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Advanced Therapy Medicinal Products in Retinal Diseases: A Focus on Age-Related Macular Degeneration

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Abbreviations

RPE	Retinal Pigment Epithelium
PT	Photoreceptor
DR	Diabetic Retinopathy
MD	Macular degeneration
AMD	Age-related Macular Degeneration
nAMD	neovascular Age-related Macular Degeneration
CFH	complement factor H
ARMS2	age-related maculopathy susceptibility 2
GA	Geographic Atrophy
VEGF	Vascular Endothelial Growth Factor
ANTI-VEGF	Anti-Vascular Endothelial Growth Factor
VITAMIN C	Ascorbic Acid
VITAMIN E	Tocopherol
Ω3	Omega three fatty acid
ATMPS	Advanced Therapy Medicinal Products
SC	Stem Cells
ESC	Embryonic stem cells
iPSC	induced Pluripotent Stem cells
HSCT	Hematopoietic stem cell transplantation
BrM	Bruch's membrane
oDRB	Outer blood-retinal barrier
CNV	Vhoroidal NeoVascularization
PLGA	Poly(lactic-co-glycolic acid)
hESC	human Embryonic Stem Cells
Ixo-vec	Ixoberogene Soroparvovec
AAV	Adeno-associated virus
AAV2	Adeno-associated virus type 2
AAV2.7m8	Adeno-associated virus type 2 vector
AAV8	Adeno-associated virus type 8
vg	vector genomes
VA	Visual Acuity

BCVA	Best Corrected Visual Acuity
AEs	Adverse Events
CD59	Cluster of Differentiation 59 antibodies
MAC	Membrane Attack Complex
sFLT-1	soluble ms-like tyrosine kinase-1
BMSCs	Bone Marrow Stem Cells
hUTCs	human Umbilical Tissue-derived Cells

ABSTRACT

Age-related macular degeneration (AMD) is a leading cause of visual impairment worldwide, primarily affecting the elderly population. It is characterized by a progressive degeneration of the macula, leading to the gradual loss of central vision. AMD is influenced by a combination of genetic predispositions and environmental factors, and current treatments focus primarily on pharmacological interventions, surgical options, and lifestyle modifications, which aim to manage symptoms but do not offer a definitive cure. This paper examines the potential of Advanced Therapy Medicinal Products (ATMPs) as innovative solutions for treating AMD, focusing on gene therapy, stem cell therapy, and tissue engineering as promising approaches to restore retinal function and prevent further degeneration.

A comprehensive literature review was conducted using databases such as PubMed, Google Scholar, and ClinicalTrials.gov, selecting articles published from 2014 to 2024 that focus on therapeutic advancements in AMD. The inclusion of clinical trial data from the U.S. and Europe helped ensure relevance and applicability in healthcare systems where ATMPs are actively under investigation. Key findings suggest that gene therapy holds promise in addressing genetic factors by introducing corrective genes, while stem cell therapy leverages regenerative potential to replace damaged retinal cells. Tissue engineering has advanced significantly, with engineered scaffolds and biomimetic models replicating the retinal environment, showing promise in AMD management by enhancing cell survival and integration into damaged tissues.

This study concludes that ATMPs represent a breakthrough in AMD treatment, offering innovative solutions that could overcome the limitations of current therapies. However, challenges such as safety, efficacy, and accessibility must be addressed to fully realize their clinical potential. The insights provided in this paper underline the necessity for further research and development in ATMPs for AMD, contributing to a foundation for future studies in the field.

1. Introduction

1.1 Overview of Retinal Diseases

Retinal diseases have become one of the main causes of visual impairment on a global scale, affecting millions of people and frequently resulting to blindness. These disorders include a plethora of abnormalities in the retina's anatomy and physiology. As the retina consists the eye's light-sensitive tissue at the posterior part of the eye, it is reasonable to think that this particular structure is vital for the vision. The photoreceptors of the retina are responsible for converting the visible light wavelengths to neural signals which then will be sent to the visual cortex for further processing. Anatomically speaking, the retina is divided into Neuroretina and Retinal Pigment Epithelium, with Neuroretina consists of 9 layers, which from inner to outer, are: 1) Inner Limiting Membrane, 2) Retinal Nerve Fiber Layer, 3) Ganglion Cell Layer, 4) Inner Plexiform Layer, 5) Inner Nuclear Layer, 6) Outer Plexiform Layer, 7) Outer Nuclear Layer, 8) External Limiting Membrane, 9) Photoreceptor Layer (Cones and Rods) [1].

Among the most common entities that affect the Retina are Diabetic Retinopathy (DR), Retinal detachment, and Macular degeneration (MD), with DR and MD going unnoticed until a significant visual impairment occurs. That type of progression underlines the importance of screening so early detection and treatment can be available for the patient. Although these topics are equally important for public health, in this Master's thesis, we will focus only on Age-related Macular degeneration (AMD), which affects mainly older people (above 65 years old) and can lead to vision loss, with nearly 200 million patients around the world [2].

1.2 Age-Related Macular Degeneration (AMD)

Age-related Macular Degeneration (AMD) is a degenerative ocular disease that affects the Macula, the central part of the Retina, whose purpose is the accurate and detailed vision. The hallmark of AMD is the accumulation of extracellular deposits, known as drusen, between RPE and the photoreceptor layer, which leads to inflammation and finally to the loss of these layers (Atrophy), resulting in severe central vision impairment [2-3].

AMD is subcategorized into two types based on the presence or absence of neovascularization: The Neovascular AMD (Wet AMD) and the non-Neovascular AMD (or, more commonly, Dry AMD). The second one is the most common type and the initial form of both types, with about 10-15% present neovascularization in later stages [4]. Although wet AMD is less common compared to dry AMD, it is progressing faster and will end up in

blindness if not properly treated. AMD is the leading cause of blindness for patients above the age of 60 in the developed world, making it a significant burden for public health and the economy [5]. Exploring the underlying pathophysiological mechanisms, the genetic background and the contribution of environmental factors to the genesis of AMD is crucial for developing effective treatments.

1.2.1 Pathophysiology of Age-Related Macular Degeneration

1.2.1.1 Genetic Factors

Age-related Macular Degeneration development depends on different genetic factors. Mutations in genes such as CFH and ARMS2 have been linked with increased risk for AMD because they participate in and regulate the complement system. Overactivation of the complement cascade results in inflammation and subsequent degeneration of Retinal cells [5-6]. The polymorphisms of the CFH gene, more specifically, influence the Alternative pathway of the Complement system. By that, they increase the risk for the advanced stages of AMD (Geographic Atrophy-GA or Neovascular AMD) [7].

1.2.1.2 Environmental

Genetics is the basis of the pathophysiologic mechanism, but other factors also contribute. Environment plays a crucial role in the development of the disease. Smoking is the single most modifiable risk factor. It has now been proven to significantly increase the risk up to four times for the emergence of AMD and the faster development of AMD to the latest stages [7]. So smoking cessation is strongly recommended, as studies indicate that individuals who have quit smoking for more than 20 years have a risk of developing AMD comparable to that of non-smokers. Diet, UV exposure, and cardiovascular disease are other risk factors contributing to the disease's development. Based on the above, it is essential to understand that changes in living must happen when needed so the risk can be limited [5-6].

1.2.2 Current Treatment Approaches for AMD

1.2.2.1. Pharmacological Treatments

The therapeutic area for AMD has seen significant improvements in the last two decades, especially after the introduction of Anti-Vascular Endothelial Growth Factor (anti-VEGF) agents. These molecules are now the standard of care for patients with the Neovascular form of AMD due to their ability to decrease the formation of new irregular and leaky blood vessels, which stabilize the Visual Acuity for most of the patients [5]. Even though it is the current standard of practice for the Wet form, there are still limitations, such as the need for repeating intravitreal injections every few months, drug-resistant nAMD and the fact that they can be used only for the Neovascular type.

Additionally, the financial burden that AMD brings affects not only the patient and their families but also society as a whole. Before the vast use of anti-VEGF as a treatment, the medical expenses related to AMD in the United States of America were estimated at around 574 million US dollars [6]. Even though the initial high cost of Anti-VEGF treatment, these therapeutic agents have been shown to be more cost-effective regarding neovascular AMD by reducing the need for more expensive surgical interventions and vision loss in the long term [6].

1.2.2.2 Surgical Interventions

In cases of drug-resistant AMD, surgical interventions are a potential treatment option. In this category, laser treatments (thermal laser photocoagulation) and photodynamic therapy are most often included [7-8]. More invasive procedures like vitrectomy or macular displacement surgery may also be considered in some advanced cases. Although surgical interventions are more invasive, they can be used as the last resort for patients who have advanced forms of AMD or for patients who do not respond to conventional pharmacological treatments [5,9]. However, these surgical procedures' detailed efficacy, risks, and limitations will not be the primary focus of the current Master's thesis.

1.2.2.3. Lifestyle and Dietary Modifications

Along with medical and surgical treatments, lifestyle and dietary modifications play a critical role in the management of AMD, especially for the dry type, where currently no

treatments seem to stop the progression. Based on the current bibliography, diets rich in antioxidants (such as vitamins C and E), Omega-3 fatty acids (Ω3), and carotenoids (lutein and zeaxanthin), such as the Mediterranean diet, can decrease the rate at which AMD proceeds [10]. In addition, smoking cessation and management of cardiovascular risk factors (such as hypertension, diabetes, and Dyslipidemia) can also help in this cause [7].

1.3 Advanced Therapy Medicinal Products (ATMPs)

The use of Advanced Therapy Medicinal Products (ATMPs) in the 21st century has brought a revolution in the area of Degenerative disorders, especially when it comes to Retina. Under the umbrella of this term are three major categories: 1) Gene therapies, 2) Cell Therapies, and 3) Tissue engineering products. They can be utilized as a means for repair, restoration, or even replacement of damaged cells and tissues. Focusing on the applications for AMD, these novel treatments can target the core of the pathophysiologic mechanism, which is the dying cells, instead of only reducing the intensity of the symptoms, starting a new era in how we approach treatment [11]. In this Master Thesis, we will review the current status of ATMPs in treating AMD.

2. Literature review

2.1. Gene Therapy

Although the pathogenesis of AMD is due to many factors, the one that contributes the most is the genetic and epigenetic background [12]. This drives modern science to develop therapies for this cause, focusing on the neovascular form. For gene therapies to be applied, vectors must be used to transfer genetic information into retinal cells. More commonly, viral vectors are used because they are the ones that have been most studied, and more specifically, Adeno-associated virus (AAV) vectors have shown the best result until now. The target is to enhance or undermine the function of specific genes that are deficient, mutated, or overexpressed, thereby offering a more permanent solution compared to traditional treatments, such as anti-VEGF injections [13].

2.1.1. Mechanisms of Action

Neovascular AMD: For gene therapies that are made for the nAMD, the primary target is to inhibit the expression of the Vascular Endothelial Growth Factor, which is responsible for the formation of abnormal leaky blood vessels. Neovascularization is the pathognomonic sign of this type. Therapies like ADVM-022 and RGX-314 use AAV vectors to transduce the desirable genetic information that will be translated into anti-VEGF proteins when introduced to retinal cells. ADVM-022 and RGX-314 are innovative gene therapies designed for the treatment of retinal diseases, specifically targeting conditions like wet age-related macular degeneration (AMD) and diabetic retinopathy, which require frequent, lifelong intravitreal injections of anti-VEGF (vascular endothelial growth factor) drugs to manage disease progression. These therapies aim to provide a sustained, one-time solution by delivering genes that enable the continuous production of anti-VEGF proteins within the eye, potentially reducing or eliminating the need for repeated injections. ADVM-022 and RGX-314 both utilize adeno-associated viral (AAV) vectors to introduce and integrate therapeutic genes into retinal cells, ensuring long-term protein expression and providing a consistent therapeutic effect directly at the site of disease. This approach is expected to improve patient compliance, reduce treatment burden, and ultimately lead to better management of vision loss in affected individuals. These genes will allow the continuous expression of anti-VEGF, reducing or eliminating the need for

frequent intravitreal injections, which are currently the standard treatment protocol [13-14].

Dry AMD: For Dry AMD, especially the advanced atrophic form, gene therapies like GT-005 focus on regulating the overactivated complement system and especially the alternative pathway, which the latest data show contributes to AMD pathogenesis. This approach aims to reduce chronic inflammation and slow the progression of Geographic Atrophy [15].

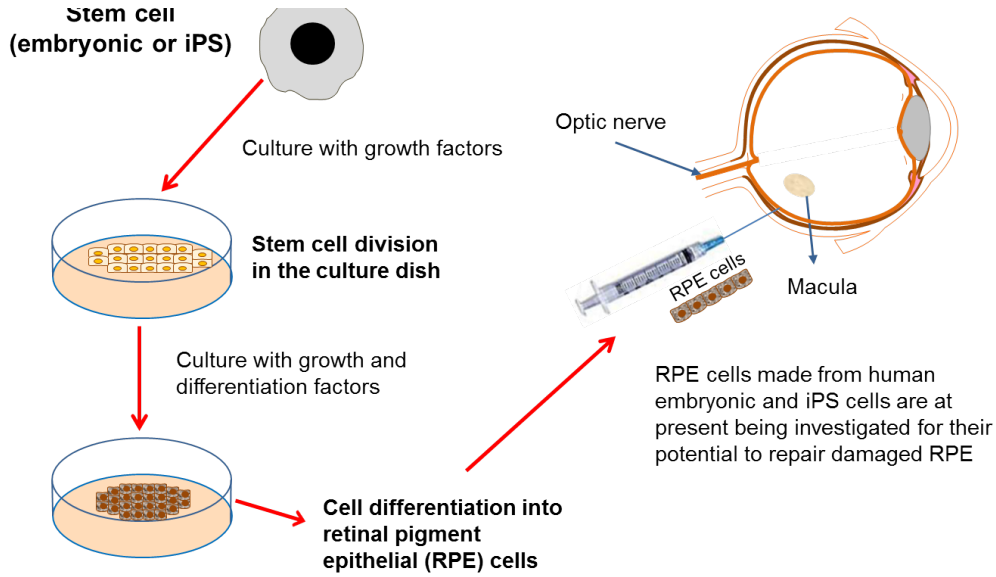
With gene therapy, potentially, only a single administration will be needed to achieve a sustained therapeutic effect, significantly reducing the treatment burden of frequent injections on patients and improving their quality of life.

2.2. Stem Cell Therapy

2.2.1 Categories of Stem Cells

Stem cells (SC) set up a unique type of cells that are characterized by two essential abilities: 1) They can be differentiated into different types of cells, and 2) They can selfrenew. Based on their origin, subsequent characteristics, and differential potential, they can be subcategorized into three major groups. The first one is the Totipotent stem cells, which can give rise to all types of cells and even to a new organism. The zygote and the early cleavage-stage blastomeres belong in this category, roughly until day three. The second group is the Pluripotent stem cells, and in contrast to Totipotent stem cells, they cannot give rise to extraembryonic tissues like the placenta. However, they can still be differentiated into any type of human cell. These kinds of cells can be retrieved from the inner cell mass of blastocysts (Embryonic stem cells, ESC) or by reprogramming differentiated cells into an Embryonic-like state (induced Pluripotent Stem cells, iPSC)[16]. The third group is the Multipotent Stem cells, which have the narrowest differential spectrum of all three groups and can give rise to different types of cells but mainly within the tissue they derive from. In practice, the most commonly used multipotent stem cells are the hematopoietic stem cells used for Hematopoietic stem cell transplantation (HSCT), often called bone marrow transplantation [17]. Understanding the different types of stem

cells, the restrictions each category bears and the pros they exhibit is critical for using them as cell therapies for
regenerative medicine [18].



"Replacing retinal pigment epithelial cells"(Source: EuroStemCell, n.d.)

2.2.2. Mechanisms of Action

Cell therapies can contribute to the treatment of AMD in various ways. One of the most basic ones is through the neuroprotection that transplanted cells achieve by secreting growth and neurotrophic factors that can fortify the retinal neurons against degeneration [19]. These agents can spread throughout the retina and promote the survival and optimal function not only of PR but also of the Retinal Pigmented Epithelium cells, which, as mentioned above, is of great importance for the pathogenesis of AMD. Cell therapies can also contribute as a neuroenhancement tool by assisting the remaining few healthy retinal neurons, leading to improved visual processing. Another fundamental mechanism is neuroregeneration, where the transplanted cells can be incorporated into the retina and then differentiated into cell types that have been impaired by the AMD, replacing the dying or severely damaged photoreceptors and/or the RPE cells. As cell deposits for these procedures can be used stem cells that belong to iPSC, ESC, and tissue-derived adult SC due to their differential potential that can end up in the replacement of the AMD-affected cell types [9,16]. The use of iPSC, as pluripotent stem cells choice, has revolutionized the therapeutic area of AMD due to their potential for ex vivo differentiation into RPE cells

without the ethical dilemmas that ESCs have. By transplanting these newly formed RPE cells, scientists have partially restored vision for patients with advanced forms of AMD. Although Cell therapies are very promising and the number of trials that apply this technology is starting to grow, there are still some challenges, such as the need for appropriate cell sourcing, effective ways to deliver the cells to the retina, and long-term safety and efficacy [20].

2.3 Tissue Engineering

Tissue engineering has emerged as a promising approach for dealing with the complicated pathology of AMD by offering solutions for both dry and wet forms. As was mentioned above, AMD affects not only the function of the retinal cells but also the anatomy of the retinal tissue and, most importantly, of the macula by disturbing the interactions between the retinal pigment epithelium, Bruch's membrane (BrM), and the choriocapillaris, leading to the formation of drusen deposits and, in severe cases, macular atrophy or neovascularization [21].

2.3.1. Mechanisms of Action

1) 3D Biomimetic Models:

The advancement in tissue engineering techniques have made possible the creation of 3D biomimetic models that replicate in a similar way the human outer blood-retinal barrier (oBRB). These newest types of models are crucial for the study of the pathogenesis of AMD, allowing for a more accurate representation of in vivo conditions in comparison to the traditional 2D cell cultures. In their study, Song et al. developed a 3D printed model of the oBRB that includes RPE cells, Bruch's membrane, and choriocapillaris, which not only had the anatomical accuracy of the outer retina but also reproduces the characteristic pathological features of AMD, which are drusen formation and choroidal neovascularization (CNV)[21,55].

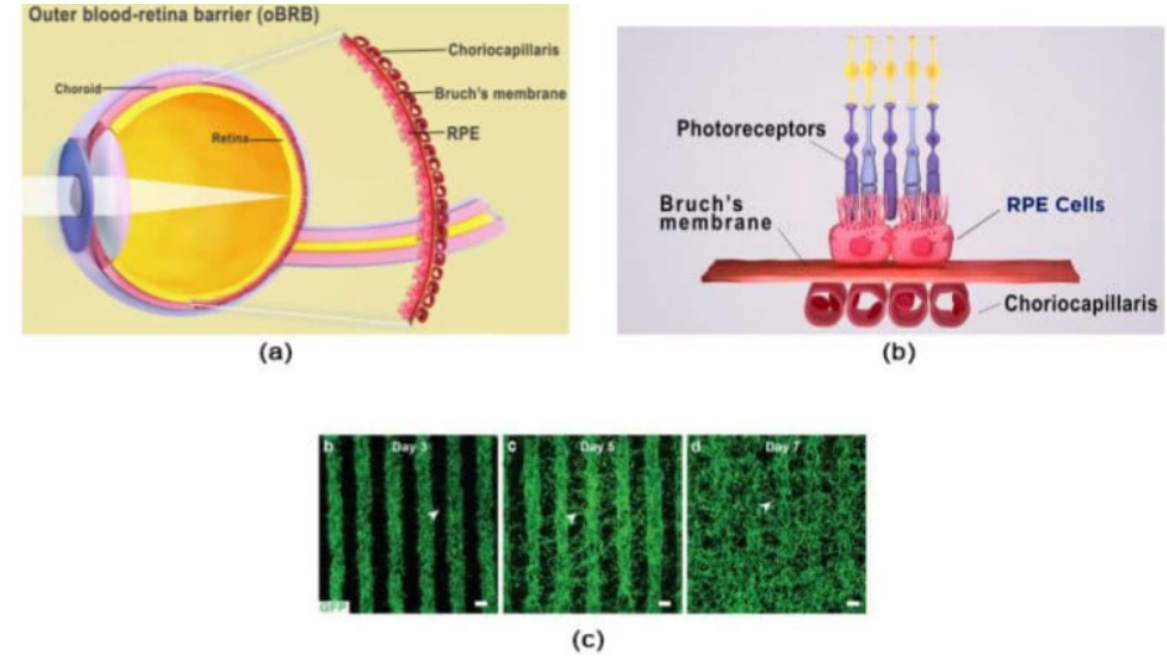


Image Reference: https://doi.org/10.1038/s41592-022-01701-1

2) Scaffold-Based Therapies:

Tissue-engineered scaffolds, which are composed of biocompatible materials like poly(lactic-co-glycolic acid) (PLGA), are a suitable substrate in order for the new retinal cells to grow and differentiate. The cells that can be exploited for seeding these platforms are stem cells, specifically human embryonic stem cells (hESC) or induced pluripotent stem cells have already been investigated, which later became RPE and Neuroretinal cells. The implantation of these cell enforced scaffolds, in the subretinal space, aims to improve vision by integrating them into the affected tissue and progressively rejuvenating the deteriorated one [21-22]. Although only a few trials have been completed, retina scaffolds have shown promising results in enhancing cell survival and maintaining cellular polarity, which is crucial for the successful integration and function of transplanted cells [21].

3) Organoid-derived Cell Sheets:

Another hopeful utilization of tissue engineering products includes the transplantations of retina-like cell sheets that come from organoids. These sheets, which may consist of various retinal cell types, have shown the advantage of integrating into the host after the transplantation. In contrast to cell therapies where only individual cells are transplanted, in this type of treatment, a structured, multi-cellular layer graft is transferred, which may

improve the overall integration and functionality of the graft [22]. In their trial, Mandai et al. revealed the dynamic of retinal organoids in AMD management by showing that transplanted retinal sheets that have been derived from iPSCs could survive and integrate into the host retina but that was based in animal models [22].

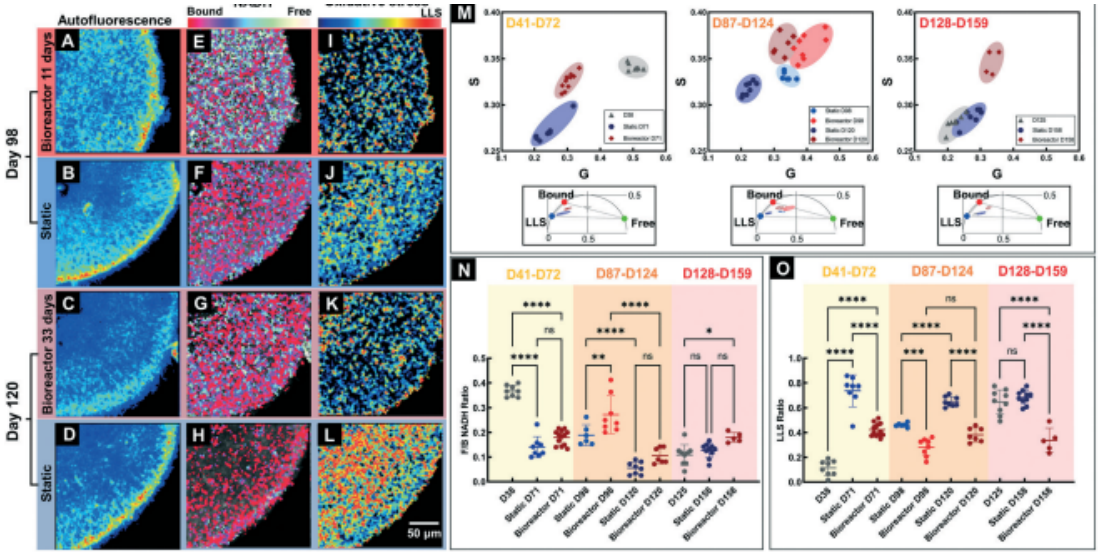


Fig. 5 Qualitative and quantitative comparison of RtOgs in two culture methods. (A–D) Total NADH autofluorescence images demonstrated the cellular structures within RtOg cross sections; pseudo color-coded free/bound NADH distribution (E–H) and LLS distribution (I–L) images were generated based on two photon lifetime within the 2-dimensional phasor space; (M) scatter plots of and the clustering of different groups of RtOgs on the FLIM phasor diagram; (N) plot of free/bound NADH ratio to evaluate metabolism (higher f/b value represented glycolysis, and lower f/b indicated greater oxidative phosphorylation). Metabolism is not significantly different between static and bioreactor RtOgs after 1 month in culture for RtOgs of different ages; (O) plot of LLS ratio to evaluate oxidative stress. LLS is significantly different between static and bioreactor maintained RtOgs of different ages after 1 month in culture. The values of f/b NADH ratio and LLS ratio reflect the average lifetimes of the organoids cross-section imaging frame (one-way ANOVA test was performed: D38, $n = 8$; static D71, $n = 8$; bioreactor D71, $n = 13$; static D98, $n = 6$; bioreactor D98, $n = 8$; static D120 $n = 8$; bioreactor D120, $n = 7$; D125, $n = 9$; static D158, $n = 10$; bioreactor D158, $n = 4$; the RtOgs placed into the bioreactor D41-72 were imaged on D38 at the outset of the experiment. The RtOgs placed into the bioreactor D128-159 were imaged on D125 at the outset of the experiment).

Image Reference: DOI 10.1039/d1lc00011j

Applications and Future Directions:

At the moment, Tissue engineering needs more time to become part of the therapeutic armamentarium for AMD. This does not apply to its use as a powerful tool for research due to its ability to replicate the dynamic human anatomy, physiology, and pathophysiologic mechanism in vitro and a three-dimensional climax. Of course, further development of advanced techniques, such as 3D bioprinting and organoid culture, could revolutionize the field by providing an even more accurate human retina models [21].

In conclusion, tissue-engineering therapies for Age-related Macular Degeneration are still in the experimental stages, with only a few clinical trials in progress. Nevertheless, these techniques offer a promising choice for understanding and treating this disease. By

addressing the result of retinal degeneration, which is the loss of cells, these therapies have the potential to provide long-lasting solutions for patients with AMD [21].

Methods

Different measures were implemented to guarantee a thorough review of the literature associated with the use of Advanced Therapy Medicinal Products (ATMPs) and their impact on the treatment of Age-related Macular Degeneration (AMD). An analytic study of academic journals and papers, was carried out using various search terms such as Advanced Therapy Medicinal Products, gene therapies, cell therapies, tissue engineering, scaffolds, Geographic Atrophy Neovascular AMD and Age-related Macular Degeneration, either individually or combined. Keywords were chosen based on their correlation with the investigated topic. AMD was selected due to the extensive volume of research and clinical trials that are currently in progress and, of course, due to personal interest. A search was conducted in three primary databases: PubMed, Google Scholar and CHEMICAL TRIALS. Only papers written in the English language and published between 01/2014 and 05/2024 were considered, with the exception of those that include information about Anatomy. In developing a literature review for the use of Advanced Therapy Medicinal Products (ATMPs) in treating Age-related Macular Degeneration (AMD), the decision to exclude the time limitation for the anatomy-focused content was intentional. This is because anatomical studies tend to cover foundational, established knowledge, which hasn't changed over the last two decades, resulting in papers that were written before 2014 still being accurate. Regarding clinical trials, the inclusion criteria for this dissertation were: the location of the trial to be either the America or Europe. The initial screening of these selected articles was performed through a careful examination of the titles and abstracts, which led to the exclusion of articles that were not relevant to the Thesis topic. More research was conducted based on the reference section of articles that met the selection criteria and these articles were further reviewed to align with the final selection and exclusion criteria.

Results

1) Gene Therapy Clinical Trials

To date, seven clinical trials on the use of gene therapy as a therapeutic tool for AMD have been successfully completed. Six of these are aimed at treating the wet form and only one for the dry form.

a) ADVM-022:

ADVM-022, or Ixoberogene Soroparvovec (Ixo-vec), is a gene therapy that uses the AAV2.7m8 capsid as a vector to deliver a codon-optimized cDNA expressing an afliberceptor-like protein (a widely used anti-VEGF agent). Based on preclinical studies, it was thought that even a single dose of this therapy could induce sustained production of anti-VEGF factor for up to thirty months after injection. This would allow a long-term inhibition of CNV.

- **OPTIC trial (NCT03748784):** This prospective, two-year, multicentre phase 1 study was designed to evaluate Ixo-vec in patients with nAMD. It includes four cohorts, with patients receiving either high doses (6×10^{11} vg/eye) or low doses (2×10^{11} vg/eye) of the therapy, with promising results . Patients receiving the high doses reduced the need for anti-VEGF injections by up to 98%, while patients on the low dose reduced their need for the same agents by 80%. Visual acuity (VA) remained stable in all groups and central retinal thickness improved, particularly in the group receiving the 6×10^{11} vg/eye dose. As a phase 1 trial, the primary outcome to be measured was the type, severity and incidence of ocular and systemic adverse events (AEs). The most common adverse event observed was ocular inflammation, which mainly affected the anterior segment of the eye, with an increased rate in the higher dose group. Inflammation was treated with topical corticosteroids and no systemic adverse events associated with Ixo-vec were reported. However, the treatment continues to be monitored for long-term safety and efficacy [23-24].

b) RGX-314

Another novel gene therapy related to nAMD was developed based on the AAV8 vector , which can deliver a gene expressing a monoclonal antibody fragment similar to ranibizumab (which is also an established anti-VEGF agent). The target site for this

treatment was the subretinal, which again aimed to reduce the need for frequent anti-VEGF injections. Ongoing clinical trials (**NCT04514653**) are investigating the suprachoroidal route as an alternative for RGX-314 [25].
The latest data show that two phase 2 clinical trials (**NCT04832724**, **NCT03066258**) [26-27], with a total of 102 participants, have been completed, and one phase II/III trial (ATMOSPHERE/ **NCT04704921**) and one phase 3 trial (ASCENT/**NCT05407636**) are ongoing [28-29]. The safety profile appears promising, with no abnormal immune responses, drug-related ocular inflammation, or postoperative complications reported so far. Efficacy is also very attractive, particularly with regard to VA and retinal thickness. . In one of the cohorts, Best Corrected Visual Acuity (BCVA) improved by +14 letters over two years, while other cohorts showed stabilization of vision.
In addition, the need for frequent anti-VEGF injections was significantly l reduced, with some patients experiencing reductions of as much as 66.7% in the average annual number of injections.Long-term follow-up trials are further evaluating the safety and durability of RGX-314 [27,30].

c) AAVCAGsCD59

The only clinical trial that has been completed for Dry-AMD until now is the one with AAVCAGsCD59.. For this treatment, an AAV2 vector is utilized to introduce a gene encoding a soluble form of CD59, a protein designed to inhibit the membrane attack complex (MAC), a component of the complement system.

The complement system can become overactive in patients with AMD resulting in chronic inflammation and retinal cell damage. The complement system has been identified as a key factor in the progression of Age-related Macular Degeneration, particularly in the dry form, leading to AMD The route of administration for both trials was intravitreal.

- **NCT03144999**: A Phase 1 trial that was designed to evaluate AAVCAGsCD59 in 17 patients with advanced dry AMD. GA is characterized by the progressive loss of RPE cells and PT, leading to visual impairment and, eventually, blindness. The trial aimed to slow the progression of these atrophic lesions by inhibiting the formation of RPE, thereby reducing the inflammatory cascade that accelerates cell death.. Early results showed a promising slowing rate of GA development, with patients

achieving slower progression of retinal degeneration. However, long-term efficacy data are still being collected, and further research is needed to determine whether AAVCAGsCD59 can stably preserve central vision for extended periods. Currently, a multicenter phase 2 trial is currently recruiting to evaluate the change in the development of geographic atrophy (GA) lesion development in eyes treated with JNJ-81201887 compared to sham control [31-33].].

AAVCAGsCD59 was tested also in 25 patients with wet-AMD in the trial **NCT03585556**.

- **NCT03585556**: This Phase 1, single-center, trial is focused on evaluating the safety, tolerability and early efficacy of AAVCAGsCD59 in patients with wet AMD, where overactivation of the complement system also plays a role in the disease progression. Similarly to NCT03144999, data from this trial suggest that AAVCAGsCD59 successfully reduces MAC formation, thereby reducing inflammation and potentially preventing further retinal tissue damage. Thus, inflammation in wet AMD may intensify the formation of abnormal blood vessels (CNV), controlling the complement pathway offers a novel therapeutic route [34-35].

d) AAV2-sFLT01

AAV 2 is also used as a transport vehicle for another gene therapy. In this case, it involves the delivery of a gene that will release a soluble form of the VEGF receptor-1 (sFLT-1). This protein binds to and neutralises VEGF, effectively preventing it from activating its original receptor and stopping the process of neovascularisation.

- **NCT01024998**: The clinical trial for AAV2-sFLT01 was a multi-center, phase 1 trial designed to evaluate its safety and transduction efficacy, after a single dose, in 19 patients with wet AMD. The treatment showed a good safety profile, with no severe immune reactions and no irreversible adverse ocular complications. Patients showed stabilization of their vision, and more than half showed a reduction in fluid leakage. However, the response to AAV2-sFLT01 varied between participants, with five having no reduction in fluid. This variation in efficacy suggests that further optimization of delivery or dosing of the gene may be necessary to achieve more consistent results. Although AAV2-sFLT01 was one of

the first gene therapies tested for nAMD and was completed in 2018, there is currently no phase 2 study to further investigate the efficacy of this therapy [36-37].

e) RetinoStat (OXB-201)

In addition to AAV, lentiviral vectors are also an alternative for gene transduction. The first ocular gene therapy to use this type of vector was RetinoStat, with the transgenes encoding two potent anti-angiogenic proteins: endostatin and angiostatin. Both of these proteins are known for their ability to suppress angiogenesis.

RetinoStat aims to provide long-term and sustained suppression of abnormal vessel growth without the need for repeated anti-VEGF injections by delivering these proteins directly to the retina via a subretinal injection.

- **NCT01301443**: This Phase 1 trial was conducted to evaluate the safety, tolerability, and potential efficacy of RetinoStat in 21 patients with advanced and poor anti-VEGF-responsive neovascular AMD. Based on results published at the annual meeting of The Association of Research in Vision and Ophthalmology in 2016, RetinoStat was well tolerated by patients, with no serious AEs or immune reactions associated with treatment and only mild inflammation observed in the high-dose group. Remarkably, the treatment succeeded in providing a sustained expression of anti-angiogenic proteins in the retina even four years after the infusion time. In some participants, a reduction in the need for additional anti-VEGF injections was observed [38-39]. Still, a follow-up study (**NCT01678872**) with the primary outcome being the incidence of adverse events and secondary outcome being the change from the baseline on the BCVA is ongoing and is expected to be completed in 2029 [40].

2) Cell Therapy Clinical Trials

Cell therapies are emerging as a promising treatment option for the dry form of AMD and particularly for the advanced atrophic form. All clinical trials completed to date have been primarily for dry AMD or AMD in general This orientation can easily be explained by the fact that, unlike Neovascular-AMD, for the dry form there is currently no treatment option that can halt the progression of RPE and photoreceptor atrophy for which AMD is characterized. The main trials completed to date, based on criteria selected for this thesis, are the follow

a) hESC-RPE

hESC-RPE (human embryonic stem cell-derived retinal pigment epithelium) trials utilize RPE cells derived from human embryonic stem cells to treat advanced cases of dry AMD. These RPE cells were transplanted into the subretinal space to replace the originally degenerated ones and slow or even stop retinal degeneration [15,4**NCT02903576**: Four patients with AMD participated in this phase 1 trial—two with dry and two with wet-AMD. The trial was designed to evaluate the safety and potential efficacy of hESC-RPE cells delivered via subretinal injections. Although the trial was completed in 2019, the results haven't been published yet [42].

b)MA09-hRPE

MA09-hRPE is another therapy derived from human embryonic stem cells and the first cell line developed as hESC-RPE. It aims to treat dry AMD and Stargardt disease. MA09-hRPE has been tested in multiple trials, with subretinal injections of these cells to replace degenerated RPE cells [43].

- **NCT01344993**: This Phase I/II multi-center clinical trial focused on 13 patients with advanced dry AMD. In this study as well, MA09-hRPE was injected subretinally in three doses (50,000, 100,000, and 150,000 cells) viasuspension form. The primary objective was safety, and secondary outcomes were focused on visual function (change in BCVA and Reading speed) and cell survival (By autofluorescence photography).

  The results published were pooled with the results from the NCT01345006 trial for Stargardt's macular dystrophy, showing that after the subretinal injection, 72% of

patients developed subretinal pigmentation compatible with engraftment of the transplanted cells. No serious safety concern associated with the treatment, such as tumor formation, immune rejection, or adverse proliferation observed. Finally, BCVA improved in 10 eyes, remained stable in 7 eyes, and decreased by more than 10 letters in a single patient [44-45].

- **NCT02463344**: This follow-up study was conducted to further evaluate the long-term safety and efficacy of MA09-hRPE transplantation. Patients from the original trial (NCT01344993) were monitored for longer periods, for longer periods of time, up to five years, to evaluate survival and integration of the cellular graft into the host retina. The results again showed maintenance of improved or stabilized vision over the extended observation period, with no significant eye-related adverse events reported and the transplanted cells demonstrated long-term survival in the subretinal space [46-47].

c)Autologous Bone Marrow Stem Cells

This Phase I/II clinical trial investigated the use of a single dose (10^6 cells) of CD34+ autologous bone marrow stem cells (BMSCs), a type of multipotent stem cells, to treat dry AMD.

- **NCT01518127:** Under this code, BMSCs were harvested from the patient's bone marrow and injected intravitreally. The primary outcome measurewas the safety of the procedure. Results were generally positive, with no significant adverse effects observed among the 10 participants with Dry AMD. However, efficacy only slightly improved the BCVA score, but it was statistically significant [48-49].

d) CNTO 2476

CNTO 2476 is a cell-based therapy developed by Janssen Pharmaceuticals involving human umbilical tissue-derived cells (hUTCs). These cells are not considered as stem cellsdue to their inability, firstly to selfrenew, secondly to differentiate into other cell types. However, , they release neurotrophic factors that help protect and maintain retinal function [50].

- **NCT01226628**: This Phase I/II trial assessed the safety and feasibility of CNTO 2476 in patients with bilateral advanced geographic atrophy. The treatment involved injecting CNTO-2476 subretinally to 32 subjects, so that it could release

neurotrophic factors into the retinal environment of one eye and use the other as a control. Based on the safety profile, CNTO-2476 was well tolerated, with no significant adverse effects. Yet, the efficacy of the therapy in halting GA progression or improving vision was limited and without statistical significance, prompting the need for further optimization of the therapeutic approach to improve clinical outcomes [50-52].

e) iPSC-RPE:

The possibility of the production of RPE cells from iPSCs and the safety of this procedure was explored in the observational clinical trial of **NCT02464956** in three patients. The donor cells derived from the patient skin or blood. Although no intervention occured in this trial, there wasn't a targeted AMD form, and both types were included in the inclusion criteria [53].

Tissue Engineering Clinical Trials

Tissue engineering is the newest sector of ocular RM due to its dependence on the evolution of cell therapies and material science technologies. Until now, only few clinical trials have been completed involving the use of hESC-RPE on a biocompatible polymeric substrate.

- **NCT02903576:**

  As we saw before, this phase I/II trial tried to explore the use of human embryonic stem cell-derived retinal pigment epithelium for AMD (Dry and Neovascular) and Stargardt's disease in two different ways. The first one was as a subretinal injection of the cell suspension, and the second one was again in the subretinal space, but this time, the cells were seeded on a polymeric membrane as a monolayer. In total, 15 patients participated in both arms. No results have been retrieved yet [54].

- **NCT02590692:**

  The findings from this study evaluating the safety and preliminary efficacy of a bioengineered retinal pigment epithelium (RPE) cell implant in patients with advanced dry age-related macular degeneration (AMD). This phase 1/2a clinical trial involves the transplantation of an allogeneic RPE cell layer on a supportive scaffold (parylene) into the subretinal space of affected individuals. Over the

course of a one-year follow-up period, the study assesses the implant's integration, survival, and potential to halt or slow the progression of geographic atrophy associated with dry AMD. The results indicate that the implant procedure was well-tolerated, with no serious adverse events directly related to the implant or surgical intervention. Some patients exhibited signs of maintained or improved visual function, suggesting potential therapeutic benefits. This study underscores the promise of tissue engineering approaches in retinal diseases, highlighting the potential of bioengineered RPE implants to restore or preserve vision in patients with advanced dry AMD. The findings support further investigation in larger clinical trials to validate efficacy and long-term safety [56].

Discussion

This present Thesis was based on 35 papers and 20 clinical trials on using Advanced Therapy Medicinal Products in Age-related macular degeneration. Based on the available bibliography, ATMPs appears to be a very promising alternative treatment for AMD. Although all three ATMPs categories (Gene therapies, Cell therapies and Tissue engineering products) can contribute to both types of AMD (Dry and Wet), each one of them, due to its specific characteristics and the different pathophysiology of the two types of Advanced AMD, may favour one more than the other. Exudative AMD is characterized by the presence of neovascularization. A key molecule to this procedure is the Vascular Endothelium Growth Factor (VEGF). Until now, anti-VEGF intravitreal injections have been the gold standard treatment leading to great results. However, the need for frequent injections is the disadvantage of this option and the point at which gene therapies can perform best, with , with early clinical trials showing that they can prolong the time intervals between the administrations by inserting genetic material that will produce an anti-VEGF factor or a VEGF receptor "decoy" or complement suppressors such as CD59 or finally proteins that inhibit angiogenesis (e.g. e.g. endostatin and angiostatin). They commonly use Adeno Associated Virus Vectors to deliver these genes, but Lentiviral Vectors have been also tested. ATMPs can also help in the treatment of Geographic Atrophy (Advance Dry-AMD). This type is characterized by the loss of Photoreceptors and Retinal Pigmented Epithelium cells, cell types l with little ability to selfrenew Currently, the only treatment options can only reduce the speed with which the atrophic lesions spread. Stem cells, with their unique ability to regenerate and to intergrade tissues, have already entered clinical trials for this entity for this entity, with four early-stage trials (I or II) already completed. Although autologous bone marrow SC or human umbilical tissue-derived cells have been used as regenerative agents, the most promising results were achieved when RPE cells were differentiated ex vivo from human embryonic stem cells. Asmall study also sought to investigate the possibility of differentiating RPE from autologous iPSCs, without intervention in patients. Tissue engineering treatment is the most complex in ATMPs, as it depends not only on the cellular science but additionally on material technologies, so that the appropriate biocompatible, non-toxic substrate can be used to achieve optimal graft integration and survival. According to the international

clinical trials database (clinicaltrials.gov), only one completed trial using a tissue engineering product has been registered to date in Europe or the USA.

In conclusion, the first steps for the introduction of ATMPs in the clinical practice of AMD have already been taken and the results show that they hold great promise for patients, as they can improve their quality of life by reducing the need for intravitreal injection or by providing a treatment option that can stabilize the vision, for patient with GA, which was not possible until now.

Conclusion

Taken together, although ATMPs are only now starting to enter the clinical practice in ophthalmology, they hold a great promise. Especially for Age-related Macular Degeneration, where the only treatment that there is, is for the neovascular form, ATMPs can provide a more stable solution. With clinical trials being already in phase three for gene therapies and successful phase I and II clinical trials for cell therapies and tissue engineering, it's only a matter of time until they become the new standard of care. Of course, there are still limitations that have to be overcome, such as the high cost of these treatments, the long-term safety profile and the need for more advanced biocompatible materials for tissue engineering.

References

1. Nguyen KH, Patel BC, Tadi P. Anatomy, Head and Neck: Eye Retina. 2023 Aug 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 31194472.
2. Pishavar, E. et al. (2021) "Nanocarriers, Progenitor Cells, Combinational Approaches, and New Insights on the Retinal Therapy," Multidisciplinary Digital Publishing Institute, 22(4),p. 1776-1776. Available at: https://doi.org/10.3390/ijms22041776.
3. Fabre, M., Mateo, L., Lamaa, D., Baillif, S., Pagès, G., Demange, L., Ronco, C. and Benhida, R. (2022) 'Recent advances in age-related macular degeneration therapies', Molecules, 27(16), p. 5089. doi: 10.3390/molecules27165089.
4. Jager, R.D., Mieler, W.F. and Miller, J.W. (2008) 'Age-related macular degeneration', *New England Journal of Medicine*, 358(24), pp. 2606–2617. doi: 10.1056/NEJMra0801537.
5. Flores, R., Carneiro, Â., Vieira, M., Tenreiro, S. and Seabra, M.C. (2021) 'Age-related macular degeneration: Pathophysiology, management, and future perspectives', *Ophthalmologica*, 244(6), pp. 495-511. doi: 10.1159/000517520.
6. Flaxel, C.J., Adelman, R.A., Bailey, S.T., et al. (2020) 'Age-related macular degeneration preferred practice pattern'®, Ophthalmology, 127(1), pp. P1-P65. doi: 10.1016/j.ophtha.2019.09.024.
7. Mettu, P.S., Allingham, M.J. and Cousins, S.W. (2021) 'Incomplete response to Anti-VEGF therapy in neovascular AMD: Exploring disease mechanisms and therapeutic opportunities', Progress in Retinal and Eye Research, 82, p. 100906. doi: 10.1016/j.preteyeres.2020.100906.
8. Kumbhar, P., Kolekar, K., Vishwas, S., et al. (2024) 'Treatment avenues for age-related macular degeneration: Breakthroughs and bottlenecks', Ageing Research Reviews, 98, p. 102322. doi: 10.1016/j.arr.2024.102322.
9. AMD Book (n.d.) Surgery for AMD. Available at: https://amdbook.org/content/surgery-amd (Accessed: 9 September 2024).

10. Buschini, E., Fea, A.M., Lavia, C.A., Nassisi, M., Pignata, G., Zola, M. and Grignolo, F.M. (2015) ‘Recent developments in the management of dry age-related macular degeneration’, Clinical Ophthalmology, 9, pp. 563–574. doi: 10.2147/OPTH.S59724.
11. Diebold, Y. and García-Posadas, L. (2021) ‘Advanced therapy medicinal products for eye diseases: goals and challenges’, Pharmaceutics, 13(11), p. 1819. doi: 10.3390/pharmaceutics13111819.
12. Gemenetzi, M. and Lotery, A.J. (2020) 'Epigenetics in age-related macular degeneration: new discoveries and future perspectives', Cellular and Molecular Life Sciences, 77(5), pp. 807-818. doi: 10.1007/s00018-019-03421-w.
13. Khanani, A.M., Thomas, M.J., Aziz, A.A., et al. (2022) 'Review of gene therapies for age-related macular degeneration', Eye (London), 36(2), pp. 303-311. doi: 10.1038/s41433-021-01842-1.
14. Bordet, T. and Behar-Cohen, F. (2019) 'Ocular gene therapies in clinical practice: viral vectors and nonviral alternatives', Drug Discovery Today, 24(8), pp. 1685-1693. doi: 10.1016/j.drudis.2019.05.038.
15. Park, Y.G., Park, Y.S. and Kim, I.B. (2021) 'Complement System and Potential Therapeutics in Age-Related Macular Degeneration', International Journal of Molecular Sciences, 22(13), p. 6851. doi: 10.3390/ijms22136851.
16. Zakrzewski, W., Dobrzyński, M., Szymonowicz, M. and Rybak, Z. (2019) ‘Stem cells: past, present, and future’, Stem Cell Research & Therapy, 10(1), p. 68. doi: 10.1186/s13287-019-1165-5.
17. Carrelha, J., Meng, Y., Kettyle, L.M., et al. (2018) 'Hierarchically related lineage-restricted fates of multipotent haematopoietic stem cells', Nature, 554(7690), pp. 106-111. doi: 10.1038/nature25455.
18. Temple, S. (2023) 'Advancing cell therapy for neurodegenerative diseases', Cell Stem Cell, 30(5), pp. 512-529. doi: 10.1016/j.stem.2023.03.017.
19. Uyama, H., Mandai, M. and Takahashi, M. (2021) 'Stem-cell-based therapies for retinal degenerative diseases: Current challenges in the establishment of new treatment strategies', Development, Growth & Differentiation, 63(1), pp. 59-71. doi: 10.1111/dgd.12704.

20. Yang, Y.P., Hsiao, Y.J., Chang, K.J., et al. (2022) 'Pluripotent Stem Cells in Clinical Cell Transplantation: Focusing on Induced Pluripotent Stem Cell-Derived RPE Cell Therapy in Age-Related Macular Degeneration', International Journal of Molecular Sciences, 23(22), p. 13794. doi: 10.3390/ijms232213794.
21. Wu, A., Lu, R. and Lee, E. (2022) 'Tissue engineering in age-related macular degeneration: a mini-review', Journal of Biological Engineering, 16(1), p. 11. doi: 10.1186/s13036-022-00291-y.
22. Dalvi, S., Chatterjee, A. and Singh, R. (2023) 'AMD recapitulated in a 3D biomimetic: A breakthrough in retina tissue engineering', Cell Stem Cell, 30(3), pp. 243-245. doi: 10.1016/j.stem.2023.02.001.
23. National Library of Medicine (2023) 'An Open Label Phase 1 Study of ADVM-022 (AAV.7m8-aflibercept) in Neovascular (Wet) Age-Related Macular Degeneration'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT03748784 (Accessed: 9 September 2024).
24. Khanani, A.M., Boyer, D.S., Wykoff, C.C., et al. (2023) '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, p. 102394. doi: 10.1016/j.eclinm.2023.102394.
25. ClinicalTrials.gov (2024) ‘A Phase 2, Randomized, Dose-escalation, Ranibizumab-controlled Study to Evaluate the Efficacy, Safety, and Tolerability of RGX-314 Gene Therapy Delivered Via One or Two Suprachoroidal Space (SCS) Injections in Participants With Neovascular Age-Related Macular Degeneration (nAMD) (AAVIATE)’ (NCT04514653). Available at: https://clinicaltrials.gov/study/NCT04514653 (Accessed: 9 September 2024).
26. ClinicalTrials.gov (2024) ‘A Phase 2, Open-label Study to Explore the Pharmacodynamics of Two Doses in Two Formulations of RGX-314 Gene Therapy Administered Via Subretinal Delivery in Participants With Neovascular Age-related Macular Degeneration’ Available at: https://clinicaltrials.gov/study/NCT04832724 (Accessed: 9 September 2024).
27. ClinicalTrials.gov (2023) ‘A Phase I/IIa (Phase 1/Phase 2a), Open-label, Multiple-cohort, Dose-escalation Study to Evaluate the Safety and Tolerability of Gene

Therapy With RGX-314 in Subjects With Neovascular AMD (nAMD)'. Available at: https://clinicaltrials.gov/study/NCT03066258 (Accessed: 8 September 2024).

28. ClinicalTrials.gov (2024) 'A Randomized, Partially Masked, Controlled, Phase 2b/3 Clinical Study to Evaluate the Efficacy and Safety of RGX-314 Gene Therapy in Participants With nAMD (ATMOSPHERE)'. Available at: https://clinicaltrials.gov/study/NCT04704921 (Accessed: 8 September 2024).
29. ClinicalTrials.gov (2023) 'A Randomized, Partially Masked, Controlled, Phase 3 Clinical Study to Evaluate the Efficacy and Safety of RGX-314 Gene Therapy in Participants With nAMD'. Available at: https://clinicaltrials.gov/study/NCT05407636 (Accessed: 8 September 2024).
30. Campochiaro, P.A., Avery, R., Brown, D.M., et al. (2024) 'Gene therapy for neovascular age-related macular degeneration by subretinal delivery of RGX-314: a phase 1/2a dose-escalation study', The Lancet, 403(10436), pp. 1563-1573. doi: 10.1016/S0140-6736(24)00310-6
31. National Library of Medicine (2021) 'A Phase 1, Open-Label, Multi-Center, Dose-Escalating, Safety and Tolerability Study of a Single Intravitreal Injection of AAVCAGsCD59 in Patients With Advanced Non-Exudative (Dry) Age-Related Macular Degeneration With Geographic Atrophy'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT03144999 (Accessed: 9 September 2024).
32. Khan, H., Aziz, A.A., Sulahria, H., Khan, H., Ahmed, A., Choudhry, N., Narayanan, R., Danzig, C. and Khanani, A.M. (2023) 'Emerging treatment options for geographic atrophy (GA) secondary to age-related macular degeneration', Clinical Ophthalmology, 17, pp. 321-327. doi: 10.2147/OPTH.S367089.
33. Janssen (2022) 'A Study of CR109236'. Global Trial Finder. Available at: https://globaltrialfinder.janssen.com/trial/CR109236 (Accessed: 9 September 2024).
34. National Library of Medicine (2022) 'A Phase 1 Proof of Concept Study Evaluating Intravitreal AAVCAGsCD59 for the Treatment of Wet Age-Related Macular Degeneration (AMD)'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT03585556 (Accessed: 9 September 2024).
35. Castro, B.F.M., Steel, J.C. and Layton, C.J. (2024) 'AAV-based strategies for treatment of retinal and choroidal vascular diseases: advances in age-related

macular degeneration and diabetic retinopathy therapies', BioDrugs, 38(1), pp. 73-93. doi: 10.1007/s40259-023-00629-y.

36. ClinicalTrials.gov (2018) ‘A Phase 1, Open-Label, Multi-Center, Dose-Escalating, Safety and Tolerability Study of a Single Intravitreal Injection of AAV2-sFLT01 in Patients With Neovascular Age-Related Macular Degeneration’. Available at: https://clinicaltrials.gov/study/NCT01024998 (Accessed: 9 September 2024).
37. Heier, J.S., Kherani, S., Desai, S., et al. (2017) 'Intravitreous injection of AAV2-sFLT01 in patients with advanced neovascular age-related macular degeneration: a phase 1, open-label trial [published correction appears in The Lancet. 2017 Jul 1;390(10089):28]', The Lancet, 390(10089), pp. 50-61. doi: 10.1016/S0140-6736(17)30979-0.
38. National Library of Medicine (2017) 'A Phase I Dose Escalation Safety Study of Subretinally Injected RetinoStat, a Lentiviral Vector Expressing Endostatin and Angiostatin, in Patients With Advanced Neovascular Age-Related Macular Degeneration'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT01301443 (Accessed: 9 September 2024).
39. Campochiaro, P.A., Lauer, A.K., Sohn, E.H., Mir, T.A., Naylor, S., Anderton, M.C., Kelleher, M., Harrop, R., Ellis, S. and Mitrophanous, K.A. (2017) 'Lentiviral vector gene transfer of Endostatin/Angiostatin for macular degeneration (GEM) study', Human Gene Therapy, 28(1), pp. 99-111. doi: 10.1089/hum.2016.117.
40. ClinicalTrials.gov (2024) ‘A Long Term Follow-up Study to Evaluate the Safety of RetinoStat® in Patients With Age-Related Macular Degeneration’. Available at: https://clinicaltrials.gov/study/NCT01678872 (Accessed: 9 September 2024)
41. Trincão-Marques, J., Ayton, L.N., Hickey, D.G., Marques-Neves, C., Guymer, R.H., Edwards, T.L. and Sousa, D.C. (2024) 'Gene and cell therapy for age-related macular degeneration: A review', Survey of Ophthalmology, 69(5), pp. 665-676. doi: 10.1016/j.survophthal.2024.05.002. Available at: https://www.sciencedirect.com/science/article/pii/S0039625724000493.
42. National Library of Medicine (2020) 'Stem Cell Derived Retinal Pigmented Epithelium Implantation in Patients With Outer Retinal Degenerations: Phase I/II Clinical Trial'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT02903576 (Accessed: 9 September 2024).

43. Qiu, T.G. (2019) 'Transplantation of human embryonic stem cell-derived retinal pigment epithelial cells (MA09-hRPE) in macular degeneration', NPJ Regenerative Medicine, 4, p. 19. doi: 10.1038/s41536-019-0081-8.
44. ClinicalTrials.gov (2021) ‘A Phase I/II, Open-Label, Multi-Center, Prospective Study to Determine the Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial (MA09-hRPE) Cells in Patients With Advanced Dry AMD’. Available at: https://clinicaltrials.gov/study/NCT01344993?cond=NCT01344993&rank=1 (Accessed: 9 September 2024)
45. Schwartz, S.D., Regillo, C.D., Lam, B.L., et al. (2015) 'Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies', The Lancet, 385(9967), pp. 509-516. doi: 10.1016/S0140-6736(14)61376-3.
46. National Library of Medicine (2020) 'Long Term Follow up to A Phase I/II, Open-Label, Multi-Center, Prospective Study to Determine the Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial (MA09-hRPE) Cells in Patients With Advanced Dry AMD'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT02463344 (Accessed: 9 September 2024).
47. Astellas Pharma Inc. (2022) 'A Phase 1/2 Study of ASP7317 for Age-Related Macular Degeneration (7316-CL-0005)', Clinical Trials Disclosure. Available at: https://www.clinicaltrials.astellas.com/study/7316-CL-0005/ (Accessed: 9 September 2024).
48. National Library of Medicine (2017) 'Intravitreal Bone Marrow-Derived Stem Cells in Patients With Macular Degeneration'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT01518127 (Accessed: 9 September 2024).
49. Siqueira, R.C., Cotrim, C.C., Messias, A., de Sousa, M.V., Toscano, L. and Jorge, R. (2016) 'Intravitreal Autologous Bone Marrow Derived Stem Cells in Dry Age-related Macular Degeneration', Investigative Ophthalmology & Visual Science, 57(12), p. 3704.
50. Ho, A.C., Chang, T.S., Samuel, M., Williamson, P., Willenbucher, R.F. and Malone, T. (2017) 'Experience with a subretinal cell-based therapy in patients with geographic

atrophy secondary to age-related macular degeneration', American Journal of Ophthalmology, 179, pp. 67-80. doi: 10.1016/j.ajo.2017.04.006.

51. National Library of Medicine (2017) 'Phase 1/2a, Multicenter, Randomized, Dose Escalation, Fellow-Eye Controlled, Study Evaluating the Safety and Clinical Response of a Single, Subretinal Administration of Human Umbilical Tissue-Derived Cells (CNTO 2476) in Subjects With Visual Acuity Impairment Associated With Geographic Atrophy Secondary to Age-related Macular Degeneration'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT01226628 (Accessed: 9 September 2024).
52. Pierson, R., Orr, S.C., Bogert, J., Ho, A., Malone, T., Crosby, R., Mathias, S. and Chang, T. (2016) 'Health-related Quality of Life in Patients with Moderate to Advanced Dry Age-related Macular Degeneration: Results from a Phase 1/2a clinical trial of CNTO 2476', Investigative Ophthalmology & Visual Science, 57(12), p. 5603.
53. National Library of Medicine (2022) 'Feasibility of Production of Induced Pluripotent Stem Cell Derived Retinal Pigment Epithelial Cells Fulfilling Regulatory Requirements for Human Transplantation in Dry Age-related Macular Degeneration'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT02464956 (Accessed: 9 September 2024).
54. National Library of Medicine (2020) 'Stem Cell Derived Retinal Pigmented Epithelium Implantation in Patients With Outer Retinal Degenerations: Phase I/II Clinical Trial'. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/study/NCT02903576?a=4 (Accessed: 9 September 2024).
55. Song, M.J., Quinn, R., Nguyen, E., et al. (2023) 'Bioprinted 3D outer retina barrier uncovers RPE-dependent choroidal phenotype in advanced macular degeneration', Nature Methods, 20(1), pp. 149-161. doi: 10.1038/s41592-022-01701-1.
56. Kashani, A.H., Lebkowski, J.S., Rahhal, F.M., Avery, R.L., Salehi-Had, H., Chen, S., Chan, C., Palejwala, N., Ingram, A., Dang, W., Lin, C.M., Mitra, D., Pennington, B.O., Hinman, C., Faynus, M.A., Bailey, J.K., Mohan, S., Rao, N., Johnson, L.V., Clegg, D.O., Hinton, D.R., & Humayun, M.S., 2021. One-Year Follow-Up in a Phase 1/2a Clinical Trial of an Allogeneic RPE Cell Bioengineered Implant for Advanced Dry Age-Related Macular Degeneration. Translational Vision Science & Technology, 10(10), p.13. Available at: https://doi.org/10.1167/tvst.10.10.13.