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Review

Diabetic Retinopathy and Ocular Melanoma: How Far We Are?

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Abstract: Diabetic retinopathy causes vascular damage to retinal neurons, presenting characteristics of chronic inflammation. The development of new therapies capable of combating vision loss involves knowledge of inflammatory retinal changes. Studies in animal models and patients with diabetes have shown a high expression of the inflammatory molecules that are involved in the progression of diabetic retinopathy. Uveal melanoma is an eye tumour that remains highly deadly, because despite the correct treatment, it still causes metastasis in about 50% of patients. This type of tumour has the ability to produce and store melanin, which may result in resistance to therapy. Over time there has been development of new therapies for this disease, such as radiotherapy and surgical resection. In this review, we discuss diabetic retinopathy and ocular melanoma, their relationship with angiogenesis and the current anti-angiogenic therapies for their treatment.

Keywords: angiogenesis; vascular endothelial growth factor; inflammation; diabetic retinopathy; uveal melanoma; anti-angiogenic therapy

1. Introduction

The eye is the organ responsible for vision and is anatomically composed of the cornea, iris, crystalline lens, vitreous body and retina [1]. Retina is a metabolically active tissue, transforming the light energy in electrical impulses, forwarding it to the brain through the optic nerve and allowing vision [2,3]. Internal and external retinal blood barriers (BRB) preserve the retina and, under ideal physiological conditions, regulate the flow of ions, proteins and water [3].

Diabetic retinopathy (DR) causes vision loss, which is the predominant cause of blindness in the population worldwide. Retinal neovascularization and oxidative stress (due to the hyperglycaemic environment) are the most common features found in this disorder [4,5]. In addition, there are also several factors (e.g., inflammation) leading to the break of both internal and external BRB and causing the progression of macular edema, which is the predominant cause of vision loss in diabetic patients. In this scenario, the cells that make up the external BRB—retinal pigmented epithelial cells (RPE)—are under hyperosmolar stress [3]. The breaking of BRB results in the increase of retinal osmotic pressure and accumulation of water, with increased risk of developing macular edema and impaired vision. RPE cells are thus subjected both to hyperosmolar stress (HOS) in the apical membrane and to hyperosmolar stimulus by enhanced plasma osmolarity [6,7]. In this context, glucose induces hyperosmolarity and causes angiogenesis and retinopathy by activating the transcription factor tonicity-responsive binding-protein (TonEBP)/nuclear factor of activated T-cells[7]. Recent studies have also proposed that increased age-related plasma osmolarity intensifies age-related macular degeneration (AMD) through the promotion of inflammation and retinal angiogenesis [8]. It is well known that AMD and choroidal neovascularization (CNV) are strongly linked to DR, as the onset of one disease can lead to the other. All these diseases are thus associated with each other. Mashayekh et al. reported that subclinical macular edema, found in 54% of uveal melanoma patients, increased the tumour thickness and diameter [9]. It has also been shown that the events causing new and abnormal blood vessels (angiogenesis or neovascularization and vasculogenesis) related to the differentiation of circulating bone-marrow-derived endothelial precursor cells are also present in uveal melanoma [10,11].

The relationship between retinopathy and ocular melanoma is well documented [12,13]. Melanoma is a primary tumour mostly affecting the eyes in adulthood, with an annual worldwide prevalence of 5.5 to 10.9 cases per million people [14]. Melanomas are classified as cutaneous and non-cutaneous. Only 5% of melanomas affect the ocular region, and these can develop in the conjunctiva, eyelid or orbit [15]. Out of the ocular melanomas, only 5% affect the conjunctiva, while the remaining 95% affect the intraocular structure of the eye and arise from the uvea (i.e., iris, ciliary body and choroid). As its name suggests, melanoma is derived from melanocytes and usually develops metastases (mainly in the liver). People with clear skin and blue eyes, together with cutaneous nevi, congenital ocular melanocytosis, uveal melanocytoma and neurofibromatosis account for the major risk factors of melanoma. The poor delayed diagnosis of this type of cancer leads to a high risk of metastatic liver cancer, favouring the development of metastases within 5–10 years upon the first diagnosis. High mortality (≈90%) associated with these sequelae is also reported in less than three months [16]. Besides the liver metastasis, the highest incidence rate is in the lung (29%), bone (17%), skin (12%) and lymph nodes (11%) [17]. Grajewski et al. used patients with metastatic cutaneous melanoma to study their range of eye diseases [18]. In 60% of patients with metastatic cutaneous melanoma, eye disease was found, especially in older patients. It has also been shown that in these patients the range of eye diseases is similar to that of the healthy age population. Bishop et al. compared the results of various tumours, including cutaneous, ocular and mucosal melanomas of specific primary sites [19]. Results were shown to be site-dependent, the external genital sites and the oral cavity being of lowest risk. This variation was attributed to the patient's gender and age, and also to tumour dimension, ciliary body ratio and extraocular extension [20]. Despite the latest developments regarding treatment and diagnosis of this type of tumour, there is no evident improvement in the survival rates. This feature is associated with the capacity of melanoma to metastasize before the primary tumour is treated, followed by a prolonged period of

latency before metastasis manifestation [21]. The main features of diabetic retinopathy and ocular melanoma are summarized in Figure 1.

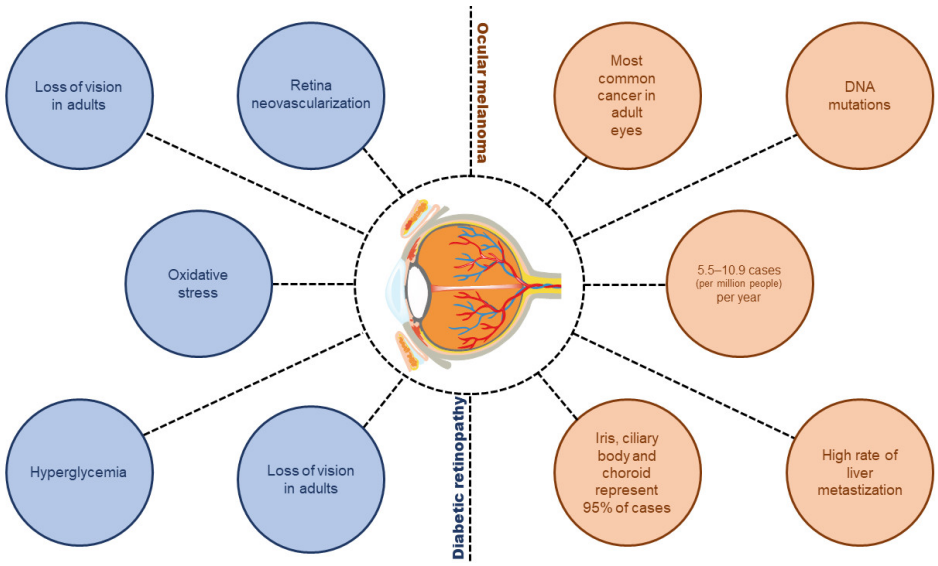

Figure 1. Main features of diabetic retinopathy (left) and ocular melanoma (right).

Uvea melanomas are highly vascularized tumours with a variety of non-random chromosomal changes. According to the gene expression profile, they can be of "Class 1" or "Class 2", the latter being of highest risk of metastasis [1]. These abnormalities can result from chromosomal instability during cell division, i.e., the abnormal separation of sister chromatids [22]. Abnormalities in uveal melanomas are characterized by total or partial loss of chromosome 3 (related to liver metastases), gains from chromosome 8 and loss of chromosome 1p (isodisomy 8q or trisomy) and gains at 6p (isochromosome formation). Loss of chromosome 3 and 1p, and gain of chromosome 8 are known to be related to a worse prognosis, larger tumour size, ciliary body involvement, existence of epithelioid cells, high mitotic count and closed connective tissue [20].

2. Angiogenesis and Anti-Angiogenic Therapy

Neovascularisation or angiogenesis is mediated by vascular endothelial growth factors (VEGFs), and these mediators are also found in retinal alterations affecting its vasculature, such as those caused by diabetes mellitus, retinal vascular occlusion, and ocular malignancies [23–25]. Vascular endothelial growth factor/receptor (VEGF/VEGFR) influences tumour neovascularization significantly, being self-secreted and by surrounding stroma [26,27]. While certain treatments such as radiotherapy also increase the secretion of VEGF by the tumour [28,29], it has been anticipated that anti-angiogenic treatment may enhance uveal melanoma's response to radiotherapy, regulating blood vessels and consequently tissue oxygenation [30,31]. Hypoxia is a trigger for the initiation of angiogenesis and results from a cascade of cellular signalling leading to vasodilation via release of nitric oxide, increased vascular network permeability by VEGF and angiopoietin-2 (Ang-2), and also due to vascular basement membrane decomposition via extracellular matrix by matrix metalloproteinases (MMPs) [32–35]. In a hypoxic environment, pericytes secrete VEGF and are involved as mediators of numerous processes related to tumour metastasis and angiogenesis. Pericytes are vascular mural cells located in the basement membrane of microvessels that also contribute to endothelial cell proliferation [36]. Although their impact in angiogenesis therapy is still unclear, it is well known that they are crucial in tumour progression. Holmgren et al. have described the molecular aspects of tumour angiogenesis, predicted anti-angiogenic therapy, and also demonstrated that tumour blood suspension influences metastatic growth [37]. The appearance of VEGF was first detailed by Criscuolo et al. [38]. Indeed, this growth factor is crucial in angiogenesis

as it controls vasopermeability and endothelial cell propagation and dispersion [39]. The serum VEGF levels are a useful biological marker of various tumours, their invasiveness and possible metastases [40]. In this review, we discuss diabetic retinopathy and ocular melanoma, their relationship with angiogenesis and also current anti-angiogenic therapies for their treatment.

3. Diabetic Retinopathy, Angiogenesis and Anti-Angiogenic Therapy

Diabetes is a chronic disease related to genetic predisposition and risk factors, i.e., obesity, aging, physical inactivity; particularly, diabetes mellitus is a chronic and progressive disease influencing a considerable percentage of the population worldwide [25,41–45]. Several works have been published on natural nutraceuticals [46–51] as well as new formulations [25,41,42] used for managing diabetes [52–54].

Diabetic retinopathy is known to lead to blindness, progressively damaging retinal microvasculature. This disease has two specifications, proliferative diabetic retinopathy (PDR) and non-proliferative diabetic retinopathy (NPDR). The first comprises retinal neovascularization under low oxygen, and the second causes changes in internal retinal microvasculature, which may be mild, moderate or severe, and is also related to diabetic macular edema (DME) [55–58]. The new blood vessels are delicate and, without proper treatment, lead to blindness. In DR there is also a high vascular permeability that can cause fluid accumulation and macula's retinal haemorrhages [59].

DR has long been seen as a microvascular problem, but now it has also been recognized that retinal microvasculature is governed by neurons and glia. These are the first cells to undergo changes, occurring before the clinical manifestation of vascular lesions. Advances have been made to treat vascular changes, but there is no therapy yet for early neuro-glial disorders in DR [5]. Many pre-clinical and clinical studies focus on vascular dysfunction in the DR, including damaged endothelial cells, pericyte death, retinal capillary basement membrane thickness and modification of tight joints. Nevertheless, diabetic microvasculopathy does not clarify the vulnerability of peripheral nerves, brain complexities and retinal impairment. The concept of the neurodegenerative aspect of RD began to emerge in the late 1990s. There are several reviews available in the literature reporting that in this disease there is vulnerability and premature death of neurons, but none of them clarify the pathophysiological mechanisms responsible for neurodegeneration [60,61]. The most affected cells—retinal ganglion cells (RGCs)—have an elevated rate of apoptosis, especially in the outer nuclear layer, with a decrease in photoreceptors in the first six months of diabetes [62–65]. Molecular analyses were also performed where it was shown that proteins relevant to photoreceptor function (e.g., rhodopsin) undergo changes prior to the onset of microangiopathy in diabetes, which is consistent with this observation [66]. Activation and dysfunction of glial cells is yet another individuality of diabetic-induced retinal neurodegeneration and has been widely studied. The relevant retinal glial cells—Müller glial cells (MGCs)—play a crucial role in retinal metabolism and are therefore vulnerable to any alteration in this metabolism. Positive regulation of glial fibrillary acidic protein (GFAP) by MGCs is indicative of retinal metabolic stress, and this occurs in animal models as well as in the tissues of diabetic patients with no to mild NPDR [67,68]. As a result, microglia cells are activated and proinflammatory mediators are produced, stimulating neuro-glial and vascular dysfunction [69]. These imbalances in neurons and glial cells also cause functional changes that occur prior to vascular lesions clinically found in RD. Thus, diabetes is known to directly affect neuroretina and is not a consequence of the breakdown of the retinal blood barrier. In diabetes, biochemical pathways related to neurodegeneration and vascular dysfunction are activated, with more angiogenic and inflammatory mediators being expressed and aberrant growth factor activated. All of this knowledge has led to the conclusion that diabetes-related retinal dysfunction is a retinal neurovascular change, highlighting the association between retinal neurons (photoreceptors, horizontal and bipolar cells, amacrine and ganglion cells), support cells (astrocytes and Müller glial cells) and vascular beds (endothelial cells and pericytes). Thus, the performance of this retinal neurovascular unit is crucial for its normal functioning, allowing its adaptation to the most diverse physiological conditions [5].

As mentioned before, DR results in microvascular changes and a failure in BRB function, succeeding the development of macular edema [70,71]. Thus, over time there has been an attempt to study and explain these mechanisms. Several pathways describing the molecular mechanisms of DR are now known, including the polyol pathway, advanced glycated-end products, protein kinase C pathway, oxidative stress, renin-angiotensin system and epigenetics, as well as VEGF [3]. Inflammation plays a crucial role in DR. The high blood glucose levels typical in diabetes activate the secondary pathway for glucose metabolism—the polyol pathway. In turn, aldose reductase reduces glucose in sorbitol, while sorbitol dehydrogenase metabolizes sorbitol in fructose. Accumulation of sorbitol inside the cells leads to osmotic changes in retinal vascular cells and in RPE cells, loss of pericytes, change in basement membrane size and oxidative stress. The activation of this pathway further promotes the breakdown of BRB that occurs in DR [72]. The resulting glycated products correspond to proteins and lipids that at the end of translation are exposed to aldose sugars, being transformed by non-enzymatic glycation and oxidation [73]. These resulting products are related to DR, as they change hormones, cytokines and extracellular matrix and further induce retinal vascular damage [74,75]. The generation of reactive oxygen species (ROS), which results from the excess of sugar in the blood, stimulates oxidative stress, which is responsible for the activation of other mechanisms promoting DR [71,76]. In this disease, the renin-angiotensin system also has a crucial role as it also leads to damage to neuronal vascular cells and retina [77]. Diabetes induces epigenetic changes (DNA methylation, histone acetylation and post-transcriptional RNA regulation) that lead to altered gene expression (genes involved in oxidation, angiogenesis, extracellular matrix degradation), which modifies the function of retinal vascular cells that are crucial in DR [78,79]. Through the analysis of in vitro and in vivo studies, several characteristics of inflammation have been detailed, such as leukostasis, neutrophil and macrophage infiltration, complement and microglial induction, positive regulation of cytokines, as well as blood flow expansion, vascular permeability and tissue edema [3]. To counter the macular edema and BRB rupture that occur in DR, steroids are usually recommended [80,81]. Regarding growth factors, these (especially VEGF-A) enhance the action of retinal ICAM-1, vascular permeability, leukostasis and BRB breakdown [82]. For the treatment of DR, anti-VEGF drugs have been commonly used, but these require several injections and are associated to limited efficacy [83]. In a rat model study, induced hyperglycaemia increased expression of VEGF, which was mediated through the protein kinase Cβ/human antigen R (HuR) and phospholipase A2 pathways [84,85]. SiRNA-containing lipid nanocarriers have also been studied, proving to be an alternative to DR therapy. These nanocarriers inhibit HuR and phospholipase A2, not allowing increased retinal VEGF levels [85,86]. Excess of glucose interferes with plasma tumour necrosis alpha (TNFα) and interleukin 6 (IL6) and VEGF plasma levels, and reduced retinal oxygen levels stimulate the release of macrophages, chemokines and microglial growth factors, as well as the expression of angiogenic factors [87–90]. In DR, RPE cells also release inflammatory cytokines, chemokines and angiogenic factors (e.g., IL6, IL8, MCP-1, TGFβ and VEGF), which are mainly responsible for the development of the disease [91]. Monocyte chemoattractant protein-1 (MCP-1) influences retinal leukocyte infiltration as well as VEGF manifestation [92,93]. The influence of MCP-1 on BRB rupture was studied in vivo. MCP-1 knockout mice injected with streptozotocin to induce diabetes showed low retinal vascular leakage, monocyte infiltration and retinal microglial stimulation [94]. It has been demonstrated that in DR, TNFα (NF-κB-transcriptionally regulated cytokine secreted by macrophages and T cells) potentiate leukostasis, lead to BRB disruption and stimulate adhesion molecules, leukocyte production, apoptosis, monocyte chemoattraction and growth factors among other inflammatory mediators [82,95]. Thus, anti-TNFα therapy tested in diabetic mice caused decreased loss of pericytes and capillary degeneration, and in humans this therapy visually improved diabetic macular edema [96]. Several inflammatory cytokines in the interleukin (IL) category also influence DR. IL6 has been shown to induce vascular permeability, angiogenesis and manifestation of VEGF in this disease [97–99]. In addition to the angiogenic function, IL8 is also chemoattractive [100]. IL1β also has a crucial role in DR. It is generated by macrophages and activates the transcription factor NF-κB. This factor commands the transcription of inflammatory cytokines (e.g., IL6 and IL8) in RPE cells. This cytokine also boosts

angiogenesis and neovascularization. In an in vivo study in a diabetic mice model it was shown that the exclusion of receptors protected the animals from the evolution of DR [101–103]. Toll-like receptors (TLRs) are a collection of receptors that respond to endogenous microbes and ligands and thus manifest in the various retinal cells. In DR, these receptors are activated through intracellular signalling cascades, which lead to the generation of proinflammatory cytokines, control costimulatory molecules, provoke oxidative DNA damage and stimulate angiogenic growth factors. High mobility group protein B1 (HMGB1) allows nucleosome stabilization and transcription of the TLR2 and TLR4 binding genes and the receptor for advanced glycation end products. This protein is discharged by monocytes, activated macrophages, natural killer cells, mature dendritic cells and also endothelial cells. In addition, it has the same function as a proinflammatory cytokine [104–106]. HMGB1 is manifested through the various cells in the retina, also promoting the manifestation of VEGF, TNFα, MCP1 and ICAM-1 and stimulating vasculopathy [107–109]. DR patients have high HMGB1 levels [107]. In vitro studies on glial cells have shown that HMGB1 favours cytotoxic effects and also promotes the death of pericytes and endothelial cells [110]. Hyperglycaemia in RPE cells promotes the generation of HMGB1 and consequently the generation of NF-κB and VEGF. In addition, in retinal endothelial cells, hyperglycaemia also induces the manifestation of TLR2 and TLR4 and the activation of NF-κB, promoting the production of IL8, TNFα, MCP1 and adhesion molecules. The expression of the receptor for advanced glycated end products TLR2, TLR4 and HMGB has been studied in vivo in the retina of mice with type 2 diabetic, and it has been shown that HMGB1 stimulates the generation of VEGF via TLR4 in RPE cells and also cytokines via TLR2 and TLR4 in endothelial cells [111,112]. In TLR7 knockout mice, it has been shown that this receptor induces inflammatory response in DR [113]. Another in vitro study also demonstrated the role of P2X7 purinergic receptors. In the presence of high glucose levels, cell lysis and ATP release occurs, stimulating the function of these receptors and inflammasome (a multimeric complex that activates IL1β-activating caspase), thereby stimulating IL1β generation in human retinal pericytes. Thus, the role of P2X7 receptors in DR was elucidated, translating into a possibility for the therapy of this disease [114].

Inflammation is the main player in retinal dysfunction associated with diabetes. Clinical studies have shown that in DR there is obstruction and increased capillary permeability; taking into account the VEGF function, it is clear that this is one of the major factors for vascular lesions of DR [115,116]. Thus, anti-VEGF therapy was shown to be successful but has limitations as repeated intraocular injections are required, maximum efficacy is in the late stages of the disease and only 50% of patients in studies respond positively to treatment [117]. It has also been shown that diabetics soon present neuroretinal changes before manifestations of microvascular lesions are visible [118]. DR is clearly proven to be closely related to inflammation, and its inhibition has already been proven to have an impact on the retinas/vitreous humour of animals and diabetic patients [5,119]. Studies in patients with DR have shown that the use of anti-inflammatory agents (e.g., salicylates and minocycline) moderates the inflammatory response, thus preventing vascular and neuronal disorders [120,121]. Currently, in order to prevent vision loss, DR is treated by laser surgery, pharmacotherapy and vitrectomy, intraocular pharmacotherapy being the most used, together with anti-inflammatory (corticosteroids) and anti-angiogenic agents (VEGF inhibitors) [122,123]. In some patients, intraocular pharmacotherapy requires only a few injections to make the treatment effective, but in patients with chronic musculoskeletal disorders, more intensive therapy is required. Intravitreal and anti-VEGF corticosteroids were shown to be effective in proliferative DR and in diabetic macular edema, but their use is still limited due to the short-term effect and adverse side effects [124]. Panretinal photocoagulation prevents vision loss in DR, but pharmacotherapy is still needed as an adjuvant treatment. To improve existing forms of therapy, anti-VEGF (aflibercept) and corticosteroids (dexamethasone and fluocinolone insertions) have been approved [125,126]. The available therapy consists of oral danazol and topical minocycline and loteprednol, which require daily administration, but these drugs have the advantage of a long-term effect. Intravitreal therapy (monotherapy or combined) is currently under development; however, its duration and efficacy have not been fully defined [123]. Thus, these new alternatives, such as long-term drugs, sustained corticosteroid and

anti-VEGF delivery systems, and also nanoencapsulation approaches, reduce the treatment burden, but may not obtain regulatory authorization for at least 5 years [59].

Inflammation is instrumental in the pathogenesis of DR and is intimately linked to angiogenesis.

4. Uveal Melanoma, Angiogenesis and Anti-Angiogenic Therapy

Malignant melanoma is a type of tumour that develops from melanocytes and is amongst those with the highest death rate [127,128]. The production and storage of high levels of melanin makes this tumour resistant to various therapies [129,130]. Choroidal melanoma is treated either with radiotherapy (which can lead to vision loss) or by the removal of the eye by surgical resection. Therefore, the treatment approach of choroidal melanoma is quite debatable [131].

VEGFs influence invasion, migration, proliferation and tumour cell metastasis. The various growth factors and cytokines have an active effect on tissues and organs, promoting tumour development [132–134]. Several studies have shown high levels of VEGFs in patients with uveal melanoma, especially those with metastases [135]. Angiogenesis influences the development of metastases, being related to hypoxia and genetic mutations [136]. The relationship between microcirculatory problems and uveal melanomas, as well as the correlation with other eye diseases, has been described in the literature since the 1990s [137]. Notting et al. have shown that VEGFs are expressed in uveal melanomas, promoting the formation and growth of new blood vessels, regulating vasopermeability and endothelial cell proliferation and migration [138]. In this study, the isoform VEGF-A is most expressed in patients with uveal melanoma, followed by VEGF-B, VEGF-C and VEGF-D isoforms. The expression of these VEGFs has a crucial role in angiogenesis, interfering with the progression and metastasis of uveal melanoma. High intraocular VEGF levels have also been shown to be associated with other diseases, such as DR, branch retinal vein occlusion, central retinal vein occlusion and uveitis [92,139–141]. Among the different isoforms, VEGF-A isoform is the one mostly occurring in aqueous and vitreous humour of uveal melanoma patients and is also overexpressed in retinopathies caused by angiogenic proliferation [142,143]. A study performed in enucleated eyes of uveal melanoma patients detected an overexpression of VEGF-A associated with the largest and more metastatic tumours [144]. The sharing of molecular pathways between ocular neovascular diseases and tumor angiogenesis has indeed been highlighted [145]. As happens with other ocular diseases, VEGF-A is the major factor stimulating angiogenesis, being associated with the activation-migration-proliferation of endothelial cells, leading to the breakdown of the extracellular matrix [146]. The association of VEGF-A and pericytes is rarely described in the literature, however that surely exists. Indeed, pericytes have an important role in angiogenesis. The function of pericytes in angiogenesis is associated to maturation of newly formed vessels [147]. Pericytes have also been observed to follow endothelial cells in newly formed capillaries [148]. Pericytes contribute to the initiation and development of angiogenesis, and consequently, they are somehow involved in uveal melanoma, while the targeting of these cells for potential therapy is being studied [149]. Ozerdem reports the possibility of neovascular pericytes in uveal melanoma deriving from the host tissue and not from the tumour, given the presence of NG-2 negative and PDGF-β-receptor positive in NG2 knockout mice [149]. However, other important molecules, such as VEGF-A and platelet-derived growth factor-BB (PDGF), are instrumental in the early stages of angiogenesis, namely in the cellular activation process, and somehow, its receptor, PDGF-β, is present in pericytes. The role of PDGF-β is mainly associated with the proliferation and migration of pericytes along the capillaries [150]. However, in the maturation stage of angiogenesis, the permeability of pericytes is decreased, inducing new blood vessels activated by PDGFR-β signalling [151]. Thus, the endothelial factor VEGF-A is the main element governing angiogenesis in ocular diseases, promoting a higher permeability and neovascularization, but it is not the only one. Recent studies reported that, when VEGF-A is down-expressed, VEGF-C is involved in the initiation and potentiation of neovascularization [152]. However, both VEGF-A and VEGF-C are overexpressed in certain physiological situations, such a hypoxia, oxidative stress, inflammation and hyperglycaemia. Paradoxically, other studies suggest that VEGF-C also incites lymphangiogenesis [153,154]. In vitro

studies have shown that tumour-associated macrophages are involved in all stages of melanomagenesis [155], in addition to the increased levels of lymphocytes and macrophages which are correlated with increased mortality. In order to stimulate angiogenesis, VEGF-C is activated by VEGFR-2 and VEGFR-3, specifically in retinal epithelial cells. Zhao et al. showed that VEGF-A supplementation stabilizes VEGF-C expression levels [156]. In DR, VEGF-C is also overexpressed by vascular endothelial cells and pericytes. Under constant hyperglycaemia levels in retinal cells, VEGF-C has been shown to promote survival of retinal vascular endothelial cells, especially via VEGFR-2 [157]. VEGF-C has been reported to control the display of VEGF-A, but the mechanisms of expression are not fully described yet [154]. VEGF is known to bind to cell surface tyrosine kinase receptors (VEGFR-1 and VEGFR-2) [158]. Some studies documented the expression of VEGFR-1 by pericytes in iris capillaries[146]. Furthermore, the secretion of VEGF and placental growth factor (PlGF) is believed to be mediated by VEGFR-1, which affects the perivascular cells directly, contributing to pericyte reduction and also to an increase of vascular leakage. Additionally, VEGFR-1 ligands have been investigated as potential therapeutic targets for cancer therapy due to their importance in the mediation of angiogenesis [158]. As mentioned previously, VEGF levels are elevated in uveal melanoma patients with metastases, in comparison to those without metastatic disease [159,160]. Tumour vasculature is crucial in uveal melanoma, because metastasis only happens through the hematogenous pathway [161]. The first VEGF-directed angiogenesis inhibitor (bevacizumab) was developed for intravenous administration to be used for the treatment of several malignancies and is being studied for other primary tumours, together with other antiangiogenic drugs [162]. These antiangiogenic agents are already widely used to treat the secondary effects of radiotherapy in uveal melanoma patients [135].

Reactive oxygen species (ROS) are products of normal cellular metabolism; however, oxidative stress occurs when the capacity to regulate their overproduction is limited. Oxidative stress damages cellular lipids, proteins and DNA. The down-regulation of ROS is instrumental, especially in tumour development, since this process maintains homeostasis and thus controls oxidative stress [163]. The role of ROS in some biological processes includes migration, differentiation, proliferation, apoptosis, stress adaptation and gene expression [164]. In this context, growth factors also play a role, as activation of their receptors (e.g., PDGF receptor) causes ROS to modulate transduction signals. In melanoma tumour cells, a set of factors strongly influencing the over-production of ROS have been described. These factors are the deregulation of antioxidant enzymes, mitochondrial dysfunction, alteration of the expression of transcription factors, disrupted signalling pathways, aberrant metabolism, alteration in proliferation and acquisition of the metastatic phenotype [165–167]. The relationship between overproduction of ROS before and during tumour development has been reported by several authors over the last two decades [168–170]. In addition, the increased ROS levels, specifically in melanoma cells, have been documented by several authors [165,171]. Melanocytes have the ability to suppress increased ROS levels, however melanoma cells do not have the same capacity [172]. Additionally, uveal melanocytes seem to a have a higher level of enzymatic antioxidant activity than normal melanocytes, the superoxide dismutase (SOD) activity being more evident. This can be explained by the high oxygen tension found in the choroid [173]. Moreover, other antioxidant enzymes have a reduced activity in melanoma cells, such as catalase, glutathione-S-transferase (GST) and manganese superoxide dismutase (MnSOD), associated with low levels of glutathione (GSH) [174–176]. An important achievement in melanoma cells is related to the enhancement of superoxide anion levels and also the decrease of hydrogen peroxide levels. The pro-oxidant intracellular environment and the activation of redox-sensitive transcription factors, which increase the high proliferative rate and drug resistance in these cells, are thought to justify these levels [177]. Another reason for the increased ROS levels is related to the ultraviolet (UV) light exposure, which is also associated with uveal melanoma development. Since melanoma results from abnormalities in melanocytes, the most harmful factor affecting these cells is UV exposure, for skin and also for ocular melanomas [178]. In uveal melanoma, UVB radiation is related to the high risk of tumour development [179]. Figure 2 illustrates the mechanism of oxidative stress and its effect in angiogenesis.

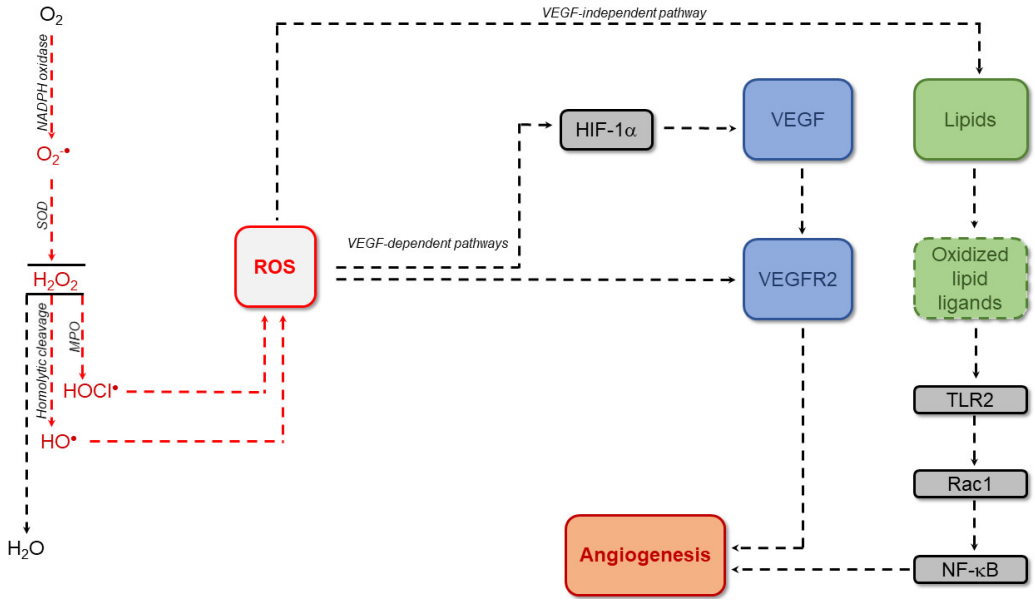

Figure 2. Oxidative stress and its effect in angiogenesis: release of reactive oxygen species and generation of endogenous antioxidant pathway in mitochondria. SOD, superoxide dismutase; MPO, myeloperoxidase; ROS, reactive oxygen species; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor/receptor; TLR, toll-like receptor; Rac1, cytosolic oxidase component; NF-κB, factor nuclear kappa B.

Several researchers have investigated the role of blood vessels in uveal melanoma growth and metastasis [40]. For metastases to occur it is necessary for uveal melanoma cells to spread beyond the primary site and to enter the surrounding tissues and then the nearby blood vessels, where they circulate to other parts of the body. Several studies show a strong association between tumour cell growth in blood vessels and extraocular extension, indicating a high risk of death [180,181]. Malignant cells circulate in the blood and reach an organ to form a metastatic tumour (in uveal melanoma it is usually the liver) [182,183].

Certainly, there is a microenvironment that allows the uveal melanoma cells to spread faster. The poor prognosis can be explained by the expression of insulin growth factor 1 receptor (IGF-1R) in this melanoma. IGF-1 leads to phosphorylation of IGF-1R, activating signal molecules involved in cell proliferation. This growth factor is mainly produced in the liver, which explains liver metastases in uveal melanoma [184,185]. It was been proven that IGF-1 promotes VEGF release in RPE cells and is also involved in tumour proliferation in liver metastases [186]. Uveal melanoma is defined by slow progression and dormant periods, which are related to the avascular phase. However, the conversion to the angiogenic phenotype has not been determined yet, and this "angiogenic change" is a consequence of the change in the balance of inhibitory/stimulating factors [187]. It is known that the VEGF gene and protein expression is observed in retinal epithelium and pigment and is controlled in retinopathies associated with angiogenic proliferation [188]. Local treatment of this melanoma allows the eye to be saved in most patients, but some of them develop other complications (neovascularization of the iris), and therefore, eye removal is required. Boyd et al. proved that melanoma patients had higher VEGF-A concentrations compared to patients with routine cataract extraction [142]. No relationship was found between aqueous VEGF levels and retinal detachment, tumour size, vascularization or immunohistochemistry. High levels of VEGF are explained by anterior radiotherapy and also by neovascularization of the iris or optic nerve head. These results show that anti-VEGF therapy can be used to treat some patients with iris neovascularization secondary to uveal melanoma [142]. Tumour microvascularity, as referred to previously, is crucial for prior knowledge of uveal melanoma diagnosis and therapy. In this tumour, angiogenesis is driven by soluble proangiogenic cytokines, especially basic fibroblast growth factor (bFGF) and VEGF-A.

VEGF is known to be present in uveal melanomas and bFGF is the autocrine growth factor in cutaneous melanoma, and therefore, through immunohistochemistry and reverse transcriptase polymerase chain reaction, Boyd et al. studied the expression of these cytokines in this type of melanoma. In the immunohistochemistry, an elevated number of tumour cells expressed bFGF at protein level, whereas way less expressed VEGF. The authors concluded that, in this tumour, the development of blood vessels resulted from the relationship between endothelial and tumour cells, with bFGF and VEGF playing a crucial role in this function [189]. The first investigation of VEGF gene expression in uveal melanoma was done in 7 different cell lines using RT-PCR [190]. Sheidow et al. have shown that enucleated eye sampling has VEGF immunostaining; the relationship between the onset of metastases and VEGF levels in uveal melanoma could not be fully disclosed [191]. Boyd et al. previously showed only moderate VEGF staining in similar samples. However, all tested uveal melanomas expressed VEGF mRNA [189]. In another study, and always using healthy eye samples as a control, high levels of VEGF were also found in vitreous and anterior chamber fluids of affected eyes [192]. In several studies, including the study by Missotten et al., higher VEGF concentrations have also been reported in the aqueous humour of the affected eyes, possibly justifying the larger basal diameter and the height of the uveal melanoma. They also found that VEGF is expressed in tumour and retinal cells [193]. Filali et al. studied the role in VEGF in uveal melanoma in vitro, demonstrating that in tumour cell cultures, hypoxia drives the expression of hypoxia-inducible factor (HIF-1α) and VEGF. On the other hand, under these conditions the antiangiogenic factor thrombospondin TSP-1 was downregulated. To respond to this situation, uvea melanoma cell lines positively regulate VEGF but do not increase cell proliferation [40,160]. By modulating VEGF expression, it is possible to initiate tumour vascularization. It is well known that this growth factor is crucial for tumour angiogenesis; thus, in vivo studies should be done on identical models of the tumour environment, considering paracrine signalling of endothelial cells. Several studies have shown that VEGF levels are indicative of the disease phase and also possible metastasis [40]. On the other hand, some studies have reported the relationship between VEGF expression and the onset of experimental metastases [194,195]. Patients with metastatic uveal melanoma have higher VEGF levels compared to patients without metastatic uveal melanoma [40,196]. In addition, in vivo studies have shown increased VEGF levels in the presence of liver metastases at liver hypoxia sites [197]. Barak et al. also showed that there is an increase in VEGF levels after the metastatic development [159]. A preclinical study conducted by Lattanzio et al. evaluated in vitro and in vivo the relationship of radiotherapy and monoclonal anti-VEGF antibody (bevacizumab) [198]. Through the viability of OCM-1 cells, an extra antitumour action of the combined treatment (bevacizumab + radiotherapy) occurs in vivo. The same effect was observed in mice inoculated with OCM-1 cells. In separately cultured cells, bevacizumab prevented a radiation-induced increase in VEGF, which may alter the radiosensitivity of tumour cells [199]. Thus, the radiosensitizing action of bevacizumab is thought to be related to the cytokine-uveal melanoma-endothelial cell interaction. Interleukin 8 (IL8 or CXCL8) is a pro-inflammatory CXC chemokine expressed in positively regulated tumours together with VEGF, and both induce the formation of new blood vessels. IL8 expression is regulated by inflammatory signs and chemical and environmental stress, among other factors [200]. The biological effects are regulated by binding of IL8 to cell surface protein G-coupled receptors (CXCR1 and CXCR2). While CXCR1 is activated by IL8, CXCR2 can bind to different CXC chemokines, for example melanoma growth stimulating activity (MGSA), macrophage inflammatory protein 1 (MIP1) and neutrophil activating protein 2 (NAP2) [200,201]. IL8 is highly expressed in many types of cancer, as it plays a crucial role in tumour development [202,203]. IL8 further induces the expression of VEGFR2 and VEGF-A. Thus, there is a synergistic relationship between IL8 and VEGF in the formation and development of new blood vessels. However, some studies report that IL8 produced by tumour cells is related to VEGF-directed therapeutic resistance. Thus, it is thought that the IL8 signalling pathway can act independently of VEGF, compensating for it [204]. It is known that in uveal melanoma patients, the IL8 levels in eye fluids are quite high, highlighting the possibility that this signalling pathway may be an alternative mechanism of resistance to radiation therapy in this tumour [205,206]. Thus, the effect of radiotherapy, bevacizumab and their relationship with cytokine

expression and the CXCR2 receptor was studied. The interaction between inflammation and angiogenesis, i.e., interaction between inflammatory and endothelial cells, is crucial for the activation of the pathways that lead to tumour development [207]. The interaction between malignant and inflammatory cells has been shown to enable endothelial cell growth, leading to neoangiogenesis, tumour development and therapeutic resistance. Radiotherapy associated with anti-VEGF therapy activates the IL8 pathway through endothelial cells and may be an alternative approach against tumour angiogenesis. These experimental results show that endothelial cells can activate different pathways, leading to therapeutic resistance. Thus, the IL8 pathway can be used together with anti-VEGF therapy, preventing the tumour from activating the cell escape mechanism, avoiding therapeutic resistance and dominating tumour neoangiogenesis [206]. Figure 3 illustrates the signalling pathways and interactions between cells in uveal melanoma proliferation.

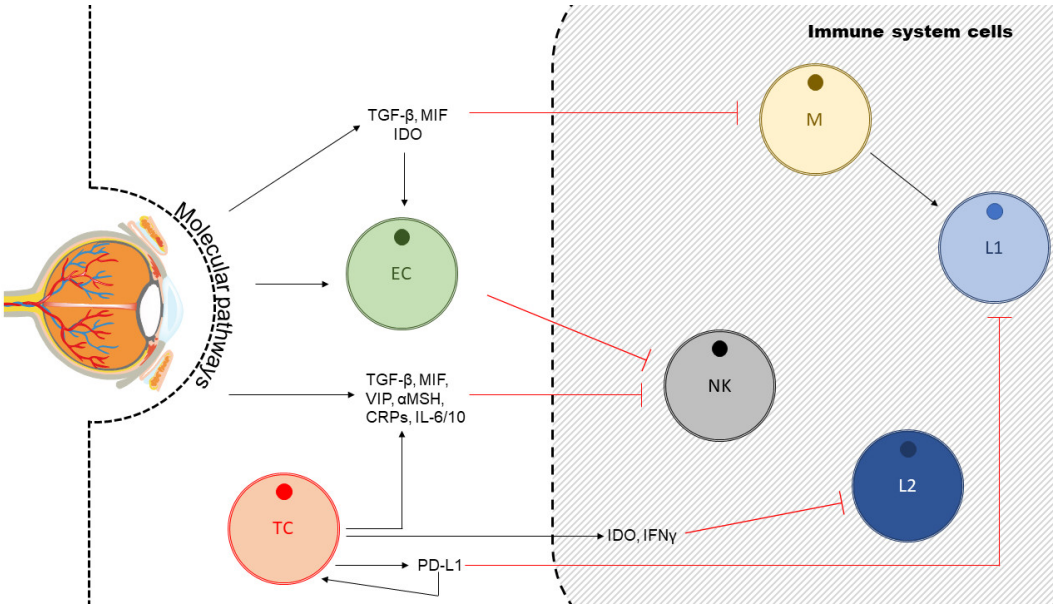

Figure 3. Signalling pathways and interactions between cells in uveal melanoma proliferation. EC, endothelial cells; M, macrophages; L1 T-cells; L2 tumour infiltrating lymphocytes; NK natural killers; red lines inhibition pathways; black lines stimulation pathways.

In oncology, the MAPK signal cascade (RAS, RAF, MEK and ERK enzymes) is a fundamental pathway. The role of RAS and BRAF in cell development and survival and also induction of oncogenesis is dependent on MEK protein kinase function [208,209]. Studies have shown that BRAF inhibitors vemurafenib and dabrafenib (the first MAPK pathway inhibitors) have relevant clinical activity in melanomas with activating mutations at position 600 [210–212]. Following this discovery, more allosteric MEK inhibitors have been clinically developed. Trametinib has a distinct pharmacodynamic from all MEK allosteric inhibitors and has a more sustained inhibition [213,214]. The clinical efficacy of trametinib as a MEK inhibitor has been reported by several studies. Falchook et al. showed a positive response in melanoma patients with BRAFV600E or BRAFV600K mutations [215]. Flaherty et al. showed greater survival in BRAF mutant melanoma patients in comparison to chemotherapy [216]. However, when comparing trametinib with the BRAF inhibitors vemurafenib and dabrafenib, the latter show a higher response rate in melanoma patients and a progression-free survival [211,216,217]. The effectiveness of MEK inhibition is clinically proven, and therefore, new combined forms of treatment are under research and development. It may be useful to study the combination of inhibition of MEK with other agents targeting different signalling cascades (e.g., via phosphatidylinositol 3 kinase). Co-inhibition of BRAF and MEK may also be useful, as BRAF

inhibitors promote paradoxical activation in normal tissues, thereby reducing the toxic effects of MEK inhibition. Thus, it will be more effective to inhibit the MAPK pathway in tumours, achieving a better clinical response [214]. Selumetinib (MEK1 and MEK2 inhibitor) was also studied and compared to chemotherapy and showed better progression-free survival and tumour response in patients with advanced uveal melanoma. It is known that in almost all cases of metastatic uveal melanoma (about 95%), patients have mutations in G proteins, which are responsible for activating most signalling pathways (e.g., MAPK, AKT and PKC). In vitro cellular studies revealed that selumetinib blocked the MEK pathway and inhibited uveal melanoma cell proliferation. As Patrick Ott commented, targeted therapy is a promising option in uveal melanoma. So far, no systemic treatment has been successful in the treatment of this disease. Several studies are currently under development. SUMIT (NCT01974752) is a phase 3 study of selumetinib in combination with dacarbazine versus chemotherapy, where previous preclinical data reveal that the effectiveness of MEK inhibition is enhanced with inhibition of AKT or PKC. A study with trametinib or in combination with GSK2141795, as well as a study of MEK162 and AEB071, is also under development. In this way, a MEK inhibitor may alter the response of a tumour and may be a promising therapeutic approach in uveal melanoma [218].

To date, anti-angiogenic drugs for the clinical treatment of uveal melanoma and its metastases are not available on the market yet. Currently, the primary tumour therapy is based on enucleation, local resection and radiotherapy (e.g., iodine or ruthenium brachytherapy, stereotactic and proton beam irradiation) [40]. Radiotherapy is considered the most effective treatment because it can almost completely dominate the tumour (about 97%) in all treated eyes [219,220]. Therefore, other anti-angiogenic drugs are in demand. It is not always possible to radiate all tumours. Irradiation is known to involve a tumour with a height greater than 10.0 mm and a diameter greater than 16.0 mm. In some cases, ultrasonography does not allow the tumour to be clearly defined as it may be diffuse or multifocal, and even when neovascularity or secondary glaucoma occurs, or in the case of extracleral extension, it is not possible to use irradiation as a treatment [221,222]. In addition, radiotherapy also has contraindications. Radiation retinopathy may occur, which is characterized by retinal ischemia, neovascularization and vessel leakage, resulting in decreased visual acuity [40].

Thus, about half of uveal melanoma patients are known to progress to metastatic disease and there is currently no effective treatment, and life expectancy in these cases is two to six months since chemotherapy and resection do not allow complete treatment but only prolong survival for a few months. Some antiangiogenic agents are being used clinically as a treatment and bring new hope for this type of tumour [40]. Table 1 summarizes some anti-angiogenic agents and their effect on tumour growth and metastases in the ocular melanoma.

Table 1. Summary of anti-angiogenic agents and their effect on tumour growth and metastases in the ocular melanoma.

Drug	Studies/Observations
Bevacizumab	Yang et al. proved that in ocular melanoma bevacizumab allows suppression of primary tumour growth, reduction of liver micrometastases and decreased VEGF levels [162]. Filali et al. observed that in vitro uveal melanoma cell proliferation did not occur, but in vivo, the use of bevacizumab caused greater intraocular tumour growth, especially under hypoxic conditions. With treatment, anterior chamber and tumour bleedings were observed in the eyes, increasing microvascular permeability due to induced VEGF expression. It is thought to be a consequence of an adaptive or evasive tumour response [40].
Sorafenib	Sorafenib inhibits VEGFR. Mangiameli et al. proved that after sorafenib therapy there is an inhibition of tumour growth and metastasis [223]. In patients with metastatic cutaneous melanoma, sorafenib monotherapy has not shown significant antitumour activity [224]. Filali et al. compared the treatment of patients with metastatic melanoma (not including uvea) with carboplatin, paclitaxel and

	sorafenib or placebo, where the results of the phase 3 study showed no relevance to overall survival [40].
Sunitinib	Sunitinib also inhibits VEGFR [225]. A preclinical phase 2 study demonstrated the benefit of sunitinib monotherapy in patients with advanced metastatic melanoma [40].

There are studies based on cutaneous metastatic melanoma, uveal melanoma and some only with patients with developing ocular melanoma-related metastasis to investigate bevacizumab, sorafenib and sunitinib as a single agent or in combination with other regimes.

Another point contributing to uveal melanoma development is the concept of immune privilege. First described in animal eyes, this mechanism of immunoregulation can be modulated by the tumour microenvironment as an escape to the immune system [226]. Tryptophan, one of the essential amino acids obtained from diet, seems to have a particular relation with the evolution of various tumour types, among which is uveal melanoma. Initially, it was stated that in normal conditions, tryptophan could be converted into serotonin and melatonin and, in pathological conditions, to kynurenines, a group of tryptophan metabolites. Unlike melatonin, and some of its derivatives, which has been shown to possess anti-proliferative activity in a uveal melanoma cell line, serotonin and kynurenines did not possess that activity, and thus did not control tumoural growth [227]. It is now known that kynurenine and other tryptophan metabolites possess anti-microbial activity but also show immunosuppressive potential, the point of interest in uveal melanoma progression. These metabolites are produced by the kynurenine pathway, which is deeply involved in the immune privilege.

This pathway starts with tryptophan and is limited by tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase 1/2 (IDO1 or IDO2) [226], enzymes that are expressed in various tumours. The first aspect concerning these enzymes and kynurenine pathway is their activity and consequent tryptophan depletion. This amino acid is required for T cell function, growth and proliferation, and therefore, the lack of tryptophan limits the action of T cells, as for example natural killer T cells, preventing tumour rejection by specific T cells. In the case of IDO, its immunosuppressive and regulatory activity allow the organism to control infections. It is also involved in the survival of transplanted allografts and, in pregnancy, prevents maternal T cells from rejecting the fetus in response to IDO produced by fetal trophoblast cells. Concerning the eye, IDO has been found in various locations (iris, lens, retina, cornea, ciliary body) and intervened in the protection against UV radiation, through the formation of UV filters based on tryptophan and antioxidant defence [228]. However, this enzyme is also strongly linked to the immune privilege encountered in uveal melanoma, while its overexpression is associated with a poorer prognosis. IDO effect in tumour-specific T cells has been reported for various carcinomas (e.g., lung, colon) and also in melanomas, as part of a microenvironment that favours tumoural growth. This enzyme upregulation has been linked to the inflammatory process, and more specifically, an IFN-γ-dependent induction of IDO has been reported. A second aspect is the interference of the overexpression of the kynurenine pathway's enzymes (IDO and TDO) and their products in other pathways and treatment targets. This is the case of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), a major target of inhibitor drugs that is induced by kynurenines, or the aryl hydrocarbon receptor (AhR), and is responsible for dendritic cells and macrophages differentiation, once again inducing immune suppression. IDO also induces the production of inhibitory cytokines by antigen-presenting cells. The same is being investigated for TDO, which has been found in uveal melanoma metastasis, and could be linked to UM proliferation and growth through the AhR pathway. Overall, products of the kynurenine pathway and its enzymes regulate the cells from the immune system, providing a tolerant environment for tumoural growth [226,228–230].

For these reasons, and differently to cutaneous melanoma, which is more susceptible to immune checkpoint inhibitors, it is very difficult to use immunotherapy in uveal melanoma, but it also makes the kynurenine pathway, and more specifically IDO and TDO, ideal targets for each preclinical study,

which has already started (Table 2). The combination with other inhibitors such as CTLA-4 is of major interest [226,228,229].

Table 2. Examples of drugs under study for the treatment of uveal melanoma or uveal melanoma-related conditions that are already approved for the treatment of other oncological pathologies.

Product	Type	Target	Status	Ref.
Bevacizumab sold as Avastin®	Monoclonal antibody Angiogenesis inhibitor	VEGF	Authorized for the treatment of various tumour types in EU and USA	[231–233]
Ipilimumab sold as Yervoy®	Monoclonal antibody Immune system modulator	CTLA-4	Authorized for melanoma and carcinoma treatment in EU and USA	[234–236]
Ranibizumab sold as Lucentis®	Monoclonal antibody fragment Angiogenesis inhibitor	VEGF-A	Authorized for macular edema and diabetic retinopathy treatment in EU and USA	[237–239]
Pembrolizumab sold as Keytruda®	Monoclonal antibody Immune system modulator	PD-1	Authorized for the treatment of melanoma and other tumour types in EU and USA	[41,240,241]
Sunitinib sold as Keytruda®	Multiple receptor tyrosine kinases inhibitor Angiogenesis inhibitor	c-KIT VEGFR PDGFR M-CSFR	Authorized for the treatment of various tumour types in EU and USA	[242–244]
Sorafenib sold as Nexavar®	Synthetic drug Multikinase inhibitor Angiogenesis inhibitor	Protein kinase	Authorized for the treatment of various carcinoma types in EU and USA	[245–248]

EU: European Union; USA: United States of America; VEGF: vascular endothelial growth factor; CTLA-4—cytotoxic T-lymphocyte-associated protein 4; PD-1: programmed cell death protein 1; VEGFR: vascular endothelial growth factor receptor; c-KIT: tyrosine-protein kinase; PDGFR: platelet-derived growth factor; M-CSFR: macrophage colony stimulating factor receptor.

5. Conclusions and Future Perspectives

Diabetic retinopathy is a compilation of vascular lesions that can be seen through ophthalmoscopy. A non-proliferative stage (vascular tortuosity, retinal haemorrhages, microaneurysms as well as lipid exudates) and a proliferative stage (development of new aberrant vessels) are shown. Diabetic macular edema is another aspect of DR, characterized by a collection of fluid in the neural retina, which in turn increases its thickness and conduct to macula cystoid edema. In the various states of DR, the DME can be verified, being the main cause for the vision loss in these patients. During DR, the BRB breaks down, the RPE cells are subjugated to HOS inducing responses in the RPE cells. HOS elicits an osmolarity-dependent response, alters cell specificities, allows collagen and elastin to develop, reduces cell growth by arresting the cell cycle and allows the release of angiogenic factors as well as pro-inflammatory mediators. In RPE cells, the impacts caused by HOS give the transcription factor TonEBP/NFAT5 a crucial role as a host for these effects. Thus, HSO and inflammation cause the lesions that occur in DR, and in order to improve therapy for this disease in the future, knowledge of the implicit molecular mechanisms is therefore required. Currently the

treatment for DR consists of corticosteroids and anti-VEGF agents, in order to prevent the progression of the disease. New medicines to reduce sugar from natural sources have recently been proposed. It is well known that long-acting drug delivery systems and proteins need to be considered for successful pharmacotherapy of DR. This review highlights the crucial function of inflammation in early DR. Several studies on diabetic animal models are available in the literature and also on diabetic patients who exhibit the influence of the diabetic environment on the increase of inflammatory molecule manifestation in the progression of DR. Glial cells are located between the vasculature and retinal neurons and thus have a crucial role in normalizing the retinal environment that causes neuroretina damage in DR. Moreover, these cells also have the ability to initiate inflammatory cascade. Scientists assume that variation in the metabolic behaviour of glial cells and its outcome in retinal neurons precedes microvascular involvement. Thus, tools have been developed to identify early changes to the neuroglial unit. In addition, there has been a development of anti-inflammatory drugs aiming to act on vascular changes and neurodegeneration. However, there is still a need to know more about the molecular mechanisms responsible for the ocular inflammation which occurs in this disease. Uveal melanoma is still a tumour with a high mortality rate and is highly metastatic. Given these percentages and although adequate treatment of the primary tumour is currently available, new ways to treat or prevent this melanoma, as well as its metastases, need to be researched. Tumour angiogenesis is crucial in tumour development and is a very complicated process that involves several pathways and molecular mediators. The therapy to combat this process is based on VEGF and is also applied to this melanoma. VEGF angiogenesis and manifestation are controlled by melanoma cells, which in turn are controlled by the environment of this tumour and may further be controlled by VEGF inhibition. It is well known that the best way not to develop systemic metastases is to sterilize or kill effective tumours while they are at an early stage of development. Plaque brachytherapy is the methodology used to combat intraocular tumours, but other methods to radiate tumours are currently being developed. Advances in technology in this area have allowed the treatment of more tumours, preventing metastasis, enucleation of the eyes or death. The knowledge of diabetic retinopathy and ocular melanoma, as well as their relationship with angiogenesis, expected in this review, may result in the future development of better antiangiogenic therapies, enabling an effective treatment of these diseases.

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Abbreviations

AMD	Age-related macular degeneration
Ang-2	Angiopoietin-2
bFGF	basic fibroblast growth factor
BRB	Blood-retinal barriers
CNV	Choroidal neovascularization
DME	Diabetic macular edema
DR	Diabetic retinopathy
GFAP	Glial fibrillary acidic protein

HMGB1	High mobility group protein B1
HOS	Hyperosmolar stress
IGF-1R	Insulin growth factor- 1 receptor
IL	Interleukins
IL6	Interleukin 6
IL8	Interleukin 8
MCP-1	Monocyte chemoattractant protein-1
MGCs	Müller glial cells
MGSA	Melanoma growth stimulatory activity
MIP1	Macrophage inflammatory protein 1
MMPs	Matrix metalloproteinases
NAP2	Neutrophil activating protein 2
NFAT5	Nuclear factor of activated T-cells 5
NPDR	Non-proliferative DR
PDGF	Platelet-derived growth factor-BB
PDR	Proliferation DR
PIGF	Placental growth factor
RGCs	Retinal ganglion cells
ROS	Reactive oxygen species
RPE	Retinal pigmented epithelial cells
RT	Radiotherapy
SOD	Superoxide dismutase
TLRs	Toll-like receptors
TNFα	Tumour necrosis alpha
TonEBP	Transcription factor tonicity-responsive binding-protein
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
VEGR	Vascular endothelial growth receptor

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