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Posted Date: 22 January 2024

doi: 10.20944/preprints202401.1524.v1

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New Generation of Gene Therapies as the Future of Wet AMD Treatment

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Abstract: Age-related macular degeneration (AMD) is an eye disease and the most common cause of vision loss in the Western World. In its advanced stage, AMD occurs in two clinically distinguished forms: dry and wet, but only wet AMD is treatable. However, the treatment based on repeated injections with vascular endothelial growth factor A (VEGFA) antagonists may at best stop the disease progression and prevent or delay vision loss but without an improvement of visual dysfunction. Moreover, it is a serious mental and financial burden for patients and may be linked with some complications. The recent first success of intravitreal gene therapy with ADVM-022, which transformed retinal cells to continuous production of aflibercept, a VEGF antagonist, after a single injection, has opened a revolutionary perspective in wet AMD treatment. Promising results obtained so far in other ongoing clinical trials support this perspective. In this narrative/hypothesis review, we present basic information on wet AMD pathogenesis and treatment, the idea of gene therapy in retinal diseases, update evidence on completed and ongoing clinical trials with gene therapy for wet AMD, and perspectives on the road to the clinic of "one and done" therapy for wet AMD to replace a lifetime of injections. Gene editing targeting the *VEGFA* gene is also presented as another gene therapy strategy to improve wet AMD management.

Keywords: age-related macular degeneration; AMD; vascular endothelial growth factor; VEGF; anti-VEGF therapy; gene therapy; clinical trials; gene editing

1. Introduction

Age-related macular degeneration (AMD) is an eye disease and a serious problem in aging societies [1]. Advanced AMD presents two clinically distinct forms: dry (atrophic) and wet (exudative, neovascular) [2]. Both forms may lead to legal blindness and sight loss, but currently, only wet AMD is treatable by intravitreal injections with antibodies to vascular endothelial growth factor A (VEGFA) and its receptor (anti-VEGFA therapy) [3,4]. However, usually wet AMD treatment does not result in the disease cure, but at best stops its progression, preventing or delaying sight loss. In addition, the treatment is troublesome for patients as it includes repeated injections into one eye or both eyes and constitutes a serious financial burden for the patients. An alternative for resistance to or intolerance of anti-VEGFA treatment is photodynamic therapy, which may be applied along with the anti-VEGFA treatment [5,6].

Although anti-VEGFA treatment is a targeted therapy, it is not free from detrimental side effects that may lead to damage to the eye. Some dietary interventions may improve these effects and the efficacy of anti-VEGFA therapy, but their effects depend on many factors that may be difficult to anticipate [7]. Moreover, some patients display resistance to this kind of therapy [8].

 doi:10.20944/preprints202401.1524.v1

In 2008, three independent groups reported a successful subretinal injection of an AAV-based expression vector carrying the retinoid isomerohydrolase RPE65 (aka retinal pigment epithelium-specific 65 KDa protein) gene to improve vision in individuals with inherited blindness (Leber's congenital amaurosis) [9–11]. This resulted in the first FDA-approved gene therapy product for the eye – Luxturna (voretigene neparvovec-rzyl). However, maybe more important was that this initial success of gene therapy in the eye contributed to the rejuvenation of gene therapy in general after its serious pitfall associated with the death of a patient inspired a deeper look into the biology of virus vectors [12,13]. These experiments set the groundwork for the development of gene therapies for AMD.

Good and bad experiences of gene therapy enabled the elaboration of the strategy of safe and efficient cargo delivery by the vectors derived from adeno-associated viruses [14]. Consequently, recent studies on gene therapy in wet AMD have revolutionized the perspective of the treatment of this disease [15]. Instead of a replacement of faulty or lacking protein with its functional counterpart, new therapies direct the eye to synthesize its anti-VEGF drugs. These therapies eliminate the burden of multiple intravitreal injections, offering a stable production of anti-VEGF antibodies for a long time. The results obtained so far in ongoing and completed clinical trials are promising, but some items concerning safety and efficacy need further studies (https://classic.clinicaltrials.gov/ct2/results?cond=Wet+Age-related+Macular+Degeneration&term=VEGF+gene+therapy&cntry=&state=&city=&dist=&Search=Search&type=Intr. Accessed January 15, 2024).

There are excellent reviews, addressing gene therapy and gene editing in eye diseases in detail, e.g., [15,16]. In this narrative/perspective review, we update information on ongoing and completed clinical trials on gene therapy for wet AMD. We shortly describe wet AMD pathogenesis and therapy and provide general information on gene therapy in ocular diseases, focusing on the AAV vector, as the main platform to deliver target DNA to retinal cells in wet AMD. Gene editing in wet AMD is also briefly presented as a further perspective in the management of this disease.

2. Wet age-related macular degeneration: pathogenesis and therapy

Age-related macular degeneration is a complex, multifactorial disease with aging, environmental/lifestyle, and genetic/epigenetic factors playing a role in its pathogenesis [17]. The complexity of this disease is also underlined in that each of the mentioned items has several variants that may interplay both within each group of factors and with factors from a different group. For instance, AMD is not a monogenic disease, and as shown in genome-wide association studies, several genetic loci may be involved in its pathogenesis, containing genes of three main pathways: the complement pathway, lipid metabolism, and extracellular matrix remodeling and variants of these genes may interact and their effect can be modulated by environmental/lifestyles AMD risk factors, including unhealthy diet [18]. The most consistently reported loci that are associated with AMD are the rs1061170 (Tyr402His/p.Y402H) single nucleotide polymorphism variant in the complement factor H (*CHF*) and the age-related maculopathy susceptibility 2 and high-temperature requirement A serine peptidase 1 (*ARMS2*/*HTRA1*) [19,20]. Therefore, many mechanisms may be involved in AMD pathogenesis, which, along with the limited possibility of studying live human eyes, results in limited treatment options for AMD.

Advanced AMD happens in two clinically distinct forms: dry and wet and individuals affected by either form may suffer from gaps or dark spots in their vision (Figure 1) Despite that wet AMD is responsible for a minority of all AMD cases, it accounts for about 90% of sight loss related to AMD and, somehow paradoxically, only wet AMD is treatable [21]. The causal relationship, if any, between these two forms of AMD is not clear, and some studies suggest that they might be considered as two distinct diseases [22]. We showed that wet AMD might correlate with mortality in a 12-year prospective case-control study, but the question, of whether wet AMD might be an independent risk factor of death is still open [23].

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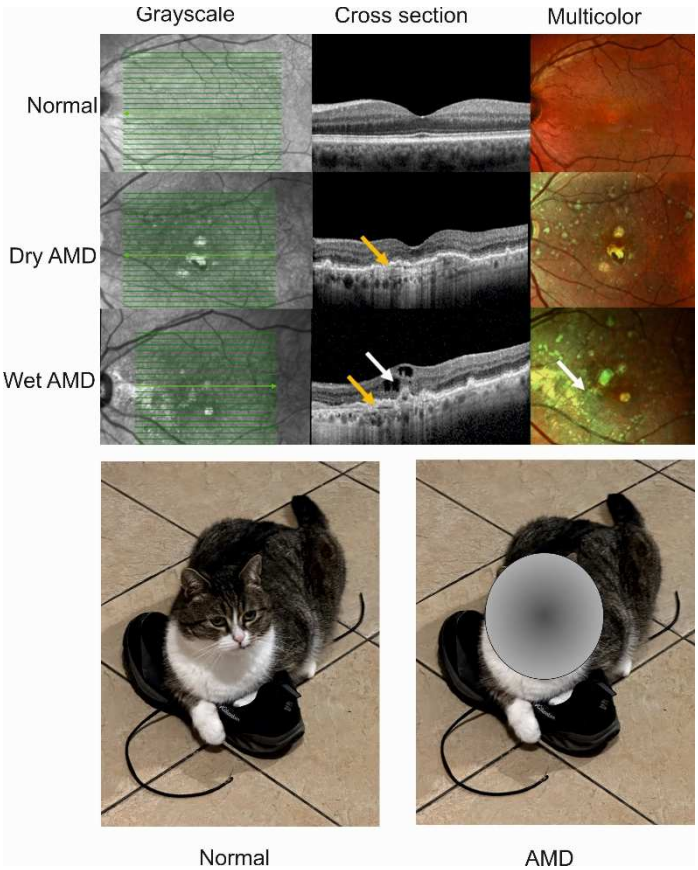

Figure 1. Advanced age-related macular degeneration (AMD) presents two clinically distinct forms: dry and wet. Optical coherence tomography of normal, dry AMD, and wet AMD eyes (upper panel). Yellow arrows indicate degenerated retinal pigment epithelial layers (green lines) and a white arrow shows edema and intraretinal fluid observed in cross-sectional view corresponding to increased intensity of green color in the multicolor image. Advanced AMD causes disturbances in or loss of central vision (lower panel).

Drusen, small light solid objects containing proteins and lipids are present in both forms of AMD but their presence in dry AMD is much more common than in wet AMD (approximately 90:10%). They can be divided into hard and soft drusen [24]. Dry AMD is characterized by a gradual deterioration of the retina following from the death of retinal cells that are not renewed [25]. Drusen and edema may be visible in the microscopic picture of the affected eye.

Choroidal neovascularization (CVN) presenting the growth of new blood vessels derived from the choroid through a break in the Bruch's membrane, is a hallmark of wet AMD [26]. In wet AMD the retinal pigment epithelium (RPE) is not broken, as in dry AMD but RPE may be detached [27]. Furthermore, the functionality of the RPE cells is changed as they overproduce VEGFA and other angiogenic factors, essential in CNV. Fresh blood vessels formed in CNV are fragile and release their content into the retina, resulting in fibrosis, with subsequent formation of disciform scar that can result in sight loss, unless treated [28]. The angiogenic effect of VEGFA in CNV is mainly mediated by the PLCγ-PKC-MAPK pathway initiated from the 1175-PY site on VEGFA receptor 2 (VEGFR-2) located on the exterior of endothelium [29].

At present, the treatment of wet AMD patients with antibodies against VEGFA and its receptor is the only accepted therapy in wet AMD, whose efficacy has been consistently confirmed and has become a routine procedure in ophthalmic clinics [30]. The anti-VEGFA treatment significantly improves the outcomes of wet AMD, although cases of improving visual acuity are rare, the treatment stops the progression of the disease and ameliorates damage in the retina [3,31]. However, the treatment per se is troublesome, as it contains repeated intravitreal injections, every 8 to 12 weeks after three monthly loading doses, with a relatively expensive formulation of monoclonal antibodies against VEGFA and its receptor.

 doi:10.20944/preprints202401.1524.v1

Many formulations of VEGFA antibodies are present in the market, including those FDA approved: aflibercept (Eylea), brolucizumab (Beovu), ranibizumab (Lucentis), and faricimab-svoa (Vabysmo); bevacizumab (Avastin) is used off-label as it is primarily designated to fight cancer [32]. Also, biosimilars to anti-VEGF have been recently approved by FDA (https://www.reviewofophthalmology.com/article/an-update-on-the-antivegf-biosimilar-pipeline; accessed January 15, 2024).

In summary, the introduction of anti-VEGFA treatment revolutionized the therapy of wet AMD. Although the treatment does not cure the disease, it may protect against sight loss and is in general safe in a long-time application. However, the necessity of lifetime intravitreal injections and the relatively high cost of anti-VEGF drugs are a serious burden for patients.

3. Gene therapy for wet age-related macular degeneration

The eye seems to be predisposed as a target for gene therapy due to its relatively small size with a compartmentalized structure and immune-privileged status [16]. These eye characteristics lower the risk of systemic exposure. Moreover, advanced non-invasive methods of imaging in the eye, including optical coherence tomography, fundoscopy, angiography, and two-photon microscopy assist a real-time monitoring of the progress of the gene therapy procedures and their safety [33]. Another advantageous feature of the aye for gene therapy is that changes in a single gene may be associated with various clinical states. For example, homozygous mutation in the "historic" *RPE65* gene may result in either Leber congenital amaurosis 2 or rare forms of retinitis pigmentosa (RP) [34].

The development of gene therapy has brought a better understanding of the biology of viral vectors as they are basic vectors used in eye gene therapy [35]. In general, virus vectors can be divided into integrating and non-integrating with the host genome. Due to safety concerns, non-integrating viral vectors may be the present and at least the near future for strategies of transgene delivery in gene therapy [36].

Among many non-integrating viral vector types, adeno-associated viral (AAV) vectors have many features of key significance for eye gene therapy [37].

Adeno-associated viruses consist of two parts: an icosahedral protein capsid and a single-stranded DNA (ssDNA) genome [38–40]. The AAV genome has 4.8 kb of ssDNA and two "T-shaped" inverted terminal repeats (ITRs), each on either end of the genome [41] (Figure 2). The terminal repeats flank two open reading frames *Rep* and *Cap* that are transcribed and translated to produce the virus life cycle proteins Rep78, Rep68, Rep52, Rep40, and VP1, VP2, and VP3 capsid proteins resulting from the use of alternate promoters and alternate splicing.

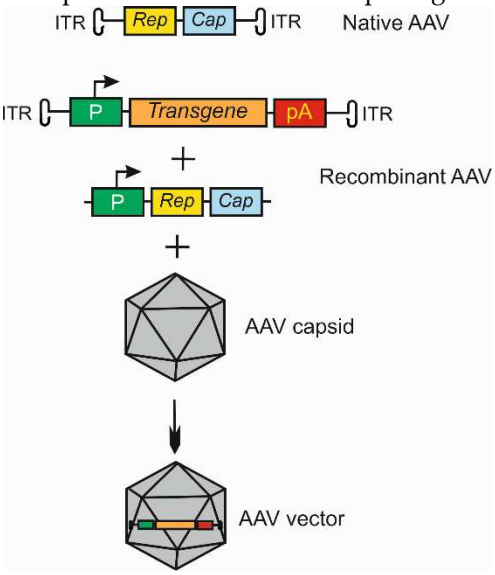

Figure 2. Basic strategy of the use of adeno-associated virus (AAV) as a vector in gene therapy. The AVV genome contains two open reading frames (ORFs) Rep and Cap flanked by two inverted terminal repeats (ITRs). Recombinant AAV is formed by replacing ORFs with an expressional cassette containing a promoter (P), a transgene that may also be an RNA molecule and terminator, here polyadenylation sequence (pA). The *Rep* and *Cap* are added in *trans*. That construct is packaged into

 doi:10.20944/preprints202401.1524.v1

the AAV capsid to form an AAV vector. Many variants of this procedure may be applied in dependence on the tropism and serotype of AAV in conjunction with target cell and tissue type.

In the transfer plasmid construct, which is to be packaged by the AAV virus, the transgene is placed between ITRs, and *Rep* and *Cap* are attached in trans (Figure 2).

AAV presents various serotypes differing in the capsid structure, immunogenicity, and cellular tropism, the most suitable serotypes can be chosen to accommodate the eye environment [42]. The parental adeno-associated virus requires a helper virus to replicate to exert its pathogenic for human action [43]. Moreover, AAV vectors were shown to elicit a limited immune response due to the restricted ability of the parent virus to infect antigen-presenting cells [44].

The virus needs the Rep protein to replicate and the open reading frame for encoding this protein, can be removed by gene editing, depriving the virus of the ability to replicate and integrate [43]. If this open reading frame is not edited, the virus integrates in a site-specific fashion with the human genome on chromosome 19 [45]. Therefore, the AAV vector offers the possibility to integrate or not with the host genome by the manipulation with the *rep* locus.

The AAV vectors have some limitations [46]. They cannot package fragment DNA longer than 4.5 kb, their long-term expression is limited to non-dividing cells, and despite their limited ability to elicit immune response, there is a high incidence of pre-existing immunity in humans [47].

If the *rep* locus is retained, one may expect a long-term, controlled expression of target DNA in chromosome 19 and accumulation of the product of that expression. Therefore, if the target DNA may encode a product that would stimulate the eye to continuously produce an antagonist of VEGFA, this would replace multiple injections with anti-VEGFA antibodies. This is a general idea of the exploitation of AAV vectors in gene therapy of wet AMD. This is a definite change of the general strategy of gene therapy, which in its standard form aims to replace a faulty or absent gene with a gene that displays normal expression. Here, nothing is replaced, but the strategy with AAV vector carriers an added value for target cells. Moreover, the recent strategies of gene therapies with the AAV vectors have created the possibility of therapeutic gene manipulations with minimal concerns for inflammatory and immune reactions with maximal efficacy [16].

The above idea is just an idea, and many details had to be elaborated before its accomplishment. The main problems to work out were the delivery route and sustained expression of the VEGFA antagonist.

In general, three routes of the delivery of a transgene are considered in ocular gene therapy delivery methods (Figure 3). The delivery to the suprachoroidal and subretinal spaces is characterized by targeted access and wide transduction of the retinal cells, low exposure to the vitreous and anterior segments, and compartmentalized delivery of the AAV vector observed in preclinical studies [48–50]. Subretinal delivery poses a low risk of immune response and inflammation [51]. Intravitreal injections are featured by limited transduction of retinal cells, a wide exposure of the vitreous and anterior segments, and a high risk of immune/inflammatory reactions [52,53].

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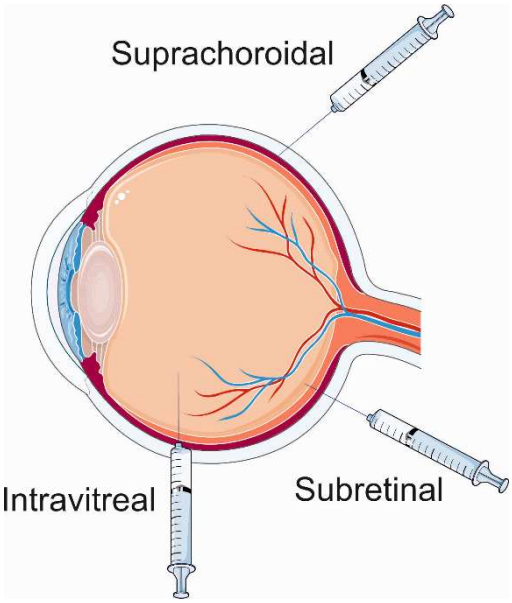

Figure 3. Ocular gene therapy delivery routes. Parts of this figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Subretinal introduction of the *RPE65* gene in patients with Leber congenital dystrophy was a fundamental work for gene therapy for AMD. The normal *RPE65* gene encodes the RPE65 protein that transforms all-trans-retinol into 11-cis-retinol in the retinal cycle, a reaction that is crucial for the conversion of visual information into electric pulses [54]. Transduction of RPE cells with the transgene resulted in the production of the functional RPE65 protein. Therefore, experiments showed that AAV vectors may be used to provide a stable expression of the target DNA in the retina, opening a perspective for the "one-and-done" in-office treatment of retinal diseases requiring a continuous production of a therapeutic protein. However, the question remained whether a subretinal injection was an optimal route of administration of the target DNA.

Surely, past, present, and likely the future of gene therapy in the eye is not limited to the use of AAV-based vectors as delivery systems, and several other types of viruses, as well as non-viral vectors, were attempted to use. More detailed analysis can be found elsewhere, e.g., in the review of Rodrigues et al. [47].

4. Clinical trials on gene therapy for wet age-related macular degeneration

ClinicalTrials.gov lists 22 studies on gene therapy in wet AMD (https://clinicaltrials.gov/search?cond=Wet%20AMD&intr=gene%20therapy, accessed January 16, 2024). These include 1 completed with results, 4 completed, 12 recruiting, 3 active, not recruiting, 1 not yet recruiting, and 1 enrolling by invitation studies. In this section, we shortly provide some details on two clinical trials ADVM-022 and RGX-314 as they present the most advanced stages of such studies. In general, these studies aim to provide a durable expression of therapeutic levels of intraocular protein and maintain vision and so safely reduce the current treatment burden.

4.1. ADVM-022

The ADVM-022 open-label clinical trial (OPTIC) by Adverum Biotechnologies was set to elaborate an in-office intravitreal gene therapy for wet AMD and diabetic macular edema (https://clinicaltrials.gov/study/NCT03748784. Accessed January 15, 2024).

The preclinical studies of the OPTIC trial were performed in mice with laser-induced CNV [55]. Those studies utilized the AAV2.7m8 capsid, which was obtained from AAV2 by directed evolution and optimizing the efficacy of transduction of the retinal cells after intravitreal administration [56]. Therefore intravitreal injection was chosen in that study as not only the vitreous body is easily accessible, but it also avoids possible barriers for target DNA delivery resulting from dense tissue

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penetration while guaranteeing a widespread delivery to the outer retina [56]. Results of several other studies contributed to the initiation and success of the project. These include works on the stabilization of the expression of aflibercept. It was shown that a single injection of ADVM-022 in non-human primates resulted in a 30-day sustained expression of aflibercept and an injection was effective in CVN inhibition even if made 13 months before laser-induction of CNV [57,58].

The expressional cassette of ADVM-022 contained the aflibercept cDNA, which was codon-optimized, following the translation start codon within the Kozak consensus sequence (Figure 4) [55]. The expression of the cassette was driven from the human cytomegalovirus promoter and several other regulatory elements optimized the expression of aflibercept, which is a recombinant chimeric protein consisting of parts of the human VEGFA binding portion of VEGFA receptors and human igG1 immunoglobulin [59].

Figure 4. The codon-optimized aflibercept cDNA expression cassette utilized in ADVM-022. The cassette is flanked by adeno-associated virus (AAV) inverted terminal repeats under the control of the human cytomegalovirus early enhancer (E)-promoter (hCMV). These elements are followed by three regulatory sequences of the AAV genome and translation start codon, symbolized here as an RNA codon AUG, within the Kozak consensus sequence and a human scaffold attachment region (SAR) and a polyadenylation termination site (pA) derived from the human growth hormone.

The main general aim of ADVM-022 was to treat wet AMD with a single intravitreal injection of the AAV2.7m8 capsid containing cDNA for an alfibercept-like protein. The Phase I trial enrolled patients previously controlled with frequent anti-VEGF injections who received high or low doses of ADVM-022, which was renamed to ixoberogene soroparvovec (ixo-vec). The high-dose population showed a 98% decrease in the number of anti-VEFGA injections, while the low-dose group – an 80% reduction [60]. Ocular treatment-emergent adverse events (TEAEs) related to ixo-vec were mild to moderate and included anterior chamber cells and vitreal cells. Five serious TEAEs (SAEs) occurring during the trial included two cases of cataract, dry AMD, retinal detachment, and a case of recurrent uveitis. However, dry AMD and recurrent uveitis could not have been attributed to ixo-vec treatment. No clinical or imaging evidence of inflammation was reported, but some patients showed the presence of anterior chamber cells and vitreal cells. Best-corrected visual acuity was stable in both groups over 2 years. The mean change in central subfield thickness decreased after ixo-vec injection and remained stable for over 2 years.

In summary, the ADVM-022 trial showed that a single injection of ixo-vec induced sustained levels of intraocular aflibercept, ameliorating retinal anatomy, no worsening of BCVA, and a significant reduction in the number of anti-VEGF injections with a majority of patients not needing such supplementation of ixo-vec treatment. The main limitation of this trial was a small sample size, which in combination with some baseline characteristics might caused a bias. The study was unmasked, creating a potential for unintentional decisions of investigators. There was no comparison between ixo-vec treatment and not-treated cases of wet AMD. Despite these and other limitations, the ADVM-022 has opened a perspective to abandon frequent anti-VEGFA injection for just a single injection of ixo-vec.

4.2. RGX-314

RGX-314 is a clinical trial also aiming at the strategy of a one-time treatment for wet AMD with an estimated enrollment of 465 participants (https://classic.clinicaltrials.gov/ct2/show/NCT05407636, accessed January 16, 2024). The trial uses an AAV8 vector carrying the Fab fragment similar to ranibizumab, a humanized antibody to VEGFA.

The preclinical study in non-human primates (NHPs) showed that mRNA and protein after subretinal delivery of anti-VEGF Fab-containing AAV8 vector were distributed widely throughout the retina [61]. A retinal demelanization was observed in all injection sites, with no intraocular

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inflammation. The low dose of the vector showed a difference in retinal thickness, whereas the high dose resulted in about a 10% decrease in total retinal thickness at the injection site. Some dose-dependent histopathological changes at 3 months were observed, including low-grade inflammation in the choroid, retina, and sclera. That study showed the presence of anti-VEGF Fab in the anterior chamber at 30 days in all RGX-314 doses and its feasibility to define an upper dose limit in further escalation studies.

Clinical trials renamed the vector to ABBV-RGX-314, likely due to the sponsor. Currently, the ATMOSPHERE (NCT04704921), and ASCENT (NCT05407636), are two pivotal, active, and enrolling patient trials evaluating the subretinal delivery of ABBV-RGX-314 in individuals with wet AMD. In addition, AAVIATE (NCT04514653), a Phase II trial for the treatment of wet AMD using suprachoroidal delivery of ABBV-RGX-314.

Phase I/IIa of RGX-314 is complete and long-term follow-up continues. It showed that RGX-314 delivered subretinally via transvitreal approach was generally well-tolerated with no abnormal immune response, or ocular inflammation (http://www.prnewswire.com/news-releases/regenxbio-announces-additional-positive-interim-phase-iiia-and-long-term-follow-up-dataof-rgx-314-for-the-treatment-of-wet-amd-301228344.html., accessed January 18, 2024). Although as many as. 20 SAEs were reported in 13 patients, only one, a significant decrease in vision, was possibly drug-related.

Phase II of ABBV-RGX-314 pharmacodynamic study with subretinal delivery of the vector was conducted to evaluate the clinical performance of ABBV-RGX-314 from the planned commercial process (BRX) vs. the initial clinical research process (Hyperstack, HS) (https://www.regenxbio.com/science-innovation/publications/; accessed January 19, 2024). Both processes showed a similar clinical profile. The primary endpoint was 6 months after the subretinal delivery of ABBV-RGX-314. The ABBV-RGX-314 protein levels were similar in cohorts administrated with both high and low doses of the drug. An improvement in BCVA between 3 and 8 letters was observed. The fraction of injection-free subjects was 73%. The study reported 5 SAEs in 4 patients, none considered drug-related. Common adverse effects were related to surgery and included conjunctival hemorrhage and inflammation.

Phase II of the AAVIATE study with suprachoroidal delivery of investigational ABBV-RGX-314 for wet AMD aimed to evaluate the mean change in BCVA for ABBV-RGX-314 compared with ranibizumab monthly injections at month 9 (https://www.regenxbio.com/science-innovation/publications/; accessed January 19, 2024). That study observed 15 SAEs but none was considered drug-related. No cases of chorioretinal vasculitis occlusion or hypotony were observed. Individual TEAEs cases included intraocular inflammation, conjunctival hemorrhage, increase in intraocular pressure, conjunctival hyperemia, and episcleritis. BCVA after ABBV-RGX-314 injection worsened as compared with ranibizumab.

In summary, the RGX-314 program includes clinical trials and preclinical studies showing that the AAV serotype 8 vector may be useful in both subretinal and subchoroidal injection of plasmid encoding an anti-VEGF protein and supporting its stable expression, resulting in a meaningful percentage of patients that do no longer require anti-VEGF injections. Although some safety issues should be addressed the procedure is generally safe and there are very few, if any, drug-related SAEs. The subretinal route of administration shows some advances over its suprachoroidal counterparts, but the clinical trials are ongoing and we expect to learn more about the efficacy and safety of RGX-314 soon.

4.2. Trials with anti-VEGFA treatment-naive patients

Completed and ongoing clinical trials mostly recruited patients who responded to anti-VEGFA therapy and the ratio of injections during the trial and before it may be one of the trial endpoints. It is difficult to enroll patients who would refuse a standard anti-VEGFA therapy without knowledge about its outcome for possibly more uncertain gene therapy.

The Phase 1, multicenter, open-label study to assess the efficacy and safety of two doses of the adeno-associated viral vector serotype 2 (AAVCAGsCD59) expressing sCD59 (CD59 molecule (CD59 blood group), soluble form) administered via intravitreal injection seven days after a single intravitreal injection of anti-VEGF enrolls patients with treatment-naive wet AMD, but no results were posted thus far (https://clinicaltrials.gov/study/NCT03585556?cond=Wet%20AMD&term=gene%20therapy&rank=4.

Accessed January 19, 2024)). another ongoing trial, entitled "A Study of Genetic and Environmental Factors and Their Effect on Response to Treatment With Lucentis (Ranibizumab) for Wet AMD" also recruited patients with the eyes of the study never treated with any anti-VEGFA drug, but no results were posted (https://clinicaltrials.gov/study/NCT00469352?cond=Wet%20AMD&term=gene%20therapy&rank=10#participation-criteria. Accessed January 19, 2024). The trial" Comparison of Treatment Regimens Using Ranibizumab: Intensive (Resolution of Intra- and Sub-retinal Fluid) vs Relaxed (Resolution of Intra-retinal Fluid and/or Sub-retinal Fluid >200µm at the Foveal Centre) (FLUID)" lists an exclusion criterion "treatment with any anti-angiogenic drugs (including any anti-VEGF agents) before baseline in study eye (allowed in fellow eye) (https://clinicaltrials.gov/study/NCT01972789?cond=Wet%20AMD&term=gene%20therapy&page=2&rank=19#participation-criteria. Accessed January 19, 2024)." That study does not compare responders to anti-VEGFA therapy with patients naive to such therapy.

The anti-VEGF responders experience a treatment burden posed by frequent injections. Therefore, trials with anti-VEGFA naive wet AMD patients may be a perspective challenge.

5. Genome editing and wet AMD

Genome editing, as its name suggests, is a method to make specific changes within the genome. The straightforward application of genome editing is the correction of mutations that are causative for inherited diseases [62]. Clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 is currently the most common platform to edit the nuclear genome with promising results for its mitochondrial counterpart [63–65]. However, CRISPR/Cas9 has several drawbacks, including genotoxicity and limited control of outcomes [66]. That is why, some modifications to the classical CRISPR-Cas system have been successfully introduced in the treatment of inherited retina diseases [67–73].

Base editors are CRISPR-Cas-based systems allowing for a targeted conversion of a single base pair that can be replaced by or supplemented with prime editing, which is a versatile tool enabling the introduction of all types of transition/transversion as well as small deletions and insertions [74,75]. Adenine base editors were used to restore visual function in mice with a de novo mutation in the *RPE65* gene [72]. The expression of the base editors occurred after the subretinal injection of a lentivirus-based vector.

The most spectacular success of prime editing in eye disease therapy was restoring visual functions in a mouse model of retinitis pigmentosa through correcting the inherited missense mutation in exon 13 of the phosphodiesterase 6B (*PDE6B*) gene, whose product is essential for the initiation of rod phototransduction [76–78]. The packaging system used in that experiment was based on the AAV virus.

Although ClinicalTrials.gov does not list any items under the entry "wet AMD genome editing". However, the NCT06031727 "CRISPR/cas13-medIated RNA tarGeting tHerapy for the Treatment of Neovascular Age-related Macular Degeneration Investigator-initiated Trial (SIGHT-I)" is a clinical trial on genome editing in wet AMD (https://clinicaltrials.gov/study/NCT06031727?cond=Wet%20AMD&term=gene%20theapy&page=3&rank=26; accessed January 18, 2024).

Intravitreal delivery of a Cas9-based system in an AAV serotype 9 vector to knockout the *VEGFA* gene to a laser-induced wet AMD mouse model resulted in a decreased level of the VEGFA protein in RPE and reduced neovascularization in the choroid [79]. That experiment showed a 45% reduction in the CNV area, while aflibercept injection decreased the area by 39%. Moreover, despite aflibercept, that genome-editing strategy ensured a long-lasting therapeutic effect with a single injection. Similar results were obtained in several other studies also with the subretinal route of vector delivery [80–82]. Several studies addressed the application of genome editing technologies to knockout the *VEGFA* gene and inhibit CNV in various research schemes (reviewed in [16]).

Even classical gene therapy, replacing a faulty gene with its normal copy, is a kind of genome editing or at least an attempt to do so. Genome editing with CRISPR/Cas 9, base editing, and prime editing represent more precise tools than classical gene therapy and opens much broader perspectives for regulating phenotype by manipulation of the genome.

 doi:10.20944/preprints202401.1524.v1

6. Conclusions, outstanding questions, and perspectives

The introduction of anti-VEGFA treatment in wet AMD revolutionized the therapy of this earlier incurable disease, but that treatment in most cases does not cure wet AMD as it mainly stops the progression of the disease preventing or delaying sight loss. Moreover, such therapy, which is realized by the lifetime intravitreal injections every 8 to 12 weeks after three monthly loading doses with relatively expensive substances, constitutes a serious burden for patients. Therefore, attempts to replace repeated injections with a single dose of a therapeutic are justified and they are a hope for a more comfortable life for millions of wet AMD patients [83].

Surely, the comfort of life should be considered in planning a therapeutic strategy, but the main points are safety and efficacy. It should be stressed that anti-VEGFA treatment in wet AMD cannot a priori be completely efficient, as VEGFA is the key regulator of normal and pathological angiogenesis, it is not the only factor responsible for neovascularization in wet AMD [84,85].

A new generation of gene therapies opens a perspective for a future "one and done" in-office treatment for wet AMD, thus replacing a lifetime of injections. It is still perspective as today we can only say that the continuous release of anti-VEGFA substance at a therapeutic level might last at least up to 3 years after a single injection of a recombinant DNA. Therefore, an outstanding question is how long can gene therapy-induced expression of a therapeutic protein last. Answering this question requires future pro- and perspective studies, but even if a 3-month time is the longest injection-free period, it would be a substantial alleviation for wet AMD patients, even if a fraction of them require additional injections.

The next outstanding question is how far is that perspective. This is the next question difficult to answer based on completed and ongoing clinical trials as real-world patients usually have worse outcomes than that reached in clinical trials [86]. Phase 3 trials are in the planning or recruiting stages and it is estimated that if these trials show results comparable with corresponding 1/2 trials, the new "one and done" in-office therapy for wet AMD may be real in 3-4 years (https://www.aao.org/eyenet/article/gene-therapy-for-amd).

Several other outstanding questions may be asked concerning future trials and preclinical studies. In general, trials of genome therapy in wet AMD do not show a breakthrough in the efficacy of the treatment and the main outcome is a reduction of the number of injections to reach a therapeutic effect. Therefore, the trials that recruited anti-VEGFA treatment naive patients may bring results that are not influenced by the burden resulting from former injections and so they seem to be a better platform for searching for a mode of more efficient anti-VEGFA therapy.

Promising results obtained in genome editing in retinal diseases and the regulation of VEGFA justify further research in this area. However, as mentioned, VEGFA is not the only protein that regulates angiogenesis, and it seems that gene editing creates more possibilities to act on more than one angiogenic protein than traditional gene therapy [87].

The use of vectors as vehicles to deliver therapeutic construct to the target site and the delivery route is the most important issue in planning any gene therapy strategy [88]. Adeno-associated viruses have an established position in therapeutic genetic manipulation in the retina [89]. However, the use of AAV vectors in the retina is not free from some obstacles, which are analyzed in a recent review of Zin [90]. They include limited tropism, penetration of the retinal inner limiting membrane, small packaging capacity, neutralizing antibody/immune response, and others. However, current works on the delivery of cargo in gene therapy in the retina focus rather on the improvement of the AAV vectors, than replacing them with others. Therefore, optimizing the construct of an AAV vector for retinal genetic manipulation is a challenge both in traditional gene therapy and genome editing in the retina and so in wet AMD.

In summary, a new generation of gene therapies in wet AMD, including genome editing, opens a perspective of a significant improvement of the comfort of life of wet AMD patients in a 3–4-year perspective to replace multiple anti-VEGFA injections with a single dose administrated in office.

Author Contributions: Conceptualization, J.B., and K.K.; writing—original draft preparation, J.B., E.P., M.D., J.C., J.S., and K.K.; writing—review and editing, J.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was not supported by any funds.

Institutional Review Board Statement: N/A

Informed Consent Statement: N/A

Data Availability Statement: N/A

Acknowledgments: None.

Conflicts of Interest: The authors declare no conflict of interest.

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