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Gene Therapy-Associated Uveitis (GTAU): Understanding and mitigating the adverse immune response in retinal gene therapy

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A B S T R A C T

Retinal gene therapy using adeno-associated viral (AAV) vectors has been a groundbreaking step-change in the treatment of inherited retinal diseases (IRDs) and could also be used to treat more common retinal diseases such as age-related macular degeneration and diabetic retinopathy. The delivery and expression of therapeutic transgenes in the eye is limited by innate and adaptive immune responses against components of the vector product, which has been termed gene therapy-associated uveitis (GTAU). This is clinically important as intraocular inflammation could lead to irreversible loss of retinal cells, deterioration of visual function and reduced durability of treatment effect associated with a costly one-off treatment. For retinal gene therapy to achieve an improved efficacy and safety profile for treating additional IRDs and more common diseases, the risk of GTAU must be minimised. We have collated insights from pre-clinical research, clinical trials, and the real-world implementation of AAV-mediated retinal gene therapy to help understand the risk factors for GTAU. We draw attention to an emerging framework, which includes patient demographics, vector construct, vector dose, route of administration, and choice of immunosuppression regime. Importantly, we consider efforts to date and potential future strategies to mitigate the adverse immune response across each of these domains. We advocate for more targeted immunomodulatory approaches to the prevention and treatment of GTAU based on better understanding of the underlying immune response.

1. Introduction

Retinal gene therapy can offer potential therapeutic benefits for patients with inherited retinal diseases (IRDs) that were previously considered incurable (Bainbridge et al., 2008; Hauswirth et al., 2008; Maguire et al., 2008). Many advanced therapeutic medicinal products have been designed to treat IRDs, each aiming to address a specific genetic mutation affecting the retina. Searching *clinicaltrials.gov* for relevant clinical trials reveals over 100 studies of retinal gene therapy that are either completed or ongoing. These trials involve a wide array of

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retinal dystrophies, including *RPE65*, *NR2E3, MYO7A, PDE6B* and *MERTK*-associated retinitis pigmentosa (RP); *RPGR*-associated X-linked RP; *GUCY2D* and *LCA5*-associated Leber congenital amaurosis (LCA); *CHM*-associated choroideremia; *RS1*-associated congenital X-linked retinoschisis; *CNGA3* and *CNGB3*-associated achromatopsia; *ABCA4*-associated Stargardt disease; and *CYP4V2*-associated Bietti's crystalline dystrophy. As the technology has matured, retinal gene therapy is also increasingly being applied to more common eye diseases such as neovascular and atrophic age-related macular degeneration (AMD) and diabetic retinopathy.

Following promising results in a landmark pivotal trial (Russell et al., 2017), the US Food & Drug Administration (FDA) and European Medicines Agency (EMA) approved the first retinal gene therapy product in 2017 and 2018 respectively. Voretigene neparvovec (Luxturna, Spark Therapeutics Inc., Philadelphia, PA, USA) is currently approved for use in IRDs associated with biallelic mutations in *RPE65* and sufficient viable retinal cells (European Medicines Agency, 2019). It is a recombinant viral vector composed of an adeno-associated viral (AAV) capsid containing the human *RPE65* transgene, delivered to the surviving retinal pigment epithelium (RPE) via subretinal injection in each eye.

Recombinant AAV, particularly serotypes AAV2, AAV5, AAV8 and increasingly AAV9, is the most common viral vector currently used to deliver exogenous DNA cargo to the retina. One of the reasons why AAV is preferred over other viral vectors, such as lentiviruses and adenoviruses, has been its relatively low immunogenicity (Arjomandnejad et al., 2023). The immune reaction seen with other viral vectors delivered systemically has sometimes been severe and even fatal in very rare cases (Raper et al., 2003). Other advantages of AAV include (Dunbar et al., 2018; Ferreira et al., 2021):

- Non-pathogenic;
- Low risk of insertional mutagenesis;
- Replication incompetent in the absence of a helper virus;
- Ability to transduce a variety of non-dividing neuronal cells, including rods, cones and RPE;
- Sustained transgene expression in non-dividing cells after a single administration.

Despite these favourable characteristics of AAV vectors, adverse ocular inflammatory reactions have been reported in up to 50 % of patients receiving Good Manufacturing Practice (GMP)-grade gene therapy products in clinical trials (Fischer et al., 2019; Reichel et al., 2022a; MacLaren et al., 2023; Pierce et al., 2024; Yang et al., 2024b; Cehajic-Kapetanovic et al., 2024; Campochiaro et al., 2024). This prevalence is also seen in post-approval studies of voretigene neparvovec (Kessel et al., 2022; Fischer et al., 2024). The intraocular inflammation is dose-dependent and can sometimes last for months or even years following treatment, leading to irreversible retinal structural damage and reduced visual function if uncontrolled (Bainbridge et al., 2015). The term *gene therapy-associated uveitis* (GTAU) has been proposed to describe this intraocular immune response.

GTAU will be used here to refer to any element of intraocular inflammation following gene therapy vector administration for convenience. However, it is worth noting that the clinical presentation of GTAU can vary significantly between patients. Furthermore, the pathophysiology of GTAU is complex and is unlikely to be entirely homogenous across all forms of AAV-mediated gene therapy. Greater understanding of the mechanisms and risk factors for GTAU is critical to developing mitigation strategies and optimising clinical outcomes from this expensive and typically one-off treatment (Bucher et al., 2021).

To this end, we conducted a search for keywords in the literature and *clinicaltrials.gov*, including 'AAV', 'gene therapy' and 'retina'. We also manually searched references to identify additional relevant literature.

In this review, we outline the clinical experience of GTAU to date and its immunological basis. We draw attention to an emerging framework with which to understand the risk of GTAU. The exact features and severity of the ocular immune response seem to depend on pre-treatment host-specific variables, vector design, dose and route of administration, and choice of immunosuppressive prophylaxis (Yang et al., 2024a). We advocate for systematic reporting of these variables in all clinical trials to facilitate meta-analysis of risk ratios and deepen our understanding of the relative contribution of each risk factor.

By collating trends in management strategies for GTAU, we also propose reconsideration of the way in which GTAU is prevented and treated. Historically, patients undergoing retinal gene therapy have received some combination of standard post-vitrectomy topical corticosteroid drops, with or without local depot, and a tapering course of systemic corticosteroids over 14–21 days around the time of treatment (Bennett et al., 2016; Fischer et al., 2019; MacLaren et al., 2023). However, severe and protracted cases of GTAU have been reported despite such immunosuppression regimes (Jalil et al., 2023). The adverse effects of high-intensity corticosteroids are also profound and well recognised (Gaballa et al., 2021). Few clinical studies to date have implemented steroid-sparing immunomodulation to prevent or treat GTAU (Cukras et al., 2018; Pennesi et al., 2022). Meanwhile, the importance of the cell-mediated immune response underlying GTAU is becoming increasingly clear. Further work to understand this may facilitate the development of targeted, steroid-sparing, immunomodulatory strategies to mitigate the risk and severity of GTAU, thus improving the clinical outcomes of gene therapy.

2. Clinical characteristics of gene therapy-associated uveitis

2.1. Intraocular inflammation reflects how AAV vector is administered to the eye

Currently, viral vectors are administered to the retina via one of three routes (Fig. 1): (i) subretinal injection concentrates most vector particles within iatrogenic blebs, potentially including the fovea; (ii) intravitreal injection delivers the vector as a bolus into the whole vitreous cavity; (iii) suprachoroidal administration enables treatment of large areas of peripheral RPE and choroid while sparing the macula.

Subretinal administration currently requires pars plana vitrectomy, a well-established technique in vitreoretinal surgery (Ladha et al., 2022). AAV particles are then deposited under the retina via a retinotomy using a 38-41G subretinal cannula. Subretinal injection generally delivers the

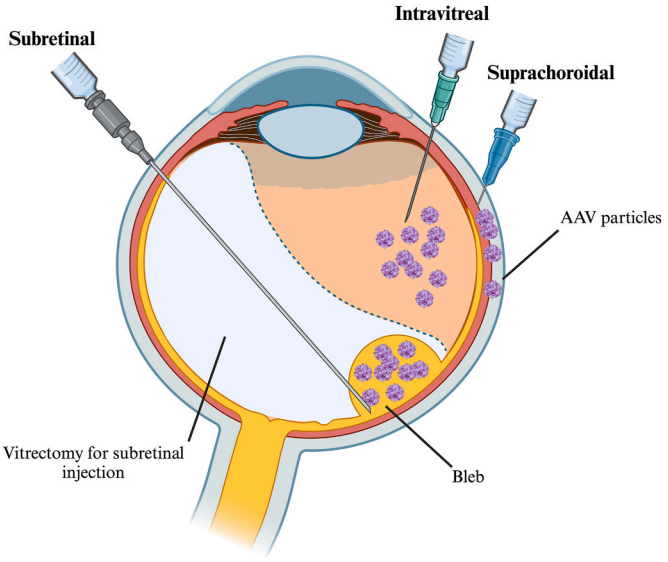

Fig. 1. Common routes of intraocular administration of AAV vector in retinal gene therapy. Subretinal and intravitreal injection are the most common delivery routes used in clinical trials of retinal gene therapy. Voretigene neparvovec is currently approved for single subretinal injection only.

highest concentration of vector particles to the photoreceptors and RPE. These are the most commonly affected cell types in IRDs (Manley et al., 2023).

In comparison, intravitreal administration of gene therapy vector is minimally invasive but faces other challenges. Firstly, vector particles are diluted by the large vitreous cavity volume (around 5 ml in an average human eye). Secondly, vector particles must traverse the inner limiting membrane (ILM) barrier to reach the outer retina, which has a mesh size of 50–100 nm (Rafael et al., 2023). Similarly, epiretinal membranes may be more prevalent in inherited and non-inherited retinal diseases and represent an additional barrier (Liew et al., 2019; Fung et al., 2021). These anatomical factors mean that a much higher dose of AAV would be required intravitreally to achieve equivalent therapeutic vector particle concentrations at the level of the photoreceptors compared with subretinal delivery.

Suprachoroidal administration of AAV vector has been made clinically feasible more recently by the first FDA-approved SCS microinjection device (Clearside Biomedical, Alpharetta, GA, USA). This route allows transduction of the peripheral RPE and choroid for the purpose of producing a secreted protein within the eye, e.g. the anti-VEGF antibody fragment protein for neovascular AMD (AAVIATE study, NCT04514653) and diabetic retinopathy (ALTITUDE study, NCT04567550).

The route of vector administration is primarily chosen to maximise transduction of the target cell population. However, the difference in the immune responses associated with each route is arguably an even more important consideration when aiming to optimise the efficacy, durability and safety of treatment.

The subretinal space, being a potential space separated from the systemic circulation by the blood-retinal barrier, provides relative immune privilege. In comparison, the vitreous cavity and suprachoroidal space are both outside the blood-retinal barrier, thus are more accessible to antibody and cell-mediated adaptive immune responses against the viral vector and transgene product (Yiu et al., 2020; Chung et al., 2021; Vignal-Clermont et al., 2023).

In line with this, a recent meta-analysis of gene therapy trials has shown that GTAU occurs in around 45 % of patients receiving intravitreal gene therapy, compared with 28 % after subretinal gene therapy and 21 % after suprachoroidal gene therapy (the latter with very limited data to date) (Buckley et al., 2024). The relative advantages and disadvantages of each route of retinal gene therapy administration in terms of the elicited immune response are summarised in Table 1.

2.2. Clinical presentations of gene therapy-associated uveitis

Fig. 2 illustrates a range of different clinical manifestations of GTAU, including anterior uveitis (cells, flare, keratic precipitates), intermediate uveitis (vitritis) and/or posterior uveitis (chorioretinitis, papillitis). Generally, the clinical presentation of GTAU appears to reflect the space where vector particles are concentrated in the eye.

2.2.1. Intravitreal injection

Vitritis and anterior chamber inflammation are typical features following intravitreal injection of AAV vector. This suggests an immune response that is directed against viral particles sequestered within the vitreous humour with some wash-through to the anterior chamber along aqueous outflow. These features are typically seen within a few weeks of

Table 1
Relative merits of different routes for retinal gene therapy. Routes of administration currently used for AAV-mediated retinal gene therapy with relative advantages, disadvantages, and examples of clinical trials (completed or ongoing) using each route. GTAU is more often seen following intravitreal delivery compared to subretinal or suprachoroidal. Suprachoroidal delivery remains a relatively uncommon approach.

Route of administration	Description	Advantages	Disadvantages	Examples of clinical trials
Intravitreal	Direct injection into the vitreous cavity, 3.5 or 4.0 mm from the limbus in pseudophakic or phakic eyes, respectively.	• Simple and convenient, minimally invasive outpatient procedure • Potentially panretinal transfection area • Better able to target inner retinal cells (e.g. ganglion cells, bipolar cells and Müller glia)	• ILM barrier restricts transduction of outer retinal cells • Off-target transduction of anterior segment structures (e.g. ciliary body, iris and corneal endothelium) • Relatively large vector dose required to achieve therapeutic concentrations in the retina • Higher systemic viral load • Elicits a high rate of GTAU, e.g. vitritis and anterior uveitis • Elicits greater neutralising antibody response against the viral capsid, which could limit the efficacy of second eye treatment and re-dosing	NCT02064569 NCT05930561 NCT05536973 NCT03144999 NCT04483440 NCT02317887 NCT02416622 NCT05228145
Subretinal	Pars plana vitrectomy followed by either 'one-step' (direct) or 'two-step' injection between the retina and RPE in a controlled, localised detachment (bleb) using a 38-41G cannula.	• Greater transgene expression in outer retina cells (i.e. photoreceptors and RPE) • A potential space that is relatively immune privileged (i.e. behind the blood-retinal barrier) • Less systemic dissemination of the viral vector thus lower incidence of neutralising antibody formation • Efficacy is not significantly affected by circulating neutralising antibodies	• Requires vitrectomy and retinotomy, which can be associated with surgical complications • Variability in vector dosing due to reflux into the vitreous cavity • Requires operating theatre and trained surgeons • Transduction and transgene expression is generally limited to the area of retinal detachment (bleb)	NCT00643747 NCT00481546 NCT00516477 NCT02610582 NCT04704921 NCT03846193 NCT01461213 NCT02341807
Suprachoroidal	Injection into the potential space between the sclera and choroid, typically using a microneedle.	• Can be performed in the outpatient setting using a specialised microneedle • Can potentially treat a large area of RPE without intraocular surgery • Relatively low rate of intraocular inflammation reported to date (but can cause episcleritis and raised intraocular pressure)	• High likelihood of eliciting systemic immune response as vector is injected into a highly vascularised space • Low efficacy of photoreceptor transduction • Need to avoid inadvertent choroidal detachment of the macula • Elicits cellular and humoral immune response directed against the transgene protein (Chung et al., 2021)	NCT04567550 NCT04514653

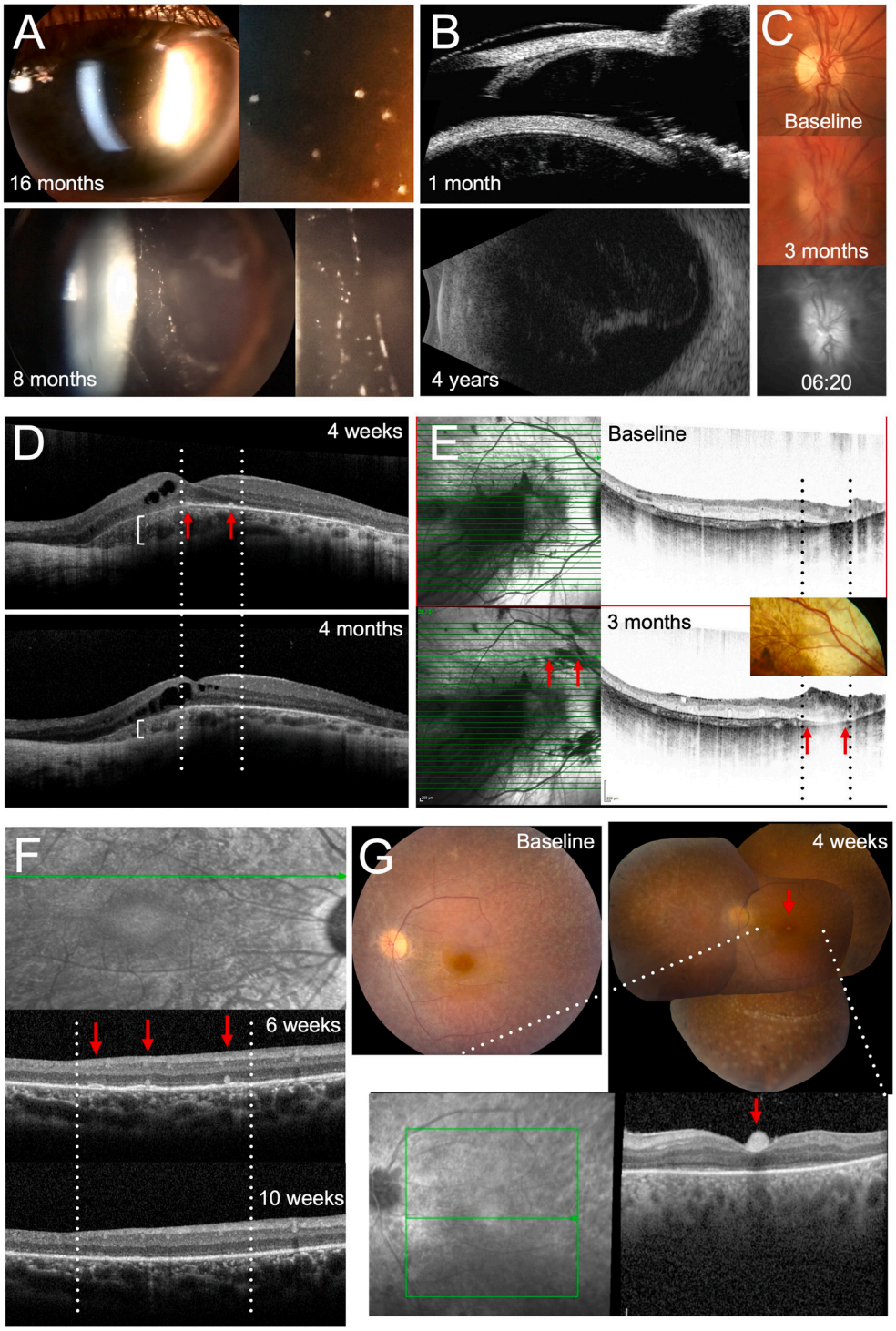

Fig. 2. Clinical examples of gene therapy-associated uveitis (GTAU). (A) Anterior uveitis in the form of numerous stellate keratic precipitates (top) and anterior vitritis (bottom) following intravitreal AAV gene therapy. **(B)** Ultrasound biomicroscopy (top) showing vitritis in the areas of intravitreal injection, and b-scan ultrasound (bottom) showing dense vitreous debris from chronic vitritis following intravitreal AAV gene therapy. **(C)** Disc swelling and disc leakage on fluorescein angiography following intravitreal AAV gene therapy. **(D)** GTAU in the form of subretinal infiltrates (arrows) and choroidal thickening (bracket) observed 4 weeks after subretinal AAV gene therapy for choroideremia (top). These changes resolved by 4 months after additional systemic corticosteroids, but some outer retinal cell loss can be seen (bottom). **(E)** Localised retinal nerve fibre layer thickening (arrows) seen 3 months following subretinal AAV gene therapy for choroideremia. **(F)** GTAU in the form of subretinal infiltrates (arrows) observed 6 weeks after subretinal AAV gene therapy for *RPGR*-associated X-linked retinitis pigmentosa, which resolved by 10 weeks after additional corticosteroid treatment. **(G)** Second eye subretinal gene therapy for *RPE65*-associated IRD performed 5 weeks after first eye treatment (due to a delay in delivery), leading to significant GTAU. Both subretinal and preretinal inflammation (including pre-foveal deposit – arrow, and inferior snowballs) were seen. This case was managed with a secondary vitrectomy washout and high-dose local and systemic corticosteroids with good final outcome.

intravitreal injection, particularly with higher vector doses (Cukras et al., 2018; Vignal-Clermont et al., 2018; Bouquet et al., 2019). This inflammation could lead to secondary cataract or raised intraocular pressure if poorly controlled (Comander et al., 2016; Newman et al., 2023).

2.2.2. Subretinal injection

Chorioretinitis, choroidal thickening and subretinal deposits (described as hyperreflective foci on optical coherence tomography, OCT), are more commonly observed following subretinal administration of AAV vector (Reichel et al., 2017; Xue et al., 2018; Cehajic-Kapetanovic et al., 2020; Rodríguez-Bocanegra et al., 2021; Kvanta et al., 2024). As well as exhibiting dose-dependence, subretinal deposits are steroid-responsive. This is indicative of an immune response that is centred around the site of maximum vector particle deposition (i.e. the subretinal space within and around the bleb area) with localised blood-retinal barrier breakdown and immune cell infiltration. These features could lead to reduced retinal sensitivity and photoreceptor and/or RPE loss if uncontrolled.

Subretinal injection may be associated with vector reflux through the retinotomy into the vitreous cavity (Reichel et al., 2021), particularly in the degenerative thin retinas of patients with IRDs. Therefore, GTAU may also present with varying degrees of anterior and intermediate uveitis following subretinal treatment (Weleber et al., 2016). Indeed, case reports describing difficulty with bleb induction have seen greater rates of vitritis despite vitreous washout, closer to the prevalence seen following intravitreal delivery (Kessel et al., 2022). This may be explained by viral particles adhering to residual cortical vitreous thus escaping standard irrigation with infusion fluid.

In contrast to the inflammatory response to intravitreal gene therapy, features of retinal inflammation following subretinal gene therapy are usually observed after one to several months (Constable et al., 2016; Fischer et al., 2020; Iannaccone et al., 2022). However, both mild and fulminant inflammation may occur as early as two weeks after subretinal injection, which appears to be more likely with higher vector doses (Weleber et al., 2016; Jalil et al., 2023; Michaelides et al., 2023).

2.2.3. Suprachoroidal injection

Suprachoroidal administration appears to be well tolerated except for reports of dose-dependent episcleritis in some patients (Dhoot, 2022; REGENXBIO, 2023; Barakat, 2023; REGENXBIO, 2024). Early therapeutic efficacy of anti-VEGF antibody fragment suprachoroidal gene therapy in neovascular AMD and diabetic retinopathy appears promising but full safety and durability data are keenly awaited.

2.3. Chorioretinal atrophy following subretinal injection of AAV vectors

Another recently described clinical presentation after gene therapy that may be related to GTAU is chorioretinal atrophy (CRA), seen in 10–20 % of patients as hypo-autofluorescent areas on fundus autofluorescence (FAF) imaging (Fischer et al., 2024).

CRA is typically visible from one to several months following subretinal injection of AAV vectors in both adults and children (Weed et al., 2019; Gange et al., 2022; Fischer et al., 2024; Kvanta et al., 2024). Subretinal infiltrates may precede the development of late CRA although many cases have not been associated with clinically detectable intraocular inflammation. Ellipsoid zone disruption a few weeks after gene therapy has been observed by OCT prior to the onset of CRA in some patients (Reichel et al., 2022b).

Surgically induced RPE trauma from cannula touch-down is visible immediately. Whereas an RPE tear may improve slowly with time, gradually enlarging areas of RPE atrophy surrounding the retinotomy site are less likely to be due to surgical trauma alone (Reichel et al., 2022b; Seitz et al., 2024). In cases of CRA, RPE changes are often noticeable at around 2 weeks as 'salt-and-pepper' mottling, which develops into a more confluent area of atrophy later on, typically located around the perimeter of the subretinal bleb or tracking along vascular arcades.

Some CRA lesions may slowly expand beyond the bleb area over time. If fluid-air exchange is performed after subretinal injection, the bleb is flattened and vector particles could be pushed peripherally, potentially contributing to atrophy expansion in the initial post-operative period. However, surgical factors alone seem insufficient to explain the pattern of atrophy spread over subsequent months which is suggestive of an immune or inflammatory component contributing to CRA.

This is supported further by the dose dependency of CRA, similar to GTAU. In a study of subretinal administration of AAV8.*PDE6A* in non-human primates (NHPs), Seitz et al. found that CRA occurred twice as often and progressed four times more rapidly in animals treated with a higher dose of viral vector (1×10^{12} viral genomes, vg, per eye versus 1×10^{11} vg) (Seitz et al., 2024). Moreover, CRA was not observed following vitrectomy and sham subretinal injection in control eyes.

Other hypotheses have been suggested to explain some features of CRA in retinal gene therapy patients, such as cellular stress resulting from transgene overexpression or excessive protein production leading to unfolded protein response (Reichel et al., 2022b). Indeed, some groups have identified a correlation between improvement in full-field stimulus threshold (FST) testing or dark adapted perimetry, measures of overall rod function, and the development of CRA (Stingl et al., 2023; Ku et al., 2024). However, this observation has not been consistently replicated in other cohorts (Fischer et al., 2024; Lorenz et al., 2024).

2.4. Monitoring for gene therapy-associated uveitis

Typical practice to detect post-operative inflammation relies on visual acuity testing, standard ophthalmic examination (e.g. slit lamp microscopy, tonometry and fundoscopy), FAF, OCT and occasionally ultrasound and fluorescein angiography. However, there is significant variability across clinical studies in the techniques and timing used to monitor patients for intraocular inflammation following AAV injection. Many trials also do not report the incidence of intraocular inflammation longitudinally at each assessment timepoint (MacLaren et al., 2023; Khanani et al., 2024a; J. J. Wang et al., 2024). This complicates identifying the true incidence and natural history of GTAU and CRA in AAV retinal gene therapy.

The value of close monitoring and early detection is illustrated by a case from a recent study of subretinal AAV gene therapy in *RPGR*-associated RP (Cehajic-Kapetanovic et al., 2020). After an initial retinal sensitivity gain following subretinal gene therapy, one participant experienced a subacute drop in retinal sensitivity from 3.4 to 0.0 dB in the treated eye between 4 and 5 weeks after treatment. OCT demonstrated scattered subretinal deposits. However, visual acuity remained unaffected throughout. A course of oral corticosteroids was re-commenced, resulting in dissolution of the subretinal deposits and overall retinal sensitivity gain by 3 months. Similar unexpected deterioration, even if small in the context of the natural history of the disease, should prompt diagnosis and treatment of GTAU to maximise the likelihood of treatment benefit.

Fundus autofluorescence is another useful non-invasive imaging modality for the detection and monitoring of GTAU. Detection of hypo-autofluorescence at two weeks after subretinal injection of AAV (beyond the features of background and progressive retinal disease), which may be an early sign of CRA, should raise the question of GTAU and whether to escalate immunosuppression. This is particularly pertinent for the standard prophylaxis strategy in the real-world use of voretigene neparvovec. In this regime, the corticosteroid taper is completed by two to three weeks after vector administration, at around the same time as when features of GTAU may appear.

Notwithstanding technological improvements and the integration of artificial intelligence in retinal imaging modalities to increase diagnostic sensitivity (Keenan et al., 2021), a purely clinical approach to

monitoring may not capture the full extent or very early signs of GTAU. Early manifestations of GTAU may be subclinical based on animal studies demonstrating microglial activation and immune cell infiltration in the outer retina without overt clinical signs of intraocular inflammation (Chan et al., 2021b; Chandler et al., 2021; Wiley et al., 2023). Serial sampling could detect pro-inflammatory cytokines and other biomarkers of cell-mediated intraocular inflammation, which are upregulated in dissected retinas following subretinal injection of viral vector in NHPs and mice (Reichel et al., 2017; Xiong et al., 2019). However, these changes may not be specific to GTAU; such a test would need to differentiate between the AAV-directed immune response, inflammation due to surgery and underlying disease activity. Understanding the mechanisms and natural history of the adverse immune response to vector delivery in the eye could aid the development of more sensitive and specific tests to detect GTAU at the earliest opportunity.

3. Immunogenicity of AAV vector preparations

3.1. Molecular interactions between AAV vectors and host cells

3.1.1. Viral structure and entry to target cells

AAV comprises an icosahedral protein shell assembled from three capsid proteins (VP1, VP2 and VP3 at a ratio of 1:1:10) encasing a single-stranded DNA (ssDNA) genome (Samulski, 1995; Snijder et al., 2014). The expression cassette of a recombinant AAV particle comprises the therapeutic transgene open reading frame (ORF) flanked by transcription regulatory elements and a 145-nucleotide viral inverted terminal repeat (ITR) sequence (Rodrigues et al., 2018). While AAV offers advantages over more complex viral vectors in terms of immunogenicity, it does have a relatively small packaging capacity of 4.7 kb of DNA.

Detail on viral entry is provided elsewhere (Dhungel et al., 2021; J.-H. Wang et al., 2024). AAV capsids gain entry into target cells by first binding to cell surface receptors. These vary by AAV serotype. For instance, the primary receptor for AAV2 is heparan sulfate proteoglycan (HSPG). Binding to a co-receptor is often required, such as the G protein-coupled receptor for AAV2. The AAV capsid is then endocytosed by the target cell.

Upon acidification of the endosome, capsid proteins undergo conformational changes and unpacking to release the ssDNA cargo. The ssDNA is trafficked to the perinuclear cytoplasm where it enters the nucleus through the nuclear pore complex. The ssDNA then undergoes second strand DNA synthesis and concatemerisation to form double-stranded episomes separate from the host genome. Here, the promoter drives transgene expression. The promoter may either be ubiquitous (e.g. the CAG promoter) or cell-specific (e.g. the rhodopsin kinase promoter, GRK1, which is photoreceptor-specific).

3.1.2. Immune response to AAV vector components

An immune response may be mounted against any AAV vector component (viral capsid proteins or genome) and/or transgene product. In addition, impurities may remain in AAV preparations from the production process, such as DNA fragments from plasmids and host cell genomes as well as empty viral capsids, which can contribute to overall immunogenicity (Wright, 2014; Clément and Grieger, 2016; Pei et al., 2018). To address this, detection of undesirable DNA fragments by quantitative PCR (qPCR) or next-generation sequencing (NGS) could enable improved purification process during vector manufacturing to reduce contaminants (Lecomte et al., 2015; Penaud-Budloo et al., 2017; Guerin et al., 2020). These methods are increasingly recognised by FDA guidelines (Food and Drug Administration, 2020). In depth reviews of vector production and purification processes can be found elsewhere (Snyder, 2016; Rodrigues et al., 2021; Davidsson and Heuer, 2022; Lothert and Wolff, 2023; Som et al., 2024; Destro et al., 2024).

Upon administration to the eye, immunogenic vector components can elicit a local innate immune response. For instance, viral vector components are detected by pattern-recognition receptors (PRRs) that are present across several retinal cell types and activate pro-inflammatory signalling cascades (Hösel et al., 2012; Cao et al., 2024). The Toll-like receptor (TLR) family are PRRs that detect various viral components. TLR2 detects the capsid proteins of extracellular viral particles, while TLR9 detects unmethylated CpG motifs within single-stranded viral DNA genomes present in endosomes (Perez et al., 2020; Wright, 2020). The TLR9-initiated pathway is a well-recognised component of the pro-inflammatory response to AAV-mediated gene therapy in the retina (Chan et al., 2021b) and elsewhere in the body (Zhu et al., 2009; Martino et al., 2011; Faust et al., 2013). To some extent, internalised AAV-derived DNA may also be recognised by cytosolic PRRs such as cGAS (Sun et al., 2012; Wu et al., 2013; Chandler et al., 2019).

The downstream intracellular cascades initiated by PRRs encourage an anti-viral state through interferon response gene expression and pro-inflammatory cytokine release. Detection of AAV components by the innate immune system also leads to the AAV-directed adaptive immune response and potential loss of transgene expression in transduced cells (Mingozzi et al., 2007; Xiang et al., 2020). AAV particles can be found in draining lymphoid tissues following injection in the eye (Seitz et al., 2017), where the adaptive immune response is primed. Emerging evidence suggests a role for the posterior ocular lymphatic drainage system in the recruitment of T cells to the retina (Yin et al., 2024).

Antigen presenting cells (APCs) contribute to this immune coordination via major histocompatibility complex (MHC) proteins (Fig. 3). It is still unclear which retinal cell types are the first responders in GTAU. Presentation of AAV-derived antigens via MHC Class II by microglia, the chief resident immune cell within the retina, may play an important role in driving the adaptive response and leukocyte infiltration, particularly early in GTAU (Okunuki et al., 2019; Chandler et al., 2021; Langer et al., 2023). Other resident APCs in the retina include Müller glia and activated RPE. The extent to which transduced cells play a role in the innate response is also unclear as AAV capsid proteins may be captured for ubiquitous peptide antigen presentation via MHC Class I.

The choice of vector serotype (capsid) and promoter determines the location and level of transgene expression within the retina. They can also influence the resulting immune response, altering how viral particles interact with resident APCs and infiltrating immune cells such as macrophages (Khabou et al., 2018; Quinn et al., 2024). For example, ubiquitous promoters have been suggested to cause a stronger immune response in the retina compared to photoreceptor-specific promoters (Xiong et al., 2019). Xiong et al. found that RPE and photoreceptor toxicity and glial activation correlated with broadly active, but not cell-specific, *cis*-regulatory sequences following subretinal administration of AAV vectors, even with no transgene expression.

3.2. Cell-mediated immune response in gene therapy-associated uveitis

The intraocular immune response to AAV vectors appears to be primarily type 1 or cell-mediated. Several independent groups have identified a preponderance of $CD45^{+}$ immune cells in the retinas of healthy murine models following treatment with AAV vectors (Tummala et al., 2021; Chandler et al., 2021; Chan et al., 2021b). This is supported by studies in large animals (NHP and canine) demonstrating activation of retinal APCs, similar immune cell infiltrates and locally increased levels of classic type 1 cytokines (IFN-γ, TNF-α, CXCL10) in eyes treated with intravitreal and subretinal AAV vectors (Vandenberghe et al., 2011; Boyd et al., 2016; Reichel et al., 2017; Timmers et al., 2020). Furthermore, the cell-mediated immune response in animal models runs a prolonged subclinical course within the retina for longer than 6 weeks (Tummala et al., 2021; Chandler et al., 2021), reflecting the above observations in clinical studies. This carries implications for the immunosuppressive prophylaxis applied in practice, which has typically ceased by one month after treatment (see below).

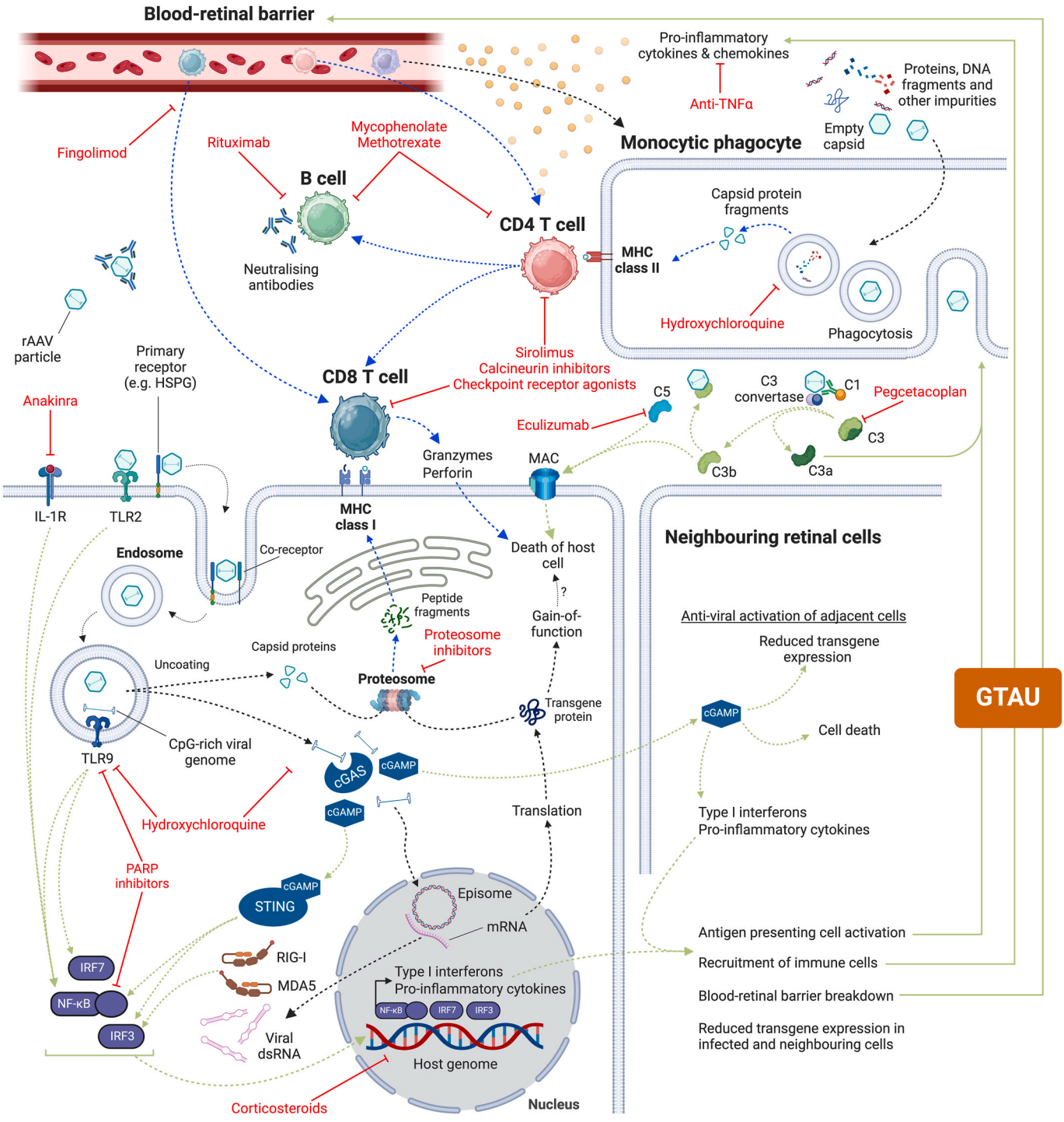

Fig. 3. Model of the immune response in gene therapy-associated uveitis (GTAU). AAV-mediated transfection of target retinal cells, e.g. photoreceptors, and transgene expression, including activation of the innate immune response (light green arrows), presentation of vector-derived antigens and activation of the adaptive immune response (dark blue arrows). GTAU is believed to be a primarily cell-mediated immune response, leading to cytotoxic destruction of transduced cells and diminishing treatment durability and long-term visual outcomes. Various anti-inflammatory, immunosuppressive and immunomodulatory agents can interfere with these pathways and may have utility in preventing and managing GTAU (indicated in red). Recombinant AAV (rAAV); Heparan sulfate proteoglycan (HSPG); Toll-like receptor 2/9 (TLR2/9); Poly (ADP-ribose) polymerase (PARP); Membrane attack complex (MAC); Major histocompatibility complex (MHC); Interleukin-1 receptor (IL-1R); Tumour necrosis factor (TNF); Cyclic GMP–AMP synthase (cGAS); Stimulator of interferon genes (STING); 2′3′ cyclic GMP–AMP (cGAMP); Interferon regulatory factor 3/7 (IRF3/7); Nuclear factor-kappa B (NF-κB); Retinoic acid-inducible gene I (RIG-I); Melanoma differentiation-associated protein 5 (MDA5). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2.1. *Cell-mediated response in intravitreal delivery*

Tummala et al. (2021) assessed eyes treated with an intravitreal injection of recombinant AAV. They monitored mice with OCT on Days 1, 7, and 29 after injection and compared findings to the untreated fellow eyes. At sacrifice, the entire intraocular contents (including aqueous humour, iris, vitreous, retina, and RPE) were dissociated and analysed by flow cytometry. Vitritis developed in all AAV-injected eyes regardless of vector preparation, peaking at Day 7 along with a

prominent CD45$^+$ immune cell presence. Importantly, the cellular immune infiltration was sustained to at least one month after treatment and persisted after clinically apparent vitritis had settled. The immune cell populations within AAV-treated eyes shifted over time, which likely reflects the identities of vitreous cells seen clinically:

i. On Day 1, the cellular infiltrate was dominated by macrophages and neutrophils;
ii. On Day 7, the majority were T cells, macrophages and natural killer cells;
iii. By Day 29, mostly CD8$^+$ T cells and microglia remained.

Moreover, the nature of the immune cell infiltrate was not significantly affected by prior AAV exposure, except that AAV-naïve eyes demonstrated a more delayed adaptive immune response.

3.2.2. Cell-mediated response in subretinal delivery

Chandler et al. (2021) also used OCT to assess the clinical response in mice but delivered the AAV vector subretinally. Whole retinas were dissociated for flow cytometric and immunohistochemical analysis on Days 3, 7, 14, and 28 after injection. In contrast to the response seen following intravitreal AAV delivery, CD45$^+$ immune cells in the retina peaked later at 14–28 days after subretinal gene therapy. Mild vitritis was only observed in three out of five eyes at Day 14 and correlated with higher levels of AAV-mediated retinal cell transduction and immune cell infiltration. Flow cytometric detection of transgene presence among monocytic phagocyte populations implicate the role of microglia and macrophages in the antigen presentation and immune activation process. Similarly, CD45$^+$ immune cell infiltration in the retina did not show signs of subsiding by one month after treatment, which indicates significantly prolonged GTAU.

Our current understanding of the potential network of events underlying the immune response to various viral vector components in the retina is summarised by Fig. 3. We are beginning to elucidate the key receptors and their downstream signalling pathways that detect intraocular gene therapy-associated antigens. Further understanding of the innate and adaptive immune pathways in GTAU, ideally in specific retinal diseases, could inform the mitigation of key risk factors. These likely include pre-treatment host and vector-related factors, administration variables such as vector dose and route, and post-treatment prophylaxis and pharmacological management. The work to date in each of these areas is presented in the following sections.

4. The potential for patient risk stratification

Several host-related factors may impact the risk of GTAU in retinal gene therapy. However, gaining greater understanding of the roles of background retinal disease, age and sex in determining the immune response should be prioritised as they are known to influence the inflammatory state of the retina and response to viruses in general (Klein, 2012; Klein and Flanagan, 2016; Chan et al., 2021a).

4.1. Background retinal disease

Retinal inflammation is a known component of several inherited and non-inherited retinal diseases and likely influences the rate of disease progression (Massengill et al., 2020; Okita et al., 2020; Adamus, 2021; Olivares-González et al., 2021; Pinilla et al., 2022; Sarici et al., 2023). For instance, immune cell infiltration occurs in the retinas of RP animal models (reviewed by Yang et al., 2024a) and pro-inflammatory cytokines have been reliably detected in the ocular fluid of patients with RP, including IL-2, IL-6, IL-8, MCP-1, MCP-2, and TARC (Yoshida et al., 2013; ten Berge et al., 2019; Lu et al., 2020; Tao et al., 2024). The pathways contributing to underlying disease progression and GTAU appear to converge on innate immunity (Yang et al., 2024a). This altered immunological state, compared with the relatively immunosuppressed state of the healthy retina, may contribute to heightened retinal inflammation in patients undergoing gene therapy.

One exploratory study examined the circulating immunological changes in 11 patients with X-linked retinoschisis as part of a retinal gene therapy trial for an AAV8.*RS1* vector (Mishra et al., 2021). Firstly, patients exhibited elevated circulating levels of pro-inflammatory cytokines (TNF-α and IFN-γ) and a greater CD4/CD8 T cell ratio at baseline compared to 12 healthy, age- and sex-matched controls. Moreover, the severity of intraocular inflammation following intravitreal administration of viral vector modestly correlated with CD4/CD8 ratios at baseline. An imbalanced CD4/CD8 ratio is a manifestation of disrupted immune homeostasis (Carvajal Alegria et al., 2017) and an elevated ratio is predictive of more severe inflammatory reactions and mortality in other viral infections (Pascual-Dapena et al., 2022).

Examination of similar relationships between immunological biomarkers, disease stage or other measures of retinal inflammation at baseline and intraocular inflammation in retinal gene therapy is warranted in larger samples and other retinal diseases. An ongoing example is the ALTITUDE study (NCT04567550) of suprachoroidal ABBV-RGX-314 in diabetic retinopathy, which is currently recruiting patients across cohorts stratified by disease severity (proliferative versus non-proliferative retinopathy) (Barakat, 2023).

This is important as the influence of background retinal inflammation on the risk of GTAU is likely to be disease specific. Thus, the same gene therapy delivered to two different conditions may lead to very different GTAU rates and safety outcomes. For instance, the INFINITY (NCT04418427) and OPTIC (NCT03748784) trials involved intravitreal administration of the same AAV2.7m8-aflibercept vector (ADVM-022) at the same doses to participants with diabetic macular oedema (DMO) and neovascular AMD, respectively. The gene therapy was well-tolerated in neovascular AMD with only mild, anterior segment inflammation reported that responded well to steroids (Busbee et al., 2021). In contrast, the INFINITY trial was halted as several patients developed panuveitis with hypotony and vision loss four to nine months after treatment (Cheng and Punzo, 2022; Ghoraba et al., 2022). These patients required vitrectomy, silicone oil, an intravitreal steroid implant and in some cases immunomodulatory therapy to control the inflammation. These were considered suspected unexpected serious adverse reactions (SUSARs) and the programme in DMO was terminated.

4.2. Age

The full impact of age on the risk of GTAU remains unclear. Anecdotally, children undergoing retinal gene therapy appear to exhibit more severe ocular responses compared to adults. Illustrating this point, AAV vectors AGTC-401 or AGTC-402 were administered subretinally to 37 adults and 18 children with achromatopsia (mutations in *CNGB3* or *CNGA3*) across two clinical trials (Iannaccone et al., 2022; Vajzovic, 2022). Four out of the five children in the highest dose group (3.2×10^{12} vg/mL/eye) developed clinically significant intraocular inflammation one month after administration. Three of these events were classed as SUSARs with some patients requiring intravitreal immunosuppression to control inflammation. In contrast, no such dose-limiting toxicities (DLTs) were observed in any adult at any vector dose.

Michaelides et al. (2023) reported a similar observation in the dose-escalation study of AAV8.CARp.*CNGB3* in achromatopsia. Twelve adults were treated with up to 1×10^{12} vg/mL/eye, delivered by subretinal injection (Michaelides et al., 2023). Treatments were well tolerated, allowing recruitment of children aged 5–13 years to the study in a safety confirmatory phase. The first child to be enrolled developed intraocular inflammation that was considered to be a DLT.

Unfortunately, the small sample sizes of paediatric cohorts in these studies and others (Le Meur et al., 2018; Yang et al., 2024b) preclude robust statistical comparison across age groups. Nevertheless, several hypotheses may explain these observations. Propagation of the subretinal bleb with vector requires higher injection pressures in children

(Scruggs et al., 2022). Consequently, there may be greater risk of retinal damage and inadvertent vector reflux into the vitreous cavity. However, Pennesi et al. also noted a greater incidence of vitritis and ocular hypertension in the treated eyes of children compared to adults that received the same vector and dose via the intravitreal route (Pennesi et al., 2022). This is indicative of immunological aetiology beyond surgical factors.

Younger patients may be more capable of mounting the interferon-driven, anti-viral innate response critical to GTAU and developing robust adaptive immunity compared to adults and the elderly (Kumar et al., 2018; Yoshida et al., 2022). Expression of Toll-like receptors 2, 4 and 9, critical in the innate immune response to PAMPs and AAV in the retina (Fig. 3), are downregulated in macrophages of aged mice (18–24 months old) compared to young mice (2–3 months old) (Renshaw et al., 2002). These changes are known to have functional consequences, including an aging-related decline in innate immune response to viral infection or vaccination. For example, macrophages from aged mice exhibit muted TNF-α release upon stimulation (Renshaw et al., 2002). Similar results have been replicated in the response to influenza vaccination across different age groups in humans (Panda et al., 2010; Bahadoran et al., 2016).

Aging also leads to a reduction in T cell function, plasticity and proliferation with subsequent impact on B cell populations, a phenomenon known as 'immunosenescence' (Makinodan and Kay, 1980; Lewis et al., 2022). Older adults demonstrate higher concentrations of circulating inflammatory mediators, constituting a low-level chronic inflammatory state coupled with diminished cell-mediated immunity, termed 'inflammaging' (Franceschi et al., 2000; Costagliola et al., 2021; Calabrò et al., 2023).

Results from ongoing retinal gene therapy trials involving participants with AMD and DMO may provide us with novel insights into the impact of immunosenescence and inflammaging on the risk of GTAU.

4.3. Sex

Differences in immune function and responses to both foreign and self antigens between males and females have been reported (Klein and Flanagan, 2016; Calabrò et al., 2023). Inflammatory responses are stereotypically greater in females, which may be dictated by greater cytokine release due to the location of cytokine and TLR genes on the X-chromosome (Oertelt-Prigione, 2012). Peripheral blood mononuclear cells (PBMCs) from females produce higher levels of pro-inflammatory cytokines and lower immunosuppressive IL-10 in the innate anti-viral response to TLR ligands and viruses compared to PBMCs from males (Torcia et al., 2012). This may lead to a stronger adaptive response in AAV gene therapy in females with greater cell and antibody-mediated immunity directed against the transgene product resulting in poorer treatment durability (Piechnik et al., 2022).

Women may also undergo changes associated with immunosenescence and inflammaging more gradually than men (Calabrò et al., 2023), widening potential differences in older age brackets. In line with this, (older) men appear more likely than women to exhibit chronically raised levels of pro-inflammatory mediators but less adaptive immune activity (Márquez et al., 2020; Huang et al., 2021).

In the retina, recent bulk RNA sequencing of human samples indicates differences in transcriptome profiles between the sexes with a distinct MC1 microglial population in the female retina that may increase susceptibility to neuroinflammation (Tan et al., 2023). Microglia in females generally demonstrate greater inflammatory phenotypes than males with heightened expression of IRF3, involved in the innate immune response to viral infection (Lynch, 2022).

Such proinflammatory priming in women may result in a greater risk of GTAU and lower durability of transgene expression following retinal gene therapy. Clare et al. explored this by administering an intravitreal injection of AAV vector encoding IL-33 to mice, modelling a retinal gene therapy approach to treat atrophic AMD (Clare et al., 2023). Female mice exhibited greater $CD45^{+}$ immune cell infiltration and poorer transgene expression compared to males. In a different experiment involving intravitreal administration of a null AAV2 vector, female mice demonstrated a more proinflammatory microglial gene expression profile at older ages compared to age-matched male mice, which was associated with more significant retinal thinning at 1 month after gene therapy (Clare et al., 2025).

On a practical level, understanding demographic differences in the risk of developing GTAU could inform the clinical delivery of retinal gene therapy, such as consenting for treatment, post-operative monitoring and prophylaxis strategies. For instance, a bias towards an initial innate immune response in older males may prompt predominantly perioperative anti-inflammatory treatment focussed on limiting innate antiviral activity in the retina. In contrast, females may be at greater risk of a robust adaptive immune response, cytotoxic clearance of transduced retinal cells and chronic GTAU, particularly in the event of bilateral or repeated retinal gene therapy. Therefore, greater emphasis could be placed on immunomodulatory strategies explored later.

For this reason and with the expansion of gene therapy approaches for more common retinal diseases, emphasis should be placed on understanding the impact of disease severity, age, and sex on the risk of GTAU.

5. Refinements in vector design

The feasibility of therapeutic gene augmentation using AAV vectors has encouraged much work over the past decade to optimise transgene delivery and limit the adverse immune response via the pathways presented above. While recombinant AAV is generally well tolerated and demonstrates best-in-class transduction rates of retinal cells, it is worth remembering that the multiplicity of infection is about 10^4 - 10^5 vector particles at the level of the RPE, depending on the disease state at the time of treatment (Trapani et al., 2014; Ramachandran et al., 2017). Targeting photoreceptors may require an even higher number of vectors to achieve sufficient transgene expression. Refining vector design to improve transduction efficiency remains a crucial effort.

Changes can be made to each of the vector's constituent parts through genetic or protein engineering of the viral capsid, therapeutic transgene or transcription/translation regulatory elements. These changes have one of two aims.

The first is to improve transduction of the target cells. This would reduce the required dose of viral vector particles to achieve therapeutic transgene expression, and thus the likelihood and severity of GTAU (Petrs-Silva et al., 2011; Leray et al., 2024). However, transgene overexpression may be toxic to target cells thus both considerations need to be balanced to optimise treatment effect.

Secondly, engineered vector properties can 'cloak' the viral vector from (or modulate) the innate and adaptive immune responses (Büning and Srivastava, 2019; Prasad et al., 2022). Both arms of the AAV-directed immune response must be targeted to achieve the goal of mitigating GTAU.

5.1. Modification of AAV capsid

5.1.1. Modifications to maximise transduction efficiency

Modifying viral peptides displayed on the capsid surface may improve vector binding and entry to target cells in the retina. This is most relevant for viral vectors targeting the outer retina and intended for intravitreal or suprachoroidal administration for which penetration of the ILM or Bruch's membrane respectively is a key obstacle.

Dalkara et al. described a peptide of 10 amino acids, referred to as '7m8', which was identified through directed *in vivo* evolution for its ability to improve transduction (Dalkara et al., 2013). When added to the capsids of AAV2 and AAV9, but not other serotypes (AAV5 and AAV8), 7m8 led to greater expression of the reporter transgene in the outer retina after intravitreal administration. When injected

subretinally, a similar result was also seen for AAV8-7m8 compared to its parental serotype (Khabou et al., 2016).

Another 'evolved' AAV capsid capable of improved ILM penetration following intravitreal administration is the R100 capsid from 4D Molecular Therapeutics (Emeryville, CA, USA), discovered using directed evolution in NHPs (Kotterman et al., 2021). The derivative AAV vector, 4D-150, encodes miRNA targeting VEGF-C and a codon-optimised version of aflibercept (Khanani et al., 2023, 2024b), which is undergoing clinical investigation as a treatment for exudative AMD (PRISM trial, NCT05197270) and diabetic retinopathy (SPECTRA trial, NCT05930561).

For improved subretinal efficacy, the novel AAV.SPR capsid from Atsena Therapeutics (Durham, NC, USA) demonstrates enhanced lateral spread of transgene expression beyond the subretinal bleb (Couto et al., 2022; Neuringer et al., 2023). This capsid variant is currently being tested in the dose-escalation LIGHTHOUSE trial in X-linked retinoschisis (NCT05878860).

AAVv128 is an additional capsid variant, discovered by rational design (Luo et al., 2024). AAVv128 exhibits an enhanced rate and area of transduction of the RPE and retina across several routes of vector delivery (subretinal, intravitreal and suprachoroidal) and animal models. However, it is yet to be translated clinically.

5.1.2. Modifications for immune evasion

Conjugation of polyethylene glycol (PEG) to the exposed viral capsid surface, or PEGylation, is one example of 'cloaking' modification. PEGylation may shield antigenic loci of AAV particles from immune recognition without affecting transduction efficiency, although this effect may be limited for intensely immunogenic or large antigens (Lee et al., 2005).

PEGylation may also facilitate 'proactive' modulation of the immune response. For instance, rapamycin or sirolimus, an inhibitor of mTOR (mammalian target of rapamycin) kinase, can be conjugated to PEGylated AAV and is released upon phagocytosis of the vector by APCs (Zhang et al., 2019). Combined systemic administration of rapamycin with AAV induces targeted suppression of APC maturation and anti-AAV cell-mediated and humoral immune responses (Meliani et al., 2018).

In a similar vein, Bentler et al. described an engineered AAV2 capsid (AAV2.MB453) containing a peptide (RDVLPGT) thought to interfere with innate TLR signalling via the MyD88 pathway (Bentler et al., 2023). The addition of RDVLPGT not only improved the transduction efficiency of the viral vector in human hepatocytes *in vitro* but also reduced the adverse immune response following systemic administration in mice *in vivo*.

Further pre-clinical studies are needed to elucidate how bioconjugation of AAV vectors affects the cell-mediated immune response in GTAU and transduction efficiency in the eye.

5.2. Modification of AAV genetic material

5.2.1. Modifications to increase transgene expression

Codon optimisation of the transgene sequence *per se* can be performed to increase transgene expression through improved translational kinetics (Foster et al., 2008). Optimisation of the human ornithine transcarbamylase codon sequence was shown to increase transgene expression in the livers of injected mice compared to self-complementary and unoptimised AAV vectors (Bell et al., 2016). Similarly, Wu et al. demonstrated that a codon-optimised vector caused 5.5- to 21.2-fold greater transgene expression, although this increase compared a single-stranded unoptimised vector to a self-complementary, optimised one (Wu et al., 2008).

In the context of retinal gene therapy, codon optimisation of the highly repetitive human *RPGR* gene ORF15 sequence has enabled successful cloning and packaging of the full-length *coRPGR* into AAV vectors (Fischer et al., 2017). This vector has demonstrated successful rescue of retinal sensitivity and structure in a Phase I/II clinical trial with a favourable immune response profile (Cehajic-Kapetanovic et al., 2020).

5.2.2. Modifications for immune evasion

Codon optimisation may also be used to reduce the number of CpG sequences in the AAV genome, particularly in the transgene region, helping vectors to evade TLR9 detection in infected cells. This has shown some promise in reducing the immunogenicity of viral vectors, reducing proliferation of naïve T cells in mice (Bertolini et al., 2021).

However, while CpG depletion of the AAV genome appears to reduce the innate immune response against the viral vector, activation of memory $CD8^+$ T cells in the presence of viral capsids, which may exist following previous natural exposure to AAV, remains relatively unaffected (Xiang et al., 2020). In addition, the extent to which the AAV genome may be depleted of CpG motifs without affecting the function of critical regulatory elements (such as promoters and ITRs) is limited.

An alternative strategy has been devised to cloak the AAV genome from TLR9 recognition by inclusion of inhibitory oligonucleotide sequences into the untranslated region of the vector construct (Chan et al., 2021b). This sequence comprises multiple copies of the 'TTAGGG' motif found in human telomeric sequences, denoted as 'TLR9i' for 'TLR9 inhibitory'. When incorporated *in cis* within an AAV2 vector construct encoding green fluorescent protein (GFP), the TLR9i-containing vectors led to improved transduction and reduced T cell infiltration in mouse retinas following intravitreal delivery. Similarly, engineered AAV8.*GFP* vectors also demonstrated evasion of innate and adaptive immune responses and photoreceptor pathologies compared to standard vectors in healthy pigs when administered subretinally. When incorporated *in cis* within an AAV2 vector encoding aflibercept, they delayed the onset of GTAU responses but not severity when delivered intravitreally in NHPs. This may be due to the higher vector dose tested, the nature of the transgene product, and/or activation of alternative immune pathways.

Another potential avenue to reduce the likelihood of a transgene-directed immune response following the intraocular administration of a high dose of viral capsids is to use a transgene cassette containing an inducible regulatory element (Willett and Bennett, 2013). Delayed expression of the transgene could potentially avoid a 'primed' immune response against the transgene product following vector delivery. This approach may be particularly useful in suprachoroidal gene therapy in which anti-transgene activity appears to be more common (Chung et al., 2021). Several pharmacological induction systems, such as rapamycin, steroid and tetracycline-responsive elements have been used in AAV design. These can provide long-term transgene expression in the retina following either subretinal or intravitreal injection, controlled by either ubiquitous or tissue-specific promoters (Sanftner et al., 2001; Stieger et al., 2006). Inducible systems are inherently limited by their requirement for co-administration of a positive regulator. However, Reid et al. illustrated the functional viability of an inducible transgene system in a murine model of wet AMD (Reid et al., 2018) while others have demonstrated its capability in IRDs (Lhériteau et al., 2010). The potential benefits of this approach for evading the immune response against the transgene product or for improving treatment durability (e.g. prevention of immune-mediated rejection of transduced cells by temporarily 'switching off' transgene expression in the weeks following surgery) remain to be explored.

6. Understanding the impact of vector dose

Irrespective of vector design or underlying retinal pathology, the likelihood and severity of GTAU appears to be dependent on vector dose. In fact, this was clear in one of the earliest clinical trials of retinal gene therapy for *RPE65*-associated IRDs, which illustrated the narrow therapeutic margin of AAV vectors (Bainbridge et al., 2015). Three out of eight participants who received the higher dose of vector (1×10^{12} vg/eye) but none in the lower dose cohort (1×10^{11} vg/eye) developed intraocular inflammation despite prophylactic peri-operative

immunosuppression. Two participants developed optic disc swelling, retinal vascular tortuosity/sheathing and mild vitritis. Notably, one participant developed anterior uveitis at 2 weeks, followed by CRA and reduced visual acuity by 6 months.

The doses of AAV vectors that have been used in retinal gene therapy trials have ranged from ~1×10^8 (Heier et al., 2017) up to ~3×10^{12} vg/eye (Yang et al., 2022; Pierce et al., 2024). In general, the likelihood of intraocular inflammation following vector injection significantly increases when the dose exceeds around 5×10^{10} to 1×10^{11} vg/eye. However, given the variation in the manifestations of intraocular inflammation following retinal gene therapy, the impact of vector dose for each route of administration is explored in turn below.

6.1. Intravitreal route

Intraocular inflammation consisting of vitritis and anterior chamber inflammation following intravitreal delivery is most often seen at viral vector doses greater than around 1 to 5×10^{10} vg/eye (Lam et al., 2022; Pennesi et al., 2022; Vignal-Clermont et al., 2018). Table 2 illustrates the growing likelihood of intraocular inflammation with increasing vector doses in intravitreal delivery across a range of vector designs and retinal diseases. The results do not take into account the severity of intraocular inflammation, patient age (both adult and paediatric populations are included), and use of prophylactic immunosuppression.

Dose-escalation trials of intravitreal gene therapy often cross this threshold to identify the maximum tolerated dose of a given viral vector to use in subsequent pivotal trials. For example, the REVEAL study (NCT02064569) explored the response to an AAV2*.2-ND4* vector (lenadogene nolparvovec) in 15 patients with LHON across four dose cohorts: 9×10^9, 3×10^{10}, 9×10^{10}, and 1.8×10^{11} vg/eye (Vignal-Clermont et al., 2018). Extended anterior chamber inflammation, vitritis or keratitis occurred in one patient in the lowest dose cohort and in all patients at higher doses. Therefore, the subsequent studies of intravitreal ledanogene nolparvovec in LHON selected a dose of 9×10^9 vg/eye (Vignal-Clermont et al., 2023). Unfortunately, 75 % of 174 patients across the three trials that followed still developed intraocular inflammation after administration (compared to 10 % of sham-injected eyes). Despite this, these studies have suggested improved best corrected visual acuity lasting to at least 3 years after treatment.

Table 2
Selected clinical trials of intravitreal gene therapy with vector dose and the proportion of patients that developed intraocular inflammation within 6 months of treatment. Clinical trials applying AAV vector to the retina via intravitreal injection are listed by clinical trial number. Only published trials that stratify the report of intraocular inflammation by a known vector dose are shown. The doses that were associated with intraocular inflammation in that clinical trial are shown in bold. There is a greater proportion of dose cohorts with patients that develop intraocular inflammation beyond a vector dose of 1×10^{10} vg/eye. *These trials are either ongoing (NCT05197270) or terminated (NCT04418427) with data available in conference presentations or a press release respectively.

Study	Number [%] of participants with intraocular inflammation after intravitreal gene therapy by AAV vector dose per eye				
	Below 5×10^9 vg	5×10^9 vg (inc.) to 1×10^{10} vg	1×10^{10} vg (inc.) to 5×10^{10} vg	5×10^{10} vg (inc.) to 1×10^{11} vg	1×10^{11} vg and above
NCT02161380	**3 [14]**		**5 [71]**		
NCT01024998	0 [0]	0 [0]	**1 [10]**		
NCT02317887	0 [0]		**1 [33]**		**4 [80]**
NCT01267422		0 [0]	0 [0]		
NCT05197270*		0 [0]	**3 [12]**	**3 [12]**	
NCT02064569		**1 [33]**	**2 [67]**	**6 [100]**	**3 [100]**
NCT02652780				**34 [92]**	
NCT02652767				**29 [74]**	
NCT03293524				**69 [70]**	
NCT04418427*					**5 [21]**
NCT03748784					**16 [53]**
NCT02416622					**25 [93]**

6.2. Subretinal route

Similarly, the results of clinical trials involving subretinal delivery of AAV vectors are shown in Table 3.

Based on the results from small cohort studies, increasing the total dose delivered to the eye but dividing AAV particles across more than one subretinal bleb appears to have little impact on the intraocular and systemic immune response compared to if the viral particles were concentrated within a single bleb (Jacobson et al., 2012; Ghazi et al., 2016; Weleber et al., 2016; Le Meur et al., 2018). This fits with the model of localised immune activation, blood-retinal barrier permeability and leukocyte infiltration underlying the retinal inflammation following subretinal treatment (Fig. 3). Moreover, this approach has the added benefit of treating a larger area of retina. It may be particularly helpful when treating children with IRDs with non-secreted transgene protein to prevent degeneration of the peripheral retina, as opposed to treating the macula alone with a single bleb. Whether therapeutic gene augmentation halts further retinal degeneration remains debated (Leroy et al., 2022).

However, caution to avoid bleb fusion is required as this may lead to retinal detachment and additional surgery (Jacobson et al., 2012). It may also increase the risk of vitritis following reflux of viral vector from

Table 3
Selected clinical trials of subretinal gene therapy with vector dose and the proportion of patients that developed intraocular inflammation within 6 months of treatment. Clinical trials applying AAV vector to the retina via a single subretinal bleb are listed by clinical trial number. Only published trials that stratify the report of intraocular inflammation by a known vector dose are shown. The doses that were associated with intraocular inflammation in that clinical trial are shown in bold. There is a greater proportion of dose cohorts with patients that develop intraocular inflammation beyond a vector dose of 5×10^{10} vg/eye. The most common vector dose across clinical trials is 1×10^{11} vg/eye. *A minority of patients in these trials received more than one retinotomy (i.e. several subretinal blebs).

Study	Number [%] of participants with intraocular inflammation after subretinal gene therapy by AAV vector dose per eye				
	Below 1×10^{10} vg	1×10^{10} vg (inc.) to 5×10^{10} vg	5×10^{10} vg (inc.) to 1×10^{11} vg	1×10^{11} vg (inc.) to 5×10^{11} vg	5×10^{11} vg and above
NCT03374657	0 [0]	**4 [67]**		**2 [67]**	
NCT03066258	**4 [67]**	**3 [50]**	**3 [50]**	**5 [21]**	
NCT03116113 (Phase I/II)	0 [0]	0 [0]	0 [0]	**5 [83]**	**2 [67]**
NCT01461213		0 [0]			
NCT00516477		0 [0]		0 [0]	
NCT03920007		0 [0]		**4 [44]**	
NCT01494805		0 [0]		**3 [13]**	
NCT03496012		**16 [47]**		**33 [48]**	
NCT02610582		0 [0]	**2 [67]**	0 [0]	
NCT04722107			**3 [25]**		
NCT02341807			0 [0]	0 [0]	
NCT01482195*			0 [0]	**1 [25]**	
NCT03116113 (Phase II/III)			**10 [91]**	**12 [100]**	
NCT03001310*			**2 [67]**	**4 [27]**	**3 [60]**
NCT01208389				0 [0]	
NCT00999609				**2 [10]**	
NCT02407678				**8 [27]**	
NCT02077361				**2 [33]**	
NCT02671539				0 [0]	
NCT03507686				**30 [45]**	
NCT02553135				**2 [33]**	
NCT03252847				**11 [34]**	
NCT00749957*				0 [0]	**3 [50]**
NCT00643747				0 [0]	**5 [63]**
NCT03872479				**2 [22]**	**2 [40]**

the first retinotomy as a result of the differential pressures of merged blebs. The latter may explain findings from a trial involving the subretinal injection of AAV2/4.RPE65p.*RPE65* at two to five extra-foveal or peripheral sites (with a constant vector titre of 6.0×10^{10} vg/mL) (Le Meur et al., 2018). Investigators also administered topical and systemic steroids for prophylaxis, which may have dampened the immune response. In general, there was little intraocular inflammation and no CRA aside from atrophy expected from surgical touch-down. Notably, however, the blebs merged in three patients, one of whom exhibited increased flare at Day 4 requiring an increase in the frequency of topical steroid administration. In another, the merged blebs may have contributed to foveal detachment and a reduced total retinal thickness and visual field coverage in the treated eye at 1-year post-treatment.

Increasing the total AAV vector dose across multiple subretinal blebs could also amplify the amount of secreted transgene product in the eye in the so-called 'biofactory' approach to retinal gene therapy. The creation of biofactories in the eye is a strategy that has thus far relied on either intravitreal (Heier et al., 2017) or single-site subretinal injection (Rakoczy et al., 2015; Campochiaro et al., 2024). Two pivotal trials of anti-VEGF biofactories in neovascular AMD are currently underway. ATMOSPHERE (NCT04704921) and ASCENT (NCT05407636) compare a single subretinal injection of ABBV-RGX-314 (REGENXBIO Inc. and AbbVie, USA) to ranibizumab and aflibercept respectively. Interestingly, neither study appears to employ peri- or post-operative prophylactic immunosuppression (REGENXBIO, 2021, 2022). Safety outcomes are therefore expected to be similar to the Phase II study in which ~30 % of patients suffered intraocular inflammation within 30 days of subretinal injection (Wykoff, 2023; Bhandari, 2024). There may be opportunity to reduce this by splitting the vector dose across several blebs, or indeed with immunosuppression or another method of vector administration.

6.3. Suprachoroidal route

Suprachoroidal administration of AAV vectors has been used in few dose-escalation clinical trials to date so it is difficult to identify a dose threshold in a similar way.

The unpublished results of studies by REGENXBIO (Rockville, MD, USA) in neovascular AMD and diabetic retinopathy suggest a rate of episcleritis in ~14 % of patients receiving a dose of 5.0×10^{11} vg/eye (Dhoot, 2022; REGENXBIO, 2023; Barakat, 2023) and ~37 % at 1.0×10^{12} vg/eye (REGENXBIO, 2024). Post-operative ocular hypertension also appeared to exhibit dose dependency and was twice as prevalent at the highest versus lowest dose in the AAVIATE study without prophylactic steroids (REGENXBIO, 2024). Full results are awaited and will inform future studies and implementation of suprachoroidal AAV delivery.

6.4. Limits to understanding the impact of vector dose

The trends described above provide some insight into the relationship between vector dose and the likelihood and severity of intraocular inflammation in retinal gene therapy. However, formal meta-analysis and multivariate linear regression across clinical studies to understand the true impact of dose is difficult for several reasons.

Firstly, one of the most commonly used methods for quantifying viral genome titres in final GMP-grade AAV vector batches – quantitative polymerase chain reaction (qPCR) – produces variable estimates depending on the exact process employed, e.g. using primers targeting the transgene versus ITRs (Werling et al., 2015; D'Costa et al., 2016; Wang et al., 2020; Martinez-Fernandez de la Camara et al., 2021). More precise methods of vector genome titre analysis, such as droplet digital PCR (ddPCR), are difficult to scale but are gaining adoption (Gimpel et al., 2021). Overall, vector titering may vary between and within manufacturing sites (Lock et al., 2014; Ayuso et al., 2014). This complicates comparison between vector lots across clinical studies without a reference standard (Kontogiannis et al., 2024), which should ideally be reported with clinical trial results if possible.

Secondly, subretinal injections may be associated with varying amounts of reflux of AAV vector into the vitreous cavity. Vector doses are often reported by the volume of vector product injected and viral genome titre, allowing estimation of the number of viral particles delivered. For instance, Michaelides et al. reported that study participants received vector doses ranging from 0.3 to 4.5×10^{11} vg/eye, based on viral vector titres ranging from 0.1 to 1.0×10^{12} vg/mL and subretinal injection volumes of up to 0.5 mL (Michaelides et al., 2023). However, it is not possible to know how many particles were contained within the subretinal bleb versus those that were lost to reflux in each case. Similarly, while information about the exact nature of intraocular inflammation in patients undergoing retinal gene therapy is sometimes available (i.e. inflammation in anterior vs. intermediate vs. posterior compartments), this is not always the case. As discussed, subretinal and intravitreal viral particles confer different risks in the diverse manifestations of GTAU, which are also associated with different consequences for vision. Detailed reporting of administration variables, which could be helped by surgical adjuncts such as intra-operative OCT for example, and the clinical manifestations of GTAU may help to elucidate the true impact of vector dose.

7. Optimising subretinal administration of AAV vector

The surgical technique for subretinal injection has reached a high level of safety with iterative refinement through numerous clinical trials and real-world delivery of voretigene neparvovec. In particular, the adoption of foot pedal-controlled viscous fluid injection systems has greatly improved injection pressure control during subretinal injection (Fischer et al., 2016; Xue et al., 2018). In contrast, intravitreal injection is straightforward and standardised, while suprachoroidal delivery is still relatively novel. A 'peel-and-puddle' surgical technique for depositing the vector solution directly over the bare inner retinal surface after ILM peeling has been shown to improve transduction, but still requires a complex surgical approach (Comander et al., 2016; Teo et al., 2018). Strategies to optimise or simplify subretinal administration of AAV vector may facilitate lower rates of vitritis and anterior uveitis in retinal gene therapy, principally by reducing the probability and amount of vector reflux (Fig. 4).

7.1. Surgical adjuncts and robotics

The recommended surgical protocol for the delivery of voretigene neparvovec is a 'one-step' (direct) subretinal injection. However, direct subretinal injection carries a significant learning curve and initial success rates can be suboptimal even among experienced surgeons (Huang et al., 2022). If the cannula is too far advanced, it may puncture the Bruch's membrane, which could result in a localised subretinal haemorrhage or lead to injection of vector into the sub-RPE space. Alternatively, if the cannula has not been advanced deep enough to penetrate the neurosensory retina before injection is initiated – an easy error to make given the lack of haptic feedback – a significant amount of viral vector may be injected into the vitreous cavity or may cause iatrogenic retinoschisis.

Intra-operative OCT (iOCT) guidance provides the surgeon with live feedback on the plane of injection and rate of bleb propagation (Xue et al., 2017; Lam et al., 2019). This is especially useful when attempting to gently detach the fovea with as small a volume as possible, for example treating photoreceptors in the central macula in X-linked RP (Lam et al., 2024). iOCT could also be used to capture cross-sectional images of the subretinal bleb immediately after vector injection and needle withdrawal. By applying the spherical cap formula (Xue et al., 2017) to iOCT images, one could estimate the volume of the subretinal bleb and therefore the doses of subretinal and refluxed vector particles to greater precision. This is not only helpful for understanding an individual's risk of different features of GTAU but also to facilitate detailed

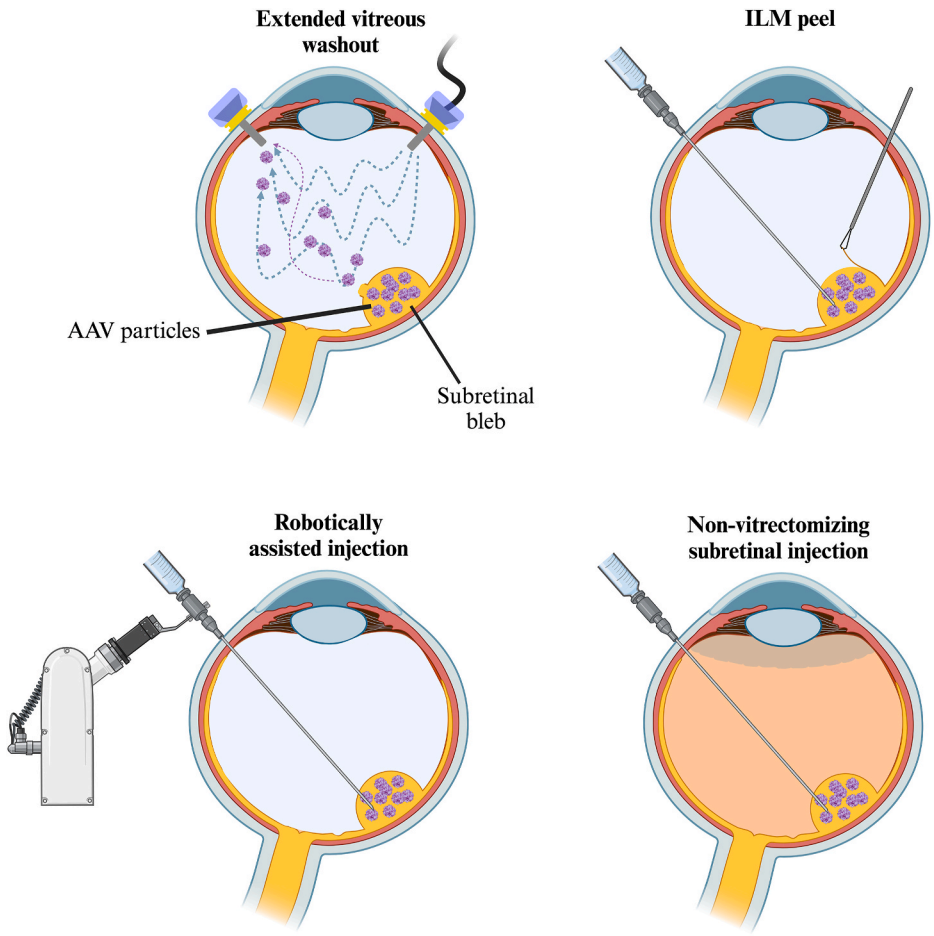

Fig. 4. Optimising subretinal administration of AAV vector to limit GTAU. Vitreous washout with infusion fluid is often performed for 3 min; extended washout may further reduce the impact of any reflux of the viral vector upon removal of the needle from the retinotomy. Surgical robotic assistance may facilitate greater precision in the creation of a retinotomy and one-step injection while limiting lateral movement of the needle and therefore the size of the retinotomy. Subretinal injection without vitrectomy or with a minimal vitrectomy may be viable especially in young patients in whom induction of posterior vitreous detachment (PVD) poses increased risk of surgical complications. The internal limiting membrane (ILM) could be removed over the intended injection site to reduce the required injection pressure and limit the risk of inadvertent injection into the vitreous, intraretinal or sub-RPE space.

reporting in clinical trials and meta-analysis as explored above.

One avenue to further improve surgical precision and to reduce reflux during direct subretinal injection is to use robotic assistance (Edwards et al., 2018; Cehajic-Kapetanovic et al., 2022). A telemanipulation system (such as the Preceyes Surgical System, Preceyes BV, now part of Zeiss Meditec AG) can provide tremor filtering, positional 'freeze', and micro-incremental advancement of the cannula tip. This enables reliable bleb induction at the precise retinal depth and slow bleb propagation with minimal vector reflux (Ladha et al., 2020). In addition, telemanipulation systems are typically compatible with intraoperative OCT, providing the surgeon with real-time feedback during injection.

7.2. Surgical technique

A different approach to subretinal injection is a 'two-step' injection with initiation of a small 'pre-bleb' using balanced saline solution (BSS) followed by slow propagation of the bleb using the viral vector (MacLaren et al., 2014). A considerable advantage of two-step injection is that it allows bleb propagation at a lower injection pressure (Scruggs et al., 2022). Alternatively, Okanouchi et al. found that removing the ILM over the site of the planned injection could reduce the injection pressure required to induce a subretinal bleb by 6 PSI (Okanouchi et al., 2016). However, the effect of this on the eventual size of the retinotomy and degree of vector reflux is unclear.

Avoiding vector reflux in a two-step approach requires the surgeon to insert the cannula through the 41G retinotomy a second time with minimal lateral movement during injection. This is technically demanding with potentially unavoidable enlargement of the retinotomy due to physiological tremor. As a result, reflux of vector could still happen during the injection and in the period after administration (Reichel et al., 2021).

It has been suggested that prolonged cannula retention in the subretinal space at the end of the injection may partially reduce reflux by plugging the retinotomy (Ladha et al., 2022). To minimise the risk of vector-induced vitritis, a thorough vitreous washout and/or additional 'shaving' of cortical vitreous can be effective (Reichel et al., 2021). In addition, supine posturing of the patient may be helpful to prevent reflux through the retinotomy in the immediate post-operative period.

The approach to subretinal injection should also take into account the specific retinal characteristics associated with the disease. For instance, various forms of RP exhibit relatively normal adhesion between the degenerate retina and the RPE (Davis et al., 2019; Chan et al., 2020); therefore, one-step injection at low pressures may be sufficient for bleb induction. Meanwhile, two-step injection is necessary in IRDs with strong adhesion between the surviving inner retina and Bruch's membrane as seen in choroideremia, which requires high injection pressures to detach a small central functional island (Xue et al., 2017, 2018).

Induction of posterior vitreous detachment (PVD) is routinely

performed as part of vitrectomy prior to subretinal vector administration but may be difficult in cases of strong adhesion. This may be important for the paediatric population, particularly as these patients stand to benefit most from retinal gene therapy (Maguire et al., 2009). Although the vitreoretinal interface may be less densely adherent in dystrophic eyes compared to the general paediatric population, PVD induction remains technically challenging and visualisation of the posterior hyaloid with intravitreal triamcinolone acetonide is almost always necessary.

Even with technical refinements, subretinal administration of gene therapy vector may be very difficult in certain IRDs. For example, X-linked retinoschisis demonstrates increased retinal fragility, making the detachment of posterior hyaloid and subretinal injection highly challenging (van der Veen et al., 2024). In this case, intravitreal gene therapy may be preferable, while accepting the higher probability of vitritis (Cukras et al., 2018; Pennesi et al., 2022). Cukras et al. observed 4 out of 9 cases of intraocular inflammation at 2–4 weeks following intravitreal injection of an AAV8.*RS1* vector to treat X-linked retinoschisis, including every patient treated with the higher dose (1×10^{11} vg/eye).

7.3. Emerging devices allowing less invasive subretinal injection

An approach that may not require PVD induction and vitrectomy involves a specially designed injection needle called the NANO SubRet Gateway Device (Vortex Surgical, MO, USA) (Wood et al., 2022). Proof-of-concept studies have been conducted in pig models with no observed post-operative intraocular inflammation, pressure changes or structural retinal damage following subretinal injection of a plasmid DNA vector. Notably, the technique also reduced operating time and vector reflux from the retinotomy compared with vitrectomized eyes (Stranak et al., 2024). Further innovation in this space may lead to simplification and improved safety of subretinal gene therapy in the future.

A second device is Orbit SDS (Gyroscope Therapeutics, acquired by Novartis in 2021), which allows subretinal injection via suprachoroidal cannulation. Orbit SDS received FDA 510(k) clearance in 2020 and has since been used in retinal gene therapy for atrophic AMD (Syncona, 2020; Gray et al., 2022; Nielsen et al., 2022; Hallam et al., 2024). Unfortunately, the programme to deliver AAV-mediated gene supplementation of complement factor I for atrophic AMD (see HORIZON NCT04566445 and EXPLORE NCT04437368, both Phase II studies), including using Orbit SDS, has since been terminated due to lack of efficacy.

8. Strategies for second eye treatment

8.1. Timing between eyes in bilateral treatment

The current protocol for voretigene neparvovec is to treat the second eye at least 6 days after the first (European Medicines Agency, 2019). Most patients have undergone second eye treatment 7 days after the first eye in real-world use (Fischer et al., 2024). This protocol was initially suggested to cover both treatments with a single course of systemic immunosuppression. However, some patients and clinicians may wish to monitor the therapeutic response in the first eye for a longer period of time before planning second eye treatment. In younger patients, this must also be balanced with the risk of amblyopia in the event of significant functional gain in the treated eye. Furthermore, there have been several cases of severe intraocular inflammation in the second eye following voretigene neparvovec administration in the first eye several weeks before, sometimes with detrimental consequences for vision (Jalil et al., 2023) (Fig. 2G, B. Leroy & F. Nerinckx, personal communication, Nov. 2024). This is potentially due to immunological memory of the adaptive arm exacerbating the response in the second eye. Controversy surrounds the optimal time interval with few comparative studies to date, which have mostly explored intervals of several years between treatments.

Bennett et al. conducted a clinical trial involving second eye subretinal gene therapy with an AAV2.CAG.*hRPE65* vector administered at an identical dose to the first eye (1.5×10^{11} vg) (Bennett et al., 2016). The trial involved 11 children and adults who had been treated 1.7–4.6 years previously. Apart from one participant who unfortunately developed culture-positive bacterial endophthalmitis after surgery, no participants demonstrated intraocular inflammation or CRA within three years of second eye treatment.

In contrast, Ku et al. reported CRA in the second treated eyes in three (out of four) patients who underwent bilateral subretinal gene therapy for *RPE65*-associated IRD (Ku et al., 2024). The first eyes of these patients were treated when they were aged 6–11 years, using a vector produced by AGTC (Applied Genetic Technologies Corporation) in a Phase I clinical trial (NCT00749957). The second eye was treated 6–10 years later with voretigene neparvovec. In this small cohort, it is difficult to assign the cause of atrophic changes to differences in vector dose or potency, as represented by the magnitude of functional improvement in these patients. Other possibilities include a sensitised cell-mediated adaptive response in the second eye or variables such as age and disease stage.

The GEMINI study also explored second eye subretinal gene therapy but used an AAV2.CAG.*REP1* vector (timrepigene emparvovec) to treat choroideremia (MacLaren et al., 2024). Three different inter-surgery intervals were explored: less than 6 months, 6–12 months, and greater than 12 months between treatments. Among 66 participants (with 53 completing the study), the rate of intraocular inflammation (45.5 %) was similar in both eyes and there was no significant correlation between the inter-surgery interval and rate of inflammation.

8.2. Implications of the humoral response for bilateral treatment

Intravitreal AAV administration is known to induce the formation of neutralising antibodies (NAb) in a dose-dependent manner (including antibodies that cross-react with several vector serotypes) (Heier et al., 2017; Ail et al., 2022; Lam et al., 2022; Pennesi et al., 2022). This is potentially problematic for retreatment for two reasons.

Firstly, NAb present in ocular fluid could block viral transduction of target cells following intravitreal vector administration, leading to reduced therapeutic effect in the second eye treated with the same dose and route (Bucher et al., 2021). In line with this, the presence of pre-existing NAb to AAV is strongly correlated with poorly sustained transgene expression following intravitreal gene therapy (Kotterman et al., 2015).

Secondly, adaptive immunological memory from intravitreal vector injection in the first eye could lead to a heightened and sustained humoral response following intravitreal treatment of the second eye with extended time between eyes (e.g. 3 months), as shown in wild-type NHPs (Bouquet et al., 2018). Antibodies could contribute to the innate immune response through antibody-opsonisation and complement activation (Fig. 3) and therefore intraocular inflammation (Ross et al., 2024). Indeed, Lam et al. demonstrated that the greatest increase of NAb titres occurred in patients with LHON that presented with GTAU after intravitreal injection of AAV vector (Lam et al., 2022). However, it must be noted that the association between the post-treatment serum antibody response and the severity of intraocular inflammation observed in patients is variable (Bouquet et al., 2019; Pennesi et al., 2022).

While simultaneous bilateral intravitreal gene therapy may be theoretically attractive, it is generally not a palatable clinical option due to concerns about the risk of detrimental bilateral vision loss in the event of complications (Willett and Bennett, 2013).

In contrast, subretinal gene therapy appears to induce NAb formation only infrequently in clinical trials and pre-clinical studies (Rakoczy et al., 2015; Reichel et al., 2018; Xue et al., 2018; Fischer et al., 2020). AAV vectors are also less susceptible to antibody neutralisation within a more immune privileged space (Li et al., 2008; Amado et al., 2010).

Repeated ipsilateral subretinal administration of AAV vector is also well tolerated in wild-type NHPs (Weed et al., 2019).

One option for second eye gene therapy regardless of the route used for the first eye is to administer vector to the second eye via the subretinal route (Bucher et al., 2021). Encouraging data for sequential bilateral subretinal administration of ABBV-RGX-314 in nAMD has recently been presented (Khanani, 2024). Second eye treatment more than one year after the first eye resulted in no intraocular inflammation and durable therapeutic effect; subretinal ABBV-RGX-314 effectively reduced the annualised anti-VEGF injection burden in second eyes by 97 %.

One potential option that could facilitate intravitreal injection in the second eye is to blunt the humoral immune response to AAV vector at the time of intravitreal injection in the first eye using targeted immunomodulation. For example, rituximab, an anti-CD20 monoclonal antibody, could be administered around the time of first eye treatment to prevent the resulting increase in NAb titres by temporarily depleting B cell populations (Mehta et al., 2021). B cell depletion has also been suggested as a strategy to limit the humoral response to vector administration elsewhere in gene therapy, although experimental models eventually developed anti-drug antibodies (ADAs) that constrained therapeutic transgene expression (Miao, 2011). Corticosteroids or other immunomodulatory drugs may be co-administered with rituximab to mitigate ADAs and improve its effect on antibody titres, a common strategy with biologics (Bitoun et al., 2023) and one that has also been effective in reducing anti-TRPM1 antibodies in cancer-associated retinopathy (Roels et al., 2017). Investigation of other immunomodulatory agents to mitigate the cell-mediated immune response in GTAU and/or the development of ADAs is of critical importance.

9. Strategies for prophylaxis and treatment of gene therapy-associated uveitis

Observations in pre-clinical studies and clinical use of AAV vectors in retinal gene therapy around the risk and natural history of GTAU highlight the need to control the immune response by adequate dosing and duration of immunosuppression.

Currently, corticosteroids are the main agent used for the prophylaxis and treatment of GTAU. Typical regimes comprise any combination of systemic corticosteroids, topical drops and/or periocular injection (e.g. conjunctival or sub-tenon) or slow-release intravitreal steroid implant (Ozurdex, AbbVie Inc., IL, USA). In some trials involving groups at potentially greater risk of GTAU, such as children, the protocol includes the option to administer several doses of intravenous methylprednisolone (Michaelides et al., 2024). As mentioned above, triamcinolone acetonide may also be used to visualise the posterior hyaloid for PVD induction prior to subretinal injection (Dimopoulos et al., 2018; Scruggs et al., 2022; Michaelides et al., 2024). Residual amounts left in the eye, albeit in small doses, may provide short-term local immunosuppression.

However, there are significant shortcomings of the corticosteroid-based approach in practice, explored in greater detail below.

9.1. Prophylaxis formulation

Firstly, the exact prophylaxis regimen used across clinical studies varies hugely or may be omitted altogether or is unreported. We analysed over 100 active or completed, unique clinical studies of AAV-mediated retinal gene therapy that were registered on *clinicaltrials.gov* and conducted worldwide. 39.4 % of studies (n = 43) documented a prophylaxis regimen in the clinical trial registration page, protocol or results published in peer-reviewed literature or available online. 79.1 % (n = 34) of these regimens included systemic corticosteroids. No steroid prophylaxis was confirmed in 8.3 % (n = 9) of studies, and the majority or 52.2 % (n = 57) had no identifiable details. Given the current variability in prophylaxis strategy, detailed reporting of regimens in clinical trials is pivotal for calculating the true prevalence of GTAU, identifying an optimal immunosuppression strategy, and elucidating relationships between host, vector and administration-related risk factors for GTAU. Few clinical trials have assessed different prophylaxis regimes as a built-in component of the study.

One example is the LUNA Phase II trial (NCT05536973), which follows the success of the Phase I OPTIC trial (Khanani et al., 2024a). The LUNA trial will assess various combinations of topical difluprednate, oral prednisolone, and the Ozurdex implant for the intravitreal administration of an AAV2-7m8.aflibercept vector at two doses to treat neovascular AMD. The unpublished results of LUNA, presented in early 2024, suggest that local immunosuppression through combined topical difluprednate and Ozurdex may be the best regimen in this patient population with no added benefit of systemic prednisolone (Kiss, 2023; Adverum, 2024).

Meanwhile, the AAVIATE trial included one cohort of 21 patients (20 %) that received either a single sub-tenon steroid injection at the time of suprachoroidal gene therapy or 6 weeks of post-operative steroid drops. No cases of intraocular inflammation were reported in the patients receiving steroid drops while sub-tenon steroid alone seemed to have little impact on the rate of inflammation (~15–25 %) (REGENXBIO, 2024).

9.2. Prophylaxis duration

The timing of immunosuppression prophylaxis is also of particular importance. Corticosteroids are currently recommended to start from 3 days prior to gene therapy with voretigene neparvovec and continued for 14 days afterwards (European Medicines Agency, 2019). This relatively modest duration of immunosuppression was intended to minimise steroid-related side effects but also to allow the sequential treatment of both eyes covered by a single course of immunosuppression (Russell et al., 2017).

However, 14–21 days of prophylactic immunosuppression does not encompass the duration of the cell-mediated immune response as seen in animal models and patients, which lasts for at least one month irrespective of administration route (Fischer et al., 2020; Tummala et al., 2021; Chandler et al., 2021). Consequently, GTAU is often diagnosed shortly after the withdrawal of systemic immunosuppression prophylaxis (Dimopoulos et al., 2018) or when the dose reaches around 15–20 mg for slower tapers, and sometimes several weeks to months after vector administration (Bainbridge et al., 2015). A longer period of effective immunosuppression or slow taper may be required to reduce the incidence of GTAU and improve treatment durability (Chan and Pepple, 2021).

9.3. Prophylaxis dose

The recommended strength of post-operative immunosuppression begins at 1 mg/kg/day of oral prednisolone up to a maximum of 40 mg/day (European Medicines Agency, 2019). This is tapered down over the subsequent two weeks. Some have implemented this dosing while others have increased it to a maximum of 80 mg/day given the potential for weight-adjusted underdosing, and slowed the taper over 6–8 weeks (Russell et al., 2017; Lam et al., 2019; Kessel et al., 2022; Pennesi et al., 2022).

In theory, one would expect a lower incidence of GTAU with a higher dose and longer duration of corticosteroid prophylaxis. Interestingly, however, this may not be the case. For example, the Phase I/II trial of cotoretigene toliparvovec (Nightstar Therapeutics, acquired by Biogen, MA, USA) in RP employed a 3-week tapering course of systemic immunosuppression (Cehajic-Kapetanovic et al., 2020). Unfortunately, 7 of 18 participants still developed intraocular inflammation following subretinal vector administration. The regime was extended to 3 months for the subsequent Phase II/III trial, yet inflammation was still reported in almost all participants at similar vector doses (Lam et al., 2024).

The evidence is similar with intravitreal delivery. The REVERSE

(NCT02652780), RESCUE (NCT02652767), and REFLECT (NCT03293524) Phase III studies in LHON all reported a similar incidence of intraocular inflammation with the same construct and dose of AAV vector despite different prophylaxis regimes. Patients in REFLECT received 4 weeks of peri-operative oral prednisolone, unlike in either REVERSE or RESCUE (Yu-Wai-Man et al., 2020; Newman et al., 2023; Vignal-Clermont et al., 2023).

The emergence of GTAU despite high-intensity corticosteroid-based immunosuppression is also not unique to AAV vectors in retinal gene therapy. Local steroids, IV methylprednisolone, and oral prednisolone unfortunately did not prevent one adult with RP associated with Usher syndrome from developing severe panuveitis following subretinal administration of a lentivirus vector (Parker et al., 2024).

9.4. Side effects of corticosteroids

In addition, the profound adverse effects of high-dose and long-term corticosteroids are well known (Gaballa et al., 2021). Briefly, gastrointestinal disturbance, osteoporosis, metabolic syndrome, cardiovascular disease, acne, sleep changes, cataracts, glaucoma, and the resulting polypharmacy (proton pump inhibitors, vitamin D, statins, antihypertensives, etc.) can severely disrupt patient quality of life. Young patients are more likely to suffer given the higher cumulative doses of corticosteroids that will be required over a lifetime, yet are also the most likely to benefit from retinal gene therapy (Maguire et al., 2009).

Compounded by the potential for GTAU that is refractory to corticosteroid immunosuppression as discussed above, it is understandable that many are now looking to steroid-sparing immunomodulation and emerging therapies to restore immune homeostasis in the eye following AAV-mediated retinal gene therapy (Chan and Pepple, 2021).

9.5. Opportunities for steroid-sparing strategies in gene therapy-associated uveitis

Fig. 3 illustrates some of the pathways through which the immune environment is disrupted in the eye following AAV delivery. Immunomodulation holds potential to restore immune homeostasis by interrupting the adverse immune response at cellular, cytokine, and blood-retinal barrier levels although it has been used in few retinal gene therapy trials to date.

In particular, immunomodulation may benefit patients with *cross-reactive immunological material (CRIM)-negative* status undergoing gene augmentation therapy (Prasad et al., 2022). The adaptive immune response may be heightened in these patients, who lack the immune tolerance of the therapeutic transgene product normally gained early in life from thymic selection. Similarly, entirely foreign cargo packaged into AAV to treat IRDs, such as the bacterial CRISPR/Cas9 gene editing system (Pierce et al., 2024) or optogenetic actuators derived from microbes (Gaub et al., 2018), may be more likely to activate innate and/or adaptive arms of the immune system and lead to severe responses that may require more aggressive immunosuppression (Mehta and Merkel, 2020; Ren et al., 2022).

Selected immunomodulatory agents are given in Table 4 along with their more commonly reported adverse effects. An important consideration is the route of administration. Ocular administration might control inflammation while lowering the risk of systemic adverse effects. However, this is currently possible only for a very limited number of agents. Future developments might include intra- or periocular, drug-

Table 4
Selected immunomodulatory agents with the potential to ameliorate the disruption to immune homeostasis in the eye following AAV-mediated retinal gene therapy.

Agent	Target (mechanism of action)	Potential use in retinal gene therapy to prevent and treat GTAU	Currently available routes of administration	Potential adverse effects (dependent on dose and route of administration)
Mycophenolate mofetil	Type II inosine monophosphate dehydrogenase (IMPDH) (inhibition)	Reduce effector T and B cell proliferation	• Oral • Intravenous	Alopecia, cytopaenias, hyperglycaemia (diabetes mellitus), abdominal pain, nausea and vomiting, diarrhoea
Tacrolimus	Calcineurin (inhibition)	Suppress IL-2 production and effector T cell differentiation	• Oral • Intravenous • Topical cutaneous ointment	Headache, alopecia, cytopaenias, hyperglycaemia (diabetes mellitus), tremor, pruritis, nausea and vomiting, diarrhoea, muscle cramps, nephrotoxicity, neurotoxicity
Ciclosporin	Calcineurin (inhibition)	Suppress IL-2 production and effector T cell differentiation	• Oral • Intravenous • Topical eye drops	Seizures, tremor, hirsutism, gingival hyperplasia, nausea and vomiting, diarrhoea, constipation, hepatotoxicity, nephrotoxicity, neurotoxicity
Hydroxychloroquine	Lysosomes in antigen-presenting cells (increases pH), cGAS and TLR9 (inhibition)	Interfere with viral antigen presentation and the intracellular innate immune response to the AAV genome	• Oral	Headache, rash and hyperpigmentation, pruritis nausea and vomiting, diarrhoea, myositis, cardiotoxicity, retinopathy
Methotrexate	Dihydrofolate reductase (DHFR) (inhibition) and IL-1β (inhibition)	Reduce immune cell proliferation and pro-inflammatory cytokine function	• Oral • Intravenous • Subcutaneous • Intramuscular • Intrathecal • Intravitreal	Flu-like symptoms, cytopaenias, mucositis, nausea and vomiting, diarrhoea, hepatotoxicity, pneumonitis, nephrotoxicity
Sirolimus (rapamycin)	Mammalian target of rapamycin (mTOR) (inhibition)	Reduce T cell sensitivity to IL-2 and therefore effector function	• Oral • *Investigation of intravitreal administration (Blair* et al., *2017*; *Nguyen* et al., *2018)*	Cytopaenias, rash, mucositis, dyslipidaemia, hyperglycaemia (diabetes mellitus), oedema, pneumonitis, nephrotoxicity
Adalimumab	Tumour necrosis factor alpha (TNFα) (inhibition)	Reduce pro-inflammatory cytokine function	• Subcutaneous • *Investigation of intravitreal administration (Leal* et al., *2018)*	Headache, hyperglycaemia, nausea, dizziness, psoriasis, tuberculosis (reactivation), cardiotoxicity, hepatotoxicity
Fingolimod	Sphingosine-1-phosphate receptor 1 (SP1R1) (inhibition)	Sequester lymphocytes in lymph nodes and reduce migration into the retina	• Oral	Headache, cytopaenias, nausea and vomiting, diarrhoea, cough and dyspnoea, hepatotoxicity, cardiotoxicity, macular oedema
Rituximab	CD20 (inhibition)	Selectively reduce B cell function and the humoral response to (intravitreal) AAV administration	• Intravenous • Subcutaneous	Headache, flu-like symptoms, cytopaenias, rash, pruritis, cough and dyspnoea, nausea and vomiting, diarrhoea, nephrotoxicity, cardiotoxicity

eluting implants, hydrogels, and micro- or nanoparticle delivery systems that can achieve therapeutic concentrations in the posterior segment for several months (Lance et al., 2015; Kim and Woo, 2021) following AAV gene therapy.

Many immunomodulatory agents are currently used to treat non-infectious uveitis, providing evidence of long-term safety data to lower the translational barrier for use in GTAU (Dick et al., 2018). Early clinical evidence further supports the use of immunomodulation to prevent and treat GTAU in the context of IRDs, discussed below.

9.5.1. Cellular immunomodulation

The antimetabolites, mycophenolate mofetil and methotrexate, are equally effective for controlling inflammation in non-infectious uveitis (Rathinam et al., 2014, 2019; Bui et al., 2022). Mycophenolate is relatively well tolerated, including in young children in whom almost 90 % are able to significantly reduce or cease systemic prednisolone use (Doycheva et al., 2007). Methotrexate has also long been used to treat paediatric uveitis and rheumatoid disease (Wieringa et al., 2019).

The successful implementation of steroid-sparing immunomodulation elsewhere in AAV gene therapy has facilitated its effective translation to treat GTAU. For example, dose-dependent, AAV-specific T cell activation was seen in patients who received intramuscular gene therapy with an AAV1 vector (alipogene tiparvovec, Glybera) for lipoprotein lipase deficiency (Mingozzi et al., 2009; Ferreira et al., 2014). A triple regimen of ciclosporin, mycophenolate and corticosteroids was used in patients receiving high vector doses (Gaudet et al., 2012), an established immunosuppression regimen used to prevent solid organ transplant rejection (Nelson et al., 2022). Initially, transient T cell activation was still seen in 64 % of patients. However, when the immunomodulation regime was introduced shortly before vector administration, Granzyme B and Fas ligand expression was reduced, indicative of a diminished $CD8^+$ T cell response against transduced cells (Ferreira et al., 2013). This would support the current clinical practice of starting immunosuppression a few days before AAV administration to capture the initial phase of the cell-mediated immune response.

Several years later, the same prophylactic regime of ciclosporin, mycophenolate and corticosteroids was used in two cases of X-linked retinoschisis treated with relatively high doses (1×10^{11} and 3×10^{11} vg/eye) of intravitreal AAV vector (Mishra et al., 2021). Prophylaxis was commenced two to three weeks before treatment. Patients subsequently demonstrated blunted or delayed intraocular inflammation compared to participants receiving lower vector doses with corticosteroid prophylaxis alone.

Meanwhile, methotrexate has effectively treated three cases of chronic uveitis after intravitreal AAV gene therapy for X-linked retinoschisis, which occurred despite systemic corticosteroids and a periocular steroid depot (Pennesi et al., 2022). Specifically, methotrexate reduced the frequency and severity of flares over 1–2 years of prolonged GTAU (P. Yang, personal communication, Nov. 2024). However, all patients elected to stop methotrexate due to the COVID-19 pandemic. Thereafter, one patient entered durable drug-free remission, whereas another patient had recurrence of chronic uveitis in the absence of maintenance therapy. The third patient was lost to follow-up. These limited results provide proof-of-concept for the efficacy of antimetabolites to prevent and treat GTAU.

T cell-specific modulatory therapies that are used in non-infectious uveitis, such as calcineurin inhibitors (e.g. tacrolimus), could also be considered (Hogan et al., 2007). Other T cell targets and agents include PD-1 (e.g. peresolimab), CD28 and ICOS (e.g. acazicolcept), and BTLA (e.g. LY3361237) (Orvain et al., 2022; Kojima et al., 2023; Tuttle et al., 2023). Acazicolcept can reduce retinal inflammation in rodent models of posterior uveitis (Wilson et al., 2023) but these agents are yet to be applied to models of GTAU.

9.5.2. Complement

Since the complement system plays important roles in phagocyte recruitment, opsonisation of immunogenic pathogens and destruction of transduced cells, modulation of complement activation may be useful to prevent and treat retinal inflammation induced by AAV gene therapy (Fig. 3). In the future, this would be ideally assessed in animal models and patients undergoing gene therapy for geographic atrophy given the potential adjunctive therapeutic benefits.

Several inhibitors of the complement cascade have already been approved by the FDA, including two intravitreal treatments for geographic atrophy: pegcetacoplan (Syfovre, Apellis Pharmaceuticals Inc., MA, USA), a complement C3 inhibitor, and avacincaptad pegol (Astellas Pharma Inc., Tokyo, Japan), a complement C5 inhibitor. Eculizumab, a monoclonal antibody complement factor 5 inhibitor, has also been used to successfully treat severe and refractory adverse effects of systemically administered AAV gene therapy vectors, such as AAV9. *SMN1* (Zolgensma, Novartis, Basel, Switzerland) for spinal muscular atrophy (Chand et al., 2021; Prasad et al., 2022).

9.5.3. Cytokine and barrier immunomodulation

Anti-TNFα therapy is currently used to treat severe, refractory uveitis in adults and children. However its efficacy may be hampered by the development of anti-drug antibodies, similar to rituximab as discussed (Bellur et al., 2023). This may also be mitigated somewhat by the co-administration of mycophenolate or methotrexate (Ungar et al., 2017; Pichi et al., 2024).

Fingolimod is thought to reduce intraocular inflammation by preventing immune cell migration into the retina (Raveney et al., 2008). It is FDA-approved as a treatment for multiple sclerosis but can also significantly reduce retinal inflammation and macrophage and $CD4^+$ T cell infiltration in murine models of uveitis (Copland et al., 2012). Investigation of its potential utility in GTAU may be beneficial given the importance of the cell-mediated adaptive immune response in the retina.

9.5.4. Inhibition of intracellular anti-viral responses

Therapeutic adjuncts that significantly improve AAV transduction efficiency can reduce the risk of GTAU without the need to modify the existing gene therapy vectors or compromise clinical efficacy by reducing the number of viral particles required for therapeutic effect.

Poly (ADP-ribose) polymerase (PARP-1) is a DNA damage response protein that can negatively affect AAV transduction and also acts as a transcriptional cofactor for genes implicated in the inflammatory processes in the retina (Hassa and Hottiger, 1999; Brady et al., 2018). Interestingly, intravitreal injection of the PARP inhibitor, olaparib, has shown promising neuroprotective effect in mouse models of IRDs (Sahaboglu et al., 2016). Moreover, PARP-1 inhibition can attenuate the AAV capsid-directed cytotoxic T cell response, downregulate TLR and NF-kB expression, and improve the transduction efficiency of AAV vectors by three-fold in the liver (Hareendran et al., 2016).

Hydroxychloroquine has been purported to interact with and antagonise the key intracellular nucleic acid sensors, TLR9 and cGAS, which likely contribute to its clinical efficacy in rheumatoid arthritis and systemic lupus erythematous (Han et al., 2020). Subretinal co-administration of hydroxychloroquine (at a relatively low concentration of 19 μM) alongside AAV vectors led to a three-fold improvement of viral transduction *in vivo* without any signs of retinal toxicity (Chandler et al., 2019). Therefore, adjunctive local administration of hydroxychloroquine may enable a significant reduction in the AAV dose required to achieve therapeutic effect.

9.5.5. Enhancing immune tolerance

Regulatory T (Treg) cells are central to maintaining immune homeostasis, limiting autoimmune activity, and can be exploited to drive antigen-specific and local immunosuppression. Endogenous Treg populations can be expanded *in situ* using clinically relevant doses of immunomodulatory agents such as sirolimus, ciclosporin, and mycophenolate and limit the severity of uveitis (Fanigliulo et al., 2015; Yuan et al., 2015; Llorenç et al., 2022; Muñoz-Melero and Biswas, 2024).

Alternatively, Tregs can be genetically engineered and expanded *ex vivo* to be applied as a cell-based therapy, a burgeoning but still experimental treatment modality (Abraham et al., 2023). Chimeric antigen receptor (CAR) Tregs achieve antigen specificity without reliance on MHC-mediated recognition (Arjomandnejad et al., 2021). Treg cell therapy has been used in early-phase clinical trials for autoimmune diseases and transplantation among other indications, demonstrating feasibility and safety. Both systemic and intravitreal administration of pre-activated Tregs can suppress cell-mediated non-infectious uveitis in animal models (Terrada et al., 2006; Grégoire et al., 2016). Immunomodulatory cell therapy is yet to be implemented in the context of retinal gene therapy due to the complexity of combining two advanced therapeutic modalities.

Antigen-specific immune tolerance could also be induced by 'subretinal-associated immune inhibition' (SRAII), a similar phenomenon to anterior chamber-associated immune deviation (ACAID) in the eye. SRAII has been described in healthy rodents and IRD models after receiving subretinal injections of immunodominant transgene peptide fragments alongside the AAV vector, significantly ameliorating the peripheral T cell response against the transgene product (Vendomèle et al., 2018, 2024). These intriguing findings open the possibility of inducing transgene-specific immune tolerance following subretinal injection without the requirement for the complexity and expense of cell therapy. The degree to which these findings would translate to humans remains unclear, especially as robust immune response against the transgene product appears to be less common following subretinal gene therapy in wild-type NHPs (Chung et al., 2021).

10. Conclusion and future outlook

The techniques underpinning AAV-mediated retinal gene therapy have undergone iterative improvement since the first-in-human studies in *RPE65*-associated IRDs (Bainbridge et al., 2008; Hauswirth et al., 2008; Maguire et al., 2008). Significant areas of optimisation include viral vector construct design and safe surgical administration. This has led us to the cusp of a major therapeutic modality applicable to a wide array of retinal diseases, including those more prevalent than IRDs, such as AMD and diabetic retinopathy.

However, concerning clinical observations of inflammatory and atrophic chorioretinal changes can influence treatment safety and durability, and at vector doses that overlap with the therapeutic threshold. This signals the need to improve the clinical delivery of this burgeoning treatment further. In order to develop effective mitigation strategies for the deleterious ocular immune response, termed *gene therapy-associated uveitis* (GTAU), we must first appreciate its immunological basis (Bucher et al., 2021) and clinical risk factors.

We provided a summary of our current understanding of the factors that contribute to GTAU, collating insights from clinical trials of AAV vector-mediated retinal gene therapy as well as real-world use of voretigene neparvovec. Factors that likely influence the risk of GTAU and are most relevant to treatment planning include host-specific (e.g. background disease severity, age and sex), vector-specific (e.g. genome identity), administration variables (dose and route) and the choice of immunosuppressive/immunomodulatory regime.

Greater understanding of the impact of these factors on an individual's risk of GTAU is an area of significant clinical need. Systematic reporting of vector production methodology, patient variables, and immunosuppression regimes would be critical for identifying trends across different patient cohorts. A related area for further improvement is the introduction of targeted prophylaxis and treatment strategies for restoring immune homeostasis in the eye after retinal gene therapy, guided by early experience of immunomodulation in a limited number of patients. This may be informed by pre-clinical research aiming to elucidate the key intracellular and cytokine signalling pathways activated among retinal and infiltrating immune cells in GTAU.

Although AAV has been the most widely used vector in retinal gene therapy to date, it is not the only available option to deliver genetic cargo to the retina. Recombinant AAV vectors have been optimised for the transduction of photoreceptors and RPE. However, AAV is limited by its small packaging capacity, which is a particular challenge for delivering CRISPR/Cas9-based gene editing constructs capable of targeted correction of pathogenic variants in a much wider range of IRDs (Quinn et al., 2021; Ng et al., 2024). This has created a need to explore alternative non-viral vector platforms for retinal gene therapy, such as liposome-based systems and synthetic nanoparticles (Salman et al., 2022). Innovation in nanoparticle delivery systems is rapidly addressing their biological challenges, including vector tropism in the retina and cargo unpackaging upon cell entry. Modulation of surface characteristics may help to improve transduction and reduce the dose required, thereby reducing the risk of GTAU (Mendes et al., 2022; Carvalho et al., 2023).

Another consideration for CRISPR/Cas9-based systems for retinal gene editing is the potential for a heightened adaptive immune response given the bacterial origin of the Cas9 enzyme and high prevalence of pre-existing immunity (Charlesworth et al., 2019). In a groundbreaking first-in-human study, EDIT-101 (Editas, Cambridge, Massachusetts, USA), comprising an AAV5 vector encoding *Staphylococcus aureus* Cas9 and two guide RNAs targeting *CEP290* associated with LCA, was well tolerated at relatively high vector doses (Pierce et al., 2024). Similarly, there is growing interest in delivering optogenetic proteins to the surviving inner retinal cells in IRDs as a disease-agnostic approach to retinal gene therapy (McClements et al., 2020). Many opsins under investigation are derived from microbes, including channelrhodopsin-2 and ChrimsonR, which have been used in early clinical trials with vectors delivered intravitreally with full results keenly awaited (Sahel et al., 2021). However, the potential immunogenicity of using non-viral vectors to deliver gene editing enzymes or opsins remains unclear and provides an important area for future investigation.

CRediT authorship contribution statement

Ryan Purdy: Writing – original draft. **Molly John:** Writing – original draft. **Alissa Bray:** Writing – review & editing. **Alison J. Clare:** Writing – review & editing. **David A. Copland:** Writing – review & editing. **Ying Kai Chan:** Writing – review & editing. **Robert H. Henderson:** Writing – review & editing. **Fanny Nerinckx:** Writing – review & editing. **Bart P. Leroy:** Writing – review & editing. **Paul Yang:** Writing – review & editing. **Mark E. Pennesi:** Writing – review & editing. **Robert E. MacLaren:** Writing – review & editing. **M Dominik Fischer:** Writing – review & editing. **Andrew D. Dick:** Writing – review & editing. **Kanmin Xue:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interests

A.B. is an employee of Oxford Biomedica.

Y.K.C. has consulted for or served as an advisor to Ally Therapeutics, AlphaSights, Anjarium Biosciences, Arthur D. Little, Celestial Therapeutics, Cirrus Therapeutics, FirstThought, Pacira Biosciences, Santé, University of Bristol, and Xora Innovation and has received consulting fees and/or equity. Y.K.C. is an employee of Cirrus Therapeutics.

R.H. declares consultancy for Novartis, Janssen, Regenxbio, Tern Therapeutics, Sparing Vision, Neurogene, Axovia.

F.N. declares consultancy for DORC, Novartis, Leica Microsystems.

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A.D.D. is a co-founder of Cirrus Therapeutics.

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Data availability

The authors do not have permission to share data.

References

Abraham, A.R., Maghsoudlou, P., Copland, D.A., Nicholson, L.B., Dick, A.D., 2023. CAR-Treg cell therapies and their future potential in treating ocular autoimmune conditions. Front. Ophthalmol. 3. https://doi.org/10.3389/fopht.2023.1184937.

Adamus, G., 2021. Importance of autoimmune responses in progression of retinal degeneration initiated by gene mutations. Front. Med. 8. https://doi.org/10.3389/fmed.2021.672444.

Adverum, 2024. Adverum biotechnologies announces positive preliminary efficacy and safety data from LUNA phase 2 trial of ixo-vec in patients with wet AMD. URL: https://investors.adverum.com/press_releases/news-details/2024/Adverum-Biotechnologies-Announces-Positive-Preliminary-Efficacy-and-Safety-Data-from-LUNA-Phase-2-Trial-of-Ixo-vec-in-Patients-with-Wet-AMD/default.aspx. accessed 10.26.24.

Ail, D., Ren, D., Brazhnikova, E., Nouvel-Jaillard, C., Bertin, S., Mirashrafi, S.B., Fisson, S., Dalkara, D., 2022. Systemic and local immune responses to intraocular AAV vector administration in non-human primates. Mol. Therapy Methods Clin. Dev. 24, 306–316. https://doi.org/10.1016/j.omtm.2022.01.011.

Amado, D., Mingozzi, F., Hui, D., Bennicelli, J.L., Wei, Z., Chen, Y., Bote, E., Grant, R.L., Golden, J.A., Narfstrom, K., Syed, N.A., Orlin, S.E., High, K.A., Maguire, A.M., Bennett, J., 2010. Safety and efficacy of subretinal readministration of a viral vector in large animals to treat congenital blindness. Sci. Transl. Med. 2. https://doi.org/10.1126/scitranslmed.3000659, 21ra16-21ra16.

Arjomandnejad, M., Dasgupta, I., Flotte, T.R., Keeler, A.M., 2023. Immunogenicity of recombinant adeno-associated virus (AAV) vectors for gene transfer. BioDrugs 37, 311–329. https://doi.org/10.1007/s40259-023-00585-7.

Arjomandnejad, M., Sylvia, K., Blackwood, M., Nixon, T., Tang, Q., Muhuri, M., Gruntman, A.M., Gao, G., Flotte, T.R., Keeler, A.M., 2021. Modulating immune responses to AAV by expanded polyclonal T-regs and capsid specific chimeric antigen receptor T-regulatory cells. Mol. Therapy Methods Clin. Dev. 23, 490–506. https://doi.org/10.1016/j.omtm.2021.10.010.

Ayuso, E., Blouin, V., Lock, M., McGorray, S., Leon, X., Alvira, M.R., Auricchio, A., Bucher, S., Chtarto, A., Clark, K.R., Darmon, C., Doria, M., Fountain, W., Gao, G., Gao, K., Giacca, M., Kleinschmidt, J., Leuchs, B., Melas, C., Mizukami, H., Müller, M., Noordman, Y., Bockstael, O., Ozawa, K., Pythoud, C., Sumaroka, M., Surosky, R., Tenenbaum, L., van der Linden, I., Weins, B., Wright, J.F., Zhang, X., Zentilin, L., Bosch, F., Snyder, R.O., Moullier, P., 2014. Manufacturing and characterization of a recombinant adeno-associated virus type 8 reference standard material. Hum. Gene Ther. 25, 977–987. https://doi.org/10.1089/hum.2014.057.

Bahadoran, A., Lee, S.H., Wang, S.M., Manikam, R., Rajarajeswaran, J., Raju, C.S., Sekaran, S.D., 2016. Immune responses to influenza virus and its correlation to age and inherited factors. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01841.

Bainbridge, J.W.B., Mehat, M.S., Sundaram, V., Robbie, S.J., Barker, S.E., Ripamonti, C., Georgiadis, A., Mowat, F.M., Beattie, S.G., Gardner, P.J., Feathers, K.L., Luong, V.A., Yzer, S., Balaggan, K., Viswanathan, A., de Ravel, T.J.L., Casteels, I., Holder, G.E., Tyler, N., Fitzke, F.W., Weleber, R.G., Nardini, M., Moore, A.T., Thompson, D.A., Petersen-Jones, S.M., Michaelides, M., van den Born, L.I., Stockman, A., Smith, A.J., Rubin, G., Ali, R.R., 2015. Long-term effect of gene therapy on leber's congenital amaurosis. N. Engl. J. Med. 372, 1887–1897. https://doi.org/10.1056/NEJMoa1414221.

Bainbridge, J.W.B., Smith, A.J., Barker, S.S., Robbie, S., Henderson, R., Balaggan, K., Viswanathan, A., Holder, G.E., Stockman, A., Tyler, N., Petersen-Jones, S., Bhattacharya, S.S., Thrasher, A.J., Fitzke, F.W., Carter, B.J., Rubin, G.S., Moore, A. T., Ali, R.R., 2008. Effect of gene therapy on visual function in leber's congenital amaurosis. N. Engl. J. Med. 358, 2231–2239. https://doi.org/10.1056/NEJMoa0802268.

Barakat, M.R., 2023. Suprachoroidal Delivery of Investigational ABBV-RGX-314 for Diabetic Retinopathy: the Phase II ALTITUDE® Study Dose Levels 1 and 2: One Year Results.

Bell, P., Wang, L., Chen, S.-J., Yu, H., Zhu, Y., Nayal, M., He, Z., White, J., Lebel-Hagan, D., Wilson, J.M., 2016. Effects of self-complementarity, codon optimization, transgene, and dose on liver transduction with AAV8. Hum. Gene Ther. Methods 27, 228–237. https://doi.org/10.1089/hgtb.2016.039.

Bellur, S., McHarg, M., Kongwattananon, W., Vitale, S., Sen, H.N., Kodati, S., 2023. Antidrug antibodies to tumor necrosis factor α inhibitors in patients with noninfectious uveitis. JAMA Ophthalmology 141, 150–156. https://doi.org/10.1001/jamaophthalmol.2022.5584.

Bennett, J., Wellman, J., Marshall, K.A., McCague, S., Ashtari, M., DiStefano-Pappas, J., Elci, O.U., Chung, D.C., Sun, J., Wright, J.F., Cross, D.R., Aravand, P., Cyckowski, L. L., Bennicelli, J.L., Mingozzi, F., Auricchio, A., Pierce, E.A., Ruggiero, J., Leroy, B.P., Simonelli, F., High, K.A., Maguire, A.M., 2016. Safety and durability of effect of contralateral-eye administration of AAV2 gene therapy in patients with childhood-onset blindness caused by RPE65 mutations: a follow-on phase 1 trial. Lancet 388, 661–672. https://doi.org/10.1016/S0140-6736(16)30371-3.

Bentler, M., Hardet, R., Ertelt, M., Rudolf, D., Kaniowska, D., Schneider, A., Vondran, F. W.R., Schoeder, C.T., Delphin, M., Lucifora, J., Ott, M., Hacker, U.T., Adriouch, S., Büning, H., 2023. Modifying immune responses to adeno-associated virus vectors by capsid engineering. Mol. Therapy Methods Clin. Dev. 30, 576–592. https://doi.org/10.1016/j.omtm.2023.08.015.

Bertolini, T.B., Shirley, J.L., Zolotukhin, I., Li, X., Kaisho, T., Xiao, W., Kumar, S.R.P., Herzog, R.W., 2021. Effect of CpG depletion of vector genome on CD8+ T cell responses in AAV gene therapy. Front. Immunol. 12. https://doi.org/10.3389/fimmu.2021.672449.

Bhandari, R., 2024. Subretinal delivery of investigational ABBV-RGX-314 for neovascular age-related macular degeneration (nAMD): a phase II pharmacodynamic study. Investig. Ophthalmol. Vis. Sci. 65, 4903.

Bitoun, S., Hässler, S., Ternant, D., Szely, N., Gleizes, A., Richez, C., Soubrier, M., Avouac, J., Brocq, O., Sellam, J., de Vries, N., Huizinga, T.W.J., Jury, E.C., Manson, J.J., Mauri, C., Matucci, A., Hacein Bey Abina, S., Mulleman, D., Pallardy, M., Broët, P., Mariette, X., ABIRISK Consortium, 2023. Response to biologic drugs in patients with rheumatoid arthritis and antidrug antibodies. JAMA Netw. Open 6, e2323098. https://doi.org/10.1001/jamanetworkopen.2023.23098.

Bouquet, C., Galy, A., Cancian, C., Thomasson, N., 2018. Impact of sequential bilateral intravitreal injection of rAAV2/2-ND4 on ocular and systemic humoral immune status in non-human primates. Investig. Ophthalmol. Vis. Sci. 59, 4537.

Bouquet, C., Vignal Clermont, C., Galy, A., Fitoussi, S., Blouin, L., Munk, M.R., Valero, S., Meunier, S., Katz, B., Sahel, J.A., Thomasson, N., 2019. Immune response and intraocular inflammation in patients with leber hereditary optic neuropathy treated with intravitreal injection of recombinant adeno-associated virus 2 carrying the ND4 gene: a secondary analysis of a phase 1/2 clinical trial. JAMA Ophthalmology 137, 399–406. https://doi.org/10.1001/jamaophthalmol.2018.6902.

Boyd, R., Boye, S.L., Conlon, T., Erger, K., Sledge, D., Langohr, I., Hauswirth, W., Komáromy, A., Boye, S.E., Petersen-Jones, S., Bartoe, J., 2016. Reduced retinal transduction and enhanced transgene-directed immunogenicity with intravitreal delivery of rAAV following posterior vitrectomy in dogs. Gene Ther. 23, 548–556. https://doi.org/10.1038/gt.2016.31.

Brady, P.N., Goel, A., Johnson, M.A., 2018. Poly(ADP-Ribose) polymerases in host-pathogen interactions, inflammation, and immunity. Microbiol. Mol. Biol. Rev.: MMBR (Microbiol. Mol. Biol. Rev.) 83, e00038. https://doi.org/10.1128/MMBR.00038-18.

Bucher, K., Rodríguez-Bocanegra, E., Dauletbekov, D., Fischer, M.D., 2021. Immune responses to retinal gene therapy using adeno-associated viral vectors – implications for treatment success and safety. Prog. Retin. Eye Res. 83, 100915. https://doi.org/10.1016/j.preteyeres.2020.100915.

Buckley, T., MacLaren, R.E., Cehajic Kapetanovic, J., 2024. Meta-analysis of gene therapy associated uveitis (GTAU). Investig. Ophthalmol. Vis. Sci. 65, 5312.
Bui, A.D., Kong, C.L., Kelly, N.K., Rathinam, S.R., Gonzales, J.A., Thundikandy, R., Kanakath, A., Murugan, B., Vedhanayaki, R., Lim, L.L., Suhler, E.B., Al-Dhibi, H.A., Doan, T., Acharya, N.R., First-Line Antimetabolites as Steroid-Sparing Treatment Research Group, 2022. Time to uveitis control with methotrexate and mycophenolate mofetil. Ophthalmology 129, 721–723. https://doi.org/10.1016/j.ophtha.2022.01.020.
Büning, H., Srivastava, A., 2019. Capsid modifications for targeting and improving the efficacy of AAV vectors. Mol. Therapy Methods Clin. Dev. 12, 248–265. https://doi.org/10.1016/j.omtm.2019.01.008.
Busbee, B., Boyer, D.S., Khanani, A.M., Wykoff, C.C., Pieramici, D.J., Regillo, C., Danzig, C.J., Joondeph, B.C., Major, J., Hoang, C., Turpcu, A., Chung, C., Kiss, S., Gasmi, M., Osborne, A., 2021. Phase 1 study of intravitreal gene therapy with ADVM-022 for neovascular AMD (OPTIC trial). Investig. Ophthalmol. Vis. Sci. 62, 352.
Calabrò, A., Accardi, G., Aiello, A., Caruso, C., Candore, G., 2023. Sex and gender affect immune aging. Frontiers in Aging 4, 1272118. https://doi.org/10.3389/fragi.2023.1272118.
Campochiaro, P.A., Avery, R., Brown, D.M., Heier, J.S., Ho, A.C., Huddleston, S.M., Jaffe, G.J., Khanani, A.M., Pakola, S., Pieramici, D.J., Wykoff, C.C., Everen, S.V., 2024. Gene therapy for neovascular age-related macular degeneration by subretinal delivery of RGX-314: a phase 1/2a dose-escalation study. Lancet 403, 1563–1573. https://doi.org/10.1016/S0140-6736(24)00310-6.
Cao, D., Byrne, B.J., de Jong, Y.P., Terhorst, C., Duan, D., Herzog, R.W., Kumar, S.R.P., 2024. Innate immune sensing of adeno-associated virus vectors. Hum. Gene Ther. 35, 451–463. https://doi.org/10.1089/hum.2024.040.
Carvajal Alegria, G., Gazeau, P., Hillion, S., Daïen, C.I., Cornec, D.Y.K., 2017. Could lymphocyte profiling be useful to diagnose systemic autoimmune diseases? Clin. Rev. Allergy Immunol. 53, 219–236. https://doi.org/10.1007/s12016-017-8608-5.
Carvalho, C., Lemos, L., Antas, P., Seabra, M.C., 2023. Gene therapy for inherited retinal diseases: exploiting new tools in genome editing and nanotechnology. Front. Ophthalmol. 3. https://doi.org/10.3389/fopht.2023.1270561.
Cehajic-Kapetanovic, J., Bellini, M.P., Taylor, L.J., Yusuf, I.H., Soomro, T., da Cruz, L., MacLaren, R.E., 2024. Gene therapy for choroideremia using an adeno-associated viral vector encoding Rab escort protein 1: the REGENERATE open-label trial. Efficacy and Mech. Evaluation; National Institute for Health and Care Res. https://doi.org/10.3310/wara5730.
Cehajic-Kapetanovic, J., Xue, K., de la Camara, C.M.-F., Nanda, A., Davies, A., Wood, L. J., Salvetti, A.P., Fischer, M.D., Aylward, J.W., Barnard, A.R., Jolly, J.K., Luo, E., Lujan, B.J., Ong, T., Girach, A., Black, G.C., Gregori, N.Z., Davis, J.L., Rosa, P.R., Lotery, A.J., Lam, B.L., Stanga, P.E., MacLaren, R.E., 2020. Retinal gene therapy in X-linked retinitis pigmentosa caused by mutations in RPGR: results at 6 months in a first in human clinical trial. Nat. Med. 26, 354–359. https://doi.org/10.1038/s41591-020-0763-1.
Cehajic-Kapetanovic, J., Xue, K., Edwards, T.L., Meenink, T.C., Beelen, M.J., Naus, G.J., de Smet, M.D., MacLaren, R.E., 2022. First-in-Human robot-assisted subretinal drug delivery under local anesthesia. Am. J. Ophthalmol. 237, 104–113. https://doi.org/10.1016/j.ajo.2021.11.011.
Chan, W.O., Brennan, N., Webster, A.R., Michaelides, M., Muqit, M.M.K., 2020. Retinal detachment in retinitis pigmentosa. BMJ Open Ophth 5. https://doi.org/10.1136/bmjophth-2020-000454.
Chan, Y.K., Dick, A.D., Hall, S.M., Langmann, T., Scribner, C.L., Mansfield, B.C., for the Ocular Gene Therapy Inflammation Working Group, 2021a. Translational Vision Sci. Technol. 10, 3. https://doi.org/10.1167/tvst.10.4.3. Inflammation in Viral Vector-Mediated Ocular Gene Therapy: A Review and Report From a Workshop Hosted by the Foundation Fighting Blindness, 9/2020.
Chan, Y.K., Pepple, K.L., 2021. Does systemic inflammation prompt gene therapy uveitis? Mol. Ther. 29, 1943–1944. https://doi.org/10.1016/j.ymthe.2021.05.001.
Chan, Y.K., Wang, S.K., Chu, C.J., Copland, D.A., Letizia, A.J., Costa Verdera, H., Chiang, J.J., Sethi, M., Wang, M.K., Neidermyer, W.J., Chan, Y., Lim, E.T., Graveline, A.R., Sanchez, M., Boyd, R.F., Vihtelic, T.S., Inciong, R.G.C.O., Slain, J.M., Alphonse, P.J., Xue, Y., Robinson-McCarthy, L.R., Tam, J.M., Jabbar, M.H., Sahu, B., Adeniran, J.F., Muhuri, M., Tai, P.W.L., Xie, J., Krause, T.B., Vernet, A., Pezone, M., Xiao, R., Liu, T., Wang, W., Kaplan, H.J., Gao, G., Dick, A.D., Mingozzi, F., McCall, M.A., Cepko, C.L., Church, G.M., 2021b. Engineering adeno-associated viral vectors to evade innate immune and inflammatory responses. Sci. Transl. Med. 13, eabd3438. https://doi.org/10.1126/scitranslmed.abd3438.
Chand, D.H., Zaidman, C., Arya, K., Millner, R., Farrar, M.A., Mackie, F.E., Goedeker, N. L., Dharnidharka, V.R., Dandamudi, R., Reyna, S.P., 2021. Thrombotic microangiopathy following onasemnogene abeparvovec for spinal muscular atrophy: a case series. J. Pediatr. 231, 265–268. https://doi.org/10.1016/j.jpeds.2020.11.054.
Chandler, L.C., Barnard, A.R., Caddy, S.L., Patrício, M.I., McClements, M.E., Fu, H., Rada, C., MacLaren, R.E., Xue, K., 2019. Enhancement of adeno-associated virus-mediated gene therapy using hydroxychloroquine in murine and human tissues. Mol. Therapy Methods Clin. Dev. 14, 77–89. https://doi.org/10.1016/j.omtm.2019.05.012.
Chandler, L.C., McClements, M.E., Yusuf, I.H., de la Camara, C.M.-F., MacLaren, R.E., Xue, K., 2021. Characterizing the cellular immune response to subretinal AAV gene therapy in the murine retina. Mol. Therapy Methods Clin. Dev. 22, 52–65. https://doi.org/10.1016/j.omtm.2021.05.011.
Charlesworth, C.T., Deshpande, P.S., Dever, D.P., Camarena, J., Lemgart, V.T., Cromer, M.K., Vakulskas, C.A., Collingwood, M.A., Zhang, L., Bode, N.M., Behlke, M. A., Dejene, B., Cieniewicz, B., Romano, R., Lesch, B.J., Gomez-Ospina, N., Mantri, S., Pavel-Dinu, M., Weinberg, K.I., Porteus, M.H., 2019. Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nat. Med. 25, 249. https://doi.org/10.1038/s41591-018-0326-x.
Cheng, S.-Y., Punzo, C., 2022. Update on viral gene therapy clinical trials for retinal diseases. Hum. Gene Ther. 33, 865–878. https://doi.org/10.1089/hum.2022.159.
Chung, S.H., Mollhoff, I.N., Mishra, A., Sin, T.-N., Ngo, T., Ciulla, T., Sieving, P., Thomasy, S.M., Yiu, G., 2021. Host immune responses after suprachoroidal delivery of AAV8 in nonhuman primate eyes. Hum. Gene Ther. 32, 682–693. https://doi.org/10.1089/hum.2020.281.
Clare, A.J., Copland, D.A., Langer, F., Ward, A., Chan, Y.K., Dick, A.D., 2023. Impact of differential sex response to the vector when developing IL-33 gene therapy for AMD. Investig. Ophthalmol. Vis. Sci. 64, 767.
Clare, A.J., Langer, P.M., Ward, A., Chan, Y.K., Dick, A.D., Copland, D.A., 2025. Characterisation of the ocular inflammatory response to AAV reveals divergence by sex and age. Mol. Ther. 33, 3. https://doi.org/10.1016/j.ymthe.2025.01.028.
Clément, N., Grieger, J.C., 2016. Manufacturing of recombinant adeno-associated viral vectors for clinical trials. Mol. Therapy Methods Clin. Dev. 3. https://doi.org/10.1038/mtm.2016.2.
Comander, J., Carvalho, L.S., Xiao, R., Langsdorf, A., Wassmer, S., Hafler, B., Wu, D.M., Eliott, D., Kim, L.A., Vandenberghe, L.H., 2016. Characterization of the limitations to gene transfer and associated inflammation following intravitreal AAV injection in nonhuman primates. Investig. Ophthalmol. Vis. Sci. 57, 771.
Constable, I.J., Pierce, C.M., Lai, C.-M., Magno, A.L., Degli-Esposti, M.A., French, M.A., McAllister, I.L., Butler, S., Barone, S.B., Schwartz, S.D., Blumenkranz, M.S., Rakoczy, E.P., 2016. Phase 2a randomized clinical trial: safety and post hoc analysis of subretinal rAAV.sFLT-1 for wet age-related macular degeneration. EBioMedicine 14, 168–175. https://doi.org/10.1016/j.ebiom.2016.11.016.
Copland, D.A., Liu, J., Schewitz-Bowers, L.P., Brinkmann, V., Anderson, K., Nicholson, L. B., Dick, A.D., 2012. Therapeutic dosing of fingolimod (FTY720) prevents cell infiltration, rapidly suppresses ocular inflammation, and maintains the blood-ocular barrier. Am. J. Pathol. 180, 672–681. https://doi.org/10.1016/j.ajpath.2011.10.008.
Costagliola, G., Spada, E., Consolini, R., 2021. Age-related differences in the immune response could contribute to determine the spectrum of severity of COVID-19. Immun. Inflamm. Dis. 9, 331–339. https://doi.org/10.1002/iid3.404.
Couto, L., Elmore, D., Peterson, J., Zhang, H., Ocelli, L., Boyd, R., Knupp, L., Boye, Sanford, Boye, Shannon, 2022. Laterally spreading AAV.SPR-hRS1 vector for treatment of XLRS. Investig. Ophthalmol. Vis. Sci. 63, 2825-A0341.
Cukras, C., Wiley, H.E., Jeffrey, B.G., Sen, H.N., Turriff, A., Zeng, Y., Vijayasarathy, C., Marangoni, D., Ziccardi, L., Kjellstrom, S., Park, T.K., Hiriyanna, S., Wright, J.F., Colosi, P., Wu, Z., Bush, R.A., Wei, L.L., Sieving, P.A., 2018. Retinal AAV8-RS1 gene therapy for X-linked retinoschisis: initial findings from a phase I/IIa trial by intravitreal delivery. Mol. Ther. 26, 2282–2294. https://doi.org/10.1016/j.ymthe.2018.05.025.
Dalkara, D., Byrne, L.C., Klimczak, R.R., Visel, M., Yin, L., Merigan, W.H., Flannery, J.G., Schaffer, D.V., 2013. In vivo–directed evolution of a new adeno-associated virus for therapeutic outer retinal gene delivery from the vitreous. Sci. Transl. Med. 5. https://doi.org/10.1126/scitranslmed.3005708, 189ra76-189ra76.
Davidsson, M., Heuer, A., 2022. Small scale adeno-associated virus-vector production for preclinical gene delivery based on chloroform precipitation. Neural Regeneration Res. 17, 99–100. https://doi.org/10.4103/1673-5374.314309.
Davis, J.L., Gregori, N.Z., MacLaren, R.E., Lam, B.L., 2019. Surgical technique for subretinal gene therapy in humans with inherited retinal degeneration. Retina 39, S2–8. https://doi.org/10.1097/IAE.0000000000002609.
D'Costa, S., Blouin, V., Broucque, F., Penaud-Budloo, M., Fçranois, A., Perez, I.C., Bec, C. L., Moullier, P., Snyder, R.O., Ayuso, E., 2016. Practical utilization of recombinant AAV vector reference standards: focus on vector genomes titration by free ITR qPCR. Mol. Therapy Methods Clin. Dev. 3. https://doi.org/10.1038/mtm.2016.19.
Destro, F., Wu, W., Srinivasan, P., Joseph, J., Bal, V., Neufeld, C., Wolfrum, J.M., Manalis, S.R., Sinskey, A.J., Springs, S.L., Barone, P.W., Braatz, R.D., 2024. The state of technological advancement to address challenges in the manufacture of rAAV gene therapies. Biotechnol. Adv. 76, 108433. https://doi.org/10.1016/j.biotechadv.2024.108433.
Dhoot, D.S., 2022. Suprachoroidal delivery of RGX-314 for diabetic retinopathy: the phase II ALTITUDETM study. Investig. Ophthalmol. Vis. Sci. 63, 1152.
Dhungel, B.P., Bailey, C.G., Rasko, J.E.J., 2021. Journey to the center of the cell: tracing the path of AAV transduction. Trends Mol. Med. 27, 172–184. https://doi.org/10.1016/j.molmed.2020.09.010.
Dick, A.D., Rosenbaum, J.T., Al-Dhibi, H.A., Belfort, R., Brézin, A.P., Chee, S.P., Davis, J. L., Ramanan, A.V., Sonoda, K.-H., Carreño, E., Nascimento, H., Salah, S., Salek, S., Siak, J., Steeples, L., Accorinti, M., Acharya, N., Adan, A., Agrawal, R., Akkoc, N., Al Ghamdi, S., Al Ghamdi, T., Al Saati, A., Alsabaani, N., Al-Shamarani, M., Bachta, A., Barisani-Asenbauer, T., Beare, N., Porto, F.B.O., Blanco, R., Yee, A.C.S., Chandran, V., Chiquet, C., Chng, H.H., Cimbalas, A., Cimino, L., Cordero-Coma, M., Cristobal, C., Cuevas, M., Eurico da Fonseca, J., de Boer, J., de la Torre, A., De Schryver, I., Derzko-Dzulynsky, L., Diaz-Valle, D., Merino, C.E.D., Facsko, A., Figueira, L., Fonollosa, A., Fortin, E., Gale, R., Galeazzi, M., Garcia, S., Garcia Ruiz de Morales, J.M., Gašperšič, N., Goldstein, D., Guedes, M., Guex-Crosier, Y., Gul, A., Hamam, R., Haroon, M., Hasegawa, K., Heiligenhaus, A., Hooper, C., Hwang, Y.-S., Hwang, D.-K., Juanola, X., Kaburaki, T., Kadayifcilar, S., Kempen, J., Kezuka, T., Kherani, A., Kirsimäe, M., Kotaniemi, K., Kraut, A., Kubicka-Trząska, A., Kuffova, L., Lightman, S., Lim, L., Lim, W.K., McCluskey, P., McGuire, M., Mirabelli, P., Miserocchi, E., Misiuk-Hojło, M., Muccioli, C., Muñoz, S., Murphy, C., Murray, P.I., Nagy, Z., Namba, K., Neri, P., Nguyen, Q., O'Gradaigh, D., Omair, M., Otsa, K., Ozyazgan, Y., Pablo, F., Paroli, M.P., Pleyer, U., Poór, G., Proença, R., Rabinovitch, T., Read, R., Rebrov, M., Recillas-Gispert, C., Rothova, A., Schwartzman, S., Seve, P., Sharma, S., Sims, J., Sohár, N., Suhler, E., Szántó, S.,

Szepessy, Z., Tappeiner, C., Thorne, J., Tugal Tutkun, I., Turno-Kręcicka, A., Van Calster, J., van der Horst-Bruinsma, I., Vidovič Valentinčič, N., Vitale, A., Voorduin Ramos, S., Vukojevic, N., Wakefield, D., Willermain, F., Yalcindag, N., Yamamoto, J. H., Yeh, S., Zemaitiene, R., Ziouzina, O., 2018. Guidance on noncorticosteroid systemic immunomodulatory therapy in noninfectious uveitis: fundamentals of care for UveitiS (FOCUS) initiative. Ophthalmology 125, 757–773. https://doi.org/10.1016/j.ophtha.2017.11.017.

Dimopoulos, I.S., Hoang, S.C., Radziwon, A., Binczyk, N.M., Seabra, M.C., MacLaren, R. E., Somani, R., Tennant, M.T.S., MacDonald, I.M., 2018. Two-year results after AAV2-mediated gene therapy for choroideremia: the alberta experience. Am. J. Ophthalmol. 193, 130–142. https://doi.org/10.1016/j.ajo.2018.06.011.

Doycheva, D., Deuter, C., Stuebiger, N., Biester, S., Zierhut, M., 2007. Mycophenolate mofetil in the treatment of uveitis in children. Br. J. Ophthalmol. 91, 180–184. https://doi.org/10.1136/bjo.2006.094698.

Dunbar, C.E., High, K.A., Joung, J.K., Kohn, D.B., Ozawa, K., Sadelain, M., 2018. Gene therapy comes of age. Science 359, eaan4672. https://doi.org/10.1126/science.aan4672.

Edwards, T.L., Xue, K., Meenink, H.C.M., Beelen, M.J., Naus, G.J.L., Simunovic, M.P., Latasiewicz, M., Farmery, A.D., de Smet, M.D., MacLaren, R.E., 2018. First-in-human study of the safety and viability of intraocular robotic surgery. Nat. Biomed. Eng. 2, 649–656. https://doi.org/10.1038/s41551-018-0248-4.

European Medicines Agency, 2019. Luxturna: EPAR - product information. URL: https://www.ema.europa.eu/en/medicines/human/EPAR/luxturna. accessed 2.8.25.

Fanigliulo, D., Lazzerini, P.E., Capecchi, P.L., Ulivieri, C., Baldari, C.T., Laghi-Pasini, F., 2015. Clinically-relevant cyclosporin and rapamycin concentrations enhance regulatory T cell function to a similar extent but with different mechanisms: an in-vitro study in healthy humans. Int. Immunopharmacol. 24, 276–284. https://doi.org/10.1016/j.intimp.2014.12.021.

Faust, S.M., Bell, P., Cutler, B.J., Ashley, S.N., Zhu, Y., Rabinowitz, J.E., Wilson, J.M., 2013. CpG-depleted adeno-associated virus vectors evade immune detection. J. Clin. Investig. 123, 2994–3001. https://doi.org/10.1172/JCI68205.

Ferreira, R.G., Gordon, N.F., Stock, R., Petrides, D., 2021. Adenoviral vector COVID-19 vaccines: process and cost analysis. Processes 9, 1430. https://doi.org/10.3390/pr9081430.

Ferreira, V., Petry, H., Salmon, F., 2014. Immune responses to AAV-vectors, the Glybera example from bench to bedside. Front. Immunol. 5. https://doi.org/10.3389/fimmu.2014.00082.

Ferreira, V., Twisk, J., Kwikkers, K., Aronica, E., Brisson, D., Methot, J., Petry, H., Gaudet, D., 2013. Immune responses to intramuscular administration of alipogene tiparvovec (AAV1-LPLS447X) in a phase II clinical trial of lipoprotein lipase deficiency gene therapy. Hum. Gene Ther. 25, 180. https://doi.org/10.1089/hum.2013.169.

Fischer, M.D., Hickey, D.G., Singh, M.S., MacLaren, R.E., 2016. Evaluation of an optimized injection system for retinal gene therapy in human patients. Hum. Gene Ther. Methods 27, 150–158. https://doi.org/10.1089/hgtb.2016.086.

Fischer, M.D., McClements, M.E., de la Camara, C.M.-F., Bellingrath, J.-S., Dauletbekov, D., Ramsden, S.C., Hickey, D.G., Barnard, A.R., MacLaren, R.E., 2017. Codon-optimized RPGR improves stability and efficacy of AAV8 gene therapy in two mouse models of X-linked retinitis pigmentosa. Mol. Ther. 25, 1854–1865. https://doi.org/10.1016/j.ymthe.2017.05.005.

Fischer, M.D., Michalakis, S., Wilhelm, B., Zobor, D., Muehlfriedel, R., Kohl, S., Weisschuh, N., Ochakovski, G.A., Klein, R., Schoen, C., Sothilingam, V., Garcia-Garrido, M., Kuehlewein, L., Kahle, N., Werner, A., Dauletbekov, D., Paquet-Durand, F., Tsang, S., Martus, P., Peters, T., Seeliger, M., Bartz-Schmidt, K.U., Ueffing, M., Zrenner, E., Biel, M., Wissinger, B., 2020. Safety and vision outcomes of subretinal gene therapy targeting cone photoreceptors in achromatopsia: a nonrandomized controlled trial. JAMA Ophthalmology 138, 643–651. https://doi.org/10.1001/jamaophthalmol.2020.1032.

Fischer, M.D., Ochakovski, G.A., Beier, B., Seitz, I.P., Vaheb, Y., Kortuem, C., Reichel, F. F.L., Kuehlewein, L., Kahle, N.A., Peters, T., Girach, A., Zrenner, E., Ueffing, M., MacLaren, R.E., Bartz-Schmidt, K.U., Wilhelm, B., 2019. Efficacy and safety of retinal gene therapy using adeno-associated virus vector for patients with choroideremia: a randomized clinical trial. JAMA Ophthalmology 137, 1247–1254. https://doi.org/10.1001/jamaophthalmol.2019.3278.

Fischer, M.D., Simonelli, F., Sahni, J., Holz, F.G., Maier, R., Fasser, C., Suhner, A., Stiehl, D.P., Chen, B., Audo, I., Leroy, B.P., 2024. Real-world safety and effectiveness of voretigene neparvovec: results up to 2 Years from the prospective, registry-based PERCEIVE study. Biomolecules 14, 122. https://doi.org/10.3390/biom14010122.

Food and Drug Administration, 2020. Chemistry, manufacturing, and control (CMC) information for human gene therapy investigational new drug applications (INDs). URL: https://www.fda.gov/media/113760/download. accessed 11.8.24.

Foster, H., Sharp, P.S., Athanasopoulos, T., Trollet, C., Graham, I.R., Foster, K., Wells, D. J., Dickson, G., 2008. Codon and mRNA sequence optimization of microdystrophin transgenes improves expression and physiological outcome in dystrophic mdx mice following AAV2/8 gene transfer. Mol. Ther. 16, 1825–1832. https://doi.org/10.1038/mt.2008.186.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging: an evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci. 908, 244–254. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x.

Fung, A.T., Galvin, J., Tran, T., 2021. Epiretinal membrane: a review. Clin. Exp. Ophthalmol. 49, 289–308. https://doi.org/10.1111/ceo.13914.

Gaballa, S.A., Kompella, U.B., Elgarhy, O., Alqahtani, A.M., Pierscionek, B., Alany, R.G., Abdelkader, H., 2021. Corticosteroids in ophthalmology: drug delivery innovations, pharmacology, clinical applications, and future perspectives. Drug Deliv. and Transl. Res. 11, 866–893. https://doi.org/10.1007/s13346-020-00843-z.

Gange, W.S., Sisk, R.A., Besirli, C.G., Lee, T.C., Havunjian, M., Schwartz, H., Borchert, M., Sengillo, J.D., Mendoza, C., Berrocal, A.M., Nagiel, A., 2022. Perifoveal chorioretinal atrophy after subretinal voretigene neparvovec-rzyl for RPE65-mediated leber congenital amaurosis. Ophthalmology Retina 6, 58–64. https://doi.org/10.1016/j.oret.2021.03.016.

Gaub, B.M., Berry, M.H., Visel, M., Holt, A., Isacoff, E.Y., Flannery, J.G., 2018. Optogenetic retinal gene therapy with the light gated GPCR vertebrate rhodopsin. Methods Mol. Biol. 1715, 177–189. https://doi.org/10.1007/978-1-4939-7522-8_12.

Gaudet, D., Méthot, J., Déry, S., Brisson, D., Essiembre, C., Tremblay, G., Tremblay, K., de Wal, J., Twisk, J., van den Bulk, N., Sier-Ferreira, V., van Deventer, S., 2012. Efficacy and long term safety of alipogene tiparvovec (AAV1-LPLS447X) gene therapy for lipoprotein lipase deficiency: an open label trial. Gene Ther. 20, 361. https://doi.org/10.1038/gt.2012.43.

Ghazi, N.G., Abboud, E.B., Nowilaty, S.R., Alkuraya, H., Alhommadi, A., Cai, H., Hou, R., Deng, W.-T., Boye, S.L., Almaghamsi, A., Al Saikhan, F., Al-Dhibi, H., Birch, D., Chung, C., Colak, D., LaVail, M.M., Vollrath, D., Erger, K., Wang, W., Conlon, T., Zhang, K., Hauswirth, W., Alkuraya, F.S., 2016. Treatment of retinitis pigmentosa due to MERTK mutations by ocular subretinal injection of adeno-associated virus gene vector: results of a phase I trial. Hum. Genet. 135, 327–343. https://doi.org/10.1007/s00439-016-1637-y.

Ghoraba, H.H., Akhavanrezayat, A., Karaca, I., Yavari, N., Lajevardi, S., Hwang, J., Regenold, J., Matsumiya, W., Pham, B., Zaidi, M., Mobasserian, A., DongChau, A.T., Or, C., Yasar, C., Mishra, K., Do, D., Nguyen, Q.D., 2022. Ocular gene therapy: a literature review with special focus on immune and inflammatory responses. Clin. Ophthalmol. 16, 1753–1771. https://doi.org/10.2147/OPTH.S364200.

Gimpel, A.L., Katsikis, G., Sha, S., Maloney, A.J., Hong, M.S., Nguyen, T.N.T., Wolfrum, J., Springs, S.L., Sinskey, A.J., Manalis, S.R., Barone, P.W., Braatz, R.D., 2021. Analytical methods for process and product characterization of recombinant adeno-associated virus-based gene therapies. Mol. Therapy Methods Clin. Dev. 20, 740–754. https://doi.org/10.1016/j.omtm.2021.02.010.

Gray, A.P., Sato, Y., Meyer, T., Stoner, K., Beltran, W.A., 2022. Surgical procedure and applicability of the Orbit subretinal delivery system (SDS)TM in the normal adult canine eye. Investig. Ophthalmol. Vis. Sci. 63, 4118-F0355.

Grégoire, S., Terrada, C., Martin, G.H., Fourcade, G., Baeyens, A., Marodon, G., Fisson, S., Billiard, F., Lucas, B., Tadayoni, R., Béhar-Cohen, F., Levacher, B., Galy, A., LeHoang, P., Klatzmann, D., Bodaghi, B., Salomon, B.L., 2016. Treatment of uveitis by in situ administration of ex vivo–activated polyclonal regulatory T cells. J. Immunol. 196, 2109–2118. https://doi.org/10.4049/jimmunol.1501723.

Guerin, K., Rego, M., Bourges, D., Ersing, I., Haery, L., Harten DeMaio, K., Sanders, E., Tasissa, M., Kostman, M., Tillgren, M., Makana Hanley, L., Mueller, I., Mitsopoulos, A., Fan, M., 2020. A novel next-generation sequencing and analysis platform to assess the identity of recombinant adeno-associated viral preparations from viral DNA extracts. Hum. Gene Ther. 31, 664–678. https://doi.org/10.1089/hum.2019.277.

Hallam, T.M., Gardenal, E., McBlane, F., Cho, G., Ferraro, L.L., Pekle, E., Lu, D., Carney, K., Wenden, C., Beadsmoore, H., Kaiser, S., Drage, L., Haye, T., Kassem, I., Rangaswamy, N., Obeidat, M., Grosskreutz, C., Saint-Geniez, M., Steel, D.H., MacLaren, R.E., Investigators, F.P., Ellis, S., Harris, C.L., Poor, S., Jones, A.V., 2024. Ocular biomarker profiling after complement factor I gene therapy in geographic atrophy secondary to age-related macular degeneration. medRxiv. https://doi.org/10.1101/2024.06.12.24308796.

Han, J., Li, X., Luo, X., He, J., Huang, X., Zhou, Q., Han, Y., Jie, H., Zhuang, J., Li, Y., Yang, F., Zhai, Z., Wu, S., He, Y., Yang, B., Sun, E., 2020. The mechanisms of hydroxychloroquine in rheumatoid arthritis treatment: inhibition of dendritic cell functions via Toll like receptor 9 signaling. Biomed. Pharmacother. 132, 110848. https://doi.org/10.1016/j.biopha.2020.110848.

Hareendran, S., Ramakrishna, B., Jayandharan, G.R., 2016. Synergistic inhibition of PARP-1 and NF-κB signaling downregulates immune response against recombinant AAV2 vectors during hepatic gene therapy. Eur. J. Immunol. 46, 154–166. https://doi.org/10.1002/eji.201545867.

Hassa, P.O., Hottiger, M.O., 1999. A role of poly (ADP-ribose) polymerase in NF-kappaB transcriptional activation. Biol. Chem. 380, 953–959. https://doi.org/10.1515/BC.1999.118.

Hauswirth, W.W., Aleman, T.S., Kaushal, S., Cideciyan, A.V., Schwartz, S.B., Wang, L., Conlon, T.J., Boye, S.L., Flotte, T.R., Byrne, B.J., Jacobson, S.G., 2008. Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. Hum. Gene Ther. 19, 979–990. https://doi.org/10.1089/hum.2008.107.

Heier, J.S., Kherani, S., Desai, S., Dugel, P., Kaushal, S., Cheng, S.H., Delacono, C., Purvis, A., Richards, S., Le-Halpere, A., Connelly, J., Wadsworth, S.C., Varona, R., Buggage, R., Scaria, A., Campochiaro, P.A., 2017. Intravitreous injection of AAV2-sFLT01 in patients with advanced neovascular age-related macular degeneration: a phase 1, open-label trial. Lancet 390, 50–61. https://doi.org/10.1016/S0140-6736(17)30979-0.

Hogan, A.C., McAvoy, C.E., Dick, A.D., Lee, R.W.J., 2007. Long-term efficacy and tolerance of tacrolimus for the treatment of uveitis. Ophthalmology 114, 1000–1006.e4. https://doi.org/10.1016/j.ophtha.2007.01.026.

Hösel, M., Broxtermann, M., Janicki, H., Esser, K., Arzberger, S., Hartmann, P., Gillen, S., Kleeff, J., Stabenow, D., Odenthal, M., Knolle, P., Hallek, M., Protzer, U., Büning, H., 2012. Toll-like receptor 2–mediated innate immune response in human nonparenchymal liver cells toward adeno-associated viral vectors. Hepatology 55, 287–297. https://doi.org/10.1002/hep.24625.

Huang, P., Narendran, S., Pereira, F., Fukuda, S., Nagasaka, Y., Apicella, I., Yerramothu, P., Marion, K.M., Cai, X., Sadda, S.R., Gelfand, B.D., Ambati, J., 2022. The learning curve of murine subretinal injection among clinically trained ophthalmic surgeons. Translational Vision Sci. Technol. 11, 13. https://doi.org/10.1167/tvst.11.3.13.

Huang, Z., Chen, B., Liu, X., Li, H., Xie, L., Gao, Y., Duan, R., Li, Z., Zhang, J., Zheng, Y., Su, W., 2021. Effects of sex and aging on the immune cell landscape as assessed by single-cell transcriptomic analysis. Proc. Natl. Acad. Sci. USA 118, e2023216118. https://doi.org/10.1073/pnas.2023216118.

Iannaccone, A., Pennesi, M.E., Yang, P., Lauer, A., Sisk, R., Gregori, N.Z., Davis, J.L., Lam, B.L., Kay, C.N., Goldbaum, M., Ashimatey, B.S., Zhu, F., Feinsod, M., Vajzovic, L., 2022. Interim safety results in two phase 1/2 open-label, dose-escalation clinical trials of subretinal gene therapy with AGTC-401 (rAAV2tYF-PR1.7-hCNGB3) and AGTC-402 (rAAV2tYF-PR1.7-hCNGA3) in subjects with achromatopsia (ACHM). Investig. Ophthalmol. Vis. Sci. 63, 2829-A0345.

Jacobson, S.G., Cideciyan, A.V., Ratnakaram, R., Heon, E., Schwartz, S.B., Roman, A.J., Peden, M.C., Aleman, T.S., Boye, S.L., Sumaroka, A., Conlon, T.J., Calcedo, R., Pang, J.-J., Erger, K.E., Olivares, M.B., Mullins, C.L., Swider, M., Kaushal, S., Feuer, W.J., Iannaccone, A., Fishman, G.A., Stone, E.M., Byrne, B.J., Hauswirth, W. W., 2012. Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. Arch. Ophthalmol. 130, 9–24. https://doi.org/10.1001/archophthalmol.2011.298.

Jalil, A., Ivanova, T., Moussa, G., Parry, N.R.A., Black, G.C.M., 2023. Retinal gene therapy in RPE-65 gene mediated inherited retinal dystrophy. Eye 37, 1874–1877. https://doi.org/10.1038/s41433-022-02262-5.

Keenan, T.D.L., Clemons, T.E., Domalpally, A., Elman, M.J., Havilio, M., Agrón, E., Benyamini, G., Chew, E.Y., 2021. Retinal specialist versus artificial intelligence detection of retinal fluid from OCT. Ophthalmology 128, 100–109. https://doi.org/10.1016/j.ophtha.2020.06.038.

Kessel, L., Christensen, U.C., Klemp, K., 2022. Inflammation after voretigene neparvovec administration in patients with RPE65-related retinal dystrophy. Ophthalmology 129, 1287–1293. https://doi.org/10.1016/j.ophtha.2022.06.018.

Khabou, H., Cordeau, C., Pacot, L., Fisson, S., Dalkara, D., 2018. Dosage thresholds and influence of transgene cassette in adeno-associated virus–related toxicity. Hum. Gene Ther. 29, 1235–1241. https://doi.org/10.1089/hum.2018.144.

Khabou, H., Desrosiers, M., Winckler, C., Fouquet, S., Auregan, G., Bemelmans, A.-P., Sahel, J.-A., Dalkara, D., 2016. Insight into the mechanisms of enhanced retinal transduction by the engineered AAV2 capsid variant -7m8. Biotechnol. Bioeng. 113, 2712–2724. https://doi.org/10.1002/bit.26031.

Khanani, A.M., 2024. Subretinal Delivery of Investigational ABBV-RGX-314 as a Gene Therapy for nAMD: First Time Results of a Fellow Eye Bilateral Dosing Study.

Khanani, A.M., Boyer, D.S., Wykoff, C.C., Regillo, C.D., Busbee, B.G., Pieramici, D., Danzig, C.J., Joondeph, B.C., Major, J.C., Turpcu, A., Kiss, S., 2024a. Safety and efficacy of ixoberogene soroparvovec in neovascular age-related macular degeneration in the United States (OPTIC): a prospective, two-year, multicentre phase 1 study. eClinicalMedicine 67. https://doi.org/10.1016/j.eclinm.2023.102394.

Khanani, A.M., Hershberger, V.S., Kay, C.N., Hu, A., Eichenbaum, D.A., Jaffe, G.J., Chung, C., Honarmand, S., Nien, C., Lee, S., Kabra, H., Kirn, D., Kim, R., 2023. Interim results for the Phase 1/2 PRISM Trial evaluating 4D-150, a dual-transgene intravitreal genetic medicine in individuals with neovascular (wet) age-related macular degeneration. Investig. Ophthalmol. Vis. Sci. 64, 5055.

Khanani, A.M., Hu, A., Eichenbaum, D.A., Maturi, R.K., Sheth, V.S., Edwards, A.O., Gupta, S., Pearlman, J., Alam, S., Heier, J.S., Makkouk, F., Emanuelli, A., Pieramici, D., Wells, J.A., Nien, C., Kim, R., 2024b. Interim results from the PRISM randomized phase 2 dose expansion trial evaluating 4D-150 in high anti-VEGF need individuals with neovascular (wet) age-related macular degeneration. Investig. Ophthalmol. Vis. Sci. 65, 4356.

Kim, H.M., Woo, S.J., 2021. Ocular drug delivery to the retina: current innovations and future perspectives. Pharmaceutics 13, 108. https://doi.org/10.3390/pharmaceutics13010108.

Kiss, S., 2023. Ixo-Vec Intravitreal Gene Therapy for Neovascular Age-Related Macular Degeneration.

Klein, S.L., 2012. Sex influences immune responses to viruses, and efficacy of prophylaxis and therapeutic treatments for viral diseases. Bioessays 34, 1050–1059. https://doi.org/10.1002/bies.201200099.

Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. Nat. Rev. Immunol. 16, 626–638. https://doi.org/10.1038/nri.2016.90.

Kojima, M., Suzuki, K., Takeshita, M., Ohyagi, M., Iizuka, M., Yamane, H., Koga, K., Kouro, T., Kassai, Y., Yoshihara, T., Adachi, R., Hashikami, K., Ota, Y., Yoshimoto, K., Kaneko, Y., Morita, R., Yoshimura, A., Takeuchi, T., 2023. Anti-human-TIGIT agonistic antibody ameliorates autoimmune diseases by inhibiting Tfh and Tph cells and enhancing Treg cells. Commun. Biol. 6, 1–13. https://doi.org/10.1038/s42003-023-04874-3.

Kontogiannis, T., Braybrook, J., McElroy, C., Foy, C., Whale, A.S., Quaglia, M., Smales, C. M., 2024. Characterization of AAV vectors: a review of analytical techniques and critical quality attributes. Mol. Therapy Methods Clin. Dev. 32. https://doi.org/10.1016/j.omtm.2024.101309.

Kotterman, M., Beliakoff, G., Croze, R., Vazin, T., Schmitt, C., Szymanski, P., Leong, M., Quezada, M., Holt, J., Barglow, K., Hassanipour, M., Schaffer, D., Francis, P., Kirn, D.. Directed evolution of AAV targeting primate retina by intravitreal injection identifies R100, a variant demonstrating robust gene delivery and therapeutic efficacy in non-human primates. bioRxiv. https://doi.org/10.1101/2021.06.24.449775.

Kotterman, M.A., Yin, L., Strazzeri, J.M., Flannery, J.G., Merigan, W.H., Schaffer, D.V., 2015. Antibody neutralization poses a barrier to intravitreal adeno-associated viral vector gene delivery to non-human primates. Gene Ther. 22, 116–126. https://doi.org/10.1038/gt.2014.115.

Ku, C.A., Igelman, A.D., Huang, S.J., Bailey, S.T., Lauer, A.K., Duncan, J.L., Weleber, R. G., Yang, P., Pennesi, M.E., 2024. Perimacular atrophy following voretigene neparvovec-rzyl treatment in the setting of previous contralateral eye treatment with a different viral vector. Translational Vision Sci. Technol. 13, 11. https://doi.org/10.1167/tvst.13.6.11.

Kumar, B.V., Connors, T.J., Farber, D.L., 2018. Human T cell development, localization, and function throughout life. Immunity 48, 202–213. https://doi.org/10.1016/j.immuni.2018.01.007.

Kvanta, A., Rangaswamy, N., Holopigian, K., Watters, C., Jennings, N., Liew, M.S.H., Bigelow, C., Grosskreutz, C., Burstedt, M., Venkataraman, A., Westman, S., Geirsdottir, A., Stasi, K., André, H., 2024. Interim safety and efficacy of gene therapy for RLBP1-associated retinal dystrophy: a phase 1/2 trial. Nat. Commun. 15, 7438. https://doi.org/10.1038/s41467-024-51575-4.

Ladha, R., Caspers, L.E., Willermain, F., de Smet, M.D., 2022. Subretinal therapy: technological solutions to surgical and immunological challenges. Front. Med. 9. https://doi.org/10.3389/fmed.2022.846782.

Ladha, R., Smit, J., Meenink, T., Caspers, L.E., Willermain, F., De Smet, M.D., 2020. Defining parameters for robotic or manual reflux-free subretinal injections in an ex vivo animal model. Investig. Ophthalmol. Vis. Sci. 61, 4485.

Lam, B.L., Davis, J.L., Gregori, N.Z., MacLaren, R.E., Girach, A., Verriotto, J.D., Rodriguez, B., Rosa, P.R., Zhang, X., Feuer, W.J., 2019. Choroideremia gene therapy phase 2 clinical trial: 24-month results. Am. J. Ophthalmol. 197, 65–73. https://doi.org/10.1016/j.ajo.2018.09.012.

Lam, B.L., Feuer, W.J., Davis, J.L., Porciatti, V., Yu, H., Levy, R.B., Vanner, E., Guy, J., 2022. Leber hereditary optic neuropathy gene therapy: adverse events and visual acuity results of all patient groups. Am. J. Ophthalmol. 241, 262–271. https://doi.org/10.1016/j.ajo.2022.02.023.

Lam, B.L., Pennesi, M.E., Kay, C.N., Panda, S., Gow, J.A., Zhao, G., MacLaren, R.E., MacLaren, R., Aleman, T., Birch, D., Jalil, A., Lotery, A., Lam, B., Pennesi, M., Kay, C. N., Yusuf, I.H., Cehajic Kapetanovic, J., Jolly, J.K., Josan, A.S., Taylor, L.J., Xue, K., Nanda, A., Buckley, T., Salvetti, A.P., Thulasidharan, S., Kurc, M., Khandhadia, S., Dias, K.O.M., Baltmr, A., Mandal, N., Tsokolas, G., Ivanova, T., EL-Faouri, M., Shaarawy, S., Black, G., Davis, J.L., Gregori, N.Z., Mendoza-Santiesteban, C.E., Lauer, A.K., Yang, P., Bailey, S., Spencer, R., Fish, G.E., Wang, R., Chong, D., Abbey, A., Anand, R., MaGuire, A.A., Roseman, R.L., Hazariwala, K.M., Parrott, B., 2024. Assessment of visual function with cotoretigene toliparvovec in X-linked retinitis pigmentosa in the randomized XIRIUS phase 2/3 study. Ophthalmology 131, 1083–1093. https://doi.org/10.1016/j.ophtha.2024.02.023.

Lance, K.D., Good, S.D., Mendes, T.S., Ishikiriyama, M., Chew, P., Estes, L.S., Yamada, K., Mudumba, S., Bhisitkul, R.B., Desai, T.A., 2015. In vitro and in vivo sustained zero-order delivery of rapamycin (sirolimus) from a biodegradable intraocular device. Investig. Ophthalmol. Vis. Sci. 56, 7331–7337. https://doi.org/10.1167/iovs.15-17757.

Langer, P.M., Ward, A., Bell, O.H., Clare, A.J., Chu, C., Chan, Y.K., Dick, A., Copland, D., 2023. Intravitreal AAV delivery causes long-term subclinical microglia activation in mice. Investig. Ophthalmol. Vis. Sci. 64, 781.

Le Meur, G., Lebranchu, P., Billaud, F., Adjali, O., Schmitt, S., Bézieau, S., Péréon, Y., Valabregue, R., Ivan, C., Darmon, C., Moullier, P., Rolling, F., Weber, M., 2018. Safety and long-term efficacy of AAV4 gene therapy in patients with RPE65 leber congenital amaurosis. Mol. Ther. 26, 256–268. https://doi.org/10.1016/j.ymthe.2017.09.014.

Lecomte, E., Tournaire, B., Cogné, B., Dupont, J.-B., Lindenbaum, P., Martin-Fontaine, M., Broucque, F., Robin, C., Hebben, M., Merten, O.-W., Blouin, V., François, A., Redon, R., Moullier, P., Léger, A., 2015. Advanced characterization of DNA molecules in rAAV vector preparations by single-stranded virus next-generation sequencing. Mol. Ther. Nucleic Acids 4. https://doi.org/10.1038/mtna.2015.32.

Lee, G.K., Maheshri, N., Kaspar, B., Schaffer, D.V., 2005. PEG conjugation moderately protects adeno-associated viral vectors against antibody neutralization. Biotechnol. Bioeng. 92, 24–34. https://doi.org/10.1002/bit.20562.

Leray, A., Lalys, P.-A., Varin, J., Bouzelha, M., Bourdon, A., Alvarez-Dorta, D., Pavageau, K., Depienne, S., Marchand, M., Mellet, A., Demilly, J., Ducloyer, J.-B., Girard, T., Fraysse, B., Ledevin, M., Guilbaud, M., Gouin, S.G., Ayuso, E., Adjali, O., Larcher, T., Cronin, T., Le Guiner, C., Deniaud, D., Mével, M., 2024. Novel chemical tyrosine functionalization of adeno-associated virus improves gene transfer efficiency in liver and retina. Biomed. Pharmacother. 171, 116148. https://doi.org/10.1016/j.biopha.2024.116148.

Leroy, B.P., Fischer, M.D., Flannery, J.G., MacLaren, R.E., Dalkara, D., Scholl, H.P.N., Chung, D.C., Spera, C., Viriato, D., Banhazi, J., 2022. Gene therapy for inherited retinal disease: long-term durability of effect. Ophthalmic Res. 66, 179–196. https://doi.org/10.1159/000526317.

Lewis, E.D., Wu, D., Meydani, S.N., 2022. Age-associated alterations in immune function and inflammation. Prog. Neuro Psychopharmacol. Biol. Psychiatr. 118, 110576. https://doi.org/10.1016/j.pnpbp.2022.110576.

Lhériteau, E., Libeau, L., Mendes-Madeira, A., Deschamps, J.-Y., Weber, M., Meur, G.L., Provost, N., Guihal, C., Moullier, P., Rolling, F., 2010. Regulation of retinal function but nonrescue of vision in RPE65-deficient dogs treated with doxycycline-regulatable AAV vectors. Mol. Ther. 18, 1085–1093. https://doi.org/10.1038/mt.2010.46.

Li, Q., Miller, R., Han, P.-Y., Pang, J., Dinculescu, A., Chiodo, V., Hauswirth, W.W., 2008. Intraocular route of AAV2 vector administration defines humoral immune response and therapeutic potential. Mol. Vis. 14, 1760–1769.

Liew, G., Strong, S., Bradley, P., Severn, P., Moore, A.T., Webster, A.R., Mitchell, P., Kifley, A., Michaelides, M., 2019. Prevalence of cystoid macular oedema, epiretinal

membrane and cataract in retinitis pigmentosa. Br. J. Ophthalmol. 103, 1163–1166. https://doi.org/10.1136/bjophthalmol-2018-311964.
Llorenç, V., Nakamura, Y., Metea, C., Karstens, L., Molins, B., Lin, P., 2022. Antimetabolite drugs exhibit distinctive immunomodulatory mechanisms and effects on the intestinal microbiota in experimental autoimmune uveitis. Investig. Ophthalmol. Vis. Sci. 63, 30. https://doi.org/10.1167/iovs.63.3.30.
Lock, M., Alvira, M.R., Chen, S.-J., Wilson, J.M., 2014. Absolute determination of single-stranded and self-complementary adeno-associated viral vector genome titers by droplet digital PCR. Hum. Gene Ther. Methods 25, 115–125. https://doi.org/10.1089/hgtb.2013.131.
Lorenz, B., Künzel, S.H., Preising, M.N., Scholz, J.P., Chang, P., Holz, F.G., Herrmann, P., 2024. Single center experience with voretigene neparvovec gene augmentation therapy in RPE65 mutation–associated inherited retinal degeneration in a clinical setting. Ophthalmology 131, 161–178. https://doi.org/10.1016/j.ophtha.2023.09.006.
Lothert, K., Wolff, M.W., 2023. Affinity and pseudo-affinity membrane chromatography for viral vector and vaccine purifications: a review. Membranes 13, 770. https://doi.org/10.3390/membranes13090770.
Lu, B., Yin, H., Tang, Q., Wang, W., Luo, C., Chen, Xinyi, Zhang, X., Lai, K., Xu, J., Chen, Xiangjun, Yao, K., 2020. Multiple cytokine analyses of aqueous humor from the patients with retinitis pigmentosa. Cytokine 127, 154943. https://doi.org/10.1016/j.cyto.2019.154943.
Luo, S., Jiang, H., Li, Q., Qin, Y., Yang, S., Li, J., Xu, L., Gou, Y., Zhang, Y., Liu, F., Ke, X., Zheng, Q., Sun, X., 2024. An adeno-associated virus variant enabling efficient ocular-directed gene delivery across species. Nat. Commun. 15, 3780. https://doi.org/10.1038/s41467-024-48221-4.
Lynch, M.A., 2022. Exploring sex-related differences in microglia may Be a game-changer in precision medicine. Front. Aging Neurosci. 14. https://doi.org/10.3389/fnagi.2022.868448.
MacLaren, R.E., Audo, I., Fischer, M.D., Huckfeldt, R.M., Lam, B.L., Pennesi, M.E., Sisk, R., Gow, J.A., Li, J., Zhu, K., Tsang, S.-F., 2024. An open-label phase II study assessing the safety of bilateral, sequential administration of retinal gene therapy in participants with choroideremia: the GEMINI study. Hum. Gene Ther. 35, 564–575. https://doi.org/10.1089/hum.2024.017.
MacLaren, R.E., Fischer, M.D., Gow, J.A., Lam, B.L., Sankila, E.-M.K., Girach, A., Panda, S., Yoon, D., Zhao, G., Pennesi, M.E., 2023. Subretinal timrepigene emparvovec in adult men with choroideremia: a randomized phase 3 trial. Nat. Med. 29, 2464–2472. https://doi.org/10.1038/s41591-023-02520-3.
MacLaren, R.E., Groppe, M., Barnard, A.R., Cottriall, C.L., Tolmachova, T., Seymour, L., Clark, K.R., During, M.J., Cremers, F.P.M., Black, G.C.M., Lotery, A.J., Downes, S.M., Webster, A.R., Seabra, M.C., 2014. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet 383, 1129–1137. https://doi.org/10.1016/S0140-6736(13)62117-0.
Maguire, A.M., High, K.A., Auricchio, A., Wright, J.F., Pierce, E.A., Testa, F., Mingozzi, F., Bennicelli, J.L., Ying, G., Rossi, S., Fulton, A., Marshall, K.A., Banfi, S., Chung, D.C., Morgan, J.I., Hauck, B., Zelenaia, O., Zhu, X., Raffini, L., Coppieters, F., Baere, E.D., Shindler, K.S., Volpe, N.J., Surace, E.M., Acerra, C., Lyubarsky, A., Redmond, T.M., Stone, E., Sun, J., McDonnell, J.W., Leroy, B.P., Simonelli, F., Bennett, J., 2009. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. Lancet (London, England) 374, 1597. https://doi.org/10.1016/S0140-6736(09)61836-5.
Maguire, A.M., Simonelli, F., Pierce, E.A., Pugh, E.N., Mingozzi, F., Bennicelli, J., Banfi, S., Marshall, K.A., Testa, F., Surace, E.M., Rossi, S., Lyubarsky, A., Arruda, V. R., Konkle, B., Stone, E., Sun, J., Jacobs, J., Dell'Osso, L., Hertle, R., Ma, J., Redmond, T.M., Zhu, X., Hauck, B., Zelenaia, O., Shindler, K.S., Maguire, M.G., Wright, J.F., Volpe, N.J., McDonnell, J.W., Auricchio, A., High, K.A., Bennett, J., 2008. Safety and efficacy of gene transfer for leber's congenital amaurosis. N. Engl. J. Med. 358, 2240–2248. https://doi.org/10.1056/NEJMoa0802315.
Makinodan, T., Kay, M.M.B., 1980. Age influence on the immune system. In: Kunkel, H. G., Dixon, F.J. (Eds.), Advances in Immunology. Academic Press, pp. 287–330. https://doi.org/10.1016/S0065-2776(08)60047-4.
Manley, A., Meshkat, B.I., Jablonski, M.M., Hollingsworth, T.J., 2023. Cellular and molecular mechanisms of pathogenesis underlying inherited retinal dystrophies. Biomolecules 13, 271. https://doi.org/10.3390/biom13020271.
Márquez, E.J., Chung, C., Marches, R., Rossi, R.J., Nehar-Belaid, D., Eroglu, A., Mellert, D.J., Kuchel, G.A., Banchereau, J., Ucar, D., 2020. Sexual-dimorphism in human immune system aging. Nat. Commun. 11, 751. https://doi.org/10.1038/s41467-020-14396-9.
Martinez-Fernandez de la Camara, C., McClements, M.E., MacLaren, R.E., 2021. Accurate quantification of AAV vector genomes by quantitative PCR. Genes 12, 601. https://doi.org/10.3390/genes12040601.
Martino, A.T., Suzuki, M., Markusic, D.M., Zolotukhin, I., Ryals, R.C., Moghimi, B., Ertl, H.C.J., Muruve, D.A., Lee, B., Herzog, R.W., 2011. The genome of self-complementary adeno-associated viral vectors increases Toll-like receptor 9–dependent innate immune responses in the liver. Blood 117, 6459–6468. https://doi.org/10.1182/blood-2010-10-314518.
Massengill, M.T., Ash, N.F., Young, B.M., Ildefonso, C.J., Lewin, A.S., 2020. Sectoral activation of glia in an inducible mouse model of autosomal dominant retinitis pigmentosa. Sci. Rep. 10, 16967. https://doi.org/10.1038/s41598-020-73749-y.
McClements, M.E., Staurenghi, F., MacLaren, R.E., Cehajic-Kapetanovic, J., 2020. Optogenetic gene therapy for the degenerate retina: recent advances. Front. Neurosci. 14. https://doi.org/10.3389/fnins.2020.570909.
Mehta, A., Merkel, O.M., 2020. Immunogenicity of Cas9 protein. JPharmSci 109, 62–67. https://doi.org/10.1016/j.xphs.2019.10.003.
Mehta, N., Robbins, D.A., Yiu, G., 2021. Ocular inflammation and treatment emergent adverse events in retinal gene therapy. Int. Ophthalmol. Clin. 61, 151–177. https://doi.org/10.1097/IIO.0000000000000366.
Meliani, A., Boisgerault, F., Hardet, R., Marmier, S., Collaud, F., Ronzitti, G., Leborgne, C., Costa Verdera, H., Simon Sola, M., Charles, S., Vignaud, A., van Wittenberghe, L., Manni, G., Christophe, O., Fallarino, F., Roy, C., Michaud, A., Ilyinskii, P., Kishimoto, T.K., Mingozzi, F., 2018. Antigen-selective modulation of AAV immunogenicity with tolerogenic rapamycin nanoparticles enables successful vector re-administration. Nat. Commun. 9, 4098. https://doi.org/10.1038/s41467-018-06621-3.
Mendes, B.B., Conniot, J., Avital, A., Yao, D., Jiang, X., Zhou, X., Sharf-Pauker, N., Xiao, Y., Adir, O., Liang, H., Shi, J., Schroeder, A., Conde, J., 2022. Nanodelivery of nucleic acids. Nat Rev Methods Primers 2, 24. https://doi.org/10.1038/s43586-022-00104-y.
Miao, C.H., 2011. Advances in overcoming immune responses following hemophilia gene therapy. J. Genet Syndr Gene Ther Suppl. 1, 7.
Michaelides, M., Besirli, C.G., Yang, Y., De guimaraes, T.A.C., Wong, S.C., Huckfeldt, R. M., Comander, J.I., Sahel, J.-A., Shah, S.M., Tee, J.J.L., Kumaran, N., Georgiadis, A., Minnick, P., Zeldin, R., Naylor, S., Xu, J., Clark, M., Anglade, E., Wong, P., Fleck, P. R., Fung, A., Peluso, C., Kalitzeos, A., Georgiou, M., Ripamonti, C., Smith, A.J., Ali, R.R., Forbes, A., Bainbridge, J., 2024. Phase 1/2 AAV5-hRKp.RPGR (botaretigene sparoparvovec) gene therapy: safety and efficacy in RPGR-associated X-linked retinitis pigmentosa. Am. J. Ophthalmol. 267, 122–134. https://doi.org/10.1016/j.ajo.2024.05.034.
Michaelides, M., Hirji, N., Wong, S.C., Besirli, C.G., Zaman, S., Kumaran, N., Georgiadis, A., Smith, A.J., Ripamonti, C., Gottlob, I., Robson, A.G., Thiadens, A., Henderson, R.H., Fleck, P., Anglade, E., Dong, X., Capuano, G., Lu, W., Berry, P., Kane, T., Naylor, S., Georgiou, M., Kalitzeos, A., Ali, R.R., Forbes, A., Bainbridge, J., 2023. First-in-Human gene therapy trial of AAV8-hCARp.hCNGB3 in adults and children with CNGB3-associated achromatopsia. Am. J. Ophthalmol. 253, 243–251. https://doi.org/10.1016/j.ajo.2023.05.009.
Mingozzi, F., Maus, M.V., Hui, D.J., Sabatino, D.E., Murphy, S.L., Rasko, J.E.J., Ragni, M. V., Manno, C.S., Sommer, J., Jiang, H., Pierce, G.F., Ertl, H.C.J., High, K.A., 2007. CD8+ T-cell responses to adeno-associated virus capsid in humans. Nat. Med. 13, 419–422. https://doi.org/10.1038/nm1549.
Mingozzi, F., Meulenberg, J.J., Hui, D.J., Basner-Tschakarjan, E., Hasbrouck, N.C., Edmonson, S.A., Hutnick, N.A., Betts, M.R., Kastelein, J.J., Stroes, E.S., High, K.A., 2009. AAV-1–mediated gene transfer to skeletal muscle in humans results in dose-dependent activation of capsid-specific T cells. Blood 114, 2077–2086. https://doi.org/10.1182/blood-2008-07-167510.
Mishra, A., Vijayasarathy, C., Cukras, C.A., Wiley, H.E., Sen, H.N., Zeng, Y., Wei, L.L., Sieving, P.A., 2021. Immune function in X-linked retinoschisis subjects in an AAV8-RS1 phase I/IIa gene therapy trial. Mol. Ther. 29, 2030–2040. https://doi.org/10.1016/j.ymthe.2021.02.013.
Muñoz-Melero, M., Biswas, M., 2024. Role of FoxP3+ regulatory T cells in modulating immune responses to adeno-associated virus gene therapy. Hum. Gene Ther. 35, 439–450. https://doi.org/10.1089/hum.2023.227.
Nelson, J., Alvey, N., Bowman, L., Schulte, J., Segovia, M.C., McDermott, J., Te, H.S., Kapila, N., Levine, D.J., Gottlieb, R.L., Oberholzer, J., Campara, M., 2022. Consensus recommendations for use of maintenance immunosuppression in solid organ transplantation: endorsed by the American college of clinical pharmacy, American society of transplantation, and the international society for heart and lung transplantation. Pharmacotherapy. J. Human Pharmacol. Drug Therapy 42, 599–633. https://doi.org/10.1002/phar.2716.
Neuringer, M., Ryu, J., Hennebold, J.D., Hanna, C., Burch, F., Mishler, E., Peterson, S., Ferguson, B., Spears, R., Woolard, H., Lauer, A.K., Wilson, D., Pennesi, M.E., Boye, S. L., Boye, S., Renner, L., 2023. Rhesus macaque model of Bardet-Biedl syndrome: progression and response to gene therapy. Investig. Ophthalmol. Vis. Sci. 64, 3854.
Newman, N.J., Yu-Wai-Man, P., Subramanian, P.S., Moster, M.L., Wang, A.-G., Donahue, S.P., Leroy, B.P., Carelli, V., Biousse, V., Vignal-Clermont, C., Sergott, R.C., Sadun, A.A., Rebolleda Fernández, G., Chwalisz, B.K., Banik, R., Bazin, F., Roux, M., Cox, E.D., Taiel, M., Sahel, J.-A., the LHON REFLECT Study Group, 2023. Randomized trial of bilateral gene therapy injection for m.11778G>A MT-ND4 Leber optic neuropathy. Brain 146, 1328–1341. https://doi.org/10.1093/brain/awac421.
Ng, B.W.J., Kaukonen, M.K., McClements, M.E., Shamsnajafabadi, H., MacLaren, R.E., Cehajic-Kapetanovic, J., 2024. Genetic therapies and potential therapeutic applications of CRISPR activators in the eye. Prog. Retin. Eye Res. 102, 101289. https://doi.org/10.1016/j.preteyeres.2024.101289.
Nielsen, J., MacLaren, R.E., Heier, J.S., Steel, D., Ivanova, T., Sivaprasad, S., Stanga, P., Bailey, C., Charbel Issa, P., Mendonca, L., Francis, J., Curtiss, D., Hughes, J., Waheed, N.K., 2022. Preliminary results from a first-in-human phase I/II gene therapy study (FOCUS) of subretinally delivered GT005, an investigational AAV2 vector, in patients with geographic atrophy secondary to age-related macular degeneration. Investig. Ophthalmol. Vis. Sci. 63, 1504.
Oertelt-Prigione, S., 2012. The influence of sex and gender on the immune response. Autoimmunity Reviews, Special Issue on Gender, Sex Hormones, Pregnancy and Autoimmunity 11, A479–A485. https://doi.org/10.1016/j.autrev.2011.11.022.
Okanouchi, T., Toshima, S., Kimura, S., Morizane, Y., Shiraga, F., 2016. Novel technique for subretinal injection using local removal of the internal limiting membrane. Retina 36, 1035. https://doi.org/10.1097/IAE.0000000000001029.
Okita, A., Murakami, Y., Shimokawa, S., Funatsu, J., Fujiwara, K., Nakatake, S., Koyanagi, Y., Akiyama, M., Takeda, A., Hisatomi, T., Ikeda, Y., Sonoda, K.-H., 2020. Changes of serum inflammatory molecules and their relationships with visual function in retinitis pigmentosa. Investig. Ophthalmol. Vis. Sci. 61, 30. https://doi.org/10.1167/iovs.61.11.30.

Okunuki, Y., Mukai, R., Nakao, T., Tabor, S.J., Butovsky, O., Dana, R., Ksander, B.R., Connor, K.M., 2019. Retinal microglia initiate neuroinflammation in ocular autoimmunity. Proc. Natl. Acad. Sci. U. S. A. 116, 9989–9998. https://doi.org/10.1073/pnas.1820387116.

Olivares-González, L., Velasco, S., Campillo, I., Rodrigo, R., 2021. Retinal inflammation, cell death and inherited retinal dystrophies. Int. J. Mol. Sci. 22, 2096. https://doi.org/10.3390/ijms22042096.

Orvain, C., Cauvet, A., Prudent, A., Guignabert, C., Thuillet, R., Ottaviani, M., Tu, L., Duhalde, F., Nicco, C., Batteux, F., Avouac, J., Wang, N., Seaberg, M.A., Dillon, S.R., Allanore, Y., 2022. Acazicolcept (ALPN-101), a dual ICOS/CD28 antagonist, demonstrates efficacy in systemic sclerosis preclinical mouse models. Arthritis Res. Ther. 24, 13. https://doi.org/10.1186/s13075-021-02709-2.

Panda, A., Qian, F., Mohanty, S., Duin, D. van, Newman, F.K., Zhang, L., Chen, S., Towle, V., Belshe, R.B., Fikrig, E., Allore, H.G., Montgomery, R.R., Shaw, A.C., 2010. Age-associated decrease in toll-like receptor function in primary human dendritic cells predicts influenza vaccine response. J. Immunol. 184. https://doi.org/10.4049/jimmunol.0901022, 10.4049/jimmunol.0901022.

Parker, M., Yang, P., Choi, D., Mohand-Said, S., Aravind, S., Faridi, A., Chegarnov, E., Butler, C., Barale, P.-O., Lauer, A.K., Stout, T., Wilson, D., Weleber, R., Pennesi, M.E., Sahel, J.A., Audo, I.S., 2024. Six-year results of a Phase I/IIa gene therapy trial in patients with Usher Syndrome Type-1B (USH1B). Investig. Ophthalmol. Vis. Sci. 65, 5327.

Pascual-Dapena, A., Chillaron, J.J., Llauradó, G., Arnau-Barres, I., Flores, J., Lopez-Montesinos, I., Sorlí, L., Luis Martínez-Pérez, J., Gómez-Zorrilla, S., Du, J., García-Giralt, N., Güerri-Fernández, R., 2022. Individuals with higher CD4/CD8 ratio exhibit increased risk of acute respiratory distress syndrome and in-hospital mortality during acute SARS-CoV-2 infection. Front. Med. 9. https://doi.org/10.3389/fmed.2022.924267.

Pei, X., Earley, L.F., He, Y., Chen, X., Hall, N.E., Samulski, R.J., Li, C., 2018. Efficient capsid antigen presentation from adeno-associated virus empty virions in vivo. Front. Immunol. 9. https://doi.org/10.3389/fimmu.2018.00844.

Penaud-Budloo, M., Lecomte, E., Guy-Duché, A., Saleun, S., Roulet, A., Lopez-Roques, C., Tournaire, B., Cogné, B., Léger, A., Blouin, V., Lindenbaum, P., Moullier, P., Ayuso, E., 2017. Accurate identification and quantification of DNA species by next-generation sequencing in adeno-associated viral vectors produced in insect cells. Hum. Gene Ther. Methods 28, 148–162. https://doi.org/10.1089/hgtb.2016.185.

Pennesi, M.E., Yang, P., Birch, D.G., Weng, C.Y., Moore, A.T., Iannaccone, A., Comander, J.I., Jayasundera, T., Chulay, J., Chulay, J., Halliman, D., Feinsod, M., Pennesi, M., Yang, P., Birch, D., Bennett, L., Weng, C.Y., Scholle, T., Channa, R., Baker, L., Stewart, J., Moore, A., Iannaccone, A., Mettu, P., Vajzovic, L., Jayasundera, K.T., Comander, J., Bressler, N., Lam, B., 2022. Intravitreal delivery of rAAV2tYF-CB-hRS1 vector for gene augmentation therapy in patients with X-linked retinoschisis: 1-year clinical results. Ophthalmology Retina 6, 1130–1144. https://doi.org/10.1016/j.oret.2022.06.013.

Perez, B.A., Shutterly, A., Chan, Y.K., Byrne, B.J., Corti, M., 2020. Management of neuroinflammatory responses to AAV-mediated gene therapies for neurodegenerative diseases. Brain Sci. 10, 119. https://doi.org/10.3390/brainsci10020119.

Petrs-Silva, H., Dinculescu, A., Li, Q., Deng, W.-T., Pang, J., Min, S.-H., Chiodo, V., Neeley, A.W., Govindasamy, L., Bennett, A., Agbandje-McKenna, M., Zhong, L., Li, B., Jayandharan, G.R., Srivastava, A., Lewin, A.S., Hauswirth, W.W., 2011. Novel properties of tyrosine-mutant AAV2 vectors in the mouse retina. Mol. Ther. 19, 293–301. https://doi.org/10.1038/mt.2010.234.

Pichi, F., Smith, S.D., AlAli, S.H., Neri, P., 2024. Adalimumab drug monitoring and treatment adjustment to drug antibodies in noninfectious uveitis. Am. J. Ophthalmol. 268, 306–311. https://doi.org/10.1016/j.ajo.2024.09.008.

Piechnik, M., Amendum, P.C., Sawamoto, K., Stapleton, M., Khan, S., Fnu, N., Álvarez, V., Pachon, A.M.H., Danos, O., Bruder, J.T., Karumuthil-Melethil, S., Tomatsu, S., 2022. Sex difference leads to differential gene expression patterns and therapeutic efficacy in mucopolysaccharidosis IVA murine model receiving AAV8 gene therapy. Int. J. Mol. Sci. 23, 12693. https://doi.org/10.3390/ijms232012693.

Pierce, E.A., Aleman, T.S., Jayasundera, K.T., Ashimatey, B.S., Kim, K., Rashid, A., Jaskolka, M.C., Myers, R.L., Lam, B.L., Bailey, S.T., Comander, J.I., Lauer, A.K., Maguire, A.M., Pennesi, M.E., 2024. Gene editing for cep290-associated retinal degeneration. N. Engl. J. Med. 390, 1972–1984. https://doi.org/10.1056/NEJMoa2309915.

Pinilla, I., Maneu, V., Campello, L., Fernández-Sánchez, L., Martínez-Gil, N., Kutsyr, O., Sánchez-Sáez, X., Sánchez-Castillo, C., Lax, P., Cuenca, N., 2022. Inherited retinal dystrophies: role of oxidative stress and inflammation in their physiopathology and therapeutic implications. Antioxidants 11, 1086. https://doi.org/10.3390/antiox11061086.

Prasad, S., Dimmock, D.P., Greenberg, B., Walia, J.S., Sadhu, C., Tavakkoli, F., Lipshutz, G.S., 2022. Immune responses and immunosuppressive strategies for adeno-associated virus-based gene therapy for treatment of central nervous system disorders: current knowledge and approaches. Hum. Gene Ther. 33, 1228–1245. https://doi.org/10.1089/hum.2022.138.

Quinn, J., Musa, A., Kantor, A., McClements, M.E., Cehajic-Kapetanovic, J., MacLaren, R. E., Xue, K., 2021. Genome-editing strategies for treating human retinal degenerations. Hum. Gene Ther. 32, 247–259. https://doi.org/10.1089/hum.2020.231.

Quinn, J., Salman, A., Paluch, C., Jackson-Wood, M., McClements, M.E., Luo, J., Davis, S. J., Cornall, R.J., MacLaren, R.E., Dendrou, C.A., Xue, K., 2024. Single-cell transcriptomic analysis of retinal immune regulation and blood-retinal barrier function during experimental autoimmune uveitis. Sci. Rep. 14, 20033. https://doi.org/10.1038/s41598-024-68401-y.

Rafael, D., Guerrero, M., Marican, A., Arango, D., Sarmento, B., Ferrer, R., Durán-Lara, E. F., Clark, S.J., Schwartz, S., 2023. Delivery systems in ocular retinopathies: the promising future of intravitreal hydrogels as sustained-release scaffolds. Pharmaceutics 15, 1484. https://doi.org/10.3390/pharmaceutics15051484.

Rakoczy, E.P., Lai, C.-M., Magno, A.L., Wikstrom, M.E., French, M.A., Pierce, C.M., Schwartz, S.D., Blumenkranz, M.S., Chalberg, T.W., Degli-Esposti, M.A., Constable, I. J., 2015. Gene therapy with recombinant adeno-associated vectors for neovascular age-related macular degeneration: 1 year follow-up of a phase 1 randomised clinical trial. Lancet 386, 2395–2403. https://doi.org/10.1016/S0140-6736(15)00345-1.

Ramachandran, P.S., Lee, V., Wei, Z., Song, J.Y., Casal, G., Cronin, T., Willett, K., Huckfeldt, R., Morgan, J.I.W., Aleman, T.S., Maguire, A.M., Bennett, J., 2017. Evaluation of dose and safety of AAV7m8 and AAV8BP2 in the non-human primate retina. Hum. Gene Ther. 28, 154–167. https://doi.org/10.1089/hum.2016.111.

Raper, S.E., Chirmule, N., Lee, F.S., Wivel, N.A., Bagg, A., Gao, G., Wilson, J.M., Batshaw, M.L., 2003. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol. Genet. Metabol. 80, 148–158. https://doi.org/10.1016/j.ymgme.2003.08.016.

Rathinam, S.R., Babu, M., Thundikandy, R., Kanakath, A., Nardone, N., Esterberg, E., Lee, S.M., Enanoria, W.T.A., Porco, T.C., Browne, E.N., Weinrib, R., Acharya, N.R., 2014. A randomized clinical trial comparing methotrexate and mycophenolate mofetil for noninfectious uveitis. Ophthalmology 121, 1863–1870. https://doi.org/10.1016/j.ophtha.2014.04.023.

Rathinam, S.R., Gonzales, J.A., Thundikandy, R., Kanakath, A., Murugan, S.B., Vedhanayaki, R., Lim, L.L., Suhler, E.B., Al-Dhibi, H.A., Doan, T., Keenan, J.D., Rao, M.M., Ebert, C.D., Nguyen, H.H., Kim, E., Porco, T.C., Acharya, N.R., FAST Research Group, 2019. Effect of corticosteroid-sparing treatment with mycophenolate mofetil vs methotrexate on inflammation in patients with uveitis: a randomized clinical trial. JAMA 322, 936–945. https://doi.org/10.1001/jama.2019.12618.

Raveney, B.J.E., Copland, D.A., Nicholson, L.B., Dick, A.D., 2008. Fingolimod (FTY720) as an acute rescue therapy for intraocular inflammatory disease. Arch. Ophthalmol. 126, 1390–1395. https://doi.org/10.1001/archopht.126.10.1390.

REGENXBIO, 2024. Suprachoroidal Delivery of Investigational ABBV-RGX-314 for Neovascular AMD: Results from the Phase II AAVIATE Study.

REGENXBIO, 2023. REGENXBIO presents positive one year data from phase II ALTITUDE® trial of ABBV-RGX-314 for treatment of diabetic retinopathy using suprachoroidal delivery. URL: https://regenxbio.gcs-web.com/news-releases/news-release-details/regenxbio-presents-positive-one-year-data-phase-ii-altituder/. accessed 10.19.24.

REGENXBIO, 2022. REGENXBIO announces initiation of second pivotal trial in RGX-314 clinical program for the treatment of wet AMD using subretinal delivery. URL: https://regenxbio.gcs-web.com/news-releases/news-release-details/regenxbio-announces-initiation-second-pivotal-trial-rgx-314/. accessed 10.26.24.

REGENXBIO, 2021. REGENXBIO announces update on RGX-314 and pivotal program for the treatment of wet AMD and new gene therapy program for the treatment of duchenne muscular dystrophy. URL: https://www.prnewswire.com/news-releases/regenxbio-announces-update-on-rgx-314-and-pivotal-program-for-the-treatment-of-wet-amd-and-new-gene-therapy-program-for-the-treatment-of-duchenne-muscular-dystrophy-301201378.html. accessed 10.26.24.

Reichel, F.F., Dauletbekov, D.L., Klein, R., Peters, T., Ochakovski, G.A., Seitz, I.P., Wilhelm, B., Ueffing, M., Biel, M., Wissinger, B., Michalakis, S., Bartz-Schmidt, K.U., Fischer, M.D., 2017. AAV8 can induce innate and adaptive immune response in the primate eye. Mol. Ther. 25, 2648–2660. https://doi.org/10.1016/j.ymthe.2017.08.018.

Reichel, F.F., Michalakis, S., Wilhelm, B., Zobor, D., Muehlfriedel, R., Kohl, S., Weisschuh, N., Sothilingam, V., Kuehlewein, L., Kahle, N., Seitz, I., Paquet-Durand, F., Tsang, S.H., Martus, P., Peters, T., Seeliger, M., Bartz-Schmidt, K.U., Ueffing, M., Zrenner, E., Biel, M., Wissinger, B., Fischer, D., 2022a. Three-year results of phase I retinal gene therapy trial for CNGA3-mutated achromatopsia: results of a non randomised controlled trial. Br. J. Ophthalmol. 106, 1567–1572. https://doi.org/10.1136/bjophthalmol-2021-319067.

Reichel, F.F., Peters, T., Wilhelm, B., Biel, M., Ueffing, M., Wissinger, B., Bartz-Schmidt, K.U., Klein, R., Michalakis, S., Fischer, M.D., for the RD-CURE Consortium, 2018. Humoral immune response after intravitreal but not after subretinal AAV8 in primates and patients. Investig. Ophthalmol. Vis. Sci. 59, 1910–1915. https://doi.org/10.1167/iovs.17-22494.

Reichel, F.F., Seitz, I., Wozar, F., Dimopoulos, S., Jung, R., Kempf, M., Kohl, S., Kortüm, F.C., Ott, S., Pohl, L., Stingl, Krunoslav, Bartz-Schmidt, K.U., Stingl, Katarina, Fischer, M.D., 2022b. Development of retinal atrophy after subretinal gene therapy with voretigene neparvovec. Br. J. Ophthalmol. 107, 1331–1335. https://doi.org/10.1136/bjophthalmol-2021-321023.

Reichel, F.F.L., Wozar, F., Seitz, I., Ochakovski, A., Bartz-Schmidt, K.U., Peters, T., Fischer, M.D., 2021. An optimized treatment protocol for subretinal injections limits intravitreal vector distribution. Ophthalmology Science 1, 100050. https://doi.org/10.1016/j.xops.2021.100050.

Reid, C.A., Nettesheim, E.R., Connor, T.B., Lipinski, D.M., 2018. Development of an inducible anti-VEGF rAAV gene therapy strategy for the treatment of wet AMD. Sci. Rep. 8, 11763. https://doi.org/10.1038/s41598-018-29726-7.

Ren, D., Fisson, S., Dalkara, D., Ail, D., 2022. Immune responses to gene editing by viral and non-viral delivery vectors used in retinal gene therapy. Pharmaceutics 14, 1973. https://doi.org/10.3390/pharmaceutics14091973.

Renshaw, M., Rockwell, J., Engleman, C., Gewirtz, A., Katz, J., Sambhara, S., 2002. Cutting edge: impaired toll-like receptor expression and function in aging. J. Immunol. 169, 4697–4701. https://doi.org/10.4049/jimmunol.169.9.4697.

Rodrigues, G.A., Shalaev, E., Karami, T.K., Cunningham, J., Rivers, H.M., Feng, S., Shah, D., Slater, N.K.H., 2021. Manufacturing considerations and challenges for AAV

ocular gene therapy. In: Neervannan, S., Kompella, U.B. (Eds.), Ophthalmic Product Development: from Bench to Bedside. Springer International Publishing, Cham, pp. 613–633. https://doi.org/10.1007/978-3-030-76367-1_22.
Rodrigues, G.A., Shalaev, E., Karami, T.K., Cunningham, J., Slater, N.K.H., Rivers, H.M., 2018. Pharmaceutical development of AAV-based gene therapy products for the eye. Pharm. Res. 36, 29. https://doi.org/10.1007/s11095-018-2554-7.
Rodríguez-Bocanegra, E., Wozar, F., Seitz, I.P., Reichel, F.F.L., Ochakovski, A., Bucher, K., Wilhelm, B., Bartz-Schmidt, K.U., Peters, T., Fischer, M.D., for the RD-CURE Consortium, 2021. Longitudinal evaluation of hyper-reflective foci in the retina following subretinal delivery of adeno-associated virus in non-human primates. Translational Vision Sci. Technol. 10, 15. https://doi.org/10.1167/tvst.10.6.15.
Roels, D., Ueno, S., Talianu, C.D., Draganova, D., Kondo, M., Leroy, B.P., 2017. Unilateral cancer-associated retinopathy: diagnosis, serology and treatment. Doc. Ophthalmol. 135, 233–240. https://doi.org/10.1007/s10633-017-9605-y.
Ross, M., Sade, K., Obolensky, A., Averbukh, E., Desrosiers, M., Rosov, A., Dvir, H., Gootwine, E., Banin, E., Dalkara, D., Ofri, R., 2024. Characterization of anti-AAV2 neutralizing antibody levels in sheep prior to and following intravitreal AAV2.7m8 injection. Gene Ther. 1–7. https://doi.org/10.1038/s41434-024-00495-5.
Russell, P.S., Bennett, P.J., Wellman, J.A., Chung, D.C., Yu, Z.-F., Tillman, A., Wittes, J., Pappas, J., Elci, O., McCague, S., Cross, D., Marshall, K.A., Walshire, J., Kehoe, T.L., Reichert, H., Davis, M., Raffini, L., George, L.A., Hudson, F.P., Dingfield, L., Zhu, X., Haller, J.A., Sohn, E.H., Mahajan, V.B., Pfeifer, W., Weckmann, M., Johnson, P.C., Gewaily, D., Drack, A., Stone, P.E., Wachtel, K., Simonelli, P.F., Leroy, P.B.P., Wright, J.F., High, K.A., Maguire, P.A.M., 2017. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet (London, England) 390, 849. https://doi.org/10.1016/S0140-6736(17)31868-8.
Sahaboglu, A., Barth, M., Secer, E., Amo, E.M. del, Urtti, A., Arsenijevic, Y., Zrenner, E., Paquet-Durand, F., 2016. Olaparib significantly delays photoreceptor loss in a model for hereditary retinal degeneration. Sci. Rep. 6, 39537. https://doi.org/10.1038/srep39537.
Sahel, J.-A., Boulanger-Scemama, E., Pagot, C., Arleo, A., Galluppi, F., Martel, J.N., Esposti, S.D., Delaux, A., de Saint Aubert, J.-B., de Montleau, C., Gutman, E., Audo, I., Duebel, J., Picaud, S., Dalkara, D., Blouin, L., Taiel, M., Roska, B., 2021. Partial recovery of visual function in a blind patient after optogenetic therapy. Nat. Med. 27, 1223–1229. https://doi.org/10.1038/s41591-021-01351-4.
Salman, A., Kantor, A., McClements, M.E., Marfany, G., Trigueros, S., MacLaren, R.E., 2022. Non-viral delivery of CRISPR/Cas cargo to the retina using nanoparticles: current possibilities, challenges, and limitations. Pharmaceutics 14, 1842. https://doi.org/10.3390/pharmaceutics14091842.
Samulski, R.J., 1995. Adeno-associated viral vectors. In: Vos, J.-M.H. (Ed.), Viruses in Human Gene Therapy. Springer, Netherlands, Dordrecht, pp. 53–76. https://doi.org/10.1007/978-94-011-0555-2_3.
Sanftner, L.H.M., Rendahl, K.G., Quiroz, D., Coyne, M., Ladner, M., Manning, W.C., Flannery, J.G., 2001. Recombinant AAV-mediated delivery of a tet-inducible reporter gene to the rat retina. Mol. Ther. 3, 688–696. https://doi.org/10.1006/mthe.2001.0308.
Sarici, K., Vyas, A., Iannaccone, A., 2023. The double-edged sword of inflammation in inherited retinal degenerations: clinical and preclinical evidence for mechanistically and prognostically impactful but treatable complications. Front. Cell Dev. Biol. 11. https://doi.org/10.3389/fcell.2023.1177711.
Scruggs, B.A., Vasconcelos, H.M., Matioli da Palma, M., Kogachi, K., Pennesi, M.E., Yang, P., Bailey, S.T., Lauer, A.K., 2022. Injection pressure levels for creating blebs during subretinal gene therapy. Gene Ther. 29, 601–607. https://doi.org/10.1038/s41434-021-00294-2.
Seitz, I.P., Michalakis, S., Wilhelm, B., Reichel, F.F., Ochakovski, G.A., Zrenner, E., Ueffing, M., Biel, M., Wissinger, B., Bartz-Schmidt, K.U., Peters, T., 2017. Superior retinal gene transfer and biodistribution profile of subretinal versus intravitreal delivery of AAV8 in nonhuman primates. Investig. Ophthalmol. Vis. Sci. 58, 5792–5801. https://doi.org/10.1167/iovs.17-22473.
Seitz, I.P., Wozar, F., Ochakovski, G.A., Reichel, F.F., Gelisken, F., Bartz-Schmidt, K.U., Peters, T., Fischer, M.D., 2024. Dose-dependent progression of chorioretinal atrophy at the injection site after subretinal injection of rAAV2/8 in nonhuman primates. Ophthalmology Science 4, 100516. https://doi.org/10.1016/j.xops.2024.100516.
Snijder, J., van de Waterbeemd, M., Damoc, E., Denisov, E., Grinfeld, D., Bennett, A., Agbandje-McKenna, M., Makarov, A., Heck, A.J.R., 2014. Defining the stoichiometry and cargo load of viral and bacterial nanoparticles by orbitrap mass spectrometry. J. Am. Chem. Soc. 136, 7295–7299. https://doi.org/10.1021/ja502616y.
Snyder, R.O., 2016. An overview of rAAV vector product development for gene therapy. In: Childers, M.K. (Ed.), Regenerative Medicine for Degenerative Muscle Diseases. Springer, New York, NY, pp. 21–37. https://doi.org/10.1007/978-1-4939-3228-3_2.
Som, M., Gikanga, B., Kanapuram, V., Yadav, S., 2024. Drug product formulation and fill/finish manufacturing process considerations for AAV-based genomic Medicines. J. Pharmaceut. Sci. 113, 1711–1725. https://doi.org/10.1016/j.xphs.2024.03.024.
Stieger, K., Meur, G.L., Lasne, F., Weber, M., Deschamps, J.-Y., Nivard, D., Mendes-Madeira, A., Provost, N., Martin, L., Moullier, P., Rolling, F., 2006. Long-term doxycycline-regulated transgene expression in the retina of nonhuman primates following subretinal injection of recombinant AAV vectors. Mol. Ther. 13, 967–975. https://doi.org/10.1016/j.ymthe.2005.12.001.
Stingl, Katarina, Stingl, Krunoslav, Schwartz, H., Reid, M.W., Kempf, M., Dimopoulos, S., Kortuem, F., Borchert, M.S., Lee, T.C., Nagiel, A., 2023. Full-field scotopic threshold improvement after voretigene neparvovec-rzyl treatment correlates with chorioretinal atrophy. Ophthalmology 130, 764–770. https://doi.org/10.1016/j.ophtha.2023.02.015.
Stranak, Z., Ardan, T., Nemesh, Y., Toms, M., Toualbi, L., Harbottle, R., Ellederova, Z., Lytvynchuk, L., Petrovski, G., Motlik, J., Moosajee, M., Kozak, I., 2024. Feasibility of direct vitrectomy-sparing subretinal injection for gene delivery in large animals. Curr. Eye Res. 49, 879–887. https://doi.org/10.1080/02713683.2024.2343335.
Sun, L., Wu, J., Du, F., Chen, X., Chen, Z.J., 2012. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type-I interferon pathway. Science (New York, N.Y.) 339. https://doi.org/10.1126/science.1232458, 10.1126/science.1232458.
Syncona, 2020. Gyroscope therapeutics granted FDA fast track designation for GT005, an investigational gene therapy for dry age-related macular degeneration. URL: https://www.synconaltd.com/news-and-insights/news/gyroscope-therapeutics-granted-fda-fast-track-designation-for-gt005-an-investigational-gene-therapy-for-dry-age-related-macular-degeneration/. accessed 10.19.24.
Tao, Y., Fukushima, M., Shimokawa, S., Zhao, H., Okita, A., Fujiwara, K., Takeda, A., Mukai, S., Sonoda, K.-H., Murakami, Y., 2024. Ocular and serum profiles of inflammatory molecules associated with retinitis pigmentosa. Transl Vis Sci Technol 13, 18. https://doi.org/10.1167/tvst.13.8.18.
ten Berge, J.C., Fazil, Z., van den Born, L.I., Wolfs, R.C.W., Schreurs, M.W.J., Dik, W.A., Rothova, A., 2019. Intraocular cytokine profile and autoimmune reactions in retinitis pigmentosa, age-related macular degeneration, glaucoma and cataract. Acta Ophthalmol. 97, 185–192. https://doi.org/10.1111/aos.13899.
Teo, K.Y.C., Lee, S.Y., Barathi, A.V., Tun, S.B.B., Tan, L., Constable, I.J., 2018. Surgical removal of internal limiting membrane and layering of AAV vector on the retina under air enhances gene transfection in a nonhuman primate. Investig. Ophthalmol. Vis. Sci. 59, 3574–3583. https://doi.org/10.1167/iovs.18-24333.
Terrada, C., Fisson, S., De Kozak, Y., Kaddouri, M., Lehoang, P., Klatzmann, D., Salomon, B.L., Bodaghi, B., 2006. Regulatory T cells control uveoretinitis induced by pathogenic Th1 cells reacting to a specific retinal Neoantigen1. J. Immunol. 176, 7171–7179. https://doi.org/10.4049/jimmunol.176.12.7171.
Timmers, A.M., Newmark, J.A., Turunen, H.T., Farivar, T., Liu, J., Song, C., Ye, G., Pennock, S., Gaskin, C., Knop, D.R., Shearman, M.S., 2020. Ocular inflammatory response to intravitreal injection of adeno-associated virus vector: relative contribution of genome and capsid. Hum. Gene Ther. 31, 80–89. https://doi.org/10.1089/hum.2019.144.
Torcia, M.G., Nencioni, L., Clemente, A.M., Civitelli, L., Celestino, I., Limongi, D., Fadigati, G., Perissi, E., Cozzolino, F., Garaci, E., Palamara, A.T., 2012. Sex differences in the response to viral infections: TLR8 and TLR9 ligand stimulation induce higher IL10 production in males. PLoS One 7, e39853. https://doi.org/10.1371/journal.pone.0039853.
Trapani, I., Colella, P., Sommella, A., Iodice, C., Cesi, G., De Simone, S., Marrocco, E., Rossi, S., Giunti, M., Palfi, A., Farrar, G.J., Polishchuk, R., Auricchio, A., 2014. Effective delivery of large genes to the retina by dual AAV vectors. EMBO Mol. Med. 6, 194–211. https://doi.org/10.1002/emmm.201302948.
Tummala, G., Crain, A., Rowlan, J., Pepple, K.L., 2021. Characterization of gene therapy associated uveitis following intravitreal adeno-associated virus injection in mice. Investig. Ophthalmol. Vis. Sci. 62, 41. https://doi.org/10.1167/iovs.62.2.41.
Tuttle, J., Drescher, E., Simón-Campos, J.A., Emery, P., Greenwald, M., Kivitz, A., Rha, H., Yachi, P., Kiley, C., Nirula, A., 2023. A phase 2 trial of peresolimab for adults with rheumatoid arthritis. N. Engl. J. Med. 388, 1853–1862. https://doi.org/10.1056/NEJMoa2209856.
Ungar, B., Kopylov, U., Engel, T., Yavzori, M., Fudim, E., Picard, O., Lang, A., Williet, N., Paul, S., Chowers, Y., Bar-Gil Shitrit, A., Eliakim, R., Ben-Horin, S., Roblin, X., 2017. Addition of an immunomodulator can reverse antibody formation and loss of response in patients treated with adalimumab. Aliment. Pharmacol. Ther. 45, 276–282. https://doi.org/10.1111/apt.13862.
Vajzovic, L., 2022. Interim Subretinal Gene Therapy Safety Results in Two Phase 1/2 Open-Label, Dose-Escalation Clinical Trials to Treat Achromatopsia - Presentation at. American Society of Retina Specialists.
van der Veen, I., Heredero Berzal, A., Koster, C., ten Asbroek, A.L.M.A., Bergen, A.A., Boon, C.J.F., 2024. The road towards gene therapy for X-linked juvenile retinoschisis: a systematic review of preclinical gene therapy in cell-based and rodent models of xlrs. Int. J. Mol. Sci. 25, 1267. https://doi.org/10.3390/ijms25021267.
Vandenberghe, L.H., Bell, P., Maguire, A.M., Cearley, C.N., Xiao, R., Calcedo, R., Wang, L., Castle, M.J., Maguire, A.C., Grant, R., Wolfe, J.H., Wilson, J.M., Bennett, J., 2011. Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey. Sci. Transl. Med. 3, 88ra54. https://doi.org/10.1126/scitranslmed.3002103.
Vendomèle, J., Chauveau, G.A., Dalkara, D., Galy, A., Fisson, S., 2024. Peripheral cellular immune responses induced by subretinal adeno-associated virus gene transfer can Be restrained by the subretinal-associated immune inhibition mechanism. Hum. Gene Ther. 35, 464–476. https://doi.org/10.1089/hum.2023.191.
Vendomèle, J., Dehmani, S., Khebizi, Q., Galy, A., Fisson, S., 2018. Subretinal injection of HY peptides induces systemic antigen-specific inhibition of effector CD4+ and CD8+ T-cell responses. Front. Immunol. 9. https://doi.org/10.3389/fimmu.2018.00504.
Vignal-Clermont, C., Uretsky, S., Fitoussi, S., Galy, A., Blouin, L., Girmens, J.-F., Bidot, S., Thomasson, N., Bouquet, C., Valero, S., Meunier, S., Combal, J.-P., Gilly, B., Katz, B., Sahel, J.-A., 2018. Safety of rAAV2/2-ND4 gene therapy for leber hereditary optic neuropathy. Ophthalmology 125, 945–947. https://doi.org/10.1016/j.ophtha.2017.12.036.
Vignal-Clermont, C., Yu-wai-man, P., Newman, N.J., Carelli, V., Moster, M.L., Biousse, V., Subramanian, P.S., Wang, A.-G., Donahue, S.P., Leroy, B.P., Sadun, A.A., Klopstock, T., Sergott, R.C., Rebolleda fernandez, G., Chwalisz, B.K., Banik, R., Taiel, M., Roux, M., Sahel, J.-A., 2023. Safety of lenadogene nolparvovec gene therapy over 5 Years in 189 patients with leber hereditary optic neuropathy. Am. J. Ophthalmol. 249, 108–125. https://doi.org/10.1016/j.ajo.2022.11.026.

Wang, J., Zhang, J., Yu, S., Li, Hongyan, Chen, S., Luo, J., Wang, Haibo, Guan, Y., Zhang, H., Yin, S., Wang, Huili, Li, Heping, Liu, J., Zhu, J., Yang, Q., Sha, Y., Zhang, C., Yang, Y., Yang, X., Zhang, X., Zhao, X., Wang, L., Yang, L., Wei, W., 2024. Gene replacement therapy in Bietti crystalline corneoretinal dystrophy: an open-label, single-arm, exploratory trial. Signal Transduct. Targeted Ther. 9, 1–10. https://doi.org/10.1038/s41392-024-01806-3.

Wang, J.-H., Gessler, D.J., Zhan, W., Gallagher, T.L., Gao, G., 2024. Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduct. Targeted Ther. 9, 1–33. https://doi.org/10.1038/s41392-024-01780-w.

Wang, Y., Menon, N., Shen, S., Feschenko, M., Bergelson, S., 2020. A qPCR method for AAV genome titer with ddPCR-level of accuracy and precision. Mol. Therapy Methods Clin. Dev. 19, 341–346. https://doi.org/10.1016/j.omtm.2020.09.017.

Weed, L., Ammar, M.J., Zhou, S., Wei, Z., Serrano, L.W., Sun, J., Lee, V., Maguire, A.M., Bennett, J., Aleman, T.S., 2019. Safety of same-eye subretinal sequential readministration of AAV2-hRPE65v2 in non-human primates. Mol. Therapy Methods Clin. Dev. 15, 133–148. https://doi.org/10.1016/j.omtm.2019.08.011.

Weleber, R.G., Pennesi, M.E., Wilson, D.J., Kaushal, S., Erker, L.R., Jensen, L., McBride, M.T., Flotte, T.R., Humphries, M., Calcedo, R., Hauswirth, W.W., Chulay, J. D., Stout, J.T., 2016. Results at 2 Years after gene therapy for RPE65-deficient leber congenital amaurosis and severe early-childhood–onset retinal dystrophy. Ophthalmology 123, 1606–1620. https://doi.org/10.1016/j.ophtha.2016.03.003.

Werling, N.J., Satkunanathan, S., Thorpe, R., Zhao, Y., 2015. Systematic comparison and validation of quantitative real-time PCR methods for the quantitation of adeno-associated viral products. Hum. Gene Ther. Methods 26, 82–92. https://doi.org/10.1089/hgtb.2015.013.

Wieringa, W.G., Armbrust, W., Legger, G.E., Los, L.I., 2019. Efficacy of high-dose methotrexate in pediatric non-infectious uveitis. Ocul. Immunol. Inflamm. 27, 1305–1313. https://doi.org/10.1080/09273948.2018.1529800.

Wiley, L.A., Boyce, T.M., Meyering, E.E., Ochoa, D., Sheehan, K.M., Stone, E.M., Mullins, R.F., Tucker, B.A., Han, I.C., 2023. The degree of adeno-associated virus-induced retinal inflammation varies based on serotype and route of delivery: intravitreal, subretinal, or suprachoroidal. Hum. Gene Ther. 34, 530. https://doi.org/10.1089/hum.2022.222.

Willett, K.L., Bennett, J., 2013. Immunology of AAV-mediated gene transfer in the eye. Front. Immunol. 4. https://doi.org/10.3389/fimmu.2013.00261.

Wilson, L., Lewis, K.E., Evans, L.S., Dillon, S.R., Pepple, K.L., 2023. Systemic administration of acazicolcept, a dual CD28 and inducible T cell costimulator inhibitor, ameliorates experimental autoimmune uveitis. Translational Vision Sci. Technol. 12, 27. https://doi.org/10.1167/tvst.12.3.27.

Wood, E.H., Rao, P., Mahmoud, T.H., 2022. Nanovitreoretinal subretinal Gateway device to displace submacular hemorrhage: access to the subretinal space without vitrectomy. Retina 42, 2225. https://doi.org/10.1097/IAE.0000000000002669.

Wright, J.F., 2020. Codon modification and PAMPs in clinical AAV vectors: the tortoise or the hare? Mol. Ther. 28, 701–703. https://doi.org/10.1016/j.ymthe.2020.01.026.

Wright, J.F., 2014. Product-related impurities in clinical-grade recombinant AAV vectors: characterization and risk assessment. Biomedicines 2, 80–97. https://doi.org/10.3390/biomedicines2010080.

Wu, J., Sun, L., Chen, X., Du, F., Shi, H., Chen, C., Chen, Z., 2013. Cyclic-GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science 339. https://doi.org/10.1126/science.1229963.

Wu, Z., Sun, J., Zhang, T., Yin, C., Yin, F., Dyke, T.V., Samulski, R.J., Monahan, P.E., 2008. Optimization of self-complementary AAV vectors for liver-directed expression results in sustained correction of hemophilia B at low vector dose. Mol. Ther. 16, 280–289. https://doi.org/10.1038/sj.mt.6300355.

Wykoff, C.C., 2023. Subretinal Delivery of RGX-314 for Neovascular AMD: A Phase II Pharmacodynamic Study.

Xiang, Z., Kurupati, R.K., Li, Y., Kuranda, K., Zhou, X., Mingozzi, F., High, K.A., Ertl, H.C. J., 2020. The effect of CpG sequences on capsid-specific CD8+ T cell responses to AAV vector gene transfer. Mol. Ther. 28, 771–783. https://doi.org/10.1016/j.ymthe.2019.11.014.

Xiong, W., Wu, D.M., Xue, Y., Wang, S.K., Chung, M.J., Ji, X., Rana, P., Zhao, S.R., Mai, S., Cepko, C.L., 2019. AAV cis-regulatory sequences are correlated with ocular toxicity. Proc. Natl. Acad. Sci. USA 116, 5785–5794. https://doi.org/10.1073/pnas.1821000116.

Xue, K., Groppe, M., Salvetti, A.P., MacLaren, R.E., 2017. Technique of retinal gene therapy: delivery of viral vector into the subretinal space. Eye 31, 1308–1316. https://doi.org/10.1038/eye.2017.158.

Xue, K., Jolly, J.K., Barnard, A.R., Rudenko, A., Salvetti, A.P., Patrício, M.I., Edwards, T. L., Groppe, M., Orlans, H.O., Tolmachova, T., Black, G.C., Webster, A.R., Lotery, A.J., Holder, G.E., Downes, S.M., Seabra, M.C., MacLaren, R.E., 2018. Beneficial effects on vision in patients undergoing retinal gene therapy for choroideremia. Nat. Med. 24, 1507–1512. https://doi.org/10.1038/s41591-018-0185-5.

Yang, P., Lauer, A., Pennesi, M.E., Birch, D.G., Spencer, R., Sisk, R., Iannaccone, A., Feinsod, M., Goldbaum, M., 2022. 18 Month analysis of macular structure using optical coherence tomography (OCT) from a phase 1/2 clinical study of subretinal gene therapy drug AGTC-501 for X-linked retinitis pigmentosa. Investig. Ophthalmol. Vis. Sci. 63, 4399-F0078.

Yang, P., Mustafi, D., Pepple, K.L., 2024a. Immunology of retinitis pigmentosa and gene therapy-associated uveitis. Cold Spring Harb Perspect Med 14, a041305. https://doi.org/10.1101/cshperspect.a041305.

Yang, P., Pardon, L.P., Ho, A.C., Lauer, A.K., Yoon, D., Boye, S.E., Boye, S.L., Roman, A. J., Wu, V., Garafalo, A.V., Sumaroka, A., Swider, M., Viarbitskaya, I., Aleman, T.S., Pennesi, M.E., Kay, C.N., Fujita, K.P., Cideciyan, A.V., 2024b. Safety and efficacy of ATSN-101 in patients with Leber congenital amaurosis caused by biallelic mutations in GUCY2D: a phase 1/2, multicentre, open-label, unilateral dose escalation study. Lancet 404, 962–970. https://doi.org/10.1016/S0140-6736(24)01447-8.

Yin, X., Zhang, S., Lee, J.H., Dong, H., Mourgkos, G., Terwilliger, G., Kraus, A., Geraldo, L.H., Poulet, M., Fischer, S., Zhou, T., 2024. Compartmentalized ocular lymphatic system mediates eye–brain immunity. Nature 628, 204–211. https://doi.org/10.1038/s41586-024-07130-8.

Yiu, G., Chung, S.H., Mollhoff, I.N., Nguyen, U.T., Thomasy, S.M., Yoo, J., Taraborelli, D., Noronha, G., 2020. Suprachoroidal and subretinal injections of AAV using transscleral microneedles for retinal gene delivery in nonhuman primates. Mol. Therapy Methods Clin. Dev. 16, 179–191. https://doi.org/10.1016/j.omtm.2020.01.002.

Yoshida, M., Worlock, K.B., Huang, N., Lindeboom, R.G.H., Butler, C.R., Kumasaka, N., Dominguez Conde, C., Mamanova, L., Bolt, L., Richardson, L., Polanski, K., Madissoon, E., Barnes, J.L., Allen-Hyttinen, J., Kilich, E., Jones, B.C., de Wilton, A., Wilbrey-Clark, A., Sungnak, W., Pett, J.P., Weller, J., Prigmore, E., Yung, H., Mehta, P., Saleh, A., Saigal, A., Chu, V., Cohen, J.M., Cane, C., Iordanidou, A., Shibuya, S., Reuschl, A.-K., Herczeg, I.T., Argento, A.C., Wunderink, R.G., Smith, S. B., Poor, T.A., Gao, C.A., Dematte, J.E., Reynolds, G., Haniffa, M., Bowyer, G.S., Coates, M., Clatworthy, M.R., Calero-Nieto, F.J., Göttgens, B., O'Callaghan, C., Sebire, N.J., Jolly, C., De Coppi, P., Smith, C.M., Misharin, A.V., Janes, S.M., Teichmann, S.A., Nikolić, M.Z., Meyer, K.B., 2022. Local and systemic responses to SARS-CoV-2 infection in children and adults. Nature 602, 321–327. https://doi.org/10.1038/s41586-021-04345-x.

Yoshida, N., Ikeda, Y., Notomi, S., Ishikawa, K., Murakami, Y., Hisatomi, T., Enaida, H., Ishibashi, T., 2013. Clinical evidence of sustained chronic inflammatory reaction in retinitis pigmentosa. Ophthalmology 120, 100–105. https://doi.org/10.1016/j.ophtha.2012.07.006.

Yuan, L.-F., Li, G.-D., Ren, X.-J., Nian, H., Li, X.-R., Zhang, X.-M., 2015. Rapamycin ameliorates experimental autoimmune uveoretinitis by inhibiting Th1/Th2/Th17 cells and upregulating CD4+CD25+ Foxp3 regulatory T cells. Int. J. Ophthalmol. 8, 659–664. https://doi.org/10.3980/j.issn.2222-3959.2015.04.03.

Yu-Wai-Man, P., Newman, N.J., Carelli, V., Moster, M.L., Biousse, V., Sadun, A.A., Klopstock, T., Vignal-Clermont, C., Sergott, R.C., Rudolph, G., La Morgia, C., Karanjia, R., Taiel, M., Blouin, L., Burguière, P., Smits, G., Chevalier, C., Masonson, H., Salermo, Y., Katz, B., Picaud, S., Calkins, D.J., Sahel, J.-A., 2020. Bilateral visual improvement with unilateral gene therapy injection for Leber hereditary optic neuropathy. Sci. Transl. Med. 12, eaaz7423. https://doi.org/10.1126/scitranslmed.aaz7423.

Zhang, P., Jain, P., Tsao, C., Wu, K., Jiang, S., 2019. Proactively reducing anti-drug antibodies via immunomodulatory bioconjugation. Angew. Chem. 131, 2455–2458. https://doi.org/10.1002/ange.201814275.

Zhu, J., Huang, X., Yang, Y., 2009. The TLR9-MyD88 pathway is critical for adaptive immune responses to adeno-associated virus gene therapy vectors in mice. J. Clin. Investig. 119, 2388–2398. https://doi.org/10.1172/JCI37607.

Tan, Q., Liu, H., Bian, B., Liu, Y., 2023. The sexed retina: a combined single-cell and bulk RNA sequencing analysis of healthy aging human retina. doi: 10.21203/rs.3.rs-3365102/v1.