (57), provides another advantage by potentially reducing the number of required administrations. Finally, no significant changes were observed with regards to the secondary endpoints, which is an expected outcome given the small cohort size and short observation period. Given these limitations and the nature of this study as a Phase 1 trial primarily assessing safety and tolerability, it may be difficult to ascertain the efficacy of ISTH0036 in controlling IOP. Future Phase 2 clinical trials intend to assess repeat dosing up to 1 year for continued antifibrotic effects in clinically relevant time periods. Such prospective clinical trials will be critical in assessing the efficacy of ISTH0036 as an antifibrotic therapy with the potential of inhibiting glaucoma pathophysiological

mechanisms via selective suppression of *TGF- β 2* when used both with MMC or as a monotherapy.

2.2 siRNA SYL040012 targeting *ADR β 2* expression (2014) – Phase 1 trial

A main risk factor for glaucoma is increased IOP, which is a result of an imbalance between the production and outflow of the aqueous humour (AH). It has been reported that the adrenergic receptors located in the eye, such as the β 2-adrenergic receptor (*ADR β 2*), positively affect AH secretion (58), thus making them great targets for therapeutic approaches.

SYL040012 is an siRNA aimed at specifically blocking the expression and synthesis of β 2-adrenergic receptors (*ADR β 2*) without affecting other members of the adrenergic receptor family. A Phase 1 clinical trial (59) primarily assessed the ocular safety, tolerability and bioavailability of SYL040012, aimed at reducing elevated IOP due to glaucoma by diminishing local *ADR β 2* expression using topical eye drop administration (**Table 1**). The primary endpoint of this trial was local ocular surface tolerance (cornea and conjunctiva), assessed using ocular examination and tolerance tests. Detailed bilateral ocular examinations were conducted during the initial screen, 72 hours after drug administration (interval 1), and 1 hour after the final installation (interval 2). Secondary endpoints included the frequency and incidence of AEs, ocular evaluation, pharmacokinetic assessment using regular haematological sampling and physical evaluation to assess the safety and systemic tolerability of the compound. Clinical parameters were assessed using physical examinations, vital signs monitoring, clinical biochemistry, urinalysis and electrocardiography. Two treatment approaches were assessed in this study; the first interval involved a single topical ocular administration of 600 μ g/eye/day of SYL040012, whilst the second utilised a repeated administration regime of either 600 or 900 μ g/eye/day over 7 consecutive days. Drops were

applied to only one eye, with the contralateral eye taken as control for the study. The single-dose regime reported no AEs, whilst six individuals reported mild AEs in the repeated administration schedule approach (**Table 2**).

Results of this Phase 1 clinical trial showed that, with regards to the primary endpoint, topical ocular installation of SYL040012 is well tolerated locally at all dose limits and administration regimes tested. Of the three drug-related adverse effects reported, all were found to be minor, reversible and associated with the repeated administration protocol. No systemic presence of the compound could be detected in any blood samples taken from all subjects, whilst accumulation of metabolites after administration was also negative. The study utilised a lower limit of quantification of 44.2 ng/ml, with results showing a lack of systemic presence of the drug either during single or repeated instillations. Animal studies assessing biodistribution of SYL040012 using bioanalysis with a lower limit of detection (0.25 ng/ml) found only trace concentrations of the compound in the blood post-topical instillation, whilst detection of its metabolite SYL040012-5'-P could only be found in ocular structures after topical instillation with none present in the systemic circulation. This may explain the absence of systemic side-effects, likely due to the activity of RNases that are naturally abundant in the bloodstream and thus cause rapid degradation of the nucleotide upon its entry into the systemic circulation (60). Therefore, this highlights SYL040012 as a drug that is only active in ocular cells and may thus make it an effective addition or alternative to current anti-glaucoma therapies.

With regards to the secondary endpoints, observation of IOP found no significant differences between the screening and post-administration values following the single 600 µg installation. Conversely, regimes using repeated dose administration demonstrated a decrease in IOP values, regardless of the dose administered. Greater efficacy of IOP control using lower dosage-regimes may be explained by oversaturation of the cellular RNAi machinery

with administration of higher doses, thus reducing the therapeutic action of SYL040012 compared to the lower doses (61). Finally, it is worth noting that this study was conducted in healthy individuals with normal IOPs and future clinical trials would need to be carried out on patients with elevated IOPs due to glaucoma to validate the results of this siRNA approach.

A 2014 Phase 2 clinical trial further assessed the tolerability and IOP-lowering effect of repeated SYL040012 (bamosiran) when administered to 89 patients with glaucoma or elevated IOP across 14 days at four different dose levels: 80 µg/eye/day, 300 µg/eye/day, 900 µg/eye/day or placebo (62). Results showed a statistically significant reduction in IOP in the 300 µg/eye/day cohort by day 14 as compared to the placebo cohort and to values measured at initial screening. Of the 14.6% of patients reporting AEs, 80% were mild and none were considered to be related to SYL040012 administration, thus the drug was well-tolerated both locally and systemically. This further highlights the compound as a promising treatment for glaucoma as it identifies its efficacy at lowering IOP whilst having a good safety profile.

3 Dry Eye Syndrome

Dry eye syndrome (DES) is a multifactorial disease associated with tear film disruption and ocular surface damage (63). Even though the signs and symptoms of DES can vary greatly, it can be classified in two distinct categories: (1) tear-deficient dry eyes (e.g. Sjögren's syndrome) and (2) evaporative dry eyes (64). Current treatment for mild to moderate DES is limited to the administration of artificial tears to lubricate the eyes and to give relief to the symptoms, whereas more severe cases are treated with autologous serum eye drops and anti-inflammatory compounds (65). However, the efficacy of these treatments is low, especially in patients with moderate to severe DES. The efficacy of cyclosporine emulsions (Restasis and Ikervis) for the management of chronic DES resulting from inflammation, and of the integrin antagonist (Xiidra) for symptom relief remain uncertain when compared to placebo (66,67). These drugs are also associated with undesirable side-effects, such as pain on instillation (66,67). Thus, there is a need for new therapeutic approaches for the treatment and relief of symptoms associated with DES, regardless of the underlying aetiology.

3.1 siRNA SYL1001 targeting *TRPVI* expression (2016) – Phase 1 and 2 trials

One such treatment approach currently being investigated is the compound SYL1001 (Tivanisiran). This siRNA is designed to specifically inhibit the expression and synthesis of the transient receptor potential cation channel subfamily V member 1 (*TRPVI*). This capsaicin receptor is involved in sensing and transmitting pain stimuli and in modulating the inflammatory response. *TRPVI*, a nociceptive transducer, is expressed in ocular tissues, including the corneal epithelium and conjunctival basal layer, and thus represents an important factor involved in mediating ocular pain. This results from corneal epithelial

damage associated with DES, which in turn stimulates corneal neural endings and causes eye discomfort.

A Phase 1 and a Phase 2 clinical trials of topical SYL1001 administration were performed to target and reduce *TRPV1* expression on ocular surfaces (**Table 1**) (68). The initial Phase 1 study aimed to test the safety, tolerability and bioavailability of ocular SYL1001 administration in a cohort of healthy volunteers. This study was divided into two distinct treatment groups. Group 1 involved a single instillation of SYL1001 followed by a 72-hour observation period, whereas group 2 involved multiple doses of SYL1001 once a day for a total of 7 days. The drug was administered to one randomly allocated eye with the contralateral eye acting as control for the observation of ocular tolerance and safety evaluation. The primary endpoints of the Phase 1 study were to assess ocular surface tolerability, particularly pertaining to the cornea and conjunctiva. Secondary endpoints were local and systemic tolerability for each dose of SYL1001, the impact of treatment on other ocular parameters, the frequency of AEs, and the evaluation of the drug's pharmacokinetics.

The Phase 2 clinical trial, involving the use of placebo as control, aimed at assessing the safety and efficacy of SYL1001 in patients with a diagnosis of mild to moderate DES. Repeated instillations of either the placebo or four different doses of SYL1001 (0.375%, 0.75%, 1.125%, 2.25%) were administered once a day for 10 days in both eyes, with assessment of all ocular and safety parameters one hour after the final instillation on day 10. Primary endpoints for the Phase 2 clinical trial included assessment of changes in ocular pain according to VAS (0 = no discomfort and 10 = maximum pain) and the Ocular Surface Disease Index (OSDI) to assess the analgesic impact. The evaluation of these parameters was carried out at screening prior to ophthalmic evaluation to provide a baseline assessment. Ocular surface tolerance was assessed using extent of conjunctival hyperaemia and corneal fluorescein staining. Secondary endpoints included systemic tolerance, impact of treatment

on other ocular parameters, and AE occurrence. These were tested using Schirmer's test and Tear Break-Up Time (TBUT). The presence of conjunctival hyperaemia, blepharitis and tear meniscus was used to assess the safety of SYL1001.

Overall, the Phase 1 and 2 clinical trials demonstrated that the topical administration of SYL1001 targeting ocular TRPV1 gene expression was effective and safe in reducing ocular pain and conjunctival hyperaemia in DES. Specifically, the Phase 1 clinical trial demonstrated no significant changes of the parameters assessed during either treatment regimes. Regarding safety, all reported AEs were unrelated to SYL1001 administration, and no significant differences were observed in the occurrence or number of AEs between treated and untreated eyes ($P=0.317$) (**Table 2**). The Phase 2 trial evaluating tolerability and efficacy displayed similar results regarding the safety of SYL1001 use in humans, reporting no serious AEs, with most being defined as mild to moderate and resolved without intervention or serious lasting impact, thus demonstrating good local and systemic SYL1001 tolerance at all dose levels tested.

When assessing the efficacy of SYL1001 in reducing ocular pain, OSDI tests demonstrated a significant decrease of 30% between baseline as compared to day 10 across all treatment groups ($P<0.05$). Additionally, assessment of pain using VAS also demonstrated a significant decrease in scores on day 10 compared to day 1 across all groups ($P<0.05$), with a statistically significant decrease in ocular pain, when compared to the placebo group, after 1.125% SYL1001 administration. Moreover, ocular surface tolerance assessment showed an improvement in conjunctival hyperaemia from abnormal to normal grade in 50% of eyes tested 10 days post-1.125% SYL1001 instillation, compared to only 20% in the placebo group ($P<0.05$). Finally, TBUT results showed a significant increase after administration of 0.75% and 1.125% SYL1001 doses at day 10 compared to baseline levels ($P<0.05$). However, no significant changes were observed in terms of Schirmer's test, blepharitis, tear

meniscus, IOP or BCVA at any tested dose levels of SYL1001. However, it should be noted that these dose-finding studies were completed in small cohort sizes with short treatment protocols. Future studies should aim to address these shortcomings and further investigate the efficacy and tolerability of SYL1001 in patients with more severe cases of DES.

4 Retinal Disorders

Retinal diseases represent one of the fields in which significant progress has been achieved regarding the use of RNAi therapeutics. This was first seen following the development of the antiviral Fomivirsen by Isis Pharmaceuticals, which was the first ASO to be approved by the FDA in 1998 for treatment of cytomegalovirus retinitis in immunocompromised individuals, including those with HIV/AIDS (21). A major blinding disease worldwide is diabetic retinopathy (69,70), which represents 8% of all incidences of legal blindness and 12% of newly diagnosed blindness (71). Although severe loss of vision is attributable to proliferative diabetic retinopathy, diabetic macular oedema (DME) is a microvascular complication resulting in functional visual loss in the majority of patients diagnosed with diabetes (72,73). The efficacy of focal/grid photocoagulation laser therapy in reducing the risk of visual loss due to DME was demonstrated in an early treatment diabetic retinopathy study (ETDRS) (73), and represents the current standard of care (74). Recent studies have demonstrated statistically significant efficacy of intravitreal anti-VEGF injections in improving visual acuity and in reducing retinal thickness in patients with DME (75–77). This is likely to be attributable to the role of VEGF in increasing vascular permeability as DME patients presenting with greater macular leakage have elevated levels of VEGF (78,79). *VEGF* is regulated by the hypoxia-inducible factor 1 (*HIF-1*) (80), thus suppression of such genes via therapeutic intervention may suppress *VEGF* expression, extracellular secretion and macular leakage.

With regards to ocular pathologies, Bevasiranib was the first siRNA therapeutic developed by Opko Health in 2004 for the treatment of DME and age-related macular degeneration (AMD) (81). It was designed to target and knockdown *VEGF* expression. Phase 1 (NCT00722384) and 2 (NCT00259753) trials demonstrated promising results with regards to the safety profile and efficacy of the drug, with evident biological activity (82). However, the

Phase 3 COBALT trial (NCT00499590) was discontinued following lack of efficacy in reducing visual loss despite treatment and thus an inability to achieve the primary objective of the trial. The Phase 3 CARBON trial (NCT00557791) aimed to assess the safety and efficacy profiles of three different doses of the drug via IVT administration when combined with ranibizumab as a maintenance treatment for AMD, but the trial was withdrawn prior to enrolment following the decision that the trial was unlikely to attain its primary objective (83). Following the development of Bevasiranib, other siRNAs have been developed with the aim of targeting the pathological processes underpinning various retinal disorders.

4.1 siRNA PF-04523655 targeting *RTP801* expression (2012) – Phase 1 trial

PF-04523655 is an siRNA inhibiting the expression of the hypoxia-inducible *RTP801* gene as a treatment for choroidal neovascularisation (CNV) resulting from age-related macular degeneration (AMD), diabetic retinopathy and macular oedema. *RTP801* gene expression is upregulated during hypoxic events or oxidative stress, resulting in neuronal cell apoptosis. The PF-04523655 siRNA blocks this pathway via mechanisms unrelated to existing anti-angiogenic pathways utilised by anti-VEGF therapies, and may thus have additive effects to the current therapies. PF-04523655 has a short length that may enhance cellular endocytosis of the compound and prevent *in vivo* degeneration.

The aim of this Phase 1 clinical trial was to assess the safety, tolerability, pharmacokinetics and dose-limiting toxicities of single intravitreal injections of PF-04523655, with dose levels between 50 and 3000 µg tested in patients with advanced neovascular AMD presenting with CNV (**Table 1**) (84). A two-pronged approach was used in this study; protocol 1 was a dose-escalating safety study performed in patients identified as unlikely to have any improvement in BCVA or those who had failed to respond to previous treatments. Protocol 2 was carried

out in patients likely to experience improvements in BCVA using the ≥ 1000 μg dose tested in protocol 1 and with a BCVA between 20/100 and 20/800 inclusive.

The primary endpoints for this study involved assessment of the safety and tolerability of PF-04523655 administration across a 24-month observation period. Protocol 1 assessed systemic tolerance by measuring baseline serum levels during initial screening and then at various timepoints following instillation to assess the pharmacokinetics of PF-04523655. Protocol 2 assessed BCVA (Early Treatment for Diabetic Retinopathy Study protocol) and central retinal thickness during initial screening and then at various timepoints as previously outlined. IOP, biomicroscopy, fundoscopy and time-domain optical coherence tomography (OCT) were also performed.

Overall, no dose limiting toxicities were observed in either cohort during this study, with only mild to moderate AEs reported by patients in both protocols (**Table 2**). However, these were ascertained to be unrelated to PF-04523655 administration. Moreover, a dose-dependent relationship was identified when analysing plasma PF-04523655 concentrations at various timepoints post-intravitreal injection of the drug. Maximal plasma concentrations were reached 1 to 4 hours after injection at all doses except 50 and 200 μg . Finally, the half-life of PF-04523655 could not be determined due to the lack of information regarding plasma concentrations 24 hours post-dose instillation.

4.2 siRNA PF-04523655 targeting *RTP081* expression (2012) – Phase 2 DEGAS trial

Based upon preclinical trials and the aforementioned Phase 1 trial assessing the safety and efficacy of PF-04523655, this Phase 2 dose-escalation clinical trial was completed over a 3-year period to assess the same parameters following administration of varying dose levels of the drug as compared to current data regarding focal/grid laser photocoagulation in patients

with visual loss due to DME (**Table 1**) (85). These parameters were assessed at 3, 6 and 12 months' post-administration to ascertain the safety and efficacy over an extended period. The primary endpoint measured in this study was the average change in BCVA at the end of the 12-month period compared to baseline. Secondary endpoints included assessment of occurrence and severity of ocular and systemic AEs, the proportion of patients with >10 and >15-letter BCVA improvement, the percentage of patients with >15-letter decline in BCVA, the average change in central subfield retinal thickness, area of fluorescein leakage compared to baseline, and the average alteration in self-reported visual functioning and quality of life with regards to the impact of vision as measured using NEI-VQF-25 composite.

This trial administered either PF-04523655 doses of 0.4 mg, 1 mg or 3 mg via intravitreal injection once a month or applied laser therapy on the study eye every 4 weeks for a total of 6 months. Following the visit at month 6 of study, the compound was still administered as required provided the central subfield retinal thickness was over 250 μm and the patient did not have an OCT or BCVA in line with the rescue criteria. With regards to the primary endpoint, the average change in BCVA at month 12 was observed to have a greater trend towards improvement in the 3 mg PF-04523655 group compared to those receiving laser therapy (5.77 and 2.39 letters, respectively, $P=0.08$). No statistically significant differences were found between the laser and PF-04523655 0.4 mg and 1 mg groups, with $P=0.93$ and $P=0.35$, respectively. However, a dose-response correlation was evident in patients receiving PF-04523655 treatment with an improvement of over 15 letters more than the laser cohort. Overall, patients treated with PF-04523655 intravitreal injections with screening values of <55 letters demonstrated a greater change in BCVA when compared to baseline values than those with screening BCVAs of ≥ 55 letters.

Assessment of other efficacy endpoints showed that central subfield retinal thickness decreased in all cohorts treated with PF-04523655 across the 12-month study period.

However, no dose-response trend was evident. Results from month 12 showed that the average CSRT-reduction for PF-04523655 doses of 0.4 mg, 1 mg, 3 mg and laser therapy cohorts to be 47 μ m, 20 mm, 63 mm and 104 mm, respectively, when compared to baseline values. In addition, a gradual decline in total retinal volume and fluorescein leakage was observed when comparing month 12 results to baseline values in the laser cohort, whereas PF-04523655 treatment cohorts did not display any distinct correlation with no dose-response trend observed. No change in the degree of diabetic retinopathy was evident in any treatment groups. Groups treated with 0.4 mg and 3 mg PF-04523655 doses showed a general improvement in NEI-VFQ-25 scores, whilst little change was reported in the PF-04523655 1 mg and laser therapy groups.

With regards to safety, AEs were reported in all cohorts, with percentages being greater in those treated with PF-04523655 0.4 mg, 1 mg and 3 mg compared to those who underwent laser therapy (91.3%, 93.5%, 95.7% and 84.8%, respectively) (**Table 2**). The majority of AEs were considered to be unrelated to the use of the drug or the injection procedure. Mild AEs possibly related to PF-04523655 use included posterior subcapsular cataracts, whereas serious AEs were typical of patients in the age demographic present in the study with diabetes mellitus.

Overall, this clinical trial demonstrated minimal efficacy of PF-04523655 administration of up to 3 mg when compared to the impact of focal/grid laser photocoagulation. Discontinuation rates were also greater amongst cohorts treated with PF-04523655 in contrast to the laser therapy group at the 3-month and 6-month analysis intervals. However the study was continued due to evidence of BCVA improvement in patients. Upon observing a trend of minimal BCVA improvement and increasing patient discontinuation rates, the trial was terminated at the 12-month interval; overall only 8 patients completed the 24-month interval and none remained in the study by the 36-month analysis point. An inversely-

proportional dose-related trend was observed between the patient discontinuation rate and the dose of PF-04523655 administered, with the rate for the 3 mg dose being 40% less than that of the 0.4 mg group.

4.3 siRNA PF-04523655 targeting *RTP801* expression (2012) – Phase 2 MONET trial

Another ocular pathology potentially treatable by PF-04523655 is neovascular AMD, which is a major cause of irreversible blindness affecting between 30 and 50 million people worldwide (86), particularly in those over 70 years of age with an incidence rate of 4-8% (87,88).

The primary endpoints of this Phase 2 study was to evaluate the efficacy and safety of PF-04523655 administered as a monotherapy or in combination with ranibizumab compared to ranibizumab administration alone in patients with neovascular AMD, by inhibiting the hypoxia-inducible RTP801 gene (**Table 1**) (89). This was assessed using changes in BCVA at week 16 compared to baseline. Secondary endpoints included assessment of changes in the central subfield retinal thickness using OCT. Secondary efficacy endpoints assessed the average change in BCVA from baseline to 16 weeks, the proportion of patients gaining ≥ 15 letters in BCVA at 16 weeks compared to baseline, changes in the central subfield retinal thickness and retinal lesion thickness according to OCT from baseline on assessment at 4, 8, 12 and 16 weeks, and changes from baseline in CNV area on FFA when assessed at week 16. Study protocol involved the random allocation of 151 patients with subfoveal CNV secondary to neovascular AMD to one of five intravitreal injection regimes. All cohorts received 0.5 mg ranibizumab at baseline; further stratification of subgroups was implemented according to the creation of varying PF-04523655 dose administrations as followed: (a) PF-04523655 1 mg every 4 weeks (Q4W) between weeks 4 and 12; (b) PF-04523655 3 mg Q4W

from weeks 4 to 12; (c) PF-04523655 3 mg every 2 weeks (Q2W) from weeks 4 to 12; (d) PF-04523655 1 mg combined with ranibizumab administered Q4W from baseline to week 12; (e) ranibizumab Q4W until week 12. Regimes (a) to (c) administered 100 µl volume-doses whilst protocols (d) and (e) used 50 µl doses. Results from this trial demonstrated changes in average BCVA values at week 16 compared to baseline in all groups treated with PF-04523655 monotherapy regimes [(a) = 1 mg Q4W, (b) = 3 mg Q4W, (c) = 3 mg Q2W] than observed in the ranibizumab group. Regime (e) administering PF-04523655 1 mg plus ranibizumab Q4W showed a greater improvement in BCVA of 9.5 letters compared to that achieved with ranibizumab alone (6.8 letters), however this result was not statistically significant ($P=0.33$). This group represented the greatest average changes in BCVA over the follow-up period compared to other cohorts. The proportion of patients gaining at least 10 letters by week 16 in the combination and ranibizumab monotherapy cohorts were 40% and 32%, respectively, with 33% and 26% gaining at least 15 letters in these subgroups. All PF-04523655 monotherapy regime groups demonstrated a lower proportion of patients gaining ≥ 15 letters compared to the ranibizumab group. Compared to the baseline values, combination and ranibizumab groups showed similar average reductions in central subfield retinal thickness and total CNV area when assessed at week 16, both of which were greater than that in the PF-04523655 monotherapy group. No statistical significance was observed between treatment cohorts in the average alterations of retinal lesion thickness.

With regards to the safety of PF-04523655 use, most AEs that occurred over the course of this trial were mild to moderate, with only a few attributed to the treatment administered (**Table 2**). Of the 3 deaths, none were attributable to PF-04523655 use, whilst of the 14 reported serious AEs, PF-04523655 administration could not be held accountable for any of them.

In summary, the Phase 1 and 2 clinical trials collectively illustrated that the development of PF-04523655 as either a monotherapy or an additive intervention may present an effective therapeutic avenue, particularly when combined with current anti-VEGF drugs such as ranibizumab. This was particularly evident in the Phase 2 clinical trial, which demonstrated the greatest BCVA improvement of 9.5 letters at week 16 compared to baseline in the PF-04523655 1 mg plus ranibizumab therapy cohort. This is significantly greater than the ranibizumab monotherapy group, in which an improvement of 6.8 letters was observed.

Of note, a further Phase 2 clinical trial enrolling 258 participants was undertaken by Quark Pharmaceuticals in 2013. The MATISSE Study (NCT01445899) evaluated the safety, tolerability and pharmacokinetic profile of IVT PF-04523655 following dose-escalation. The study was split into two distinct arms, with part one involving administration of the drug to all patients in order to establish the maximally tolerated dose and assess dose-limiting toxicities for a 6-month follow-up period. The second stratum consisted of a multi-centre double-masked study aimed at assessing the safety and efficacy of the drug at various escalating doses. PF-04523655 was administered both as a monotherapy and when combined with ranibizumab as compared to the use of ranibizumab alone in patients with DME, with patient follow-up undertaken for 30 days. The results for this study are yet to be published.

4.4 Sirna-027 targeting *VEGFR1* expression (2010) – Phase 1 trial

The current therapeutic drug target for the treatment of AMD is to inhibit one of the two VEGFA tyrosine kinase receptors: VEGFR 1 and 2 (90,91). Sirna-027 (also known as AGN 211745) is an siRNA developed for targeting VEGFR1 mRNA molecules.

This Phase 1 clinical trial was a prospective dose-escalation study assessing the safety, tolerability, pharmacokinetics and dose-limiting toxicity as primary endpoints following

single intravitreal Sirna-027 injections of escalating dose levels in patients with CNV in AMD (**Table 1**) (92). As it was a Phase 1 trial, no clinical efficacy endpoints were assessed in this study. Drug pharmacokinetics were measured according to plasma concentrations following instillation, and dose-limiting toxicity was assessed to provide data for future clinical trials regarding potential dosage levels. Secondary endpoints included assessment of the retina for changes in thickness observed on OCT, CNV size according to changes in lesion size and leakage on fluorescein angiography (FA) and alterations to VA post-injection. Having established safety of single 100 µg intravitreal Sirna-027 injections in the first cohort, subsequent cohorts underwent instillations of 200, 400, 800, 1200 and 1600 µg per eye. Twenty-six patients were enrolled in this study, with all participants completing through to day 84. All reported AEs were of mild to moderate severity, most commonly in the eye but other body systems were also affected (**Table 2**). Of all AEs reported, only conjunctivitis, increased IOP and eye pain were considered as potentially related to Sirna-027 administration. All other AEs were unrelated to the drug. Moreover, no serious AEs were reported; hence the study was unable to determine the maximally tolerated dose.

Clinically significant improvements in BCVA (quantified by more than 3 lines of ETDRS letters) were observed in all dose groups but no statistically significant trend was observed between the dose administered and the BCVA changes; this also proved true for the relationship between the numbers of letters able to be read at baseline and the magnitude of change after drug administration. All patients were stable or demonstrated improvement until at least day 56; by day 84, 92.3% of patients were stable or had improved, with 4 patients maintaining a clinically significant improvement and 2 patients demonstrating clinically significant deterioration in BCVA.

Adjusted average foveal thickness decreased within 2 weeks of drug administration, with this being most prevalent in the 100 and 200 µg dose groups. Improvements in BCVA and foveal

thickness observed in this study indicated beneficial effects of Sirna-027 in targeting VEGFR1 activity for the mediation of angiogenesis. Finally, pharmacokinetic analysis found that the plasma levels of the compound at 1, 4 and 24 hours' post-instillation were below the lower limit of detection (100 ng/mL) in all patients.

Despite promising results regarding the safety profile and clinical improvements observed following Sirna-027 administration, the Allergan Phase 2 clinical trial (NCT00395057) was discontinued following an inability to achieve the desired visual acuity objective (7) and an off-target effect associated with the direct activation of toll-like receptor 3 (TLR3) by siRNAs, which in turn may have undesirable impacts on the vascular and immune systems (93,94).

5 Inherited Retinal Disorders

Inherited retinal diseases (IRDs) are genetic disorders that affect the eye, and as such constitute attractive targets for gene therapy. Jointly, they have an estimated prevalence of 1:2000, making them the leading cause of blindness between 15 and 45 years of age (95–97). They can be stationary disorders, such as achromatopsia that results from cellular dysfunction generally without cell death, or progressive disorders, such as retinitis pigmentosa (RP) that involves photoreceptor degeneration and cell death (98). There are ongoing clinical trials utilising siRNAs and ASOs for the treatment of inherited retinal diseases, which have demonstrated promising results.

5.1 Antisense oligonucleotide QR-110 (Sepofarsen) targeting CEP290 splicing (2018) – Phase 1 and 2 trial

The most severe form of IRD is Leber congenital amaurosis (LCA), a predominantly autosomal recessive disorder that appears at birth or within the first few months of life and represents almost 5% of all retinal dystrophies (99). To date, twenty five genes associated with LCA have been identified, with Centrosomal protein of 290 kDa (*CEP290*) accounting for 15-20% of all known cases (100). *CEP290* is a centrosomal protein with an important role in centrosome and cilia development. The most common defect in *CEP290*-associated LCA (LCA10) is an intronic mutation (c.2991+1655A→G) that creates a strong splice-donor site and inserts a cryptic exon (exon X) in the *CEP290* mRNA, which contains a premature termination codon p.(Cys998*) (101).

Recently, a Phase 1/2 clinical trial utilising the ASO QR-110 (Sepofarsen) has demonstrated promising results in treating LCA10 (102). QR-110 is a single-stranded oligonucleotide, with

a fully phosphorothioated backbone and 2' O-methyl modified nucleotides, that binds directly on the defective splice site to prevent recognition of the cryptic splice site to exclude exon X and restore the correctly spliced CEP290 mRNA transcript (103). The aim of this Phase 1/2 clinical trial was to assess the safety and tolerability of QR-110 intravitreal injections in patients who were compound heterozygotes for the c.2991+1655A→G mutation. An initial 50 µl dose of either 160 µg or 320 µg was administered unilaterally, followed by a 50 µl maintenance dose of 80/160 µg, administered trimonthly, for a maximum of 4 doses (**Table 1**). At 3 months after the initial intervention, the treated eyes showed a statistically significant and clinically meaningful improvement of 0.54 log₁₀ minimum angle of resolution (MAR) in visual acuity over the untreated eyes (0 log MAR corresponds to Snellen acuity of 20/20, 2 log MAR corresponds to 20/2000). In particular, a patient, who was an exceptional responder, had a large 2.7 log₁₀ MAR improvement, shifting from simple perception of light to a Snellen acuity of 20/400. In addition, in all patients, this improvement corresponded to a quantifiable improvement in fixation stability and a similar improvement in red and blue full-field stimulus testing (FST) thresholds (-0.37 ± 0.72 log₁₀ cd/m² for red, -0.82 ± 0.83 log₁₀ cd/m² for blue), which are indicative of improved cone function. Finally, no serious adverse effects were reported in the 9-month follow-up period and pharmacokinetic measures showed the serum level of the study drug to be below the level of quantification (1.02 ng/ml), supporting negligible systemic exposure in all patients (**Table 2**).

Based on these results, a Phase 2/3 trial (NCT03913143) to evaluate the efficacy, safety, tolerability and systemic exposure of Sepofarsen in patients with LCA10 after 24 months of treatment is currently ongoing, with an estimated completion date of December 2021. In addition, patients who have participated in the Phase 1/2 study are able to continue the treatment in an extension of the original study (NCT03913130) if their data support current and/or future benefits.

5.2 Antisense oligonucleotide QR-1123 targeting the P23H mutation of the *RHO* gene (2019) – Phase 1 and 2 AURORA trial

Mutations in the rhodopsin gene (*RHO*) are a cause of autosomal dominant RP (adRP), a congenital eye disorder resulting in the progressive loss of peripheral vision and eventually blindness by middle age (104). Misfolded rhodopsin accumulates in the endoplasmic reticulum or reaches the cell surface but is not functional, eventually causing the death of rod cells. This later results in the death of cone cells and is a cause of RP. *RHO* mutations are the most common cause of adRP, accounting for 20-30% of all cases, and over 150 *RHO* mutations have been identified to date. In adRP cases, the first and most frequently reported *RHO* mutation is a C-to-A transversion in codon 23 of *RHO* (c.68C>A), which causes a Pro-23-His single amino acid substitution (P23H) (105). However, due to the dominant nature of *RHO* mutations, gene replacing therapeutic approaches cannot be utilised.

In December 2019, a Phase 1/2 clinical trial was launched to evaluate the safety, tolerability and efficacy of ASO QR-1123 intravitreal injections in patients with adRP due to the P23H mutation. QR-1123 is a 2'-O-methoxy-ethyl (2'-MOE) modified, phosphorothioate oligonucleotide, designed to bind to the P23H mutated RNA, induce RNase H-mediated degradation and reduce the levels of the misfolded protein in the cells. A two-pronged approach was used in this study; one open label group will receive a single dose-escalating IVT injection, whereas a second, randomised, double masked group will receive either a unilateral IVT injection of QR-1123 or a sham procedure every 3 months (**Table 1**). The follow-up period is 12 months, with the main objective being the assessment of the incidence and severity of ocular and non-ocular AEs. The secondary outcomes include effects on visual function (visual acuity, visual field and FST) as well as retinal structure (OCT) and changes in quality-of-life (**Table 2**). Interim results are expected in early 2021, but the preclinical

animal data are promising. Studies in relevant mouse models demonstrate a measurable reduction in levels of mutant *RHO* and slowing of the rate of photoreceptor degeneration, with greater preservation of cell structure and function following ASO treatment (104).

5.3 Antisense oligonucleotide QR-421a targeting exon 13 mutations of the *USH2A* gene (2019) – Phase 1 and 2 STELLAR trial

Usher syndrome (USH) is an autosomal recessive genetic disorder that is characterised by both hearing and vision loss, making it the leading cause of deaf-blindness, with a prevalence of ~1/25000 (106). Based on the different clinical phenotypes, USH is classified into three distinct types; Usher syndrome type II (USH2), and more specifically subtype IIa (USH2A), is the most common of the three types, accounting for over half of all USH cases (107), and is characterised by moderate to severe hearing loss and the onset of RP within the second decade of life (106). The *USH2A* gene encodes two alternatively spliced usherin transcripts, consisting of 21 and 72 exons, respectively (108). To date, 692 pathogenic and likely pathogenic unique variants of the *USH2A* gene have been reported in the Leiden Open Variation Database (109), with c.2299delG in exon 13, p.Glu767Serfs*21 (originally reported as c.2314delG) being the most common molecular defect causing the disorder (107,108,110). This frameshifting mutation results in a premature termination codon and leads to nonsense-mediated mRNA decay.

In December 2018, a Phase 1/2 clinical trial began to evaluate the safety and tolerability of single-dose QR-421a intravitreal injections in patients with RP due to mutations in exon 13 of the *USH2A* gene. QR-421a is a 21-mer, single-stranded oligonucleotide, with a fully phosphorothioated backbone and 2' O-methyl modified nucleotides (111) that is designed to induce skipping of *USH2A* exon 13 during pre-mRNA splicing, resulting in a slightly shorter, albeit functional, usherin protein. In this randomised sham-controlled study, three different

doses (50, 100 and 200 µg) of QR-421a will be tested for their safety and tolerability, however based on the ongoing data monitoring additional dose levels (e.g. 25 or 400 µg) might be tested (**Table 1**). The monitoring period is 24 months, with the main objective being to assess the incidence and severity of ocular and non-ocular AEs. The secondary outcomes include effects on visual function (visual acuity, visual field and full-field stimulus test (FST)) as well as retinal structure (OCT) and QR-421a pharmacokinetics.

In March 2020, ProQR published non-peer reviewed 3-month interim results of this study (112), which suggested that two out of the eight participants receiving low (50 µg) and middle (100 µg) doses of the ASO demonstrated improvements in visual function across multiple outcome measures. Additionally, QR-421a was observed to be well tolerated, with no serious AEs noted so far (**Table 2**). The estimated completion date of this study is June 2022.

5.4 Antisense oligonucleotide IONIS-FB-L_{RX} targeting *CFB* expression (2020) – Phase 1 trial

An advanced form of AMD, geographic atrophy (GA) is a progressive and irreversible degenerative condition characterised by bilateral visual loss following depletion of the retinal pigment epithelium, photoreceptors and choriocapillaris layer (113). It is estimated that 5 million people have at least unilateral GA, with half of the patients developing the syndrome bilaterally within 7 years of initial diagnosis (114,115). Early stages of AMD present with drusen, which is considered to be a prognostic factor for GA. Eyes with multiple and larger drusen are more likely to develop the disease than those with smaller drusen (116). The pathogenesis of GA is considered to be multifocal and triggered in particular by oxidative stress, which in turn can be affected by both internal and external stressors. The resulting inflammation is thought to be triggered by pathways, such as the complement cascade, which

eventually result in retinal cell death and causes GA. Dysfunction of complement cascade has been implicated in the pathogenesis of GA (117), and a strong genetic association has been found through the identification of 4 single nucleotide polymorphisms in the complement cascade genes which conferred an increased risk of AMD development (118). These have been found to increase activation or reduce deactivation of the complement pathways and thus exaggerate inflammatory events.

There are currently no treatments available for the treatment of GA, however a recent Phase 1 clinical placebo-controlled clinical trial was completed which investigated the safety, pharmacokinetic and pharmacodynamic profiles of the antisense oligonucleotide IONIS-FB-L_{RX} which targets the complement factor B (*CFB*) gene involved in the alternative pathway (119). The compound aims to knockdown *CFB* gene expression, particularly in hepatocytes, and thus reduce circulating CFB protein levels, which would therefore decrease the activity of the alternative pathway in the choriocapillaris layer. The study enrolled 54 healthy volunteers and assessed the aforementioned parameters across multiple ascending doses via monitoring of AEs, drug plasma concentrations, laboratory parameters and clinical examinations. Two arms were employed in this study; the first cohort were given either 10 mg of the compound or placebo, whilst the second were given 20 mg of compound or placebo (0.9% saline). Volunteers were given 2 doses of the drug per week via subcutaneous injection for the first 2 weeks, followed by a single dose per week over the following 4 weeks for a total of 8 doses. Screening was carried out at regular intervals until day 127 of the study. Following the administration of IONIS-FB-L_{RX}, plasma CFB levels reduced by 56% in the 10 mg cohort and 72% in the 20 mg subgroup after 36 days of administration. A reduction in complement cascade screening components (Bb, AH50 and CH50) was observed in the 20 mg cohort. No significant AEs were reported and no clinically relevant changes were observed in any of the assessments. These results indicate a good safety profile for IONIS-

FB-L_{RX} and demonstrate promising initial findings with regards to the efficacy of the compound in reducing the circulating levels of CFB and concomitantly the overactivity of the alternative complement cascade pathway.

A Phase 2 multicentre double-masked placebo-controlled clinical trial is currently underway to further assess the safety and efficacy of multiple IONIS-FB-L_{RX} doses when administered in patients with GA secondary to AMD (GOLDEN study, NCT03815825). The trial aims to evaluate the effect of the compound on the rate of change in the area of GA as measured by fundus autofluorescence (FAF) in up to 330 patients at 3 dose levels at early stages, after which the cohorts of 2 dose levels will be expanded. Administration protocol initially involves subcutaneous administration of the compound every 4 weeks for 45 weeks; after expansion of 2 of the dose level cohorts, the same 4-weekly administration regime will be employed for another 45 weeks, with the primary outcome assessing the absolute change from baseline in GA area by week 49 using retinal imaging. Secondary outcomes involve assessment of changes from baseline in levels of plasma CFB, serum AH50 and low luminance visual acuity, which can be particularly affected by GA.

6 Discussion

Throughout the years, the fields of gene therapy and RNAi therapeutics had their big successes and great failures. In basic research as well, gene knockdown experiments have fallen in and out of favour with researchers. We are now in a new era where several gene therapies have reached clinical trials and are showing promising results to be translated to patients. For example, Patisiran is the first FDA-approved siRNA therapeutic for the treatment of polyneuropathy in patients with hereditary transthyretin-mediated amyloidosis (120).

Ophthalmology has also been the initial route to market for many gene therapy technologies (Spark Therapeutics, Nightstar Therapeutics), and Luxturna is the first FDA-approved gene therapy for inherited RPE65 retinal dystrophies. The eye presents the distinct advantage of being a closed compartmentalised organ where local delivery of siRNA and ASO therapeutics can be achieved, either by using topical eye drops, subconjunctival, intracameral or intravitreal injections (38–41,121). Moreover, localised delivery to the eye presents the added advantage of increased drug bioavailability due to the proximity of administration to the desired target tissue, and fewer adverse side-effects compared to systemic delivery (42). Other body systems have also shown positive responses to siRNA and ASO therapies, namely in neurodegenerative conditions, inflammatory diseases, cancer, cardiovascular disorders and viral infections (7,122).

Several key ophthalmic pathologies have been shown to respond encouragingly to such drugs with favourable safety and tolerability characteristics. However, despite all these recent successes, they still have a long way to go to become the new standard of care, with several challenges facing the translation of such interventions into clinical practice. Longer term studies are required in future clinical trials to establish the specificity and safety of such

compounds when used in humans, building upon preclinical and early clinical trial studies. With regards to the compounds discussed here, Phase 2 clinical trials have been completed for SYL040012 and PF-04523655, whilst this is planned for ISTH0036 and IONIS-FB-L_{RX}. A Phase 3 clinical trial was completed in 2018 for SYL1001 (results pending) and Phase 2/3 trials are currently pending completion for QR-110 (Sepofarsen), whilst the Phase 2 study for Sirna-027 was discontinued following a lack of efficacy and off-target effects. Interim results remain pending for QR-1123 and QR-421a, however preliminary studies have shown promising features.

Although the progression to further clinical trials highlights a promising future for the integration of siRNAs into clinical practice, treatments administered using multiple intravitreal injections carry potential deleterious side-effects, such as elevated risks of developing cataract, retinal detachment, endophthalmitis and vitreous haemorrhage (123). The development of improved delivery techniques resulting in sustained release of RNAi compounds would help to circumvent such complications and enhance their clinical efficacy. The requirement for administration of multiple doses of compounds also presents a problem with regards to the cost-benefit analysis of its use, an issue particularly pertaining to RNAi using nanoparticle technology for administration.

Furthermore, there are particular challenges associated with RNAi technology. Although they are designed to knockdown specific genes, long-sequence compounds can have unintentional and potentially undesirable off-target effects following the stimulation of unintended gene targets. This was exemplified in the Sirna-027 study following activation of the TLR3 pathway and consequent potentially adverse impacts on the vascular and immune systems (94). This in turn can impact the system of innate immunity and introduces the potential for toxicity. The delivery of RNAi to target tissues also remains a challenge, particularly *in vivo* given the rapid excretion of compounds from the kidney and the activity of systemic RNases

which may limit the half-life and thus efficacy of these drugs (124,125). Towards these ends, there has been a plethora of research publications and pre-clinical trials utilising well-established and new technologies, such as lipid nanoparticles (LNPs) and backbone modifications, to achieve safer and more efficient results (71,72). Another potential disadvantage of using siRNAs may be that long-term use could theoretically out compete the endogenous action of miRNAs in certain cells, which much like siRNAs are involved in the regulation of gene expression (4). In the future, we could see RNAi therapeutics being used as monotherapies, but more likely we will see RNAi being used in combination with the current established treatment regimens as they confer several additional benefits to current standards of care.

As has been exemplified by several of the discussed compounds, these treatments are generally well tolerated and have good safety profiles, with few AEs attributable to their use. When combined with their extended *in vivo* half-life and lack of local and systemic side effects, these compounds have the potential to reduce the requirement for repeated administration regimes and improve overall treatment adherence and patient quality of life to a greater extent, especially when combined with current standards of care. This could further help to reduce the costs associated with later complications associated with disease progression; this effect could be further amplified by the potential enhancement of improved clinical outcomes over an extended period of time due to the mechanisms of RNAi technology in targeting the underlying pathological mechanisms. Overall, when combined with the effectiveness observed in several clinical trials, the use of RNAi technology for gene silencing exhibits great promise for incorporation into therapeutic interventions for the treatment of a range of ocular diseases. More than a decade of research has preceded the transition of siRNAs and ASOs from preclinical studies to clinical trials; further investigation

into their mechanisms of action, improved stability and better drug delivery methods will help to accelerate their clinical translation to benefit patient care in the future.

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References

1. Kole R, Krainer AR, Altman S. RNA therapeutics: Beyond RNA interference and antisense oligonucleotides. *Nat Rev Drug Discov.* 2012;11(2):125–40.
2. van der Vlugt RAA, Ruiter RK, Goldbach R. Evidence for sense RNA-mediated protection to PVYN in tobacco plants transformed with the viral coat protein cistron. *Plant Mol Biol.* 1992;20(4):631–9.
3. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *caenorhabditis elegans*. *Nature.* 1998;391(6669):806–11.
4. Dorsett Y, Tuschl T. siRNAs: Applications in functional genomics and potential as therapeutics. *Nat Rev Drug Discov.* 2004;3(4):318-29.
5. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature.* 2001;411(6836):494–8.
6. Stark GR, Kerr IM, Williams BRG, Silverman RH, Schreiber RD. How cells respond to interferons. *Annu Rev Biochem.* 1998;67(1):227–64.
7. Titze-de-Almeida R, David C, Titze-de-Almeida SS. The race of 10 synthetic RNAi-based drugs to the pharmaceutical market. *Pharm Res.* 2017;34(7):1339–63.
8. Pratt AJ, MacRae IJ. The RNA-induced silencing complex: A versatile gene-silencing machine. *J Biol Chem.* 2009;284(27):17897–901.
9. Jinek M, Doudna JA. A three-dimensional view of the molecular machinery of RNA interference. *Nature.* 2009;457(7228):405-12
10. Hamilton AJ, Baulcombe DC. A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science (80-).* 1999;286(5441):950–2.
11. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature.* 2000;404(6775):293–6.
12. Zamore PD, Tuschl T, Sharp PA, Bartel DP. RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell.* 2000;101(1):25–33.
13. Elbashir SM, Martinez J, Patkaniowska A, Lendeckel W, Tuschl T. Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo

- lysate. *EMBO J.* 2001;20(23):6877–88.
14. Martinez J, Patkaniowska A, Urlaub H, Lührmann R, Tuschl T. Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. *Cell.* 2002;110(5):563–74.
 15. Meister G, Tuschl T. Mechanisms of gene silencing by double-stranded RNA. *Nature.* 2004;431(7006):343–9.
 16. Caplen NJ, Parrish S, Imani F, Fire A, Morgan RA. Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. *Proc Natl Acad Sci USA.* 2001;98(17):9742–7.
 17. Bitko V, Barik S. Phenotypic silencing of cytoplasmic genes using sequence-specific double-stranded short interfering RNA and its application in the reverse genetics of wild type negative-strand RNA viruses. *BMC Microbiol.* 2001;1:1–11.
 18. Behlke MA. Chemical modification of siRNAs for in vivo use. *Oligonucleotides.* 2008;18(4):305–19.
 19. Kenski DM, Butora G, Willingham AT, Cooper AJ, Fu W, Qi N, Soriano F, Davies IW, Flanagan WM. SiRNA-optimized modifications for enhanced in vivo activity. *Mol Ther - Nucleic Acids.* 2012;1(1):e5.
 20. Stephenson ML, Zamecnik PC. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. *Proc Natl Acad Sci USA.* 1978;75(1):285–8.
 21. Henahan S. Fomivirsen focuses on the future in CMV retinitis. *Inpharma Wkly.* 1998;1138:11–12.
 22. Rinaldi C, Wood MJA. Antisense oligonucleotides: The next frontier for treatment of neurological disorders. *Nat Rev Neurol.* 2018;14(1):9–22.
 23. Dhuri K, Bechtold C, Quijano E, Pham H, Gupta A, Vikram A, Bahal R. Antisense oligonucleotides: an emerging area in drug discovery and development. *J Clin Med.* 2020;9(6):2004.
 24. Rifai A, Brysch W, Fadden K, Clark J, Schlingensiepen KH. Clearance kinetics, biodistribution, and organ saturability of phosphorothioate oligodeoxynucleotides in mice. *Am J Pathol.* 1996;149(2):717–25.
 25. Freier SM, Altmann KH. The ups and downs of nucleic acid duplex stability: Structure-stability studies on chemically-modified DNA:RNA duplexes. *Nucleic Acids Res.* 1997;25(22):4429–43.
 26. Crooke ST. Progress in antisense technology. *Annu Rev Med.* 2004;55:61–95.
 27. Karaki S, Paris C, Rocchi P. Antisense oligonucleotides, a novel developing targeting therapy. *IntechOpen - Antisense Therapy.* 2019.

28. Lundin KE, Højland T, Hansen BR, Persson R, Bramsen JB, Kjems J, Koch T, Wengel J, Smith CE. Biological activity and biotechnological aspects of locked nucleic acids. *Advances in Genetics*. 2013;82:47–107.
29. Summerton J, Stein D, Huang S Ben, Matthews P, Weller D, Partridge M. Morpholino and phosphorothioate antisense oligomers compared in cell-free and in-cell systems. *Antisense Nucleic Acid Drug Dev*. 1997;7(2):63–70.
30. Dzierlega K, Yokota T. Optimization of antisense-mediated exon skipping for Duchenne muscular dystrophy. *Gene Ther*. 2020;27(9):407–16.
31. Quemener AM, Bachelot L, Forestier A, Donnou-Fournet E, Gilot D, Galibert MD. The powerful world of antisense oligonucleotides: From bench to bedside. *Wiley Interdiscip Rev RNA*. 2020;11(5):1–22.
32. Rossor AM, Reilly MM, Sleigh JN. Antisense oligonucleotides and other genetic therapies made simple. *Pract Neurol*. 2018;18(2):126–31.
33. Müller S, Appel B, Balke D, Hieronymus R, Nübel C. Thirty-five years of research into ribozymes and nucleic acid catalysis: Where do we stand today?. *F1000Research*. 2016;5(0):1–11.
34. Kotterman MA, Chalberg TW, Schaffer DV. Viral vectors for gene therapy: translational and clinical outlook. *Annu Rev Biomed Eng*. 2015;17:63–89.
35. Wu SS, Li QC, Yin CQ, Xue W, Song CQ. Advances in CRISPR/Cas-based gene therapy in human genetic diseases. *Theranostics*. 2020;10(10):4374–82.
36. Chery J. RNA therapeutics: RNAi and antisense mechanisms and clinical applications. *Postdoc J*. 2016 ;4(7):35.
37. Watts JK, Corey DR. Silencing disease genes in the laboratory and the clinic. *J Pathol*. 2012;226(2):365-79.
38. Yu-Wai-Man C, Tagalakakis AD, Manunta MD, Hart SL, Khaw PT. Receptor-targeted liposome-peptide-siRNA nanoparticles represent an efficient delivery system for MRTF silencing in conjunctival fibrosis. *Sci Rep*. 2016;6:21881.
39. Tagalakakis AD, Maeshima R, Yu-Wai-Man C, Meng J, Syed F, Wu LP, Aldossary AM, McCarthy D, Moghimi SM, Hart SL. Peptide and nucleic acid-directed self-assembly of cationic nanovehicles through giant unilamellar vesicle modification: Targetable nanocomplexes for in vivo nucleic acid delivery. *Acta Biomater*. 2017;51:351–62.
40. Fernando O, Tagalakakis AD, Awwad S, Brocchini S, Khaw PT, Hart SL, Yu-Wai-Man C. Development of targeted siRNA nanocomplexes to prevent fibrosis in experimental glaucoma filtration surgery. *Mol Ther*. 2018;26(12):2812–22.

41. Liang Y, Peng J, Li N, Yu-Wai-Man C, Wang Q, Xu Y, Wang H, Tagalakakis AD, Du Z. Smart nanoparticles assembled by endogenous molecules for siRNA delivery and cancer therapy via CD44 and EGFR dual-targeting. *Nanomedicine Nanotechnology, Biol Med.* 2019;15(1):208–17.
42. Guzman-Aranguez A, Loma P, Pintor J. Small-interfering RNAs (siRNAs) as a promising tool for ocular therapy. *Br J Pharmacol.* 2013;170(4):730–47.
43. Akhtar S, Benter IF. Nonviral delivery of synthetic siRNAs in vivo. *J Clin Invest.* 2007;117(12):3623–32.
44. Quigley H, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol.* 2006;90(3):262–7.
45. Lama PJ, Fechtner RD. Antifibrotics and wound healing in glaucoma surgery. *Surv Ophthalmol.* 2003;48(3):314–46.
46. Saeed AM. Comparative study between trabeculectomy with photodynamic therapy (BCECF-AM) and trabeculectomy with antimetabolite (MMC) in the treatment of primary open angle glaucoma. *Clin Ophthalmol.* 2012;6:1651–64.
47. Bindlish R, Condon GP, Schlosser JD, D'Antonio J, Lauer KB, Lehrer R. Efficacy and safety of mitomycin-C in primary trabeculectomy: Five-year follow-up. *Ophthalmology.* 2002;109(7):1336–41.
48. De Fendi LI, Arruda GV, Scott IU, Paula JS. Mitomycin C versus 5-fluorouracil as an adjunctive treatment for trabeculectomy: A meta-analysis of randomized clinical trials. *Clin Exp Ophthalmol.* 2013;41(8):798–806.
49. (No Title). Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/022572Orig1s000MedR.pdf
50. Inatani M, Tanihara H, Katsuta H, Honjo M, Kido N, Honda Y. Transforming growth factor- β 2 levels in aqueous humor of glaucomatous eyes. *Graefes Arch Clin Exp Ophthalmol.* 2001;239(2):109–13.
51. Ochiai Y, Ochiai H. Higher concentration of transforming growth factor-beta in aqueous humor of glaucomatous eyes and diabetic eyes. *Jpn J Ophthalmol.* 2002;46(3):249–53.
52. Min SH, Lee T Il, Chung YS, Kim HK. Transforming growth factor-beta levels in human aqueous humor of glaucomatous, diabetic and uveitic eyes. *Korean J Ophthalmol.* 2006;20(3):162–5.
53. Trivedi RH, Nutaitis M, Vroman D, Crosson CE. Influence of race and age on aqueous

- humor levels of transforming growth factor-beta 2 in glaucomatous and nonglaucomatous eyes. *J Ocul Pharmacol Ther.* 2011;27(5):477–80.
54. Fuchshofer R. The pathogenic role of transforming growth factor- β 2 in glaucomatous damage to the optic nerve head. *Exp Eye Res.* 2011;93(2):165-9.
 55. Pfeiffer N, Voykov B, Renieri G, Bell K, Richter P, Weigel M, Thieme H, Wilhelm B, Lorenz K, Feindor M, Wosikowski K . First-in-human phase I study of ISTH0036, an antisense oligonucleotide selectively targeting transforming growth factor beta 2 (TGF- β 2), in subjects with open-angle glaucoma undergoing glaucoma filtration surgery. *PLoS One.* 2017;12(11):1–18.
 56. Okimoto S, Kiuchi Y, Akita T, Tanaka J. Using the early postoperative intraocular pressure to predict pressure control after a trabeculectomy. *J Glaucoma.* 2014;23(6):410–4.
 57. Wosikowski K, Hasenbach K, Allais L, Fant P, Truchot N. Preclinical profile of ISTH0036, a potent and selective antisense oligonucleotide targeting transforming growth factor beta 2 (TGF- β 2) for the treatment of ophthalmic diseases. *Investig Ophthalmol Vis Sci.* 2015;56(7):5714.
 58. Wax MB, Molinoff PB. Distribution and properties of β -adrenergic receptors in human iris-ciliary body. *Investig Ophthalmol Vis Sci.* 1987;28(3):420–30.
 59. Moreno-Montañés J, Sádaba B, Ruz V, Gómez-Guiu A, Zarranz J, González MV, Pañeda C, Jimenez AI. Phase I clinical trial of SYL040012, a small interfering RNA targeting β -adrenergic receptor 2, for lowering intraocular pressure. *Mol Ther.* 2014;22(1):226–32.
 60. Haupenthal J, Baehr C, Kiermayer S, Zeuzem S, Piiper A. Inhibition of RNase A family enzymes prevents degradation and loss of silencing activity of siRNAs in serum. *Biochem Pharmacol.* 2006;71(5):702–10.
 61. Bian Y, Zhou W, Zhao Y, Li X, Geng W, Hao R, Yang Q, Huang W. High-dose sirnas upregulate mouse Eri-1 at both transcription and posttranscription levels. *PLoS One.* 2011;6(10).
 62. Gonzalez V, Palumaa K, Turman K, Muñoz FJ, Jordan J, García J, Ussa F, Antón A, Gutierrez E, Moreno-Montanes J. Phase 2 of bamosiran (SYL040012), a novel RNAi based compound for the treatment of increased intraocular pressure associated to glaucoma. *Invest Ophthalmol Vis Sci.* 2014;55(13):564.
 63. Lemp MA, Baudouin C, Baum J, Dogru M, Foulks GN, Kinoshita S, Laibson P, McCulley J, Murube J, Pflugfelder SC, Rolando M, Toda I. The definition and

- classification of dry eye disease: Report of the definition and classification subcommittee of the international Dry Eye WorkShop. *Ocul Surf.* 2007;5(2):75-92.
64. Phadatare SP, Momin M, Nighojkar P, Askarkar S, Singh KK. A comprehensive review on dry eye disease: diagnosis, medical management, recent developments, and future challenges. *Adv Pharm.* 2015;2015(2):1–12.
 65. Pflugfelder SC, Geerling G, Kinoshita S, Lemp MA, McCulley J, Nelson D, Novack GN, Shimazaki J, Wilson CI. Management and therapy of dry eye disease: Report of the management and therapy subcommittee of the international Dry Eye WorkShop. *Ocul Surf.* 2007;5(2):163-78.
 66. Schultz C. Safety and efficacy of cyclosporine in the treatment of chronic dry eye. *Ophthalmol Eye Dis.* 2014;6:37–42.
 67. Leonardi A, Van Setten G, Amrane M, Ismail D, Garrigue JS, Figueiredo FC, Baudouin C. Efficacy and safety of 0.1% cyclosporine A cationic emulsion in the treatment of severe dry eye disease: A multicenter randomized trial. *Eur J Ophthalmol.* 2016;26(4):287–96.
 68. Benitez-Del-Castillo JM, Moreno-Montañés J, Jiménez-Alfaro I, Muñoz-Negrete FJ, Turman K, Palumaa K, Sádaba B, González MV, Ruz V, Vargas B, Paneda C. Safety and efficacy clinical trials for SYL1001, a novel short interfering RNA for the treatment of dry eye disease. *Investig Ophthalmol Vis Sci.* 2016;57(14):6447–54.
 69. Aiello LP. Angiogenic pathways in diabetic retinopathy. *N Engl J Med.* 2005;353(8):839-41.
 70. Zimmet P, Alberti KGMM, Shaw J. Global and societal implications of the diabetes epidemic. *Nature.* 2001;414(6865):782-7.
 71. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, Mariotti SP. Global data on visual impairment in the year 2002. *Bull World Health Organ.* 2004;82(11):844–51.
 72. Moss SE, Klein R, Klein BEK. The 14-year incidence of visual loss in a diabetic population. *Ophthalmology.* 1998;105(6):998–1003.
 73. Early photocoagulation for diabetic retinopathy. ETDRS report number 9. Early Treatment Diabetic Retinopathy Study Research Group. *Ophthalmology.* 1991;98(5 Suppl):766–85.
 74. Aiello LP, Edwards AR, Beck RW, Bressler NM, Davis MD, Ferris F, Glassman AR, Ip MS, Miller KM, Diabetic Retinopathy Clinical Research Network. Factors associated with improvement and worsening of visual acuity 2 years after focal/grid

- photocoagulation for diabetic macular edema. *Ophthalmology*. 2010;117(5):946–53.
75. Arevalo JF, Fromow-Guerra J, Quiroz-Mercado H, Sanchez JG, Wu L, Maia M, Berrocal MH, Solis-Vivanco A, Farah ME, Pan-American Collaborative Retina Study Group. Primary intravitreal bevacizumab (Avastin) for diabetic macular edema: results from the Pan-American Collaborative Retina Study Group at 6-month follow-up. *Ophthalmology*. 2007;114(4):743–50.
 76. Nguyen QD, Shah SM, Khwaja AA, Channa R, Hatf E, Do DV, Boyer D, Heier HS, Abraham P, Thach AB, Lit ES. Two-year outcomes of the ranibizumab for edema of the macula in diabetes (READ-2) study. *Ophthalmology*. 2010;117(11):2146–51.
 77. Nguyen QD, Shah SM, Heier JS, Do D V., Lim J, Boyer D, Abraham P, Campochiaro PA, READ-2 Study Group. Primary end point (six months) results of the Ranibizumab for edema of the macula in diabetes (READ-2) study. *Ophthalmology*. 2009;116(11):2175–81.
 78. Funatsu H, Yamashita H, Sakata K, Noma H, Mimura T, Suzuki M, Eguchi S, Hori S. Vitreous levels of vascular endothelial growth factor and intercellular adhesion molecule 1 are related to diabetic macular edema. *Ophthalmology*. 2005;112(5):806–16.
 79. Funatsu H, Yamashita H, Ikeda T, Mimura T, Eguchi S, Hori S. Vitreous levels of interleukin-6 and vascular endothelial growth factor are related to diabetic macular edema. *Ophthalmology*. 2003;110(9):1690–6.
 80. Forsythe JA, Jiang BH, Iyer N V, Agani F, Leung SW, Koos RD, Semenza GL. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol*. 1996;16(9):4604–13.
 81. Dejneka NS, Wan S, Bond OS, Kornbrust DJ, Reich SJ. Ocular biodistribution of bevasiranib following a single intravitreal injection to rabbit eyes. *Mol Vis*. 2008;14:997–1005.
 82. Singerman L. Combination therapy using the small interfering RNA bevasiranib. *Retina*. 2009;29(6):49–50.
 83. Garba AO, Mousa SA. Bevasiranib for the treatment of wet, age-related macular degeneration. *Ophthalmol Eye Dis*. 2010;2:OED.S4878.
 84. Nguyen QD, Schachar RA, Nduaka CI, Sperling M, Basile AS, Klamerus KJ, Chi-Burris K, Yan E, Paggiarino DA, Rosenblatt I, Khan A . Phase 1 dose-escalation study of a siRNA targeting the RTP801 gene in age-related macular degeneration patients. *Eye*. 2012;26(8):1099–105.

85. Nguyen QD, Schachar RA, Nduaka CI, Sperling M, Basile AS, Klamerus KJ, Chi-Burris K, Yan E, Paggiarino DA, Rosenblatt I, Aitchison R. Dose-ranging evaluation of intravitreal siRNA PF-04523655 for diabetic macular edema (the DEGAS study). *Investig Ophthalmol Vis Sci*. 2012;53(12):7666–74.
86. Chopdar A. Age related macular degeneration. *BMJ*. 2003;326(7387):485–8.
87. Klein R, Klein BEK, Linton KLP. Prevalence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology*. 1992;99(6):933–43.
88. Jonasson F, Arnarsson A, Eirksdottir G, Harris TB, Launer LJ, Meuer SM, Klein BE, Klein R, Gudnason V, Cotch MF. Prevalence of age-related macular degeneration in old persons: Age, gene/environment susceptibility Reykjavik study. *Ophthalmology*. 2011;118(5):825–30.
89. Nguyen QD, Schachar RA, Nduaka CI, Sperling M, Klamerus KJ, Chi-Burris K, Yan E, Paggiarino DA, Rosenblatt I, Aitchison R, Erlich SS. Evaluation of the siRNA PF-04523655 versus ranibizumab for the treatment of neovascular age-related macular degeneration (MONET Study). *Ophthalmology*. 2012;119(9):1867–73.
90. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med*. 2003;9(6):669-76.
91. Shibuya M. Differential roles of vascular endothelial growth factor receptor-1 and receptor-2 in angiogenesis. *J Biochem Mol Biol*. 2006;39(5):469-78.
92. Kaiser PK, Symons RCA, Shah SM, Quinlan EJ, Tabandeh H, Do DV, Reisen G, Lockridge JA, Short B, Guercioli R, Nguyen QD, Sirna-027 Study Investigators. RNAi-Based Treatment for Neovascular Age-Related Macular Degeneration by Sirna-027. *Am J Ophthalmol*. 2010;150(1).
93. Kleinman ME, Yamada K, Takeda A, Chandrasekaran V, Nozaki M, Baffi JZ, Albuquerque RJ, Yamasaki S, Itaya M, Pan Y, Appukuttan B. Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. *Nature*. 2008;452(7187):591–7.
94. Cho WG, Albuquerque RJC, Kleinman ME, Tarallo V, Greco A, Nozaki M, Green MG, Baffi JZ, Ambati BK, De Falco M, Alexander JS. Small interfering RNA-induced TLR3 activation inhibits blood and lymphatic vessel growth. *Proc Natl Acad Sci USA*. 2009;106(17):7137–42.
95. Krumpaszky HG, Lüdtkke R, Mickler A, Klauss V, Selbmann HK. Blindness incidence in Germany: A population-based study from Wurttemberg-Hohenzollern. *Ophthalmologica*. 1999;213(3):176–82.

96. Rattner A, Sun H, Nathans J. Molecular genetics of human retinal disease. *Annual Review of Genetics*. 1999;33(1):89-131.
97. Hamel C. Retinitis pigmentosa. *Orphanet J Rare Dis*. 2006;1(1):40.
98. Cremers FPM, Boon CJF, Bujakowska K, Zeitz C. Special issue introduction: Inherited retinal disease: Novel candidate genes, genotype-phenotype correlations, and inheritance models. *Genes (Basel)*. 2018;9(4):1–10.
99. Chacon-Camacho OF. Review and update on the molecular basis of Leber congenital amaurosis. *World J Clin Cases*. 2015;3(2):112.
100. Sheck L, Davies WIL, Moradi P, Robson AG, Kumaran N, Liasis AC, Webster AR, Moore AT, Michaelides M. Leber congenital amaurosis associated with mutations in CEP290, clinical phenotype, and natural history in preparation for trials of novel therapies. *Ophthalmology*. 2018;125(6):894–903.
101. Den Hollander AI, Koenekoop RK, Yzer S, Lopez I, Arends ML, Voesenek KEJ, Zonneveld MN, Strom TM, Meitinger T, Brunner HG, Hoyng CB. Mutations in the CEP290 (NPHP6) gene are a frequent cause of leber congenital amaurosis. *Am J Hum Genet*. 2006;79(3):556–61.
102. Cideciyan AV, Jacobson SG, Drack AV, Ho AC, Charng J, Garafalo AV, Roman AJ, Sumaroka A, Han IC, Hochstedler MD, Pfeifer WL. Effect of an intravitreal antisense oligonucleotide on vision in Leber congenital amaurosis due to a photoreceptor cilium defect. *Nat Med*. 2019;25(2):225–8.
103. Dulla K, Aguila M, Lane A, Jovanovic K, Parfitt DA, Schulkens I, Chan HL, Schmidt I, Beumer W, Vorthoren L, Collin RW. Splice-modulating oligonucleotide QR-110 restores CEP290 mRNA and function in human c.2991+1655A>G LCA10 models. *Mol Ther - Nucleic Acids*. 2018;12:730–40.
104. Murray SF, Jazayeri A, Matthes MT, Yasumura D, Yang H, Peralta R, Watt A, Freier S, Hung G, Adamson PS, Guo S. Allele-specific inhibition of rhodopsin with an antisense oligonucleotide slows photoreceptor cell degeneration. *Investig Ophthalmol Vis Sci*. 2015;56(11):6362–75.
105. Athanasiou D, Aguila M, Bellingham J, Li W, McCulley C, Reeves PJ, Cheetham ME. The molecular and cellular basis of rhodopsin retinitis pigmentosa reveals potential strategies for therapy. *Prog Retin Eye Res*. 2018;62:1–23.
106. Mathur P, Yang J. Usher syndrome: Hearing loss, retinal degeneration and associated abnormalities. *Biochim Biophys Acta - Mol Basis Dis*. 2015;1852(3):406–20.
107. Eudy JD, Weston MD, Yao SF, Hoover DM, Rehm HL, Ma-Edmonds M, Yan D,

- Ahmad I, Cheng JJ, Ayuso C, Cremers C. Mutation of a gene encoding a protein with extracellular matrix motifs in Usher syndrome type IIa. *Science* (80-). 1998;280(5370):1753–7.
108. Lenassi E, Saihan Z, Bitner-Glindzicz M, Webster AR. The effect of the common c.2299delG mutation in USH2A on RNA splicing. *Exp Eye Res.* 2014;122:9–12.
 109. Usher Syndrome Database. Available from: https://grenada.lumc.nl/LOVD2/Usher_montpellier/USHbases.html
 110. Weston MD, Eudy JD, Fujita S, Yao SF, Usami S, Cremers C, Greenburg J, Ramesar R, Martini A, Moller C, Smith RJ. Genomic structure and identification of novel mutations in Usherin, the gene responsible for usher syndrome type IIa. *Am J Hum Genet.* 2000;66(4):1199–210.
 111. Xue K, MacLaren RE. Antisense oligonucleotide therapeutics in clinical trials for the treatment of inherited retinal diseases. *Expert Opin Investig Drugs.* 2020;29(10):1163–70.
 112. ProQR. Interim findings of QR-421a phase 1/2 clinical trial for Usher Syndrome and nsRP. 2020. p1–5. Available from: <https://www.proqr.com/wp-content/uploads/2020/03/ProQR-Patient-Community-update-QR-421a-interim-three-month-analysis-March-2020.pdf>
 113. Holz FG, Strauss EC, Schmitz-Valckenberg S, Van Lookeren Campagne M. Geographic atrophy: Clinical features and potential therapeutic approaches. *Ophthalmology.* 2014;121(5):1079-91.
 114. Wong WL, Su X, Li X, Cheung CMG, Klein R, Cheng CY, Wong TY. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: A systematic review and meta-analysis. *Lancet Glob Heal.* 2014;2(2).
 115. AREDS Research Group. Change in area of geographic atrophy in the age-related eye disease study: AREDS report number 26. *Arch Ophthalmol.* 2009;127(9):1168–74.
 116. Chew EY, Clemons TE, Agrón E, Sperduto RD, SanGiovanni JP, Davis MD, Ferris FL. Ten-year follow-up of age-related macular degeneration in the age-related eye disease study: AREDS report No. 36. *JAMA Ophthalmol.* 2014;132(3):272–7.
 117. Hollyfield JG, Bonilha VL, Rayborn ME, Yang X, Shadrach KG, Lu L, Ufret RL, Salomon RG, Perez VL. Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med.* 2008;14(2):194–8.
 118. Fritsche LG, Chen W, Schu M, Yaspan BL, Yu Y, Thorleifsson G, Zack DJ, Arakawa S, Cipriani V, Ripke S, Igo Jr RP. Seven new loci associated with age-related macular

- degeneration. *Nat Genet.* 2013;45(4):433–9.
119. Jaffe GJ, Sahni J, Fauser S, Geary RS, Schneider E, McCaleb M. Development of IONIS-FB-LRx to treat geographic atrophy associated with AMD. *Investig Ophthalmol Vis Sci.* 2020;61(7):4305.
 120. Adams D, Gonzalez-Duarte A, O’Riordan WD, Yang CC, Ueda M, Kristen AV, Tournev I, Schmidt HH, Coelho T, Berk JL, Lin KP. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med.* 2018;379(1):11–21.
 121. De Fougerolles AR. Delivery vehicles for small interfering RNA in vivo. *Hum Gene Ther.* 2008;19(2):125-32.
 122. Guo J, Fisher KA, Darcy R, Cryan JF, O’Driscoll C. Therapeutic targeting in the silent era: Advances in non-viral siRNA delivery. *Mol Biosyst.* 2010;6(7):1143-61.
 123. Kurz D, Ciulla TA. Novel approaches for retinal drug delivery. *Ophthalmol Clin North Am.* 2002;15(3):405–10.
 124. Bartlett DW, Davis ME. Effect of siRNA nuclease stability on the in vitro and in vivo kinetics of siRNA-mediated gene silencing. *Biotechnol Bioeng.* 2007;97(4):909–21.
 125. Juliano R, Alam MR, Dixit V, Kang H. Mechanisms and strategies for effective delivery of antisense and siRNA oligonucleotides. *Nucleic Acids Res.* 2008;36(12):4158–71.
 126. Filipowicz W. RNAi: The nuts and bolts of the RISC machine. *Cell.* 2005;122(1):17-20.
 127. Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. *Cell.* 2009;136(4):642-55.
 128. Malpettes L, Fussenegger M. Impact of RNA interference on gene networks. *Metab Eng.* 2006;8(6):672–83.

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Figure Legends

Figure 1. Illustration showing the mechanism of siRNA gene silencing in a cell.

1) Double-stranded RNA (dsRNA) undergoes division via the action of the cell-mediated enzyme Dicer; **2A)** dsRNA is cleaved into smaller double-stranded fragments of 21-23 base pair length, known as small interfering RNAs (siRNAs). Each is formed of a guide (antisense, red) and passenger (sense, blue) strand; **2B)** Alternatively, siRNAs are artificially synthesised and pair duplexes are delivered directly into the cell; **3A)** siRNAs are incorporated into a nuclease-containing multiprotein complex known as RNA Induced Silencing Complex (RISC); **3B)** Degradation of the passenger (sense) strand occurs simultaneously (126), whilst the guide (antisense) strand forms a functional siRNA-RISC complex with the siRNA bound to the Ago protein (127); **4)** Target recognition of specific messenger RNA (mRNA) sequences occurs using the siRNA-RISC formation using complementary Watson-Crick base-pairing (127) and mRNA is bound by the siRNA-RISC;

5) mRNA degradation occurs via cleaving action and the target mRNA is released from the complex whilst the siRNA-RISC formation is recycled for further processing of specific mRNA targets and gene silencing (128); **6)** Consequently, there is no further synthesis or expression of pathogenetic proteins due to the lack of mRNA and thus translation.

Figure 2. Illustration showing the mechanism of ASO gene silencing in a cell.

1) ASOs bind by complementary base pairing to mRNAs, forming RNA-DNA duplexes. These hybrids attract RNase H, which then catalyses the cleavage of mRNA in the duplexes; 2) By binding to mRNA, ASOs can block ribosomal access (steric block), which results in decreased protein expression; 3) Lastly, by binding to intron-exon junctions ASOs can destabilise the splicing sites and hence include or exclude particular exons in the mature mRNA.

Table Legends

Table 1. Protocols and drug dosages in the clinical trials

Ocular disease	Phase and year of clinical trial	Target gene	Drug	Protocol	Doses administered (number of patients)	Monocentre vs Multicentre	Masked vs Unmasked
Glaucoma (POAG)	Phase 1 (2017)	<i>TGFβ2</i>	ISTH0036 (ASO)	Single dose-escalating	6.75 µg (3), 22.5 µg (3), 67.5 µg (3), 225 µg (3)	Multicentre	Not mentioned
Glaucoma (healthy volunteers studied)	Phase 1 (2014)	<i>ADRB2</i>	SYL040012 (siRNA)	Protocol 1	Single 600 µg dose (6)	Monocentre	Not mentioned
				Protocol 2	600 µg/eye/day (12); 900 µg/eye/day (12)		
Dry Eye Syndrome (DES)	Phase 1 (2016)	<i>TRPVI</i>	SYL1001 (siRNA)	Protocol 1	Single 2.25% dose, 26.6 µL (6)	Monocentre	Masked
				Protocol 2	Multiple 2.25% doses, 26.6 or 40.0 µL (24)		
	Phase 2	<i>TRPVI</i>	SYL1001 (siRNA)	Protocol 1	Placebo (20); Multiple 1.125% (20); Multiple 2.25% (20)	Multicentre	Double masked
				Protocol 2	Placebo (24); Multiple 0.375% (21); Multiple 0.75% (21)		
Age-related Macular Degeneration (AMD)	Phase 1 (2012)	<i>RTP801</i>	PF-04523655 (siRNA)	Protocol 1	50 µg (3); 100 µg (3); 200 µg (3); 400 µg (3); 670 µg (3); 1000 µg (3); 1500 µg (3); 2250 µg (3); 3000 µg (3)	Multicentre	Unmasked
				Protocol 2	1000 µg (3); 1500 µg (3); 2250 µg (3); 3000 µg (18)	Multicentre	Masked
AMD	Phase 1/2 (2010)	<i>VEGFR1</i>	Sirna-027 (siRNA)	Single dose-escalating	100 µg (4); 200 µg (3); 400 µg (3); 800 µg (6); 1200 µg (6); 1600 µg (4)	Multicentre	Masked
AMD	Phase 2 (2012)	<i>RTP801</i>	PF-04523655 (siRNA)	Single dose-escalating	a) 1mg/4 weeks, weeks 4-12 (29); b) 3mg/4 weeks, weeks 4-12 (31); c) 3mg/2 weeks, weeks 4-12 (30); d) 1mg + ranibizumab/ 4 weeks, 0-12 weeks (30); e) Ranibizumab/ 4 weeks, week 0-12 (31)	Multicentre	Masked
Diabetic Macular Oedema	Phase 2 (2012)	<i>RTP801</i>	PF-04523655 (siRNA)	Single dose-escalating	0.4 mg (46); 1 mg (46); 3 mg (46); Focal/grid laser photocoagulation (46)	Multicentre	Masked
Leber Congenital Amaurosis 10 (LCA10)	Phase 1/2 (2018)	<i>CEP290</i>	QR-110 / Sepofarsen (ASO)	Multiple doses	Initial dose of 160 µg (5) or 320 µg (5); Maintenance dose of 80 µg or 160 µg, respectively, administered trimonthly	Multicentre	Unmasked

Retinitis Pigmentosa (RP)	Phase 1/2 (2019)	<i>RHO</i>	QR-1123 (ASO)	Protocol 1	Single dose-escalating; 5 dose levels	Multicentre	Unmasked
				Protocol 2	Sham Procedure; Multiple doses, trimonthly		Double masked
Usher Syndrome IIa (USH2A)	Phase 1/2 (2019)	<i>USH2A</i>	QR-421a	Single dose-escalating	Sham Procedure; 50µg; 100µg; 200µg	Multicentre	Masked
Geographic Atrophy (GA) – Advanced AMD	Phase 1	<i>CFB</i>	IONIS-FB-L _{RX}	Single/multiple dose-escalating	10 mg or placebo (27); 20 mg or placebo (27)	Multicentre	Masked

Table 2. Results and adverse events in the clinical trials

Drug	Target Disease	Phase / Clinical Trial Number	Study duration	Delivery route	Protocol	Control group	Primary outcomes and results	Main adverse events (AEs)
ISTH0036	Glaucoma	Phase 1 (NCT02406833)	12 weeks	Intravitreal injection	Single post-operative administration in affected eye undergoing trabeculectomy + MMC	None	Safety: 36 AEs reported, none related to drug/procedure. Tolerability: No dose limiting toxicities observed. Efficacy: dose-response trend in IOP reduction, dose cohorts 3 and 4 = consistently <10 mmHg postop. Other parameters: no significant trends for interventions post-trabeculectomy, visual field, slit lamp microscopy, optic disc status. Increase in bleb score, BCVA deterioration (expected post-trabeculectomy).	Main AEs due to primary surgery (n=16), include corneal erosion, corneal epithelial defect, extremes of IOP and other ocular disorders.
SYL040012	Glaucoma	Phase 1 (NCT00990743)	3 days	Topical eye drops	Single administration in healthy volunteers, 72-hour observation period	Contralateral eye	Safety: No AEs for single dose regime, 6 mild AEs due to repeat administration. Other parameters: No significant changes in clinical evaluations, vital signs, ECGs. Statistically significant reduction in diastolic BP in 600µg/eye/day cohort. No changes in haematology, biochemistry, urinalysis.	900 µg/eye/day cohort: muscle spasm, grade 1 conjunctival hyperaemia in contralateral eye, bilateral itching/difficulty focusing. 3 = stinging/grade 1 conjunctival hyperaemia in study eye (900µg/eye/day group), sporadic headache ~24 hrs.
			7 days	Topical eye drops	Administration once a day for 7 days in healthy volunteers	Contralateral eye	Tolerability: Both single and repeat administration regimes were well tolerated. Bioavailability: undetectable in blood samples after single or repeat instillations, undetectable in systemic circulation.	No ocular changes or underlying causes for symptoms detected on examination.
SYL1001	Dry Eye Syndrome (DES)	Phase 1 (NCT01438281)	3 days	Topical	Single administration in healthy volunteers, 72-hour observation period	Contralateral eye	Phase 1: Ocular tolerance: no significant changes in ocular parameters. Improvement in conjunctival hyperaemia after 1.125% instillation compared to placebo (50% vs 20%, P<0.05). No differences in AE rates, good tolerability. Other parameters: No significant changes in physical examination, laboratory assessments, vital signs, ECG. Pharmacokinetics: Compound undetectable in plasma post-instillation.	Phase 1: 16 mild AEs in 13 volunteers, all resolved without intervention and deemed unrelated to study drug. No intra-cohort differences observed in AE frequency between treated and untreated groups (P=0.317).
			7 days	Topical	Single administration in healthy volunteers once a day for 7 days	Contralateral eye		

		Phase 1/2 (NCT01776658) Phase 2 (NCT02455999)	10 days, follow-up until day 20	Topical	Single administration in patient with mild/moderate DED once a day in both eyes	Placebo (n=40)	<p>Phase 2: Safety: No serious AEs, mild intensity, resolved without intervention. No significant differences in AE frequency between cohorts. Good local/systemic tolerance.</p> <p>Efficacy: Topical 1.125% OD significantly decreased VA scores compared to placebo from day 4 until treatment end. Decrease in ocular pain after 1.125% instillation compared to placebo</p> <p>OSDI scores: All cohorts showed significant reduction in OSDI scores from initial visit to day 10 (P<0.05), no intra-treatment differences. Significant reduction compared to baseline (P<0.01), no significant intra-cohort changes.</p>	<p>Phase 2 Protocol 1: 5/60 experienced possibly AEs related to compound use.</p> <p>Phase 2 Protocol 2: 7/66 experienced AEs possibly/probably related to drug use. 1 patient receiving the 0.75% dose discontinued the study due to abnormal eye sensation and headache of moderate severity, possibly due to use of study drug.</p>
PF-04523655	Age-related Macular Degeneration (AMD)	Phase 1 (NCT00725686)	84 days dose-regime, 24 months follow-up	Intravitreal injection	Single administration in patients advanced AMD with CNV, unlikely to improve VA or failed response to previous treatment	None	<p>Protocol 1: Safety: 74.1% reported at least 1 AE, most were mild-moderate and unrelated to compound.</p> <p>Protocols 1 and 2: Tolerability: No dose limiting toxicities, could not establish maximally tolerated dose. Pharmacokinetics: Plasma concentrations after single intravitreal injection ≥ 400 μg measurable at 1, 4 and 24 hours. 1 patient (3000 μg cohort) = measurable at 7 days. None with measurable levels at day 14. Excretion of compound/metabolites likely via urine.</p>	<p>Protocol 1: Eye disorders: contralateral eye developed CNV, study eye developed conjunctival haemorrhage and hyperaemia, irritation, pain, punctate keratitis, and pruritus (both eyes). GI disorders: dry mouth. Infections: bronchitis and nasopharyngitis.</p>
					Single administration in CNV patients secondary to advanced AMD, likely to improve VA	None	<p>Protocol 2: Safety: 70.4% reported ≥ 1 AE, mild-moderate, unrelated to compound use.</p>	<p>Protocol 2: AEs for: eye: CNV, retinal pigment epithelial detachment, foreign body sensation, increased IOP, eye pain in study eye. GI: nausea. MSK/connective tissue: pain in extremities. Nervous system: headache.</p>
Sirna-027	AMD	Phase 1/2 (NCT00363714)	Safety assessed after 14 days	Intravitreal injection	Single ocular administration in patients with CNV secondary to AMD	None	<p>Safety: 96.2% of patients reported AEs of mild-moderate severity, majority unrelated to study drug use. Tolerability: no dose limiting toxicities reported during 84 days post-administration; maximally tolerated dose could not be established. Pharmacokinetics: Plasma levels 1, 4 and 24 hours post-administration</p>	<p>88.5% AEs in eye; eye pain (30.8%), corneal epithelial defect (23.1%), irritation (15.4%), discomfort (15.4%). Nervous system disorders (23.1%, dizziness = 15.4%), GI disorders (15.4%), infections (15.4%).</p>

							were below limit of detection (100 ng/ml) in all patients.	Conjunctivitis, eye pain, increased IOP related to drug use (n=1 each).
PF-04523655	AMD	Phase 2 (NCT00701181)	12 months total: 3 months treatment regime, 9 months follow-up	Intravitreal injection	All patients received 0.5mg ranibizumab, then ocular administration of siRNA according to allocated regime in eye of study	None	Efficacy: mean change in BCVA at 16 weeks compared to baseline: greatest improvement observed in PF 1mg + ranibizumab combination cohort (9.5 letters) compared to ranibizumab monotherapy cohort (6.8 letters), not statistically significant. BCVA improvement in PF monotherapy cohorts = less than in ranibizumab monotherapy cohort.	Few due to compound use, majority = mild-moderate severity. 14 subjects = SAEs; 2 (mild ischaemic stroke and moderate TIA) due to ranibizumab use. None attributable to PF. Frequent AEs = worsening retinal oedema (10.6%, AMD progression), reduced VA (9.3%), conjunctival (9.3%)/retinal haemorrhage (8.6%) in study eye. All intravitreal injection-related = mild-moderate severity, mostly in 3mg/2 week cohort.
PF-04523655	Diabetic Macular Oedema	Phase 2 (NCT00713518)	12 months, originally, 24 months	Intravitreal injection/laser therapy	Single ocular administration in one eye of patients with DME every 4 weeks for 6 months total, 12 month follow-up	None	Efficacy: Change in BCVA compared to baseline: cohorts tested at all doses demonstrated BCVA improvement at month 12 compared to baseline. 3mg cohort showed greatest improvement compared to laser (5.77 vs 2.39 letters).	>90% AE incidence in PF cohorts, 84.8% in laser cohort. Majority unrelated to PF. Most common = increased IOP post-injection, conjunctival/retinal haemorrhage in eye of study, nasopharyngitis, systemic hypertension, mild posterior subcapsular cataracts due to compound use. 37 serious AEs (SAEs), none related. 3 due to injection procedure: 1 = transient vision loss, 1 = endophthalmitis 3 days after 1st injection, emergency evisceration 1.5 weeks later. Laboratory abnormalities (irrespective of baseline): elevated HbA1c, glycaemic levels, serum creatinine, blood urea nitrogen, protein.

QR-110 (Sepofarsen)	Leber Congenital Amaurosis 10 (LCA10)	Phase 1/2 (NCT03140969)	12 months	Intravitreal injection	Single unilateral administration in patients heterozygote for the c.2991+1655A→G mutation, every 3 months for a maximum of 4 doses	None	<p>Safety: No serious AEs, mild intensity, resolved without intervention. No intra-ocular inflammation after 4 doses.</p> <p>Pharmacokinetics: Undetectable in serum post-administration</p> <p>Efficacy: Improved fixation stability and red and blue full-field stimulus testing (FST) thresholds. One exceptional responder had a large MAR improvement, shifting from simple perception of light to a Snellen acuity of 20/400</p>	5/10 patients had a subconjunctival haemorrhage at the site of injection. Mild lens modifications and an air bubble for one patient. One patient presented mild parafoveal intraretinal cysts at OCT detected at 4 months after the first injection.
QR-1123	Retinitis Pigmentosa (RP)	Phase 1/2 (NCT04123626)	12 months follow-up	Intravitreal injection	<p>Single unilateral administration in patients with adRP due to the P23H mutation</p> <p>Single unilateral administration in patients with adRP due to the P23H mutation every 3 months</p>	Sham Procedure	No Data	No Data
QR-421a	Usher Syndrome IIa (USH2A)	Phase 1/2 (NCT03780257)	24 months follow-up	Intravitreal injection	Single unilateral administration in patients with RP due to mutations in exon 13 of the USH2A gene	Sham Procedure	<p>Preliminary Data: Well tolerated with no serious AEs reported. 2/8 patients in the low and middle dose groups demonstrated improvements in visual function across multiple outcome measures.</p>	No Data
IONIS-FB- L _{RX}	Geographic Atrophy (GA)	Phase 1 ACTRN12616000335493	6 weeks	Subcutaneous injection	Administration of 2 doses per week for the Weeks 1 and 2, then 1 per week for the next 4 weeks	Placebo	<p>Safety: No serious AEs reported</p> <p>Other parameters: No clinically relevant changes in blood chemistry, haematology urinalysis, ECG or vital signs</p> <p>Efficacy: Reduction in CFB levels by 56% in 10 mg cohort and 72% in 20 mg. Reduction in complement-associated proteins in the 20 mg cohort above that observed in 10 mg cohort</p>	No serious AEs reported

Highlights

- Small interfering RNAs (siRNA) and antisense oligonucleotides (ASO) are two major types of gene therapy, utilising oligonucleotides for targeting and downregulating genes associated with specific pathologies.
- We are now in a new era where several gene therapies have reached clinical trials and are showing promising results to be translated to patients.
- The eye presents the distinct advantage of being a closed compartmentalised organ where local delivery of siRNA and ASO therapeutics can be achieved, either by using topical eye drops or by subconjunctival, intracameral or intravitreal injections.
- Several challenges, including target specificity, safety and efficacy, will need to be overcome before they can become part of standard of care in ophthalmology.