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Teaser Diabetic retinopathy is the leading cause of blindness in young adults. Current treatments target late-stage disease symptoms. This review highlights the need for new therapies that target early stages of the disease.

Recent advances in the management of diabetic retinopathy

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Diabetic retinopathy (DR) is a microvascular complication of diabetes and is the leading cause of vision loss in people with diabetes. The current treatments do not target early stages of disease or impede disease progression. Therefore, the identification of new therapeutic targets, the development of novel therapies targeting early stages of the disease and accurate models that simulate pathological characteristics of this disorder are crucial. This review provides an overview of the pathological mechanisms underlying the development of DR, highlighting the recent advances in current and emerging treatments for DR.

Introduction

Diabetic retinopathy (DR) is one of the most common neurovascular complications of diabetes and it is currently the leading cause of vision loss in working-age adults in the developed world. Approximately 30% of people with diabetes develop some degree of DR. The social and financial impact of visual impairment is significant to those affected and, more broadly, their communities [1,2]. Diabetes affects >30 cell types in the retina, therefore the classification and grading of DR have been based on the degree of damage to the vasculature. The Early Treatment of Diabetic Retinopathy Study (ETDRS) scale is used to visualise the number of photographically detectable microvascular lesions in the retina. The ETDRS is anatomy-based and hence might not provide a true quantitative measure or reflect important functional deficits [3]. DR can be broadly classified into two stages based on the level of microvascular- and ischemic-related damage: nonproliferative DR (NPDR) and advanced proliferative DR (PDR). NPDR can be further subdivided into mild, moderate and severe. Mild NPDR occurs at an early stage and is characterised by microaneurysms that tend to leak fluid in the retina. Moderate NPDR is the progression of the disease where the blood vessels that nourish the retina swell, distort and lose their ability to transport blood [4–6]. These changes distort the retina and can contribute to diabetic macular oedema (DMO). During severe NPDR, more blood vessels become obstructed, depriving this area of the retina of blood. In

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this stage growth factors are secreted, signalling to the retina to form new blood vessels. PDR is the most advanced stage of DR whereby growth factors secreted by the retina induce the proliferation of new blood vessels along the inside surface of the retina and in the vitreous gel. These new blood vessels are fragile and leak fluid and blood into the retina. Scar tissue is then formed and can cause retinal detachment, which can eventually lead to permanent vision loss. DMO, also termed diabetic maculopathy, occurs when DR affects the macular region of the retina. The leakage from the blood vessels promotes swelling of the macula and this also contributes to vision loss in diabetic patients. DMO can be classified into 'peripheral' or 'central' if the fovea is affected and, depending on the extent of the oedema, DMO can also be divided into 'focal' or 'diffuse' [4,6]. Management of this debilitating disease requires a thorough understanding of the disease pathogenesis and identification of therapeutic agents that can target the early stages of the disease. This review provides an overview of the pathogenesis of DR and a comprehensive review of the current and novel management strategies.

Pathogenesis of diabetic retinopathy

Retinal stress in DR can be induced by many factors. These include inflammation, oxidative stress, epigenetic mechanisms and neurodegeneration (Fig. 1) [7].

Inflammation

The pathogenesis of DR is complex but is mainly triggered by hyperglycaemia (≥150 mg/dl) and subsequent metabolic stress on the retinal cells. Prolonged elevation of blood glucose induces the production of advanced glycation end-products (AGEs). AGEs are principally formed through the Maillard reaction and deposition of AGEs has been shown to have detrimental effects on cells and tissues. The role of AGEs in the pathology of DR can be attributed to the crosslinking effects (with proteins, lipids and nucleic acids) and the binding of AGEs to receptors of AGE (RAGE) that in turn trigger multiple cell-signalling pathways [8]. AGEs and AGE–RAGE interactions are important contributors to pericyte drop-out, endothelial dysfunction and vascular inflammation, leading to the increase in transcription of vascular endothelial growth factor (VEGF), adhesion molecules and proinflammatory cytokines and chemokines such as tumour necrosis factor (TNF)α, interleukin (IL)-6 and IL-1β [9].

In addition, hyperglycaemia is known to be responsible for chronic low-grade inflammation that leads to upregulation of adhesion molecules such as intercellular cell adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, as well as the upregulation of proinflammatory cytokine VEGF, which controls angiogenesis, abnormal vascular permeability and inflammation. Consequently, retinal hypoxia and ischemia will proceed and cause loss of pericytes, endothelial dysfunction and retinal mitochondrial degeneration. Hypoxia-activated macrophages and microglia migrate to the hypoxic areas and stimulate the release of hypoxia-inducible factor (HIF)-1, monocyte chemotactic protein (MCP)-1, TNFα, IL-6 and IL-8, and further increase the levels of VEGF. The inflammatory state of DR can also be aggravated by modifications in the retinal fatty-acid metabolism via the

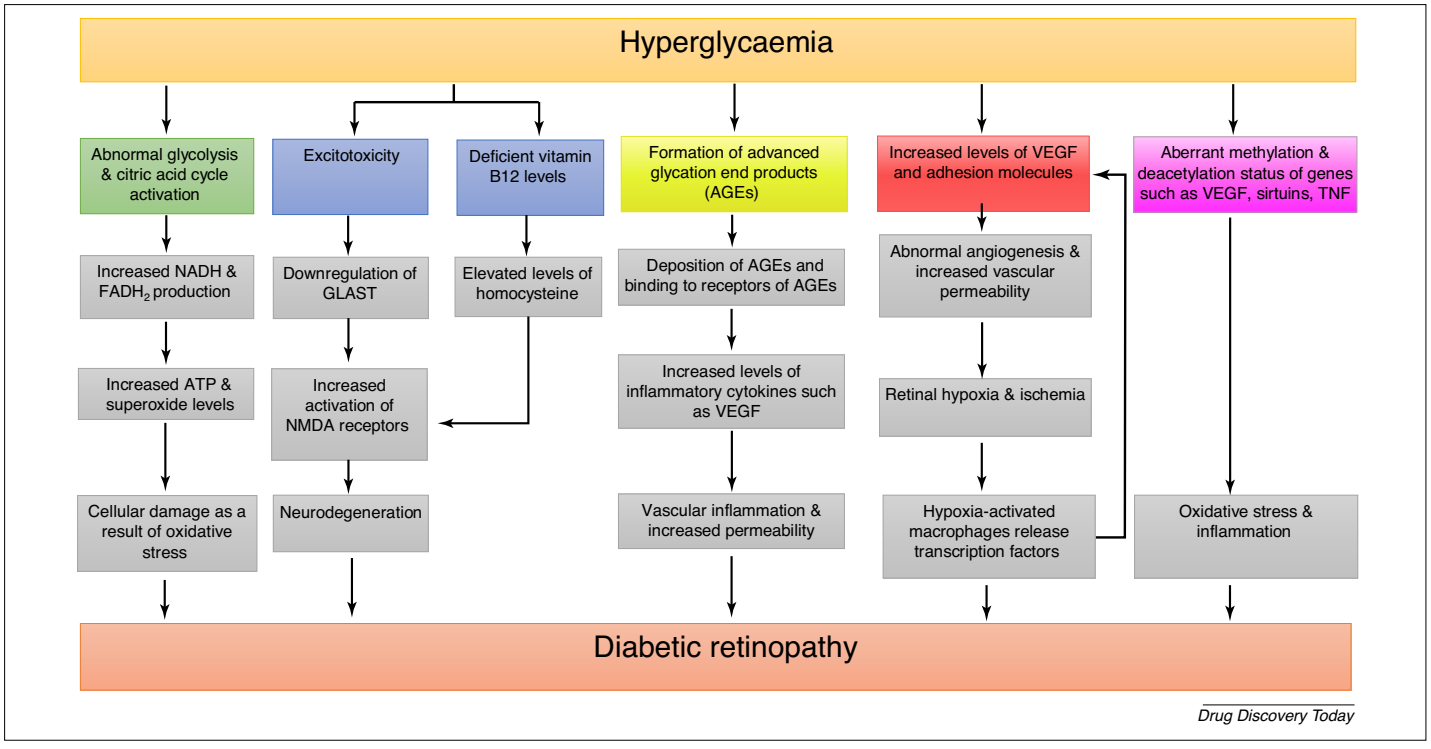

FIGURE 1

Pathophysiological pathways of diabetic retinopathy as a consequence of hyperglycaemia. The main pathological features include: inflammation, oxidative stress, neurodegeneration and epigenetic changes. Abbreviations: AGEs, advance glycation end products; $FADH_2$, redox cofactor flavin adenine dinucleotide; GLAST, glutamate transporter protein; NADH, reduced form of nicotinamide adenine dinucleotide; NMDA, *N*-methyl D-aspartate receptor; TNF, tumour necrosis factor; VEGF, vascular endothelium growth factor.

mitogen-activated protein (MAP) kinase pathway. Oxidised glycated low-density lipoprotein has been demonstrated to cause death of retinal pigment epithelium, Müller cells and pericytes seen in early DR [6,10].

Oxidative stress

Prolonged hyperglycaemia also results in the overproduction of reactive oxygen species (ROS) that lead to the disruption of normal cellular physiology. Increased ROS levels result in cellular damage via lipid peroxidation, DNA modification, protein misfolding and mitochondrial damage [7]. The increased ROS production can be attributed to increased glucose metabolism by the glycolysis and citric acid cycle pathways. Disturbances in these pathways results in increased production of reduced forms of nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FADH). The reduced forms, NADH and FADH2, are vital in the generation of ATP and superoxide radicals in the mitochondria [11]. This increase in superoxide levels has been demonstrated in *in vivo* and *ex vivo* models of DR [12–14]. In addition, the activity of enzyme superoxide dismutase (SOD) which is responsible for the conversion of superoxide into oxygen and hydrogen peroxide has been shown to be downregulated in streptozotocin (STZ)-induced diabetic rats [14]. These models also demonstrated an upregulation of pro-oxidant and proapoptotic enzymes that exacerbate this oxidative stress. Moreover, increased oxidative stress has been linked to neurodegeneration in DR [15,16].

Neurodegeneration

Several studies have demonstrated that dysregulation of excitotoxic metabolites such as glutamate, homocysteine, endogenous peptides and neurotrophic factors is closely linked to the neurodegeneration seen in early DR [17,18]. Glutamate is the most important excitatory neurotransmitter in the brain and retina and numerous studies have shown that disparity in glutamate homeostasis is associated with neuronal damage and initiation of DR. Owing to the activation of the *N*-methyl D-aspartate (NMDA) receptors by glutamate, there is an influx of calcium and sodium within the cells leading to the generation of free radicals and death of retinal ganglion cells (RGCs) [19,20]. Müller cells are specialised glial cells in the inner retina and control the intra- and extracellular uptake of excess glutamate. They aid in lowering postsynaptic excitotoxicity seen in progressing DR. An imbalance between the influx of calcium, sodium and the amount of glutamate produced induces stress on the Müller cells; leading to their death.

Another contributor to retinal neurodegeneration seen in DR is the elevated levels of homocysteine (Hcys). Hcys is a sulfur-containing amino acid that depends on vitamin B12 and folate for efficient degradation. Vitamin B12 deficiency and hyperhomocysteinemia have been linked to early DR [21]. Hcys acts as an agonist at the glutamate site of the NMDA receptors and induces RGC apoptosis, reduces rod responses and can affect glial cell viability [22,23]. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and pigment-epithelium-derived growth factor (PEDF) play important parts in maintaining healthy interactions between neuronal, vascular and retinal cells, preventing ischemia and neuronal death. BDNF is secreted by glial cells and, in DR, absence of BDNF is associated with RGC loss. Other studies show neuroprotective effects of BDNF via the upregulation of glutamine synthetase in Müller cells [24,25]. In addition, overexpression of PEDF in Müller cells counteracts the effects of VEGF, reduces neovascularisation and aids in the survival of RGCs, axons and neurons [26,27]. Thus, strategies that can increase the levels of neurotrophic factors could assist in managing the early stages of DR.

Epigenetics

In the past decade, epigenetic processes have been reported to mediate various pathological events that occur in DR [28]. Global DNA hypermethylation patterns have been associated with increasing severity of DR and low levels of intermediates for DNA methylation (e.g., folic acid and vitamin B12) increase the risk of this ocular disease [29]. The methylation of histones can also play a part in the impaired redox pathways seen in DR.

Glutathione (GSH) is an important intracellular antioxidant and its production is mediated by the activity of the glutamate-cysteine ligase catalytic subunit (Gclc) and the transcription factor nuclear factor erythroid 2 like 2 (Nrf2). Changes in histone methylation can alter the Nrf2-Gclc-GSH cascade [30]. Dimethylation of histone H3 at lysine 4 (H3K4me2) at the Gclc-antioxidant response element region (ARE)4 is increased in retinal endothelial cells in a diabetic rat model, whereas H3K4me1 and H3K4me3 are decreased. RNA interference of histone demethylase LSD1 reverses the decreased binding of Nrf2 at Gclc–ARE4 and Gclc transcripts, thus restoring the normal activity of these key antioxidant enzymes [30]. In addition, retinal cells from hyperglycaemic diabetic rats that have been transfected with short-interfering RNA (siRNA) of the methyltransferase enzyme SetD7 promote an upregulation of Nrf2 and downregulate its cytoplasmic repressor Keap1 [30]. However, reversal of hyperglycaemia does not affect the methylation of *Nrf2* and *Keap1* genes. This indicates that epigenetic modifications might not reverse the expression of genes once the DR phenotype begins to manifest.

Hypoxic conditions in the retina account for the increased expression of VEGF which promotes neovascularisation in DR. Pisani *et al.* (2018) revealed that the astrocyte marker glial water channel aquaporin (AQP)4 can regulate the methylation status of the HIF-1 binding site [31]. In the absence of AQP4, demethylation of the HIF-1 binding site becomes impaired, HIF-1 binding to the VEGF gene promoter is prevented and VEGF-induced retinal damage as a result of hypoxia is attenuated [31]. Hypomethylation of the IL-17 receptor C gene promoter resulting in the overexpression of its gene in choroidal blood vessels and retinal pigmented epithelial (RPE) cells was also found under hypoxic conditions [32]. Thus, this gene can be a biomarker of choroidal neovascularisation and RPE cell degeneration.

In 2015, a genome-wide analysis of DNA methylation in type 1 diabetes patients revealed an association between epigenetic changes with proliferative DR [33]. A total of 349 differentially methylated phosphorylated cytosine-guanine (CpG) sequences within genes including TNF, chitinase-3-like protein 1, glycine receptor subunit alpha-1, B cell lymphoma (BCL)-6 corepressor-like protein 1 and glutathione peroxidase 1 were detected in patients with DR compared with normal healthy controls. These genes encode proteins that are involved in cell signalling, metabolism and redox reactions and most of these CpG sites experienced

DNA hypomethylation [33]. Another interesting observation was the significant positive correlation between natural-killer-cell-mediated cytotoxicity and these CpG sites; thus suggesting the influence of DNA methylation status on innate immunity [33].

Sirtuin (Sirt)1 is a deacetylase enzyme that mediates multiple cellular functions including gene transcription and it is inhibited in the diabetic retina. Mishra *et al.* (2018) investigated Sirt1-overexpressing diabetic mice and these animals showed an alleviation of hypermethylated Sirt1 promoter DNA but no changes in the retinal vasculature or degree of apoptosis were observed compared with wild-type diabetic mice [34]. By contrast, Sirt1 expression mediated by protein arginine methyltransferase 1 conferred protection to human RPE cells against oxidative damage and apoptosis [35]. A normal Sirt1 phenotype is also required to impede increases in p300, endothelin-1 and transforming growth factor (TGF)-β1 expression for the prevention of hyperglycaemic stress in the kidneys and retina of Sirt1-overexpressing mice [36]. These conflicting findings suggest that there could be differences in the responses to epigenetic stimulations between *in vivo* and *in vitro* DR models. Another Sirt family member, Sirt6, has also been implicated in the regulation of cellular aging, metabolism and degeneration. In the presence of high concentrations of glucose, retinal cells from normal wild-type rats demonstrated an increase in VEGF gene expression that was accompanied by reduced Sirt6 activity and increased H3K9 and H3K56 acetylation [37]. When Sirt6 was silenced, levels of VEGF gene expression increased [37]. The overexpression of the Sirt3 gene was also found to attenuate diabetic injury via deacetylation and activation of manganese SOD, the scavenging enzyme crucial for eliminating the build-up of ROS in the mitochondria of retina cells [38]. Additionally, concurrent elevation of the levels of matrix metalloproteinase (MMP)-9, VEGF, HIF-1α and insulin growth factor-1 were also observed [39]. Taken together, these findings indicate that normal expression of Sirt family members is required for the maintenance of healthy retinal vascular and neuronal homeostasis.

Current treatments

Current therapeutic options target the late stages of DR when vision is already significantly degraded. Current treatment for NPDR is limited and patients are advised to tightly control their blood glucose levels to reduce the risk of PDR. In severe cases patients will receive pan-retinal photocoagulation (PRP) therapy to reduce the risk of disease progression to PDR. PRP is also offered to patients who have PDR without vitreous haemorrhage to reduce the risk of severe vision loss. The only treatment option available for patients with extensive vitreous haemorrhage or tractional retinal detachment owing to severe PDR is pars plana vitrectomy surgery [40]. The majority of currently approved pharmacological treatments for DR target the resulting DMO. These established treatments aim to prevent haemorrhaging using laser therapy, intraocular injections of corticosteroids and therapeutic anti-VEGF antibodies [40,41]. Table 1 summarises the currently available treatment options for DMO.

Promising therapies in the pipeline

Currently available anti-VEGF therapy requires frequent intraocular injections and increases the risk of retinal haemorrhage, increased intraocular pressure and retinal detachment. In addition, this invasive procedure affects the patient adherence to the medication regimen and is a considerable burden to the healthcare system. Therefore, various companies are currently developing drug delivery systems and therapies that reduce the need for frequent invasive procedures. One such long-acting delivery system is the ranibizumab port delivery system (PDS) developed by Forsight Vision 4 (Menlo Park, USA) and acquired by Roche/Genentech (San Francisco, USA). This reservoir-based implant is placed under the conjunctiva in the pars plana and does not require sutures. The implant can be refilled when needed by a clinician [42] (LADDER study; NCT02510794). A Phase III clinical trial is currently recruiting participants to compare the efficacy of ranibizumab PDS implant with monthly ranibizumab injections in patients with wet age-related macular degeneration [43].

Abicipar pegol is a new anti-VEGF therapy developed by Molecular Partners (Zurich, Switzerland). It is a designed ankyrin repeat protein (DARPin®), a genetically engineered mimetic protein that has low molecular weight, high stability, high affinity and long intravitreal half-life. Abicipar is reported to suppress VEGF for up to 8–12 weeks in individuals with DMO. A Phase II clinical trial (PALM study; NCT02186119) evaluating the efficacy of abicipar in DMO in comparison to monthly ranibizumab demonstrated improved visual acuity and retinal thickness that is comparable with monthly ranibizumab injections [44,45].

Aerpio therapeutics (Cincinnati, USA) and Roche (Basel, Switzerland) have developed new therapies for DMO targeting the angiopoietin pathway. Angiopoietins are known to interact with Tie2 tyrosine kinase receptors on endothelial cells. Once

TABLE 1

Currently available treatments for diabetic macular oedema.

Drug	Mechanism of action	Formulation and route of administration	Frequency of administration	Refs
Bevacizumab (Avastin®) (off-label use)	Anti-VEGF antibody	Solution; intravitreal route	Monthly or bi-monthly	[102]
Ranibizumab (Lucentis®)	Anti-VEGF antibody	Solution; intravitreal route	Every 4 weeks	[103]
Aflibercept (Eyelea®)	Anti-VEGF antibody	Solution; intravitreal route	Every 4 weeks for the first five injections, then every 8 weeks	[104]
Dexamethasone (Ozurdex®)	Anti-inflammatory corticosteroid	Biodegradable implant; intravitreal route	Every 3–6 months	[105]
Fluocinolone (Iluvien®)	Anti-inflammatory corticosteroid	Nonbiodegradable implant; intravitreal route	Every 3 years	[106]

activated, Tie2 receptors promote vascular stability in healthy endothelial cells. However, angiopoietin-2 (Ang-2), which is upregulated in hyperglycaemia, is a negative regulator of Tie2 receptors and increases the vascular leakage and responsiveness to VEGF and inflammatory cytokines [46]. Currently, there are two molecules that target this pathway in the pipeline. These are AKB-9778 [47] (Aerpio Therapeutics; NCT01702441) and RO6867461/RG7716 (Roche; NCT02699450) [48]. AKB-9778 activates the Tie2 receptor via inhibition of vascular endothelial protein tyrosine phosphatase (VE-PTP). It is administered subcutaneously twice daily and provides the advantage of self-administration by the patient. The TIME-2 study reported subcutaneous injection of AKB-9778 combined with ranibizumab caused significantly greater reduction in DMO in comparison with ranibizumab alone [45,49]. RO6867461/RG7716 (faricimab) is a bispecific IgG1 monoclonal antibody that has one arm binding to VEGF and the other arm binding to Ang-2. The BOULEVARD study (Phase II clinical trial; NCT02699450) compared the efficacy of monthly intravitreal injections of faricimab (1.5 mg and 6 mg) with monthly intravitreal injections of ranibizumab. This study demonstrated statistically significant improvements in visual acuity gains in patients administered with faricimab (6 mg) compared with ranibizumab. In addition, both doses of faricimab resulted in considerable reduction of central retinal thickness and faricimab was well tolerated [50]. The investigators are currently recruiting participants for a Phase III clinical trial; NCT03622580 to determine the efficacy, safety and pharmacokinetics of faricimab in patients with DMO [48].

Allegro Ophthalmics (San Juan Capistrano, USA) is currently studying the efficacy of ALG-1000 (Luminate®). Luminate® is an integrin antagonist that binds to multiple integrin receptor sites to inhibit multiple integrin pathways. Integrins are located on endothelial cells and regulate angiogenesis. *In vivo* studies on Luminate® have shown that it reduces the angiogenesis and leakage from abnormal blood vessels in rodent models. Recently, the DEL MAR Phase II clinical trial (NCT02348918) demonstrated that, when used as a sequential therapy in patients with DMO, the visual acuity gains were equivalent at all time points to bevacizumab monotherapy [45].

Somatostatin (SST) is an endogenous cyclic tetradecapeptide hormone and it is one of the most important neuroprotective factors synthesised by the retina, the retinal pigment epithelium (RPE) being the main source in the human eye [51]. SST can ameliorate glutamate neurotoxicity by regulating the amount of glutamate available to glutamate receptors. SST and analogues can also exert an antiangiogenic effect by reducing endothelial cell proliferation and neovascularisation. This effect can contribute to the inhibition of post-receptor signalling events of peptide growth factors observed for IGF-1, VEGF, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) [52,53]. Studies in transgenic mice have shown that SSTR2 activation can protect against angiogenesis [54]. However, this has not been demonstrated in diabetic models. It is worth noting that nondiabetic models are usually used to mimic retinal neovascularisation and these models may not provide an accurate reflection of the disease pathogenesis [55]. Several clinical studies have investigated the efficacy of SST analogues such as lanreotide and octreotide in DR. In a case report, lanreotide Autogel®, Ipsen Pharma Biotech, Signes, France (Somatuline® Autogel®) was administered to two diabetic patients with bilateral persistent cystoid macular oedema. Authors reported improved vision in three out of four eyes. Foveal thickness was reduced and Health Related Quality of Life (HRQOL) scores increased, although visual acuity was not shown to be significantly improved [56]. However, in 2001, a larger study showed that 3 years of octreotide treatment in type 1 diabetes patients with PDR significantly reduced vitreous haemorrhage and improved visual acuity [57]. Grant *et al.* (2000) demonstrated that octreotide treatment retarded the progression and delayed the time to laser photocoagulation [58]. A more recent multicentre Phase II/III randomised controlled clinical trial has recently been completed in Europe to compare the efficacy of brimonidine and SST eyedrops in retinal neurodegeneration and microvascular impairment (EUROCONDOR; NCT01726075). This trial revealed that SST and brimonidine topical formulations only prevented the worsening of pre-existing retinal neurodysfunction [59]. It is interesting to note that SST (1 mg/ml) was delivered via the topical route and that the low bioavailability of SST in the retina might have contributed to the reported outcomes.

ASP8232 (Astellas Pharma; Northbrook, USA) is an orally administered vascular adhesion protein (VAP)-1 inhibitor that is currently being studied in patients with DMO. A Phase II study has recently been completed comparing the efficacy of ASP8232 with ranibizumab as well as combination therapy with ranibizumab; however,study results are not yet available (NCT02302079) [60]. Other novel therapeutics include KVD001 (Kalvista Pharmaceutica, Cambridge, USA), a plasma kallikrein inhibitor administered intravitreally [45], and EBI-031 (Eleven Biotherapeutics, Cambridge USA), an IL-6 antibody administered intravitreally [45]. KVD001 is currently being evaluated in Phase II clinical trials (NCT03466099) and the outcomes are expected to be revealed in 2019. The current list of therapies in the pipeline demonstrates the advances in understanding of the disease pathology and the need for targeting pathways that are involved in early stages of the disease. These exciting innovations are likely to revolutionise the management of DR in the future and provide patients with a more effective method of managing this disease. Table 2 summarises the therapies in the pipeline for the management of DR.

Experimental therapies

Although intensive diabetes treatment strategies are currently available or are in the pipeline for the management of DR, new treatment approaches focusing on antioxidants, anti-inflammatory agents, hemichannel blockers, inhibitory neuropeptides and epigenetics are being evaluated to ameliorate disease progression and adverse effects associated with current treatments.

Targeting inflammatory pathways

Inflammation (Fig. 1) is a key contributor to the progression of DR. There is evidence that inflammatory factors IL-17A, IL-18, IL-6, IL-1β, TNFα, MCP-1, ICAM-1 and nuclear factor (NF)-κB are involved in DR. In addition, the inflammatory contribution of the NOD-like receptor protein (NLRP)3 inflammasome has been investigated in an Akimba mouse model. This study demonstrated that dysregulated activation of the NLRP3 inflammasome results in an increased activation of macroglia, microglia and perivascular macrophages such as CD14 leading to an upregulation of proinflammatory (IL-1β and IL-6) and proangiogenic (ICAM-1, VEGF)

TABLE 2

Promising therapies for DR in the pipeline.

Name	Mechanism of action	Route of administration	Stage of development	Company	Refs
Ranibizumab port delivery system	Anti-VEGF antibody	Reservoir-based implant placed under conjunctiva	Phase III clinical trial	Roche/Genentech, San Francisco, USA	[42,43]
AKB-9778	Vascular endothelial protein tyrosine phosphatase inhibitor	Subcutaneous	Phase III clinical trial	Aerpio, Cincinnati, USA	[47,49]
Faricimab (RO6867461)	Anti-VEGF & anti-angiopoietin-2 antibody	Intravitreal injection	Phase III clinical trial	Roche Basel, Switzerland	[48]
Somatostatin	Neuroprotective peptide	Topical eyedrops	Phase II/III clinical trials	BCN Peptides	[59]
Abicipar Pegol	Anti-VEGF DARPin®	Intravitreal injection	Phase II clinical trial	Molecular Partners, Zurich, Switzerland	[44]
ASP8232	Vascular adhesion protein-1 inhibitor	Oral	Phase II clinical trial	Astellas Pharma, Northbrook, USA	[60]
KVD001	Plasma kallikrein inhibitor	Intravitreal injection	Phase II clinical trial	Kalvista Pharmaceutica, Cambridge, USA	[45]
Luminate® (ALG-1000)	Integrin antagonist	Intravitreal injection	Phase II clinical trial	Allegro Ophthalmics San Juan Capistrano, USA	[45]
EBI-031	Interleukin-6 antibody	Intravitreal injection	Phase I clinical trial	Eleven Biotherapeutics, Cambridge, USA	[45]

markers [61]. These inflammatory targets are currently being investigated to develop efficient treatment options for DR.

Canakinumab (Ilaris®, Novartis Pharmaceuticals, New Jersey, USA) is a human IgGκ monoclonal antibody that targets IL-1β. It is currently approved for the treatment of periodic fever syndromes and systemic juvenile idiopathic arthritis. A prospective, uncontrolled open-label pilot study of canakinumab (150 mg, SC every 8 weeks for 24 weeks) demonstrated promising effects of IL-1β inhibition on DMO and stabilisation of neovascularisation in PDR. Interestingly, canakinumab was not able to reduce the baseline neovascularisation in patients with PDR [62].

High mobility group box (HMGB)-1 is a proinflammatory cytokine linked to the inflammatory events seen in DR and is associated with RGC loss. HMGB-1 is upregulated in rodent models of diabetes and it is known to upregulate the expression of IL-1β, NOX2, caspase-3 and poly(ADP-ribose) polymerase-1 (PARP-1). Glycyrrhizin, a component of liquorice, can inhibit HMGB-1 expression, promoting RGC survival [63,64]. These data demonstrate the interaction between proinflammatory cytokines and oxidative stress pathways, highlighting the need for therapies that target multiple pathological features of DR.

Connexin 43 (Cx43) is a gap junction protein that is abundantly expressed in vascular endothelium and astrocytes. It plays a major part in the bidirectional movement of ions and metabolites between cells (hemichannels) and is involved in acute injury responses and chronic diseases. Cx43 is known to play an important part in retinal homeostasis and during acute injury, with the uncontrolled opening of hemichannels mediating inflammation, vascular permeability and cell death [65]. It has been reported that upregulation of retinal Cx43 was observed during optic nerve injury which was associated with inflammation and RGC death [66,67]. Another study showed that Cx43 expression was increased during light damage in the choroid linked to reduced neuronal responses and increased inflammation. Cx43 mimetic peptides (MP) contain asmall peptide sequence that blocks Cx43 hemichannel opening with high specificity. Several studies have demonstrated that Cx43 MP including Gap 26/27, Peptide5, L2 and Gap19 block Cx43 hemichannels and interfere with inflammatory pathways to reduce neuronal cell death, ischemia, vascular leak as well as RCG and astrocyte death [68,69]. Intravitreal injection of Peptide5 supressed choroidal- and glial-cell-related inflammatory responses [70]. A study by Chen *et al.* (2015) encapsulated Peptide5 in poly(D,L-lactide-co-glycolide) acid (PLGA) nanoparticles (NPs) for slow release to promote RGC survival [66]. More recently, Huang *et al.* (2018) used hyaluronic acid (HA) to coat NPs that encapsulated the Cx43 MP for more-efficient drug delivery. It is known that HA is a major constituent of the vitreous humour -and can bind to several cell-surface receptors including CD44, which is highly expressed in the human retina. Cx43 MP NPs coated with HA were more efficient compared to free Cx43 MP in preventing thinning of retinal layers and disruption of retinal blood vessels [71].

Another area of great interest is the identification and development of therapeutic agents that target AGE formation. Compounds such as benfotiamine, aminoguanidine, pyridozamine, carnosine and phenyl thiazaolium bromide have been investigated for their anti-AGE activity [9,72]. Identification of soluble receptors for AGE (sRAGE) that can block the association of AGE with RAGE, which in turn can inhibit the proinflammatory signalling pathways, also provide promise in managing DR. Perindopril, an angiotensin-converting enzyme (ACE) inhibitor, and benfotiamine, a synthetic thiamine derivate, can both reduce the circulating levels of AGEs by increasing sRAGE. Moreover, there is intensive research on the use of sRAGE as a biomarker of DR [9,73]. Overall, it is clear that there is a strong need for additional research to elucidate the molecular mechanisms that underpin the chronic inflammation observed in DR and to understand the crosstalk between different pathways. This will enable the identification of effective pharmacological agents to slow or halt the inflammation in early stages of the disease.

Neuroprotective agents

Retinal neurodegeneration is an early event in the development of DR and can be managed by blocking the glutamate signalling

pathway, replacing downregulated neuroprotective factors and/or improving the neurovascular coupling function [74]. Another promising therapeutic strategy is the administration of glucagon-like peptide (GLP)1. Hernandez *et al.* (2016) demonstrated the neuroprotective effects of GLP1 receptor agonist liraglutide, administered systemically (SC 400 μg/kg/day) to db/db mice. In addition, the authors reported similar neuroprotective effects following topical administration of native GLP1 and GLP1 receptor agonists (liraglutide, exenatide and lixisenatide) in diabetic mice [75]. GLP1 levels can also be upregulated by the administration of dipeptidyl peptidase IV (DPP-IV) inhibitors. In a recent study, Hernandez *et al.* (2017) reported the topical administration of DPP-IV inhibitors saxagliptin and sitagliptin to db/db mice, which resulted in a significant increase in GLP1, prevention of glial activation, apoptosis and vascular leakage induced by hyperglycaemia [76]. Interestingly, the authors reported higher rates of retinal apoptosis in db/db mice compared with other animal models of DR. Therefore, these *in vivo* effects could be dependent on the choice of animal model, and viable clinical use is yet to be evaluated.

Cell-based therapies could also provide a promising approach to manage DR at early or late stages of the disease. This technology is based on the ability of mesenchymal stem cells (MSCs) to secrete neuroprotective and neurotrophic factors such as fibroblast growth factor (FGF)-2 and ciliary neurotrophic factor (CNTF). MSCs are also able to absorb ROS and promote regeneration of neuro-retinal tissues. Other cell types used in the management of DR include endothelial progenitor cells (EPCs), which can repair damaged vasculature, and adipose stromal cells (ASCs), which can provide dual function of repairing vasculature and providing neuroprotection. Cell-based therapies have been administered via the intravitreal and periocular routes [77]. Although clinical data on the use of cell-based therapies are lacking, this technology holds promise in offering a novel method of managing DR symptoms at early stages of the disease. Currently, this technology is limited by the hostile environment created by hyperglycaemia that can hinder the migration and adhesion of these cells and the lack of success with autologous therapy. New strategies are needed to normalise the functions of diabetic progenitor cells and to provide renewable sources of stem cells that are capable of appropriate differentiation. Table 2 and 3 summarises the therapies in the clinical stages and preclinical stage for the management of DR, respectively.

Anti-oxidant-based therapies

Vitamins, green tea polyphenols, sulforaphane, α-lipoic acid and various other antioxidants have been tested *in vitro* and *in vivo* for their impact on DR. The results are variable and currently there is no clinically translatable outcome for these antioxidants [78]. More recently, there has been increased interest in the development of activators of the transcription factor Nrf2. Nrf2 is known to upregulate the transcription of antioxidant genes hemeoxygenase (HO)-1 and nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) quinine oxidoreductase (NQO)1 [79,80]. Dh404 is a synthetic small-molecule activator of the Nrf2/Kelch ECH-associating protein (Keap)1 pathway. Deliyanti *et al.* (2018) demonstrated reduced oxidative stress in the retina when diabetic rats were treated with Dh404 by oral gavage (3 mg/kg dissolved in 200 μl sesame oil). *In vitro* studies have also demonstrated that Dh404 is able to upregulate HO-1 and NQO1 as well as reduce the expression of Nox1 and Nox4 [81].

Sulforaphane is another potent Nrf2 activator that is found in cruciferous vegetables such as broccoli and radish. Li *et al.* (2019) investigated the effects of sulforaphane *in vitro* and *in vivo* in rats with STZ-induced diabetes. The authors reported an upregulation of HO-1 and NQO1 levels and a reduction of NLRP3 inflammasome indicating that sulforaphane can attenuate DR pathology by acting on antioxidant and anti-inflammatory pathways [82]. Fenofibrate, a peroxisome proliferator-activator receptor (PPAR)α, is commonly used to treat hypercholesterolemia. Recent large-scale

TABLE 3

An overview of promising therapies in the preclinical stage.

Drug	Mechanism of action	Stage of investigation	Refs
Cx43 mimetic peptides	Block Cx43 hemichannel opening	*In vivo*	[69–71]
Canakinumab	IL-1β inhibitor	Pilot study – uncontrolled open-label study	[62]
Glycyrrhizin	High mobility group box 1 inhibitor	*In vivo*	[63,64]
Pyridozamine	Anti-AGE	*In vivo*	[9,72]
Perindopril	ACE inhibitor with activity to increase soluble receptors for AGE	*In vivo*	[9,73]
Benfotiamin	Increase soluble receptors for AGE	*In vivo*	[9,73]
Liraglutide	Glucagon-like peptide 1 receptor agonist	*In vivo*	[75]
Exenatide	Glucagon-like peptide 1 receptor agonist	*In vivo*	[75]
Saxagliptin	Dipeptidyl peptidase IV inhibitor	*In vivo*	[76]
Sitagliptin	Dipeptidyl peptidase IV inhibitor	*In vivo*	[76]
Dh404	Activate Keap1 pathway/Nrf2 activator	*In vivo*	[81]
Sulforaphane	Activate Keap1 pathway/Nrf2 activator	*In vivo*	[82]
Fenofibrate	Peroxisome proliferator-activator receptor α	*In vivo*	[83–89]
Melatonin	Antioxidant	*In vivo*	[90]
Curcumin	Increase levels of DNA methyltransferase enzyme	*In vitro*	[91]
Flavonoids	Potentiator of SIRT1	*In vivo*	[92,93]
Resveratrol	Potentiator of SIRT1	*In vitro*	[95]
Plantaginis semen	Potentiator of SOD enzyme	*In vivo*	[96]
Trichostatin A	HDAC inhibitor	*In vitro*	[99]
Native glucagon-like peptide 1	HDAC inhibitor	*In vivo*	[100]

studies have shown that fenofibrate is able to stop the progression of DR [83–85]. Fenofibrate has been shown to downregulate the abnormal overexpression of basement membrane components and inflammatory mediators such as NF-κB and VEGF [86,87]. Fenofibrate can also reduce hyperglycaemia-induced oxidative stress in rat retinas and reduce the expression of thioredoxin-interacting protein (TXNIP) which is known to reduce cellular antioxidant capacity [88]. A more recent *in vivo* study has shown neuroprotective effects of fenofibrate that could be linked to improved retinal mitochondrial function [89].

Melatonin is another highly potent antioxidant that has demonstrated efficacy *in vitro* and *in vivo*. Dehdashtian *et al.* (2018) recently published a review article on the use of melatonin in DR focusing on its involvement in autophagy, inflammation and oxidative stress [90]. As per current evidence, antioxidant-based therapies could be used to preserve visual function of patients with diabetes and could have a role as an adjunct therapy in the management of DR.

Epigenetic-based therapies

Because methylation and acetylation status as well as RNA interference of genes involved in cell signalling, hypoxia, oxidative stress, inflammation and neovascularisation have been implicated in the pathogenesis of DR, much effort has been made in investigating the use of novel therapeutic compounds with epigenetic properties. Curcumin, an extract from turmeric, has been reported to restore normal levels of DNA methyltransferase enzymes that mediate DNA methylation resulting in normal production of ROS required for detoxification enzymes to function in the RPE of diabetic mice [91]. This demonstrates the antioxidant properties of curcumin potentially in a DR setting.

Another naturally occurring antioxidant compound known as flavonoid is commonly found in abundant levels in cocoa. Following the treatment of rat Müller cells previously exposed to high glucose or hydrogen peroxide with cocoa, neural cell injury was inhibited as marked by a low or negligible expression of the marker glial fibrillary acidic protein (GFAP). Cocoa treatment also reduced excessive production of active ROS and the activity of the apoptotic marker PARP-1, while increasing the expression of the oxidative-stress-sensitive SIRT1 [92]. Additionally, enriching the cocoa with polyphenols before treatment of the STZ-induced diabetic rats enhanced the SIRT1 activity; thereby protecting the retinal cells from oxidative damage [92]. One potentiator of SIRT1 is resveratrol, a phenol found in the skin of grapes and berries. SIRT1 is known to inhibit the expression of the inflammatory cytokine IL-17 commonly found to be increased in the sera of patients with PDR compared with healthy individuals [93]. Peripheral blood mononuclear cells from patients treated with resveratrol increased SIRT1 activation and decreased IL-17 expression levels [93]. The findings suggest that this phenolic compound has potential protective effects against the inflammatory damage seen in proliferative DR. Resveratrol also has the ability to induce acetylation of p65 and prevent MMP-9 activation; thus, reversing the mitochondrial damage and retinal cell apoptosis [94]. Despite these positive findings, others have reported that resveratrol does not exert vasoprotective effects on retinal vessels of Nestin-Cre or Tie2-Cre mice with oxygen-induced proliferative retinopathy and proposed that endogenous SIRT1 (rather than exogenous stimulation by resveratrol) is vital in mediating protection against retinopathy [95]. A traditional extract from plantaginis semen (PS) is known to be rich in polyphenols and has been used to treat compromised vision in certain Asian communities. When tested in an *in vivo* diabetic rat model, PS increased the activity of redox enzymes including SOD and catalase and reduced the expression of proangiogenesis and proinflammatory genes such as *VEGF*, *IL-1* and *HIF-1* in the retina of the diabetic rats [96]. This finding supports PS as a novel therapeutic option for the prevention and/or progression of DR.

Fenofibrate, a therapeutic compound used as a first-line treatment for lowering cholesterol levels in patients with cardiovascular disease, exhibits anti-inflammatory and epigenetic properties. The administration of high glucose to human retinal endothelial cells attenuated the expression and activity of SIRT1 [97]. However, this effect was reversed by fenofibrate which led to a subsequent decrease in the expression of the proinflammatory protein complex NF-κB [97]. Also, its promising antioxidant properties as revealed by clinical trial data have been previously described in this review. Therefore, fenofibrate could be a promising approach to halting the inflammation and oxidative stress responsible for the development of DR.

Trichostatin A (TSA) is a known inhibitor of histone deacetylases (i.e., HDAC). Hakami *et al.* (2016) reported that TSA successfully retarded aberrant angiogenesis, tube formation and migration by dampening the expression of NADPH oxidase (Nox)4 via ubiquitination of p300-histone acetyltransferase and by suppressing TGF1 [98]. TSA can also inhibit glycated albumin-induced changes via HDAC6 inhibition and can restore normal fluid homeostasis in the hyperglycaemic retina [99]. These mechanisms-of-action of TSA confer benefit in using HDACIs to target and ameliorate the abnormal angiogenesis and fluid imbalance in DR. Another potential HDACI is the incretin mimetic glucagon-like peptide (GLP)-1. GLP-1 functions to suppress glucagon release and increase insulin sensitivity of cells in type 2 diabetes. It was reported that GLP-1 treatment of diabetic rats decreased the expression of Nox3 and superoxide dismutase (SOD)2 and caspase 3 accompanied by reduction of HDAC6 in retinal cells of these rats [100]. Although this observation demonstrates that GLP-1 can reverse apoptosis of cells affected by DR, it is contradictory because histone hyperacetylation resulting from HDAC inhibition should open the chromatin structure and activate rather than inhibit gene expression. Perhaps, the regulation of the expression of Nox3, SOD2 and caspase 3 is not entirely mediated by HDAC6 but by other classes of HDACs that together could counteract the activity of HDAC6. Conversely, a specific GLP-1 agonist, exendin-4, induced histone H3 hyperacetylation at the SOD3 promoter in human retinal microvascular endothelial cells. This elevates the expression of the SOD gene and subsequently inhibits the oxidative stress implicated in DR [101]. Taken together, GLP-1 agonists can have differential effects in an *in vitro* versus an *in vivo* DR setting where these compounds decrease Nox3 and SOD gene expression in diabetic rat models but increase the expression of such genes in human retinal microvascular endothelial cells.

Concluding remarks

Over the past decade, our understanding of the pathogenesis of DR has broadened and there is an increased interest in targeting the

early stages of the disease. Currently, anti-VEGF therapies are used as the first-line therapy in managing late-stage disease. Frequent injections and poor response to anti-VEGF therapy by patients has increased the demand for new treatment options. These new treatment options include cytokine and chemokine inhibitors, inhibitors of adhesion molecules and inhibitors of the kallikrein–kinin system. In addition to the identification of new therapeutic targets and the development of new therapeutic molecules, it is important to develop new ways of translating these findings into clinical practice. This includes the development of new drug delivery platforms that enhance the bioavailability of the therapeutic agent, reduce adverse effects and increase patient adherence. The PDS currently investigated by Genentech could be the answer to frequent intravitreal injections. However, the most appropriate re-filling intervals and the patient acceptance of such a device remain to be explored. If successful in future clinical trials, this device will likely revolutionise drug delivery to the posterior segment of the eye. Other approaches to reduce the risk of disease progression could be to combine anti-VEGF therapy with novel therapeutic agents such as cytokine and adhesion molecule inhibitors. Novel drug delivery systems such as stimuli-responsive drug delivery systems, sustained release implants and stem cell therapy are also on the horizon.

Future studies should also focus on identifying appropriate biomarkers for this debilitating disease. Patient response to anti-VEGF therapy and other novel therapies could be dependent on genetic and epigenetic factors. Therefore, identification of biomarkers and other factors that influence the success of a therapy could assist in reducing or preventing disease progression. Novel treatment development and biomarker identification also require the use of well-aligned animal and *in vitro* models of DR. Currently, a wide range of models is used to study the disease pathogenesis and efficacy of pharmacological agents. The lack of translatability of some of the novel pharmacological agents into the clinic could be related to the choice of *in vivo* model. Therefore, it is important to use a model that includes genetic and environmental factors that influence the disease progression. In conclusion, novel treatments in the clinical trial pipeline and emerging therapies resulting from advances in understanding of the disease pathology will expand the management options available for patients with DR in the near future.

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References

1 Ting, D.S.W. *et al.* (2016) Diabetic retinopathy: global prevalence, major risk factors, screening practices and public health challenges: a review. *Clin. Exp. Ophthalmol.* 44, 260–277
2 Yau, J.W.Y. *et al.* (2012) Global prevalence and major risk factors of diabetic retinopathy. *Diab. Care* 35, 556–564
3 Stitt, A.W. *et al.* (2016) The progress in understanding and treatment of diabetic retinopathy. *Prog. Retin. Eye Res.* 51, 156–186
4 Cai, X. and McGinnis, J.F. (2016) Diabetic retinopathy: animal models, therapies, and perspectives. *J. Diab. Res.* 2016, 3789217
5 Fogli, S. *et al.* (2016) Pathophysiology and pharmacological targets of VEGF in diabetic macular edema. *Pharmacol. Res.* 103, 149–157
6 Semeraro, F. *et al.* (2015) Diabetic retinopathy: vascular and inflammatory disease. *J. Diab. Res.* 2015, 582060
7 Eshaq, R.S. *et al.* (2017) Diabetic retinopathy: breaking the barrier. *Pathophysiology* 24, 229–241
8 Pusparajah, P. *et al.* (2016) Molecular markers of diabetic retinopathy: potential screening tool of the future? *Front. Physiol.* 7, 200
9 Xu, J. *et al.* (2018) Involvement of advanced glycation end products in the pathogenesis of diabetic retinopathy. *Cell. Physiol. Biochem.* 48, 705–717
10 Capitao, M. and Soares, R. (2016) Angiogenesis and inflammation crosstalk in diabetic retinopathy. *J. Cell Biochem.* 117, 2443–2453
11 Behl, T. *et al.* (2016) Implication of oxidative stress in progression of diabetic retinopathy. *Survey Ophthalmol.* 61, 187–196
12 Wang, Z. *et al.* (2018) Metabolic memory in mitochondrial oxidative damage triggers diabetic retinopathy. *BMC Ophthalmol.* 18, 258
13 Du, Y. *et al.* (2013) Photoreceptor cells are major contributors to diabetes-induced oxidative stress and local inflammation in the retina. *Proc. Natl. Acad. Sci. U. S. A.* 110, 16586–16591
14 Eshaq, R.S. *et al.* (2014) Oxygen delivery, consumption, and conversion to reactive oxygen species in experimental models of diabetic retinopathy. *Redox Biol.* 2, 661–666
15 Sasaki, M. *et al.* (2010) Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. *Diabetologia* 53, 971–979
16 Ozawa, Y. *et al.* (2011) Neural degeneration in the retina of the streptozotocin-induced type 1 diabetes model. *Exp. Diab. Res.* 2011, 108328
17 Ola, M.S. *et al.* (2011) Regulation of glutamate metabolism by hydrocortisone and branched chain keto acids in cultured rat retinal Muller cells (TR-MUL). *Neurochem. Int.* 59, 656–663
18 Ola, M.S. *et al.* (2012) Recent advances in understanding the biochemical and molecular mechanism of diabetic retinopathy. *J. Diab. Complications* 26, 56–64
19 Wilding, C. *et al.* (2014) c-Synuclein antibodies have neuroprotective potential on neuroretinal cells via proteins of the mitochondrial apoptosis pathway. *PLOS One* 9, e90737
20 Zhang, Y. and Bhavnani, B.R. (2006) Glutamate-induced apoptosis in neuronal cells is mediated via caspase-dependent and independent mechanisms involving calpain and caspase-3 proteases as well as apoptosis inducing factor (AIF) and this process is inhibited by equine estrogens. *BMC Neurosci.* 7, 49
21 Aroda, V.R. *et al.* (2016) Long-term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. *J. Clin. Endocrinol. Metab.* 101, 1754–1761
22 Lee, J.Y. *et al.* (2017) Relationship between plasma homocysteine level and glaucomatous retinal nerve fiber layer defect. *Curr. Eye Res.* 42, 918–923
23 Sadiqulla, M. *et al.* (2014) Serum homocysteine as a risk factor in diabetics with colour vision deficiency and for developing retinopathy. *Int. J. Med. Sci. Public Health* 3, 165
24 Sanchez-Migallon, M.C. *et al.* (2016) Apoptotic retinal ganglion cell death after optic nerve transection or crush in mice: delayed RGC loss with BDNF or a caspase 3 inhibitor. *Invest. Ophthalmol. Vis. Sci.* 57, 81–93
25 Ola, M.S. *et al.* (2013) Reduced levels of brain derived neurotrophic factor (BDNF) in the serum of diabetic retinopathy patients and in the retina of diabetic rats. *Cell. Mol. Neurobiol.* 33, 359–367
26 Unterlauft, J.D. *et al.* (2012) Pigment epithelium-derived factor released by Muller glial cells exerts neuroprotective effects on retinal ganglion cells. *Neurochem. Res.* 37, 1524–1533
27 Haurigot, V. *et al.* (2012) Long-term retinal PEDF overexpression prevents neovascularization in a murine adult model of retinopathy. *PLoS One* 7, e41511
28 Kwa, F.A. and Thrimawithana, T.R. (2014) Epigenetic modifications as potential therapeutic targets in age-related macular degeneration and diabetic retinopathy. *Drug Discov. Today* 19, 1387–1393
29 Berdasco, M. *et al.* (2017) DNA methylomes reveal biological networks involved in human eye development, functions and associated disorders. *Sci. Rep.* 7, 11762
30 Mishra, M. *et al.* (2014) Epigenetic modifications of Keap1 regulate its interaction with the protective factor Nrf2 in the development of diabetic retinopathy. *Invest. Ophthalmol. Vis. Sci.* 55, 7256–7265
31 Pisani, F. *et al.* (2018) Potential role of the methylation of VEGF gene promoter in response to hypoxia in oxygen-induced retinopathy: beneficial effect of the absence of AQP4. *J. Cell. Mol. Med.* 22, 613–627
32 Alivand, M.R. *et al.* (2016) Probable chemical hypoxia effects on progress of CNV through induction of promoter CpG demethylation and overexpression of IL17RC in human RPE cells. *Curr. Eye Res.* 41, 1245–1254

33 Agardh, E. *et al.* (2015) Genome-wide analysis of DNA methylation in subjects with type 1 diabetes identifies epigenetic modifications associated with proliferative diabetic retinopathy. *BMC Med.* 13, 182

34 Mishra, M. *et al.* (2018) Sirt1: a guardian of the development of diabetic retinopathy. *Diabetes* 67, 745–754

35 Kim, D.I. *et al.* (2015) PRMT1 and PRMT4 regulate oxidative stress-induced retinal pigment epithelial cell damage in SIRT1-dependent and SIRT1-independent manners. *Oxid. Med. Cell Longev.* 2015, 617919

36 Mortuza, R. *et al.* (2015) SIRT1 reduction causes renal and retinal injury in diabetes through endothelin 1 and transforming growth factor beta1. *J. Cell. Mol. Med.* 19, 1857–1867

37 Zorrilla-Zubilete, M.A. *et al.* (2018) Epigenetic control of early neurodegenerative events in diabetic retinopathy by the histone deacetylase SIRT6. *J. Neurochem.* 144, 128–138

38 Gao, J. *et al.* (2016) Deacetylation of MnSOD by PARP-regulated SIRT3 protects retinal capillary endothelial cells from hyperglycemia-induced damage. *Biochem. Biophys. Res. Commun.* 472, 425–431

39 Mao, X.B. *et al.* (2017) Potential suppression of the high glucose and insulin-induced retinal neovascularization by Sirtuin 3 in the human retinal endothelial cells. *Biochem. Biophys. Res. Commun.* 482, 341–345

40 Tolentino, M.S. *et al.* (2016) Current and investigational drugs for the treatment of diabetic retinopathy. *Exp. Opin. Invest. Drugs* 25, 1011–1022

41 Miller, K. and Fortun, J.A. (2018) Diabetic macular edema: current understanding, pharmacologic treatment options, and developing therapies. *Asia Pacific J. Ophthalmol.* 7, 28–35

42 Study of the Efficacy and Safety of the Ranibizumab Port Delivery System (RPDS) for Sustained Delivery of Ranibizumab in Participants With Subfoveal Neovascular Age-Related Macular Degeneration (AMD) (LADDER). Available at: https://clinicaltrials.gov/ct2/show/NCT02510794.

43 A Phase III Study to Evaluate the Port Delivery System Implant With Ranibizumab Compared With Monthly Ranibizumab Injections in Participants With Wet Age-Related Macular Degeneration (Archway). Available at: https://clinicaltrials.gov/ct2/show/NCT03677934.

44 Campochiaro, P.A. *et al.* (2013) Treatment of diabetic macular edema with a designed ankyrin repeat protein that binds vascular endothelial growth factor: a Phase I/II study. *Am. J. Ophthalmol.* 155, 697–704

45 Urias, E.A. *et al.* (2017) Novel therapeutic targets in diabetic macular edema: beyond VEGF. *Vision Res.* 139, 221–227

46 Ung, C. *et al.* (2017) Current and emerging treatment for diabetic macular edema. *Int. Ophthalmol. Clin.* 57, 165–177

47 The TIME-2b Study: A Study of AKB-9778, a Novel Tie 2 Activator, in Patients With Non-Proliferative Diabetic Retinopathy (NPDR) (TIME-2b). Available at: https://clinicaltrials.gov/ct2/show/NCT03197870.

48 A Study to Evaluate the Efficacy and Safety of Faricimab (RO6867461) in Participants With Diabetic Macular Edema (RHINE). Available at: https://clinicaltrials.gov/ct2/show/NCT03622593.

49 Campochiaro, P.A. *et al.* (2016) Enhanced benefit in diabetic macular edema from AKB-9778 Tie2 activation combined with vascular endothelial growth factor suppression. *Ophthalmology* 123, 1722–1730

50 F. Hoffmann-La Roche Ltd. Investor Update. Available at: https://www.roche.com/investors/updates/inv-update-2018-02-12.htm.

51 Hernandez, C. *et al.* (2013) Somatostatin replacement: a new strategy for treating diabetic retinopathy. *Curr. Med. Chem.* 20, 3251–3257

52 Wilson, S.H. *et al.* (2001) Modulation of retinal endothelial cell behaviour by insulin-like growth factor I and somatostatin analogues: implications for diabetic retinopathy. *Growth Hormone IGF Res* 11 (suppl. A), 53–59

53 Baldysiak-Figiel, A. *et al.* (2004) Octreotide prevents growth factor-induced proliferation of bovine retinal endothelial cells under hypoxia. *J. Endocrinol.* 180, 417–424

54 Dal Monte, M. *et al.* (2007) Antiangiogenic role of somatostatin receptor 2 in a model of hypoxia-induced neovascularization in the retina: results from transgenic mice. *Invest. Ophthalmol. Vis. Sci.* 48, 3480–3489

55 Ramos, D. *et al.* (2013) Mimicking microvascular alterations of human diabetic retinopathy: a challenge for the mouse models. *Curr. Med. Chem.* 20, 3200–3217

56 Hernaez-Ortega, M.C. *et al.* (2008) Lanreotide Autogel for persistent diabetic macular edema. *Diab. Res. Clin. Pract.* 80, e8–e10

57 Boehm, B.O. *et al.* (2001) Octreotide reduces vitreous hemorrhage and loss of visual acuity risk in patients with high-risk proliferative diabetic retinopathy. *Hormone Metabol. Res.* 33, 300–306

58 Grant, M.B. *et al.* (2000) The efficacy of octreotide in the therapy of severe nonproliferative and early proliferative diabetic retinopathy: a randomized controlled study. *Diab. Care* 23, 504–509

59 Simó, R. *et al.* (2019) Effects of topically administered neuroprotective drugs in early stages of diabetic retinopathy: results of the EUROCONDOR clinical trial. *Diabetes* 68, 457–463

60 A Study to Evaluate ASP8232 in Reducing Central Retinal Thickness in Subjects With Diabetic Macular Edema (DME) (VIDI). Available at: https://clinicaltrials.gov/ct2/show/NCT02302079.

61 Chaurasia, S.S. *et al.* (2018) The NLRP3 inflammasome may contribute to pathologic neovascularization in the advanced stages of diabetic retinopathy. *Sci. Rep.* 8, 2847

62 Stahel, M. *et al.* (2016) SYSTEMIC INTERLEUKIN 1β INHIBITION IN PROLIFERATIVE DIABETIC RETINOPATHY: a prospective open-label study using canakinumab. *Retina* 36, 385–391

63 Abu El-Asrar, A.M. *et al.* (2014) The proinflammatory cytokine high-mobility group box-1 mediates retinal neuropathy induced by diabetes. *Mediators Inflamm.* 2014, 716415

64 Mohammad, G. *et al.* (2015) Mutual enhancement between high-mobility group box-1 and NADPH oxidase-derived reactive oxygen species mediates diabetes-induced upregulation of retinal apoptotic markers. *J. Physiol. Biochem.* 71, 359–372

65 Mugisho, O.O. *et al.* (2018) The inflammasome pathway is amplified and perpetuated in an autocrine manner through connexin43 hemichannel mediated ATP release. *Biochim. Biophys. Acta* 1862, 385–393

66 Chen, Y.S. *et al.* (2015) Sustained intravitreal delivery of connexin43 mimetic peptide by poly(D,L-lactide-co-glycolide) acid micro- and nanoparticles—closing the gap in retinal ischaemia. *Eur. J. Pharm. Biopharm.* 95, 378–386

67 Mat Nor, N. *et al.* (2018) Sustained connexin43 mimetic peptide release from loaded nanoparticles reduces retinal and choroidal photodamage. *Invest. Ophthalmol. Vis. Sci.* 59, 3682–3693

68 O'Carroll, S.J. *et al.* (2008) Connexin 43 mimetic peptides reduce swelling, astrogliosis, and neuronal cell death after spinal cord injury. *Cell Commun. Adhes.* 15, 27–42

69 Danesh-Meyer, H.V. *et al.* (2012) Connexin43 mimetic peptide reduces vascular leak and retinal ganglion cell death following retinal ischaemia. *Brain* 135, 506–520

70 Guo, C.X. *et al.* (2016) Connexin43 mimetic peptide improves retinal function and reduces inflammation in a light-damaged albino rat model. *Invest. Ophthalmol. Vis. Sci.* 57, 3961–3973

71 Huang, D. *et al.* (2018) Hyaluronic acid coated albumin nanoparticles for targeted peptide delivery in the treatment of retinal ischaemia. *Biomaterials* 168, 10–23

72 Schalkwijk, C.G. and Miyata, T. (2012) Early- and advanced non-enzymatic glycation in diabetic vascular complications: the search for therapeutics. *Amino Acids* 42, 1193–1204

73 Mohamed, Q. and Wong, T.Y. (2008) Emerging drugs for diabetic retinopathy. *Expert Opin. Emerg. Drugs* 13, 675–694

74 Simó, R. and Hernández, C. (2015) Novel approaches for treating diabetic retinopathy based on recent pathogenic evidence. *Prog. Retin. Eye Res.* 48, 160–180

75 Hernández, C. *et al.* (2016) Topical administration of GLP-1 receptor agonists prevents retinal neurodegeneration in experimental diabetes. *Diabetes* 65, 172

76 Hernandez, C. *et al.* (2017) Topical administration of DPP-IV inhibitors prevents retinal neurodegeneration in experimental diabetes. *Diabetologia* 60, 2285–2298

77 Kramerov, A.A. and Ljubimov, A.V. (2016) Stem cell therapies in the treatment of diabetic retinopathy and keratopathy. *Exp. Biol. Med.* 241, 559–568

78 Li, C. *et al.* (2017) Oxidative stress-related mechanisms and antioxidant therapy in diabetic retinopathy. *Oxidative Med. Cell. Longevity* . http://dx.doi.org/10.1155/2017/9702820

79 Fu, J. *et al.* (2015) Protective role of nuclear factor E2-related factor 2 against acute oxidative stress-induced pancreatic β-cell damage. *Oxidative Med. Cell. Longevity* 2015, 639191

80 Sharma, A. *et al.* (2017) The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) activator dh404 protects against diabetes-induced endothelial dysfunction. *Cardiovas. Diabetol.* 16. http://dx.doi.org/10.1186/s12933-017-0513-y

81 Deliyanti, D. *et al.* (2018) Nrf2 activation is a potential therapeutic approach to attenuate diabetic retinopathy. *Invest. Ophthalmol. Vis. Sci.* 59, 815–825

82 Li, S. *et al.* (2019) Protective effects of sulforaphane on diabetic retinopathy: activation of the Nrf2 pathway and inhibition of NLRP3 inflammasome formation. *Exp. Anim.* . http://dx.doi.org/10.1538/expanim.18-0146

83 Keech, A. *et al.* (2007) Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial. *Lancet* 370, 1687–1697

84 Sharma, N. *et al.* (2015) The use of fenofibrate in the management of patients with diabetic retinopathy: an evidence-based review. *Austr. Fam. Phys.* 44, 367–370

85 Simó, R. *et al.* (2015) Non-traditional systemic treatments for diabetic retinopathy: an evidence-based review. *Curr. Med. Chem.* 22, 2580–2589

86 Abcouwer, S.F. (2013) Direct effects of PPARα agonists on retinal inflammation and angiogenesis may explain how fenofibrate lowers risk of severe proliferative diabetic retinopathy. *Diabetes* 62, 36–38

87 Roy, S. *et al.* (2015) Beneficial effects of fenofibric acid on overexpression of extracellular matrix components, COX-2, and impairment of endothelial permeability associated with diabetic retinopathy. *Exp. Eye Res.* 140, 124–129

88 Li, J. *et al.* (2018) Fenofibrate ameliorates oxidative stress-induced retinal microvascular dysfunction in diabetic rats. *Curr. Eye Res.* 43, 1395–1403

89 Pearsall, E.A. *et al.* (2019) Neuroprotective effects of PPARα in retinopathy of type 1 diabetes. *PLOS One* 14, e0208399

90 Dehdashtian, E. *et al.* (2018) Diabetic retinopathy pathogenesis and the ameliorating effects of melatonin; involvement of autophagy, inflammation and oxidative stress. *Life Sci.* 193, 20–33

91 Maugeri, A. *et al.* (2018) Curcumin modulates DNA methyltransferase functions in a cellular model of diabetic retinopathy. *Oxid. Med. Cell. Longev.* 2018, 5407482

92 Duarte, D.A. *et al.* (2015) Polyphenol-enriched cocoa protects the diabetic retina from glial reaction through the sirtuin pathway. *J. Nutr. Biochem.* 26, 64–74

93 Liu, S. *et al.* (2016) Protective effects of SIRT1 in patients with proliferative diabetic retinopathy via the inhibition of IL-17 expression. *Exp. Ther. Med.* 11, 257–262

94 Kowluru, R.A. *et al.* (2014) Sirt1, a negative regulator of matrix metalloproteinase-9 in diabetic retinopathy. *Invest. Ophthalmol. Vis. Sci.* 55, 5653–5660

95 Michan, S. *et al.* (2014) Sirtuin1 over-expression does not impact retinal vascular and neuronal degeneration in a mouse model of oxygen-induced retinopathy. *PLOS One* 9, e85031

96 Tzeng, T.F. *et al.* (2016) Antioxidant-rich extract from plantaginis semen ameliorates diabetic retinal injury in a streptozotocin-induced diabetic rat model. *Nutrients* 8, 572

97 Zhao, S. *et al.* (2015) Fenofibrate suppresses cellular metabolic memory of high glucose in diabetic retinopathy via a sirtuin 1-dependent signalling pathway. *Mol. Med. Rep.* 12, 6112–6118

98 Hakami, N.Y. *et al.* (2016) Trichostatin A, a histone deacetylase inhibitor suppresses NADPH oxidase 4-derived redox signalling and angiogenesis. *J. Cell. Mol. Med.* 20, 1932–1944

99 Desjardins, D. *et al.* (2016) Histone deacetylase inhibition restores retinal pigment epithelium function in hyperglycemia. *PLOS One* 11, e0162596

100 Cai, X. *et al.* (2017) GLP-1 treatment improves diabetic retinopathy by alleviating autophagy through GLP-1R-ERK1/2-HDAC6 signaling pathway. *Int. J. Med. Sci.* 14, 1203–1212

101 Yasuda, H. *et al.* (2016) Exendin-4 induces extracellular-superoxide dismutase through histone H3 acetylation in human retinal endothelial cells. *J. Clin. Biochem. Nutr.* 59, 174–181

102 Arevalo, J.F. *et al.* (2018) Intravitreal bevacizumab in diabetic retinopathy. Recommendations from the Pan-American Collaborative Retina Study Group (PACORES): the 2016 knobloch lecture. *Asia Pacific J. Ophthalmol* 7, 36–39

103 Dervenis, N. *et al.* (2017) Ranibizumab in the treatment of diabetic macular edema: a review of the current status, unmet needs, and emerging challenges. *Adv. Ther.* 34, 1270–1282

104 Brown, D.M. *et al.* (2015) Intravitreal aflibercept for diabetic macular edema: 100-week results from the VISTA and VIVID studies. *Ophthalmology* 122, 2044–2052

105 Boyer, D.S. *et al.* (2014) Three-year, randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with diabetic macular edema. *Ophthalmology* 121, 1904–1914

106 Campochiaro, P.A. *et al.* (2011) Long-term benefit of sustained-delivery fluocinolone acetonide vitreous inserts for diabetic macular edema. *Ophthalmology* 118, 626–635