INTRODUCTION

The eye is the organ in charge of vision. It is a means by which the human being communicates with the outside. This function is performed by converting, through photoreceptors, the energy of the visible spectrum from the periphery into action potentials that the optic nerve conducts towards the cerebral cortex. On this site, it is interpreted to form an image of what is happening within the visual field. The vision is a sense that people fear losing, being the target of various systemic and local pathologies. Worldwide, about 2.2 billion people are visually impaired or blind. Of these cases, 1 billion could have been avoided or not yet treated.

Different disorders (associated with the posterior segment of the human eye) lead to visual impairment and blindness. They include glaucoma, age-related macular degeneration (AMD), diabetic retinopathy, uveitis, and retinoblastoma. The World Health Organization (WHO) reported that three of the nine leading causes of visual impairment were disorders associated with the posterior segment of the eye (glaucoma, AMD, and diabetic retinopathy). Related to uveitis, it is one of the five leading causes of blindness in developed countries and represents up to 10% of all cases in the United States. Retinoblastoma is also relevant, being the most common ocular cancer in childhood. Around 8,000 children per year develop this disease globally.

The most utilized route for pharmacological treatment is intravitreal, providing direct administration. First-line treatments include corticosteroids, steroids, prostaglandin analogs, beta-blockers, diuretics, cholinergic agonists, and alpha agonists.
However, its pharmacokinetics is complicated. There is no uniformity because of variations in the vitreous, such as viscosity or loss of collagen fibril links\textsuperscript{11,12}. Therefore, it has been decided to employ new therapeutic options such as monoclonal antibodies based on the comprehension of the molecular biology of these ocular diseases\textsuperscript{13}. An example is the vascular endothelial growth factor (VEGF), related to AMD\textsuperscript{14}.

The Food and Drug Administration (FDA) approved the first monoclonal antibody in 1986. Since then, the most widely utilized therapeutic proteins are immunoglobulins G (IgGs). These products primarily work by blocking target receptors or ligands and reducing the activity of specific pathways involved in various ophthalmological diseases' pathogenesis, making them a crucial therapeutic alternative in severe eye conditions\textsuperscript{15,16}. Given the tremendous progress in recent years, this work's objective was to check information about monoclonal antibody treatments for the most recurrent ophthalmic pathologies in the eye's posterior segment.

ANATOMIC OVERVIEW OF THE EYE

An image of the eye's anatomy is shown in Figure 1. The eyeball occupies approximately one-third of the orbit volume, while the other two-thirds are fat, muscles, nerves, and vasculature\textsuperscript{17}. The organ can be divided into two segments: anterior and posterior. The former comprises cornea, conjunctiva, aqueous humor, iris, ciliary body, and crystalline lens. Together they represent one-third of the eye. The remaining two-thirds (posterior segment) include the sclera, choroid, Bruch's membrane, retinal pigment epithelium, neural retina, and vitreous humor\textsuperscript{18}.

When making a lateral eyeball cut, three main layers are distinguished: a fibrous outer, a vascular/muscular medium, and a neural inner. The fibrous outer layer is what surrounds the organ and protects it. It includes the cornea (positioned in the anterior fraction) and the sclera (it extends back to the optic nerve). Both are formed of collagen and elastin. Their difference is the structural organization of the collagen fibers. They are arranged in very regular laminae in the cornea, allowing light rays to pass through without interference. In the sclera, they appear interwoven and extend in all directions\textsuperscript{19}. The cornea is thin, convex, transparent, smooth, avascular, and highly innervated. Therefore, it is the most sensitive tissue in the body directly exposed to the external environment, constituting 20% of the outer layer. The sclera, commonly known as the eye's white, is a hard, avascular muscle with elastic tissue\textsuperscript{20}.

The middle or uveal layer comprises three pigmented tissue structures: choroid, ciliary body, and iris. They have a nutritional function. In the anterior part is the iris, in the form of a muscular ring. Longitudinal muscle fibers allow the pupil
to dilate when they contract at the edge. In an intermediate position is the ciliary body (formed by the ciliary muscle), in charge of adjusting the shape of the lens and by the ciliary processes, whose function is to produce aqueous humor. Finally, posteriorly and in contact with the retina, the choroid is located. It has a vascular arrangement, which supplies oxygen and nutrients to the outer and inner layers.

As a complement, the inner or neural layer is the retina. It has photoreceptors (rods and cones), which detect light impulses from the environment. In addition, there are first- and second-order neurons (ganglion cells) and neuroglial elements in command of transmitting impulses to the visual cortex. On the outside is the pigment epithelium. It consists of a single layer of cells with adjacent nuclei joined together by tight junctions. Together, they form the retinal blood barrier.

Inside the eyeball are two fluid media: the aqueous humor and the vitreous body, separated by the crystalline lens and the suspensory ligament. Aqueous humor is a clear liquid secreted by the ciliary epithelium. It helps form the eye’s anterior and posterior chambers as a blood substitute for the lens and cornea. This element provides nutrition, eliminates excretory products of metabolism, transports neurotransmitters, stabilizes the ocular structure, and contributes to these ocular tissues’ homeostasis regulation. Its main components are carbohydrates, glutathione, urea, proteins, oxygen, carbon dioxide, water, and inorganic ions.

For its part, the vitreous humor is a transparent gel that provides structural support. It occupies the eye’s posterior segment, between the lens and the retina, and consists of 99% water. The remaining 1% is a mixture of collagen fibers, hyaluronic acid, hyalocytes, inorganic salts, lipids, and proteins (albumin being the main one, with 60 to 70% of the total protein concentration).

DISEASES THAT COMMONLY AFFECT THE POSTERIOR SEGMENT OF THE EYE

The pathologies associated with this anatomical region are very diverse. Some are specific to each component or may be related to a secondary condition. The most relevant ones are detailed below:

Uveitis

It refers to inflammation of the uveal tract. It can also produce inflammation of adjacent tissues (cornea, sclera, retina, and even optic nerve). About 5 to 10% of cases appear in children. Around 30% are associated with juvenile idiopathic arthritis. Furthermore, it has been linked to other autoimmune diseases such as Behcet’s syndrome and sarcoidosis. Common symptoms are blurred or distorted vision, pain, photophobia, floaters, photopsia, blind spots, and haloes. Cataracts, macular edema, epiretinal membrane, and glaucoma are common complications. Other signs include ciliary flush, corneal or scleral thinning, keratic precipitates, and anterior or posterior synechiae. Some chronic forms are asymptomatic.

Most non-infectious uveitis is mediated by helper T lymphocytes (CD4+) through a T helper 1 (Th1) phenotype. Th1 cells induce cytotoxic cells and inflammatory reactions mediated by interleukin-2 (IL-2), interferon-gamma (INF-γ), and tumor necrosis factor-alpha (TNF-α). The primary function of IL-2 is the proliferation and activation of B and T cells. Understanding the ocular inflammation pathology is limited, and most cases are indistinct (inflammatory, infectious, traumatic, genetic, neoplastic, ischemic, or drug-induced mechanisms). There is a transposition between them because there is not likely a single reason.

Retinoblastoma

It is a tumor located in the nuclear layer of the primary retinal photoreceptor cells. The disease originates from an alteration on chromosome 13, specifically in the q14 band. For its initiation, mutations of both alleles are necessary, usually called Knudson’s “two-hit” hypothesis. This malignant neoplasm is the most common in childhood, being equivalent to 10 to 15% of cancer cases that occur in one-year-old children and 2.5 to 4% of all pediatric cancers. It develops very quickly and metastasizes if it is not treated. A good
prognosis occurs with an early diagnosis. Otherwise, the retina is destroyed within a few weeks, and the tumor spreads within the eye. It should be noted that retinoblastoma was first cancer for which it was demonstrated that genetic factors influence its development, with two clinical forms. The bilateral or multifocal hereditary form occurs in 25% of events. The mean onset age is nine months earlier than in unilateral situations. Germine mutations of the RB129 gene are observed. This gene is a tumor suppressor, transmitted with recessive autonomic inheritance. It encodes the Rb protein in the cell nucleus and regulates the cell cycle. The mutation can be inherited from an affected person (25%) or be a new germline one (75%). Additionally, trilateral retinoblastoma corresponds to bilateral retinoblastoma association with a primary intracranial tumor (less than 10% of cases).

The unilateral or unifocal form is equivalent to 75% of events. The average onset age is 2 to 3 years. Usually, the illness does not develop in the other eye. Metachronous retinoblastoma occurs when a new lesion in the contralateral eye appears more than 30 days after the unilateral retinoblastoma diagnosis. This situation occurs only in 1.5 to 3% of the cases. It is usually discovered in two-year-old kids. Still, it can be detected from birth. The first symptoms occur in the first year of life but sometimes can be asymptomatic for a period. In the non-hereditary form, neoplastic changes can occur for up to 5 years. Mostly, leukocoria is seen in children under two years. It can be noticed after a flash photo. Another common sign is strabismus (related to macular involvement). Moreover, advanced intraocular tumors can become painful due to secondary glaucoma. Common symptoms are redness, tenderness, pain in the eyeball, choroidal inflammation of the eye, and bleeding into the ocular chamber.

**Diabetic retinopathy**

It damages the retina microvasculature, a common diabetes complication derived from its increased duration and chronic hyperglycemia. The disease is one of the leading causes of visual impairment, affecting around 4.2 million people worldwide. As the diabetes duration augments, chronic hyperglycemia damages the retina's blood vessels, and the pericytes are lost. Consequently, involution in the microcirculation occurs. Besides, loss of regular capillary exchange and leakage of endovascular products are facilitated. The disease progresses from the nonproliferative type to the proliferative one. The first condition is aneurysms, hemorrhages, and exudation in the retinal circulation. The other implies ocular neovascularization in the iris, retina, or optic nerve. This retinopathy generally does not originate symptoms significantly if only one eye is affected. The internal mechanism includes producing advanced glycated end products, creating a pro-inflammatory microenvironment, and inducing oxidative stress. Visual acuity is gradually lost because of preretinal or intraretinal hemorrhages, diabetic macular edema, and retinal detachment.

**AMD**

It is an acquired disease of the retina. It produces progressive loss of central vision through non-vascular (drusen and atrophy) and neovascular (choroidal neovascular membranes) disorders. Disease evolution presents diverse stages. The early is characterized by extracellular material deposit between the retinal pigment epithelium (RPE) and Bruch's membrane (outer layer close to the choriocapillaris), allowing the passage of nutrients towards the retina while acting as a barrier) known as drusen. The drusen are medium size (63 to 125 μm) at this stage. Another feature is the pigmentary changes of the retina (hyper or hypopigmentation) in the macular region. There is a slight central distortion and a reduced ability to read in low light. The stage is often asymptomatic.

In the intermediate one, the drusen size exceeds 125 μm in diameter (large), and there is a greater risk of progressing to the late stage. In this phase, a severe and permanent visual impairment and legal blindness occur (visual acuity of 20/200 or worse). It is characterized by neovascular or atrophic AMD signs. The manifestations can coexist in the same eye or one in each organ.

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The late stage progresses faster in the neovascular form (weeks or months) than the atrophic one (years or decades). The first symptoms may be a distorted vision when reading, driving, or watching television and difficulty recognizing faces. If only one eye is affected, the pathology may be asymptomatic until it progresses to the other \(^44\).

Age is a risk factor. Most late cases occur in people over 60 years old. Also, non-genetic and environmental factors involve smoking and diet. The former is the most substantial modifiable risk factor, generating twice the possibility of developing the late disease. In 2017, 52 common and rare variants were identified at 34 genetic loci independently associated with late AMD\(^44\).

Glaucoma

It is a group of eye disorders associated with damage to the retinal ganglion cells (RGCs) and optic nerve degeneration. Changes in the optic disc and progressive visual field loss are observed\(^45\). It is the most frequent cause of irreversible blindness worldwide\(^46\). Primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG) are common. The angle is the junction between the iris and the cornea, where the trabecular meshwork drains the aqueous humor from the anterior chamber\(^47\).

The angle remains open in the POAG as the iris tissue unblocks the trabecular meshwork. Intraocular pressure is transmitted to the RGCs axons at the optic nerve as mechanical stress, causing cell death. Nevertheless, about 50% of cases have normal intraocular pressure when diagnosed. After losing 30% of the RGCs, visual field damage is seen in perimetric tests\(^47\). PACG implies that the peripheral iris obstructs the exit of aqueous humor, leading to intraocular pressure increase and optic nerve damage. Shorten eyes with a shallower anterior chamber are at higher risk. The disease can have a subacute or acute (after a sudden increase in intraocular pressure) or chronic (insidious and mostly asymptomatic) development\(^47\).

Most patients are previously diagnosed with a chronic disease of both types and are unaware of any visual field loss. When left untreated, chronic, progressive, and irreversible loss occurs, moving to tunnel vision and the central one. Patients remain asymptomatic even as the disease progresses because the gradual loss is peripheral and asymmetric. This development generates compensation given by the other eye\(^47\).

The main risk factor is increased intraocular pressure (greater than 21 mmHg), frequently observed in POAG. Vascular factors, oxidative stress, and elevated glutamate or nitric oxide levels are also considered. Furthermore, there is an immunologic component involved\(^45\). Other risk factors include advanced age, ethnic origin, positive family history of glaucoma, disease stage, high myopia, and thin central cornea\(^46\).

PRINCIPAL CHARACTERISTICS OF MONOCLONAL ANTIBODIES

Its discovery began at the end of the 19th century from studies seeking defense mechanisms against microbial agents. These investigations found that serum produces substances capable of antagonizing different toxins\(^48\). Antitoxin is generated by blood cells, producing side chains that react against toxins specifically, like a key with its lock\(^49\). Subsequently, the term toxin was replaced by antigen and antitoxins by antibodies. These molecules come from B lymphocytes. Each one has its specificity, given by mutations in B cells' maturation\(^50\).

Structure and isotypes

As shown in Figure 2, antibodies are made up of two light chains and two heavy ones, identical to each other and linked by disulfide bridges. Together, they form two binding sites for the antigen. Additionally, they have an amino-terminal end (binds and recognizes the antigen) and a carboxyl-terminal end (effector function). Both chains have variable and constant portions. The variable fragment provides the antibody specificity, and the constant determines the class and the isotype. The five classes are IgA, IgD, IgE, IgG, and IgM\(^50\).

The light chains have two domains (each with 110 amino acids) with beta sheets, one in the variable portion and the other in the constant fragment. Heavy chains have one domain in the variable portion and three or four in the constant one,
depending on the Ig class. Between the domains of the constant portion is a hinge region, which generates flexibility and a better adaptive coupling. This area gives the antibody a Y shape.\(^{30}\)

The variable regions of the heavy and light chains generate the antigen-binding site. It consists of three hypervariable segments of 10 amino acids that produce space on the antibodies' surface and interact with antigens.\(^{30}\) This part, and the constant region of the light chain and the heavy chain's first constant domain, are known as the antigen-binding fragment (Fab). The heavy chain's last two domains are the crystallizable fragment (Fc).\(^{31}\) This section has the immunological capacity, mainly cytotoxic functions. Therefore, it mediates antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC).\(^{52,53}\)

![Figure 2. Structure of an antibody. The constant region of the heavy chain is shown in gray and that of the light in white. The dark gray portions are the variable regions of both chains.](image)

**Types of monoclonal antibodies**

In 1975, monoclonal antibodies were discovered by Köhler and Milstein.\(^{48}\) For its development, mouse myeloma cell lines and spleen cells of an immunized mouse were used to fuse the heavy and light chains of antibodies from both cell types, creating hybrid molecules capable of expressing both parents' characteristics and new ones. It resulted from the DNA translocation and the ordering during their transcription. Thus, antibodies were generated toward a specific antigen. This technique is called a hybridoma.\(^{54,55}\)

The procedure combines B lymphocytes from an immunized animal spleen with immortalized myeloma cells that cannot produce the enzyme hypoxanthine-guanine-phosphoribosyltransferase (HGPRT), which allows nucleotide production. Their mixture (those of the hybridoma and those that did not fuse) is cultivated in a culture media with aminopterin, inhibiting de novo nucleotide production. Because myeloma cells have a blocked nucleotide production pathway, they will not be viable. In contrast, B lymphocytes can produce them even if this pathway is useless, thus selecting hybridomas.\(^{55}\)

Monoclonal antibodies have a specific target and are produced from a single cellular clone.\(^{51}\) They are generated to restore, imitate, or improve the immune system's attack by binding to antigens found in the body cells.\(^{96}\) The first ones were made from murine proteins (they are identified with the -omab suffix). However, they generated many allergic reactions and antibodies against the drug. Furthermore, they showed weak binding to the Fc region in humans and were unsuitable for promoting ADCC and CDC. Therefore, other antibodies were developed.\(^{55,57,58}\)

1. Chimeric monoclonal antibodies: chimerization is a technique related to these proteins where the murine variable region (antigen-binding), but they have the constant portions of human heavy and light chains, obtaining 65% human antibodies. They are on the market with the -ximab suffix.
2. Humanized monoclonal antibodies: are generated from a human antibody framework and the murine hypervariable region (approximately 95% human). Its suffix is -zumab.

3. Fully human antibodies: these molecules were created by animals carrying human immunoglobulin genes. These drugs are less antigenic than the others and have the -umab.

As a result of the immunogenicity decrease, the antibodies' half-life progressively increases, with entirely human ones having the most extended values\textsuperscript{69}.

The advancement for the humanization of monoclonal antibodies has been linked to various techniques. One was the creation of phage display libraries from hybridoma technology. The procedure generates clones that encode the Fab region of B lymphocytes in bacteriophage plasmid vectors. Then, the bacteria express genes from a viral capsid. The library obtained can generate new antibodies \textit{in vitro}. Similarly, more antigens can be tested by presenting the ability to engineer and manipulate genes and quickly obtain antibodies. As a complement, the molecules are more stable since the phages can withstand adverse conditions, including temperature, pH, and others\textsuperscript{69}.

There are various antibody libraries: immune, naive, semi-synthetic, and synthetic. Immune libraries are made from IgG mRNA from infected or recovered people. They consist of specific antibodies and can be used as direct therapy or diagnosis, generally infectious agents such as viruses. Naive, "single-pot," or universal libraries are made from IgM mRNA of B cells from non-immunized healthy people and are employed to obtain antigen binders, regardless of the person's condition. The last two consists of synthetic or semi-synthetic sequences and are utilized to select antibodies against autoantibodies\textsuperscript{69}. They can be highly defined, and natural antibodies are not required\textsuperscript{69}.

Other methods are antigen-specific single B cell sorting strategies and B cell culturing methods. Techniques with B lymphocytes present a significant impediment since they require sophisticated instrumentation and great personnel experience\textsuperscript{69}. For its part, transgenic mice generate antibodies from the hybridoma technique. Endogenous Ig genes are silenced in rodents, and portions of human heavy and light chain genes are inserted, yielding human antibodies. These humanized mice are immunized against the antigen of interest. Later, the B cells with specificity for this antigen are isolated, generating the desired proteins\textsuperscript{64,65}.

**INDUSTRIAL PRODUCTION**

Cells suitable for the process must secrete the desired membrane protein for production. Mammalian cells can produce complex molecules and patterns compatible with the human immune system. Some are Chinese hamster ovary (CHO), human embryonic kidney (HEK293), mouse myeloma (NSO), and transformed human embryo retina (PER.C6) cells. These cell lines have been modified to express a specific membrane protein through transient transfection of expression vectors or stable integration of a transgene. Therefore, they can produce humanized and chimeric antibodies in large quantities. Other cells come from genetically modified plants, insects, and microorganisms. The latter offer ease of handling and modification and reproducible production\textsuperscript{64,66}.

Regarding the culture media, they should be free of any animal component. Its conditions are already established. Typically, when the temperature and pH decrease to lower values than usual, the compound's production increases. Additionally, the CHO cell line generated antibodies glycosylation by the presence of N-acetylglucosaminyltransferase III in the cell. Without this enzyme, they will have a lower ADCC\textsuperscript{67}. Furthermore, glycosylation can affect antibody stability, receptor binding, effector functions, clearance, and half-life\textsuperscript{68}.

The current processes for monoclonal antibody production are upstream cell culture and downstream purification. These procedures are not the same for all since they have various properties. However, there is a general method to perform its production. Upstream cell culture refers to the rapid growth and high-specific-productivity manufacture of cell cultures with determined media. Thus, effective expression systems must be defined, and markers within the cell line development vectors must be previously determined. They are genes that encode dihydroxy folate reductase and glutamine synthetase, using promoters that enhance cell messenger RNA (mRNA) transcription\textsuperscript{64}.
The selection of cell lines with high specific productivity can be made by fluorescence-activated cell sorting, choosing those that produce the highest antibody levels. For its large-scale production (upstream cell culture), a bioreactor with controlled dissolved oxygen, pH, and temperature conditions must be employed. The types comprise stirred tanks, airlifts, hollow fiber bioreactors, and rotatory cell culture systems. The usually chosen for antibody production is the stirred tank bioreactor.

One way to accomplish production is by fed-batch mode. There are two methods. First, a near-optimal basal media is added, and its concentration is maintained by putting concentrated nutrients as cell growth occurs. The second way is to incorporate concentrated nutrients into the complete media with or without standard amino acids, glucose, and glutamine, increasing antibody production at the beginning. This fed-batch technique allows the product concentration to augment and has given the best manufacture and yield results. Still, other strategies are perfusion and fed-perfusion culture.

Perfusion feeding involves retaining cells in a culture vessel while the spent culture medium is removed and an equal volume of fresh one is incorporated. As only the media is renewed, dead cells accumulate, and toxic metabolites are released. Then, a small stream containing cells is removed. In contrast, fed-perfusion culture involves replenishing depleted components and keeping nutrients constant, minimizing the toxic metabolite generation.

For the second part, the downstream purification is based on a filtration sequence of the bioreactor harvest through various chromatographic columns. This process depends on the components' physicochemical properties, so that the chromatography type may differ. Filtration is usually done through a series of depth filters or by centrifuging the bioreactor harvest. The first step is capture chromatography, where the impurities binding is generated with their subsequent elution, increasing the product safety.

The column's stationary phase is protein A, for which the antibody exhibits affinity and interacts with the column. Cellular proteins, DNA, and other impurities pass through it. The pure antibody is obtained by its Fc region affinity with the protein A ligand at low pH. This protein A comes from Staphylococcus aureus, which is highly immunogenic. The process is precise. After performing the chromatography, the sample is further purified, and impurities are removed. Then, viral elimination and inactivation must be ensured by filtration. Finally, ultrafiltration/diafiltration is executed to reduce the volume.

Other proteins such as G and L can be considered in the stationary phase, depending on the type of antibody purified, the matrices employed, and the available culture supernatant. G is derived from Streptococcus sp, and L comes from Peptostreptococcus magnus.

The possibility of purification without a protein related to them should be noted because it dramatically increases production costs. They are more complicated techniques based on small-molecule ligands with similar selectivity to protein A. They are presented as resins. Its ability to bind with the antibody depends on its density and concentration in the load material—moreover, some work by its ionic strength.

One consideration is the microheterogeneity of these molecules, which produces structural variations, affecting their biological activity and presenting more adverse effects. They could be generated by post-translational modifications during production or by physicochemical modifications during the purification, formulation, or storage process. Therefore, quality control is focused on verifying its physicochemical properties throughout the production process. Some tests to ensure drug quality are capillary electrophoresis, liquid chromatography techniques (size exclusion, ion exchange, reversed-phase), polyacrylamide-gel electrophoresis, capillary zone electrophoresis, and capillary isoelectric focusing. These methods allow the determination of the size and charge variants and antibody glycosylation through the procedure.

Its administration in the eye can be done by direct intravitreal, subconjunctival, or systemic intravenous injections. Each has its advantages and limitations. The intravitreal option is the most used with the most significant number of studies. This pathway reduces pro-inflammatory agents and retinal edema, preserves the retinal structure, and prevents ganglion cell neuronal death.
APPLICATIONS

Ophthalmic monoclonal antibody offers many advantages over traditional treatments due to a considerable reduction in side effects and a better therapeutic response. The main molecular targets are TNF and VEGF, whose three-dimensional structure and main features appear in Table I. Likewise, intravitreal products against these molecular targets offer better safety and efficacy in treating the previously described diseases. In the first place, commercialized monoclonal antibodies, whose molecular target is TNF, will be discussed. Then, those active principles made against VEGF will be addressed. Finally, these diseases' products in different development phases will be mentioned.

Table I. Three-dimensional structure and principal characteristics of TNF and VEGF

<table>
<thead>
<tr>
<th>Target</th>
<th>Three-dimensional structure</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>TNF</td>
<td><img src="https://www.rcsb.org" alt="Image" /></td>
<td>It is a pleiotropic cytokine synthesized mainly by monocytes, macrophages, and T lymphocytes and, to a lesser extent, by neutrophils, mast cells, and fibroblasts, in response to infection or immune impairment. It promotes inflammation by direct cytotoxicity and through indirect mechanisms, including the production of pro-inflammatory cytokines, arachidonic acid mediators, metalloproteinases, chemokines, adhesion molecules, and angiogenesis factors.</td>
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<tr>
<td>VEGF</td>
<td><img src="https://www.rcsb.org" alt="Image" /></td>
<td>The protein was identified as a potent promoter of vascular permeability and endothelial cell proliferation and, later, as the angiogenesis master regulator. Its increased concentration at the ocular level has been associated with diabetic retinopathy, AMD, glaucoma, and retinoblastoma. As a complement, it augments inflammation by inducing the expression of vascular cell adhesion molecule 1 (VCAM-1). Besides, it promotes endothelial cell proliferation with a signaling cascade mediated by tyrosine kinases. VEGF-A regulates angiogenesis and vascular permeability through the VEGF receptors 1 and 2 (VEGFR-1 and VEGFR-2), while VEGF-C/VEGF-D regulates lymphangiogenesis through the VEGFR-3.</td>
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Note: Images were taken from the Protein Data Bank (https://www.rcsb.org)

TNF

In the case of autoimmune ocular inflammation, TNF-α is the central molecule to consider. The target receptors are TNFR-1 or p55 (involved in pro-apoptotic and inflammatory signals) and TNFR-2 or p75 (participated in cell growth and proliferation). The ophthalmic drugs used against this target are adalimumab, infliximab, certolizumab pegol, and golimumab. They are utilized for the treatment of uveitis.

Adalimumab

It is a fully human IgG1 monoclonal antibody. It interacts with TNF and prevents its binding to the p55 and p75 receptors. This drug showed a lower risk of failure than a placebo in clinical studies. Furthermore, it controlled many uveitis aspects without glucocorticoid support. Nonetheless, the vitreous haze was the primary cause of failure in the placebo group compared to those receiving adalimumab. Likewise, chorioretinal lesions were more frequent in patients receiving a placebo concerning the management of said antibody.

Infliximab

Chimeric IgG1 antibody that has two murine antigen-binding sites. It neutralizes the biological activity of TNF. Therefore, the drug has been used to treat non-infectious uveitis. A single intravitreal dose of infliximab (15 μg/eye) or control vehicle was applied in a preclinical investigation, and the samples were analyzed with flow cytometry. In mice who received the monoclonal antibody, a significantly reduced CD45+ infiltrate was seen on day 14, showing a decrease in CD4+ lymphocytes. In contrast, the control group presented in the same period the typical symptoms of the disease (vasculitis and choroidal lesions).
Later, a clinical study with 72 patients demonstrated efficacy since 81.8% showed clinical remission. However, 58.3% of these patients required additional therapy with immunomodulators. The most common adverse effects were skin rash and fatigue.  

**Certolizumab pegol**

Certolizumab is a humanized monoclonal antibody. It does not have the Fc portion, impeding to induce of CDC, ADCC, apoptosis, or granulocyte degranulation. In addition, it has a Fab fragment conjugated to polyethylene glycol (PEG) to enhance plasma half-life. The latter showed efficacy in a clinical study with 21 patients receiving either golimumab or certolizumab pegol. Meanwhile, some case reports show good outcomes as a therapy against refractory, non-infectious uveitis.

**VEGF**

Drugs against this target have emerged as a tool widely utilized in intravitreal therapy in recent years. This alternative offers excellent safety, although there may be systemic absorption. The main medications administered through the ophthalmic route are listed below.

**Bevacizumab**

It is a humanized IgG1 antibody. The concentrations required for its adequate pharmacological effect are deficient (around 1800 pM). Its intravitreal employment is considered for diabetic retinopathy and AMD. It can cross ocular barriers and generates an inhibitory effect of VEGF in plasma (systemic effects cannot be ruled out). Preclinical studies have shown that VEGF neutralization with bevacizumab could inhibit the differentiation of retinoblastoma cells by blocking the extracellular pathway regulated by kinases. Also, it affects cell growth and differentiation in vitro. Although this therapeutic strategy may play a role in its clinical management, further studies and tests are required to optimize therapy for patients with this illness. Moreover, safety and improved disease progress were demonstrated in a clinical trial in which 26 eyes with neovascular glaucoma were treated using intravitreal bevacizumab. The average intraocular pressure passes from 39.79 mmHg to 16.51 mmHg one week after injection.

**Ranibizumab**

It is a humanized monoclonal antibody that only has its variable fraction. This structure is endowed with activity against VEGF, binding to the active form of VEGF-A. The constant fraction absence in its structure implies the impossibility of binding to the neonatal Fc receptor and the lack of blood transport. Consequently, its systemic bioavailability is nil after intravitreal administration, avoiding effects on other human body's anatomical sites. The formulation is prepared for intravitreal administration, avoiding problems derived from handling.

In a clinical study of 5496 patients with neovascular AMD who were given bevacizumab or ranibizumab intravitreally, they gained an average of 15 letters in visual acuity, and no statistically significant difference in efficacy was shown. The most frequent adverse effects were increased intraocular pressure and ocular inflammation. Both antibodies showed similar efficacy in other clinical investigations, although bevacizumab reported a higher proportion of adverse effects, as it has a much longer half-life (20 versus 0.5 days). Nevertheless, bevacizumab is applied more widely for its lower cost.

**OTHER MONOCLONAL ANTIBODIES UNDER CLINICAL TRIALS**

In addition to those mentioned above, there are currently commercialized products to treat other pathologies. Their clinical studies are being performed for the ophthalmic diseases of the posterior segment of the eye.
Monoclonal Antibodies: A Therapeutic Option for the Treatment of...

Golimumab
The fully human monoclonal antibody of the IgG1 type selectively binds to TNF. It is approved for rheumatoid arthritis, ankylosing spondylitis, and Crohn's disease. It is currently in phase II clinical studies to treat refractory Behcet's uveitis.

Brolucizumab
Humanized, single-chain fragment antibody that targets VEGF-A. It was approved in 2019 for the treatment of AMD. It has presented efficacy similar to aflibercept in preclinical studies and with fewer adverse effects. The data obtained shows a higher affinity than other VEGF-A antagonists with scarce side effects, making it an excellent option to manage AMD and diabetic retinopathy. The most common adverse effects in clinical trials were conjunctival hemorrhage, eye pain, and hyperemia, which were mild in intensity and resolved within a few days without treatment. It is currently in phase III clinical investigations to treat diabetic retinopathy and AMD.

THERAPEUTIC TARGETS UNDER CLINICAL INVESTIGATION

Tocilizumab
It is a humanized monoclonal antibody of the IgG1 type acting as an antagonist of the IL-6 receptor. It is widely utilized in rheumatic diseases such as juvenile idiopathic arthritis. In a clinical study with 11 patients who presented refractory uveitis associated with Behcet's disease, the antibody treatment combined with traditional immunosuppressants significantly improved compared to the group that only received therapy with traditional immunosuppressants. Phase II clinical studies have been done.

Ustekinumab
It is an IgG1 human monoclonal antibody that binds to the p40 subunit of IL-12 and 23. It is employed to treat Crohn's disease. In studies made in humans, increased levels of IL-23 have been detected compared to control patients, doing it a relevant therapeutic target. Therefore, phase II clinical investigations are being done for uveitis treatment.

Faricimab
It is the first bispecific monoclonal antibody designed for intravitreal use, binding VEGF and angiopoietin-2. Its good safety profile was established in phase I clinical studies, and no toxic effects were observed up to the highest dose (6 mg). Besides, all the parameters to define visual acuity improved significantly in most patients. Also, in phase II clinical investigation, the efficacy of ranibizumab was compared with faricimab. The latter demonstrated greater efficacy and better gain in visual acuity. Phase III clinical studies are in progress.

CONCLUSION
Monoclonal antibodies have been developed to treat disorders associated with the eye's posterior segment by blocking TNF (adalimumab, infliximab, and certolizumab pegol) and VEGF (bevacizumab and ranibizumab). Other options with different targets are studied through clinical trials, like golimumab, brolucizumab, tocilizumab, ustekinumab, and faricimab. Therefore, it is expected that more research will be done in the next future to find novel molecules for the treatment of these diseases.

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AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest in this research.

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