Review Article

Novel pharmacotherapies in diabetic retinopathy: Current status and what's in the horizon?

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The blood–retinal barrier (BRB) alteration is the hallmark feature of diabetic retinopathy. Vascular endothelial growth factor (VEGF) is a potent vasopermeability factor that has been implicated in the pathogenesis of BRB alteration. Inflammation also plays a crucial role in this process with involvement of several chemokines and cytokines. Multiple anti-VEGF drugs are widely used as in the treatment of diabetic macular edema (DME) as well as proliferative diabetic retinopathy. Several clinical trials have proved the beneficial effects of these drugs in improvement of vision and prevention of vision loss. However, the response to anti-VEGF drugs in DME is not complete in a significant number of patients. The effect seems transient in this latter group, and many patients do not show complete resolution of fluid. Potential novel therapies targeting molecules beyond VEGF are being developed and examined in clinical trials.

Key words: Blood–retinal barrier, diabetes mellitus, diabetic macular edema, diabetic retinopathy, inflammation, vascular endothelial growth factor

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Diabetic retinopathy still remains one of the leading causes of blindness in the middle-aged population (20–64 years).[1,2] This microvascular complication of diabetes is prevalent in about 35% of people with diabetes.[1] Laser photocoagulation has been the mainstay of management for many decades in diabetic retinopathy patients in addition to control of systemic factors. However, the use of intravitreal pharmacotherapies in the last decade has revolutionized the management of diabetic macular edema (DME) as well as proliferative diabetic retinopathy (PDR). In this review, we will discuss the pathophysiology of diabetic retinopathy, the current pharmacologic treatment strategies for diabetic retinopathy, and also the novel treatments in the pipeline.

Pathophysiology

The hallmark of the pathogenesis of diabetic retinopathy is an alteration of the blood–retinal barrier (BRB).[3] Normally, the inner BRB at the retinal capillary level is composed of pericytes that cover the vessels outside, endothelial layer, and basement membrane in between these cells. In diabetes, three changes occur at BRB namely, (i) selective loss or drop-out of pericytes, (ii) loss of endothelial cell-cell junctions, and (iii) thickening of the basement membrane. Once BRB breaks down, it leads to intraretinal hemorrhages, hard exudates, and macular edema. Selective pericyte loss is a classic histopathological lesion seen in diabetic retinopathy.[4] Normally, pericytes function as modified smooth muscle cells, are contractile in nature, and regulate the retinal capillary blood flow.[5] Pericyte loss results in focal weakening of the vessel wall and focal endothelial cell proliferation that leads to microaneurysms.[6] Later, endothelial cells also undergo apoptosis resulting in acellular capillaries and capillary nonperfusion. The pathogenesis of diabetic retinopathy is attributed to increased activity of four major biochemical pathways such as (a) polyol pathway, (b) advanced glycation end-product pathway, (c) protein kinase C pathway, and (d) hexosamine pathway.[7] All these pathways eventually lead to increased oxidative stress and inflammation.

Many features of inflammation including leukostasis, neutrophil and macrophage infiltration, complement and microglial activation, upregulation of cytokines, increased blood flow, and vascular permeability and tissue edema have been described in animal models of diabetic retinopathy and as well as humans.[8] The inflammation in diabetes is actually a chronic process rather than acute vasculitis. Leukostasis, or adherence of leukocytes to the endothelial layer of the retinal capillaries, is an early event in diabetic retinopathy.[9] We have shown that increased monocyte/macrophage trafficking into extravascular retinal tissue occurs in early diabetes in an animal model.[10] The chemokine, Monocyte Chemoattractant protein-1 (MCP-1), also known as chemokine ligand 2 (CCL2), causes monocyte/macrophage influx into the retina. Increases in MCP-1 levels in the vitreous along with increased vascular endothelial growth factor (VEGF) levels have been described in

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patients with DME.[11] In MCP-1 knockout mice made diabetic, there is a significant reduction in retinal vascular leakage and monocyte infiltration in the retina. Activated monocytes differentiate into macrophages which along with activated microglia, secrete cytokines and growth factors including VEGF, tumor necrosis factor α (TNFα), interleukins (IL-6 and IL-1b), and matrix metalloproteinases, and all of which can alter the cell-cell junctional molecules of BRB [Fig. 1].

Hypoxia is the initiating factor in the development of retinal new vessels or angiogenesis seen in PDR. Many angiogenic factors such as VEGF, basic fibroblast growth factor (bFGF), insulin-like growth factor, and angiopoietin-2 (Ang-2) play a key role in this process. Normally, there is a balance of angiogenic factors and endogenous anti-angiogenic factors such as pigment epithelium-derived factor and endostatin. Once this balance breaks down, endothelial proliferation from existing retinal capillaries occurs resulting in new vessels as seen in PDR.

Pharmacotherapies

Anti-vascular endothelial growth factor therapy

VEGF is a potent vasopermeability factor that has been investigated extensively in relation to DME and alteration of BRB and retinal new vessel formation in PDR. The VEGF levels are significantly elevated in vitreous of DME patients when compared with nondiabetic eyes.[11] VEGF induces the phosphorylation of cell-cell junctional molecules such as VE-cadherin, occludin, and ZO-1 and thus causes a breakdown of the BRB. Out of all the isoforms, VEGF 165 is the major proinflammatory cytokine. Several anti-VEGF drugs target the molecule, VEGF. Drugs that directly inhibit the VEGF molecule are anti-VEGF aptamer, pegaptanib (Macugen, OSI), monoclonal antibody fragment ranibizumab (RBZ) (Lucentis, Genentech), full-length antibody bevacizumab (BVZ) (Avastin, Genentech) and soluble VEGF receptor analogs, aflibercept (AFB), VEGF-Trap (Regeneron). Other anti-VEGF molecules include small interfering RNAs, bevasiranib (Opko Health), and rapamycin (Sirolimus, MacuSight). Anti-VEGF drugs are injected intravitreally under topical anesthesia as office procedures. Currently, anti-VEGF agents are considered the first line of treatment in center-involving DME, where the indication of focal/grid laser is limited to noncenter-involving DME [Fig. 2].

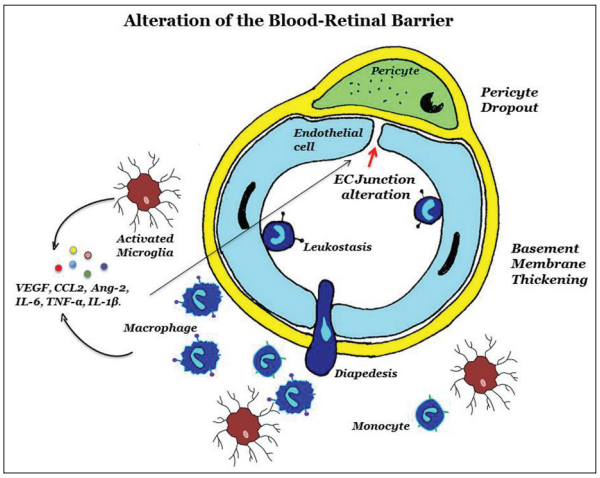

Figure 1: Alteration of the blood–retinal barrier in diabetes mellitus. Chronic inflammation in diabetes leads to production of chemokines (including monocyte chemoattractant protein-1, also known as chemokine ligand 2) that result in leukostasis, diapedesis, and influx of monocytes into the retina and extravascular space. Monocytes are differentiated into macrophages which along with activated microglia produce an array of cytokines and chemokines including vascular endothelial growth factor. These mediators then break down the cell-cell junction molecules resulting in alteration of the blood–retinal barrier

Pharmacokinetics of anti-vascular endothelial growth factor agents [Table 1]

Pegaptanib (Macugen) is a 50 kDa pegylated aptamer (28-ribonucleotide molecule) that binds to the heparin-binding domain of the proinflammatory VEGF-A 165 isoform.[12] As it is an RNA-based molecule, it has almost no toxicity or immunogenicity. Its main disadvantage is its small molecular size and rapid elimination from circulation. To address this, pegaptanib is conjugated to 20 kDa monomethoxy-polyethylene glycol molecules for its increase in molecular size to 50 kDa (pegylation). The recommended dose is 0.3 mg intravitreally every 6 weeks. It penetrates all layers of the retina. In humans, the plasma half-life after intravitreal injection of pegaptanib is 10 days.

BVZ (Avastin) is a recombinant full-length humanized monoclonal antibody (149 kDa), almost 3 times the size of the RBZ molecule, which binds to the receptor binding domain of all isoforms of VEGF-A.[12] It inhibits binding of VEGF to its receptor and thus results in inhibition of downstream proangiogenic receptor signaling. The recommended dose is 1.25 mg intravitreally every 4 weeks. BVZ can penetrate all layers of the retina. In a time-dependent penetration study in monkeys, the drug has been shown at inner limiting membrane and ganglion cell layer on day 1, at inner nuclear layer and outer plexiform layer on day 4, and at photoreceptor layer on days 7–14.[13] After intravitreal injection, its vitreous half-life is 9.8 days, and plasma half-life is 17–21 days.

RBZ (Lucentis) is a recombinant humanized monoclonal immunoglobulin IgG1 (48 kDa) that binds to the receptor binding domain of all isoforms of VEGF-A.[12] It is genetically engineered through a process of selective mutation and is thus "affinity-enhanced" to provide stronger affinity to bind to VEGF-A. RBZ does not have Fc domain. The recommended dose of RBZ is 0.3 mg intravitreally every 4 weeks. Its vitreous half-life and plasma half-life is 9 days.

AFB (Eylea) is a soluble protein (97 kDa) that contains extracellular VEGF receptor 1 and 2 sequences fused to Fc domain of an IgG molecule and blocks all isoforms of VEGF-A, VEGF-B, as well as the placental growth factor.[14] It has a prolonged biological activity and thus offers the advantage of every 2 months injections rather than monthly injections. The recommended dose of AFB is 2 mg intravitreally every 4 weeks for the first three injections and then every 8 weeks. It can also penetrate all layers of the retina. After intravitreal injection, its vitreous half-life is 7 days, and plasma half-life is 5–6 days.

AFB has the highest affinity for VEGF among all anti-VEGF drugs, almost 100-fold compared to BVZ or RBZ [Table 1]. In a systemic pharmacokinetics study in wet age-related macular degeneration (ARMD) patients, all three anti-VEGF agents

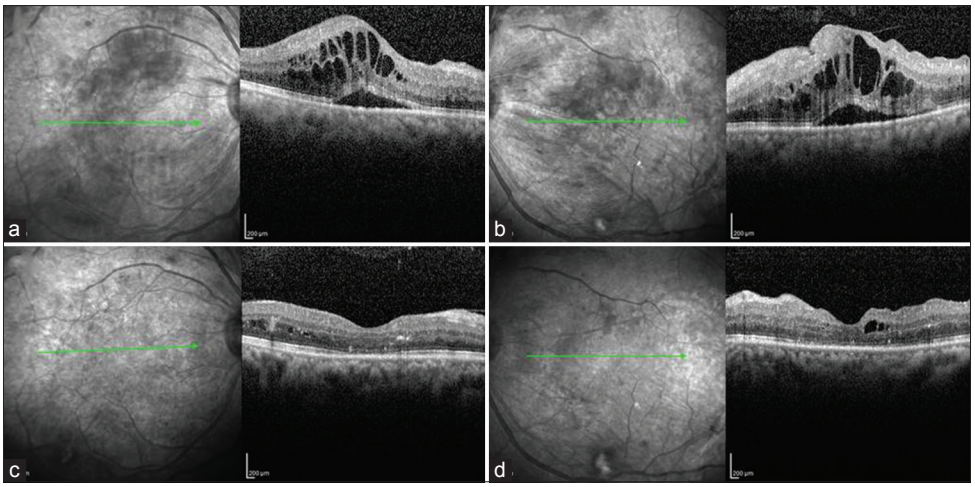

Figure 2: Optical coherence tomography images of a patient with center-involving diabetic macular edema who responded well to anti-vascular endothelial growth factor drugs. Right eye (a and c). Left eye (b and d). (a and b) Fundus and optical coherence tomography images a 57-year-old diabetic visual acuity of 20/100 and central retinal thickness of 697 um in the right eye (a) and visual acuity of 20/200 and central retinal thickness of 763 um in the left eye (b). (c and d) Just after one dose of bevacizumab injection in each eye, there was dramatic improvement, and her visual acuity and central retinal thickness were 20/40 and 266 um in the right eye and 20/40 and 280 um in the left eye, respectively

Table 1: Pharmacokinetics of anti-vascular endothelial growth factor drugs in clinical trials

Features	Pegaptanib	BVZ	RBZ	AFB
Description	Ribonucleic acid aptamer	Full length humanized monoclonal antibody	A monoclonal antibody fragment derived from BVZ (no Fc part)	A recombinant fusion protein that contains extracellular VEGF receptor 1 and 2 sequences fused to Fc domain of a human IgG1
Molecule weight (kDa)	50	149	48	97
Vitreous half-life (days)	10	9.8	9	7
Plasma half-life (days)	10	17-21	9	5-6
Affinity		Lowest affinity compared to other drugs	6-fold affinity compared to BVZ	94-fold binding affinity compared to BVZ or RBZ
Target	VEGF 165 isoform	All isoforms of VEGF	All isoforms of VEGF	All isoforms of VEGF and PlGF
Absorption	Slow systemic absorption	Detected in systemic circulation and contralateral eye	Slow systemic absorption	Very low systemic absorption
Ocular distribution	Penetrates all layers of retina	Penetrates all layers of the retina	Penetrates all layers of retina	Penetrates all layers of retina
Recommended dose	0.3 mg once every 6 weeks	1.25 mg once every 4 weeks	0.3 mg once every 4 weeks	2 mg once every 4 weeks for 3 months, once every 8 weeks

PlGF: Placental growth factor, VEGF: Vascular endothelial growth factor, BVZ: Bevacizumab, RBZ: Ranibizumab, AFB: Aflibercept

were detected in the bloodstream; however, RBZ was cleared quickly whereas BVZ and AFB showed greater systemic exposure.[15] Both BVZ and AFB caused marked reduction of plasma free VEGF levels compared to RBZ. Intravitreal injection of BVZ in PDR patients has resulted in regression of new vessels not only in the injected eye but also in the fellow eye.[16] Similarly in DME patients, decreased retinal thickness has been observed after intravitreal injection of BVZ, not RBZ.[17] These observations are of clinical significance as systemic administration of these agents, although beneficial to cancer patients, have been involved with severe side effects such as bleeding, including central nervous system hemorrhage and death. In all trials with intravitreal anti-VEGF drugs, the incidence of cerebrovascular accident, myocardial infarction, and death has not been found to be significantly elevated. Thus, small doses of anti-VEGF drugs for intravitreal injections have so far been found to be safe. The reduction of systemic VEGF level and concerns for serious adverse events may be more significant in a subset of patients such as retinopathy of prematurity babies, diabetics, elderly people, or those with recent arterial thromboembolic events (ATEs) as stroke.[18] An animal study has suggested that chronic suppression of locally synthesized VEGF in the eye may be also deleterious in terms of damage to the choriocapillaris and cone function.[19] Knocking out VEGF-A in adult mice results in damage to retinal pigment epithelial (RPE) cells, ablation of the choriocapillaris, and rapid dysfunction of cone photoreceptors. Thus, endogenous VEGF is critical for trophic support for retinal function. Fortunately, there is no strong evidence of such damage documented in human studies.

Anti-vascular endothelial growth factor agents in diabetic macular edema [Table 2]

Ranibizumab

Four large, randomized clinical trials have shown the efficacy of intravitreal RBZ in center-involving DME patients. In the READ-2 study, DME patients were randomized to receive intraocular injections of RBZ, focal or grid laser, or a combination of RBZ and focal or grid laser.[20] At month 6 primary endpoint, if retreatment criteria were met, patients could be treated with intraocular injections of 0.5 mg RBZ. At 2 years, the percentage of patients who gained three lines or more of best-corrected visual acuity (BCVA) was 21, 0, and 6 at month 6, compared with 24, 18, and 26 at month 24, respectively. Thus, intraocular injections of RBZ were found to provide long-term benefit in patients with DME. At 3 years, there was an improvement of 10.3 letters in the RBZ group, −1.6 letters in the laser group, and +2 letters in the RBZ + laser group.[21] Thus, more aggressive treatment with RBZ in the year 3 resulted in more reduction of macular thickness and improvement of vision in the RBZ group.

In the RIDE/RISE study, where patients were randomized to either two different doses of intravitreal RBZ (0.3 mg and 0.5 mg) or sham injection, the proportion of patients showing >15 letters (3 Snellen lines) improvement were 19% in the sham group compared to 37% in the 0.3 mg RBZ group and 40% in the 0.5 mg RBZ group after 3 years.[22] The mean improvement of vision was 11.8 letters in the RBZ group and 4.5 letters in the sham group. The efficacy of these two doses of RBZ was found to be similar. As diabetic patients have an underlying risk of mortality and cardiovascular events, the use of 0.3 mg RBZ may have less risk potentially related to systemic VEGF suppression. The US Food and Drug Administration (FDA) approved the use of 0.3 mg RBZ for DME. It is important to remember that a dose response curve was also seen in wet ARMD and retinal vascular occlusion patients favoring 0.5 mg RBZ over 0.3 mg RBZ. Interestingly, after crossover to 1 year of treatment with monthly RBZ injections, the average vision gain in the sham group was lower. This indicates that delayed treatment with RBZ does not result in the same extent of vision improvement. The Phase II RESOLVE trial also randomized DME patients to 0.3 mg RBZ, 0.5 mg RBZ, or sham similar to the RIDE/RISE study, but the protocol was 3 monthly injections followed by pro re nata (PRN) monthly injections.[23] In eyes with residual edema, the RBZ dose was doubled at 1st month. At month 12, the mean vision improved by 10.3 letters in the RBZ group and declined by 1.4 letters in the sham group.

The Diabetic Retinopathy Clinical Research (DRCR) Network, an NIH-sponsored multicenter, randomized clinical trial concluded that intravitreal RBZ with prompt or deferred laser was more effective through 5 years compared with prompt laser alone for center-involving DME. Five-year data from this study showed that visual improvement was more in RBZ + deferred (for >24 weeks) laser group (58% with >10 letters improvement) compared to RBZ + prompt laser (at the initiation of RBZ injection) group (46% with >10 letters improvement) and thus suggested no benefit of earlier initiation of focal/grid laser for better visual outcome.[24] However, the deferred laser group needed more RBZ injections. Most eyes treated with RBZ (prompt or deferred laser) maintain vision gains obtained by

Table 2: Comparative data of trials of different anti-vascular endothelial growth factor drugs in diabetic macular edema

Drug	Study	Compared with (vs.)	Duration of the study (years)	Results		Comments
				Mean vision change (letters)	Mean CMT change (μm)	
BVZ	BOLT	Laser	2	+8.6 versus −0.5	−146 versus −118	Median number of treatments=13 for BVZ and 4 for laser therapy Visual acuity benefit was maintained through 2 years
RBZ	RIDE/RISE	Sham injection	3	+11.8 versus +4.5	−258 versus −126	Efficacy equivalent between 0.3 and 0.5 mg doses. FDA approved 0.3 mg dose
	RESOLVE	Sham injection	1	+10.3 versus −1.4	−194 versus −48	Visual acuity improvement 3-fold higher in RBZ group
	READ-2	Laser and laser + RBZ	3	+10.3 versus −1.6 versus + 2.0	−70 versus −36 versus −24	Resolution of edema more common in RBZ group
	DRCR I	Laser	5	+7.2 (prompt) versus + 9.8 (deferred)	−167 (prompt) versus −165 (deferred)	Focal/grid laser at initiation of RBZ therapy is no better than deferring laser for 24 weeks
	RESTORE	Laser	3	+8.0 (RBZ) versus +6.7 (RBZ + laser) versus + 6.0 (laser)	−142 versus −146	Efficacy with progressively declining number of injections of PRN dosing
AFB	dA Vinci	Laser	1	+11 versus −1.3	−189 versus −58	Benefit in the 2 mg q8 weeks treatment schedule, which could reduce the number of visits by half
	VISTA/VIVID	Laser	2	+11.5 versus +0.8	−193 versus −85	Similar efficacy of AFB in q4 weeks and q8 weeks dosing

BVZ: Bevacizumab, AFB: Aflibercept, RBZ: Ranibizumab, BOLT: Bevacizumab or laser therapy, DRCR: Diabetic Retinopathy Clinical Research, CMT: Central macular thickness, PRN: Pro re nata, FDA: Food and Drug Administration

the 1st year, thus needing very little additional treatment after 3 years. The RESTORE study by the European group, a Phase III trial randomized DME patients to RBZ, RBZ plus laser, or laser.[25] In the core study, the mean vision gain at month 12 was 7.9 (RBZ), 7.1 (RBZ + laser), and 2.3 letters (laser). In the extension study, patients were allowed to receive RBZ (individualized dosing regimen). The vision was maintained from months 12 to 36 (prior RBZ: 8.0 letters; prior RBZ + laser: 6.7 letters at month 36), and the vision improved progressively in the prior laser group (6 letters at month 36).[25]

Bevacizumab

It has been used as an "off-label" drug for the treatment of DME. As the cost of this drug is much lower than that of other anti-VEGF drugs, it has become widely accepted in the clinics worldwide. The BVZ or laser therapy study compared intravitreal injections of BVZ (1.25 mg, 6 weekly) with focal/grid laser treatment for 2 years and showed a mean improvement of 8.6 letters with BVZ injections, whereas the laser group lost 0.5 letters.[26] This study was involved with 6 weekly injections of BVZ rather than 4 weekly injections in other trials. It is possible that 4 weekly injections of BVZ would provide better visual gains as seen in the RIDE/RISE RBZ trials.

Aflibercept

In the Phase II dA Vinci study, DME patients were randomized to one of the five groups: AFB 0.5 mg q4 weeks, 2 mg q4 weeks, 2 mg q4 weeks × 3 followed by q8 weeks, 2 mg q4 weeks × 3 followed by PRN treatment, and laser only.[27] At 1st year, the mean improvement of vision was 11.4 letters in the AFB groups and − 1.3 letters in the laser group, and the mean decrease in macular thickness 189 um in the AFB groups and 58 um in the laser group. In the Phase III VIVID-DME and VISTA-DME trials, patients receiving AFB (2 mg every 4 weeks or every 8 weeks compared with laser) had a mean BCVA change from baseline of 11.5 to 11.1 letters, respectively, after 2 years compared to a mean change from baseline in BCVA of 0.8 letter in patients receiving laser photocoagulation.[28] The proportion of eyes that gained ≥15 letters from baseline at week 100 was 38%, 31–33%, and 12–13% in every 4 weeks AFB, every 8 weeks AFB, and laser treatment, respectively. The efficacy of 2 mg dose every 4 weeks and every 8 weeks was very similar. The FDA has approved the use of 2 mg AFB for the treatment of DME.

The DRCR evaluated a head-to-head comparison of the efficacy and safety of these three drugs, RBZ, BVZ, and AFB in DME patients. One-year results of this trial showed that there was a little difference between these drugs in terms of efficacy when the visual acuity is 20/40 or better.[29] However, when the baseline visual acuity is 20/50 or worse, there was a clinically meaningful advantage with the use of AFB; for example, an improvement in the visual acuity of 3 Snellen lines was observed in 63% more AFB-treated eyes than BVZ-treated eyes and in 34% more AFB-treated eyes than RBZ-treated eyes [Fig. 3]. Rates of death, serious adverse events (including death), hospitalization, and systemic adverse events were similar in the three treatment groups.

There are several caveats from this study as pointed out by a report from the American Society of Retinal Specialists.[30] It is important to note that these results may not apply to eyes with persistent or recurrent DME that are already treated with anti-VEGF agents as the latter were not included in this clinical trial. If the patient has access to AFB, this drug may be used to start treatment in eyes with worse baseline visual acuity. As the cost-effectiveness of BVZ far outweighs that of AFB or RBZ, it is reasonable to start with BVZ if AFB is not available. One should remember that all three anti-VEGF drugs improve visual acuity with <5% patients developing substantial vision loss. It still remains a personal choice about which drug to choose and how frequently to inject after 3 monthly anti-VEGF injections in the center-involving DME patients.

Safety profiles

A recent Cochrane meta-analysis conducted in DME patients treated with anti-VEGF agents showed no excess risk for systemic adverse events, ATEs, and overall mortality.[31] The RIDE/RISE studies showed a higher rate of serious adverse events in the RBZ group (more in the higher dose 0.5 mg group than 0.3 mg group) compared to the sham.[22] However, the DRCR Protocol I study and the RESTORE study did not show any higher risk of systemic adverse events with the use of RBZ in DME patients. Another recent meta-analysis concluded that there is an increased risk for cerebrovascular accidents and vascular death in those patients receiving monthly injections of anti-VEGF agents for 2 years.[32] These patients had a much higher level of drug exposure than patients in other studies. This finding may not be of major concern as most patients in real life undergo less intensive therapy. For example, in many studies, patients received 9–10 injections of anti-VEGF agents in the 1st year and then 2–3 injections in the 2nd year.

Steroids

The beneficial effects of steroids in DME are due to the fact that several inflammatory cytokines and chemokines involved in the inflammatory cascade of DME are susceptible to steroids, whereas inhibition of VEGF itself may not result in neutralization of other molecules beyond VEGF [Fig. 2]. In the DRCR Protocol I, the effect of intravitreal triamcinolone and laser was equivalent to that of RBZ and laser up to 24 weeks, and then the effect of triamcinolone started to decline because of cataract formation.[33] In the subgroup of pseudophakic patients, the triamcinolone plus laser group was found to be superior to the laser alone treatment and equally effective as the RBZ group.

In another randomized, multicenter 3-year long trial, the FAME study, intravitreal inserts of fluocinolone acetonide (0.2 and 0.5 ug/day) resulted in significant visual improvement in patients with DME.[34] However, the majority of patients on fluocinolone acetonide developed cataract, and the incidence of incisional glaucoma surgery was 4.8% (low dose) and 8.1% (high dose). Recently, the FDA approved this drug for DME in those patients who have been treated with a course of corticosteroids and did not show significant intraocular pressure (IOP) rise. It is interesting to note that the FAME study showed enhanced benefits of using fluocinolone acetonide in chronic DME (>3 years duration) compared to nonchronic DME (<3 years duration). It has been hypothesized that the disease may be driven by VEGF early in the disease, but in chronic DME, micro-environmental changes necessitate targeting of multiple mediator molecules with steroids. Another steroid, dexamethasone has been investigated in the MEAD study that

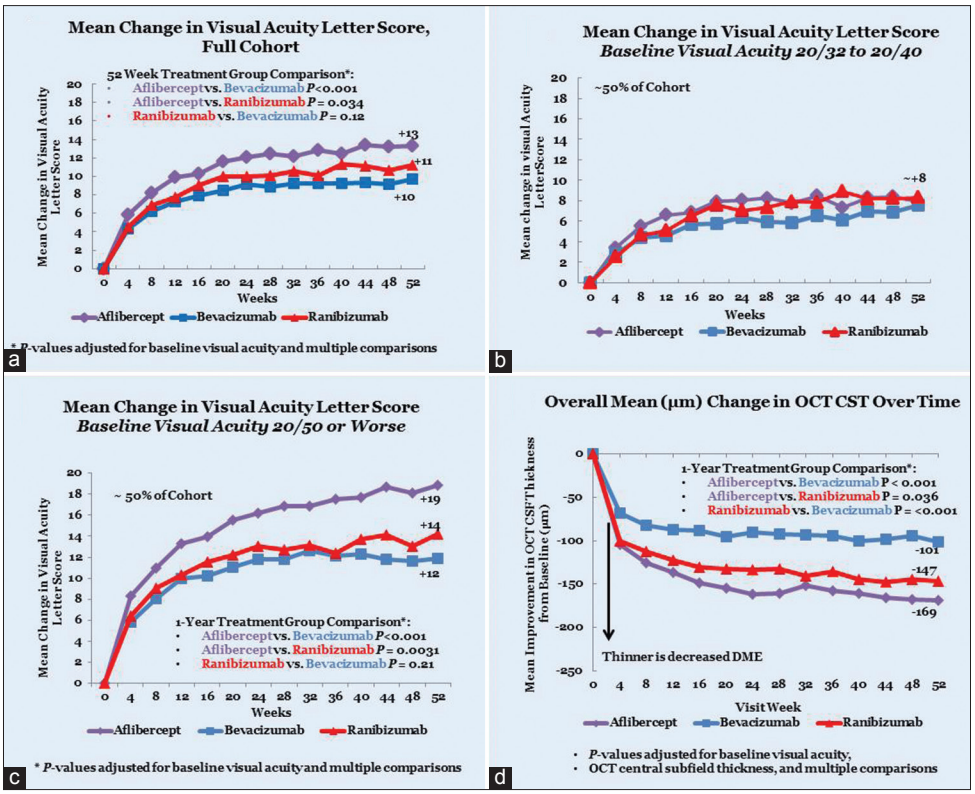

Figure 3: (a) Mean change in visual acuity letter score over time for the full cohort over a period of 1 year, the mean visual acuity letter score improved by about 13 with aflibercept, by 10 with bevacizumab, and by 11 with ranibizumab. (b) When the initial visual acuity letter score was 78–69 (equivalent to approximately 20/32–20/40), the mean improvement was about 8.0 with no significant difference between aflibercept, bevacizumab, and ranibizumab. (c) When the initial letter score was < 69 (approximately 20/50 or worse), the mean improvement was about 19 with aflibercept, 12 with bevacizumab, and 14 with ranibizumab. (d) Mean change in optical coherence tomography central subfield thickness over time the overall decrease in central subfield thickness over 1 year was 101 um for bevacizumab, 147 um for ranibizumab, and 169 um for aflibercept (courtesy of Diabetic Retinopathy Clinical Research Network)

examined slow-release intravitreal dexamethasone implants (Ozurdex, Allergan Inc.,) that release sustained levels of dexamethasone for 6 months.[35] There was more than three line visual improvement in about 22% patients (0.7 mg) and 18% (0.35 mg) in the dexamethasone group compared to the sham group (12% patients). Cataract formation was seen in up to 68% of the patients on dexamethasone, and IOP rises could be managed with topical medications. Because of an increased rate of elevated IOP and cataract formation with steroids, the use of intravitreal steroids in clinical practice is currently reserved as the second-line treatment in center-involving DME patients. The combination of anti-VEGF agents and steroids may be more effective in certain DME patients who are difficult to control with anti-VEGF agents alone.

Anti-vascular endothelial growth factor drugs in diabetic retinopathy

One of the interesting observations from the RIDE/RISE study was that RBZ-treated patients were less likely to develop PDR. Using the ETDRS retinopathy severity scale for eyes, it was revealed that more RBZ-treated eyes showed substantial (≥2- and ≥3-step) improvements in retinopathy severity and fewer showed substantial worsening.[22] The clinical significance of retinopathy improvement is still unknown. Similar benefits of RBZ in retinopathy progression were also seen with the use of AFB in DME patients in the VIVID/VISTA studies.[28] It is yet to be determined whether such beneficial effects of anti-VEGF agents last even after cessation of their use. The FDA has expanded the approved use of both RBZ (0.3 mg) and AFB (2 mg) to treat diabetic retinopathy in patients with DME.

Anti-vascular endothelial growth factor drugs in proliferative diabetic retinopathy

Panretinal photocoagulation (PRP) laser has been found to be very effective in rapid regression of retinal neovascularization seen in the majority of PDR patients. A recently completed clinical trial (DRCR Protocol S) compared the anti-VEGF therapy (RBZ monthly for 6 months) with the PRP laser in PDR patients.[36] Intravitreal RBZ was found to be no worse than (noninferior to) PRP laser treatment in PDR. There was no statistically significant visual acuity difference between the two groups. As expected, more peripheral visual field loss occurred and more vitrectomies were needed in the PRP group compared with the RBZ group. The advantages of the PRP treatment include an effective treatment that can be completed in one visit, minimal cost, and absence of risk of endophthalmitis or systemic exposure to anti-VEGF agents. The advantages of the RBZ treatment include absence of visual field loss and less eyes developing DME or needing vitrectomy. The physician's decision whether to use PRP versus anti-VEGF therapy in PDR may depend on the presence or absence of DME. In presence of DME, the anti-VEGF therapy may be preferable as it will treat both DME and PDR, provided the patient is compliant for monthly visits for injections. The DRCR suggests considering treatment cost, compliance, and frequency of follow-up and

patient preference in choosing one procedure over the other. Furthermore, one needs to be careful in using anti-VEGF agents in PDR patients with significant fibrovascular membranes as these agents may worsen traction retinal detachment.

What's in The Horizon?

As the current anti-VEGF therapies, although effective, have limitations in terms of frequent injections, treatment burdens, and complete efficacy in DME patients, novel therapies are being investigated. Strategies targeting molecules beyond VEGF are being explored along with novel drug delivery mechanisms [Fig. 4].[37]

Anti-vascular endothelial growth factor agents

High dose of anti-vascular endothelial growth factor drugs

As the VEGF level varies in the vitreous of patients with DME, it has been proposed that those poor responders with anti-VEGF therapy may need much higher doses of anti-VEGF drugs due to higher levels of VEGF. With this concept, a new study, READ-3 has examined the efficacy of RBZ at two different doses (0.5 mg and 2.0 mg) in DME patients. However, 1-year results from this study showed no additional benefit in using 4 times higher dose than the regular dose.[38] In addition, in the RIDE/RISE trials, the efficacy of both doses of RBZ (0.3 mg and 0.5 mg) was equivalent. As there is less chance of any potential side effects with the lower dose, the FDA approved the 0.3 mg RBZ for DME.[22]

Designed ankyrin repeat protein

Novel molecules such as designed ankyrin repeat proteins (DARPins) have been engineered to target VEGF-A. These agents have higher potency and longer half-life (2 weeks). A Phase II trial of DARPins (abicipar pegol) for wet AEMD patients has shown better visual gain compared to RBZ with fewer injections.[39] A randomized Phase II trial (Allergan) of abicipar pegol in DME patients is in progress.

Other anti-VEGF approaches include targeting a central regulator such as mammalian target of rapamycin, src kinase, and RTP 801 gene.

Extended drug delivery

As the monthly intravitreal anti-VEGF injections are treatment burdens for the patients, sustained release delivery systems are more useful in treating chronic diseases such as DME. Several approaches in this direction include bioerodible implants, bioerodible microspheres, encapsulated cells, and gene therapy. Recently, a Phase I clinical trial using a refillable, nonbiodegradable long-term drug delivery implant for RBZ has been completed on twenty patients with wet macular degeneration. The trial showed a constant mean improvement of vision of 10 letters throughout 1 year. The implant, based on the passive, diffusion-controlled drug delivery mechanism, can be refilled in the office as needed. A Phase II trial (LADDER) using this implant has been just initiated in wet ARMD patients. If the results are successful in ARMD patients, these implants will be useful in DME patients also.[40]

Encapsulated cells

Implants using encapsulated cells utilize an RPE cell line that produces a soluble VEGF receptor protein for at least 2 years. Cells are encapsulated in a semi-permeable membrane that allows selective passage of molecules. A Phase II clinical trial using NT-503 encapsulated cell therapy (Neurotech) has been started in wet ARMD patients with recurrent choroidal neovascularization.[41]

Inhibitor of multiple growth factors

Squalamine, a small molecule anti-angiogenic drug, targets VEGF along with platelet-derived growth factor and bFGF. This compound was discovered in tissues of dogfish sharks that has anti-angiogenic and antiviral properties. A Phase II trial (IMPACT) using a combination of squalamine eye drops and RBZ injections has shown better visual improvement in comparison to RBZ alone. A Phase II trial using squalamine is in progress in DME patients.[42]

Steroids

Several steroid preparations such as betamethasone microspheres (subtenon injection), dexamethasone-cyclodextrin (topical), loteprednol (topical), and danazol (oral) have been in different phases of clinical trials in DME patients.

Cytokine inhibitors

Angiopoietin-2

Angiopoietins belong to a family of growth factors that bind endothelial receptor tyrosine kinase Tie-2. There are two ligands, Ang-1 and -2 that bind to Tie-2. Ang-2 destabilizes blood vessels whereas Ang-1 stabilizes. Ang-2 is upregulated in retinas in an animal model of diabetes, and increased Ang-2 leads to increased retinal vascular permeability.[43] Many systemic conditions (sepsis, acute lung injury, systemic capillary leak syndrome, disseminated intravascular coagulation, and acute kidney injury) with vascular leakage have been reported with elevated Ang-2 levels in blood. This pathway has been targeted in a recent ongoing clinical trial with a Tie-2 activator (AKB-9778, Aerpio Therapeutics) in DME patients.[44] The

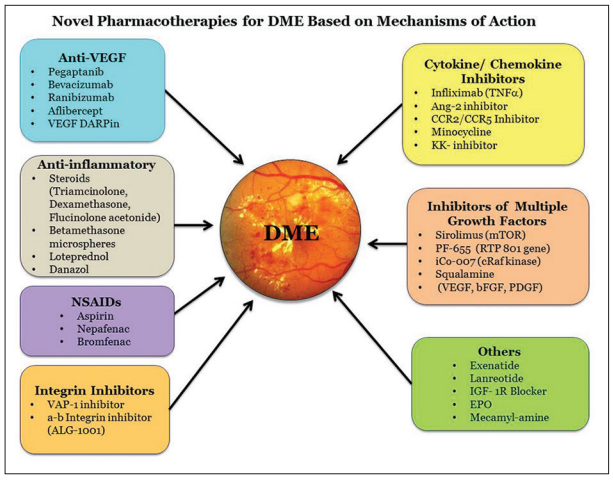

Figure 4: Novel pharmacotherapies for diabetic macular edema based on mechanisms of actions. Several anti-vascular endothelial growth factor inhibitors (ranibizumab and aflibercept) and steroids (dexamethasone and fluocinolone) are approved for use in diabetic macular edema patients while many other drugs are in clinical trials and preclinical stage for development. DARPin: Designed ankyrin repeat protein, NSAIDs: Nonsteroidal anti-inflammatory drugs, TNFα: Tumor necrosis factor α, CCR2/CCR5: Chemokine receptor 2 and 5, KK: Kallikrein-kinin, mTOR: Mammalian target of rapamycin, bFGF: Basic fibroblast growth factor, PDGF: Platelet-derived growth factor, IGF: Insulin-like growth factor

combination of AKB-9778 and RBZ provided significant benefit over RBZ monotherapy in reduction of macular edema with a trend toward improved visual acuity in 3 months. One advantage of this drug is its subcutaneous administration that can be easily done by patients themselves.

Tumor necrosis factor

TNFα is an important cytokine that has been implicated in many inflammatory diseases including rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, and Crohn's disease. A double-blind, randomized, placebo-controlled, crossover study in a small number of DME patients showed significant improved visual acuity and reduction in retinal thickness with intravenous infliximab (TNFα inhibitor) (5 mg/kg) intravenously.[45] Larger trials are needed to confirm the efficacy of these drugs in DME patients.

Interleukins

IL-6 is an important cytokine that has been consistently elevated in vitreous of patients with DME along with VEGF. A clinical trial using an intravitreal IL-6 antibody (EBI-029, Eleven Biotherapeutics, Cambridge, MA, USA) will examine its efficacy in DME patients.[46]

Chemokine inhibitors

The chemokine, CCL2, also known as monocyte chemotactic protein-1 (MCP-1) is considered to play an important role in vascular inflammation by inducing leukocyte recruitment and activation. This molecule is critical for monocyte trafficking and microglial activation in the retina seen in early diabetic retinopathy.[10] Clinical trials using inhibitors of the chemokine pathway are in progress in systemic diseases such as atherosclerosis, chronic kidney diseases, diabetes, and diabetic nephropathy. An oral inhibitor targeting the receptors for chemokine, CCR2/CCR5 (Pfizer, USA), is being currently examined in an ongoing clinical trial in DME patients in comparison with intravitreal RBZ.[47]

Kallikrein-kinin inhibitor

Vitreous proteomics has shown increased levels of plasma prekallikrein and plasma kallikrein (PKal) levels in vitreous of DME patients.[48] Intraocular activation of the PKal-kinin pathway may contribute to increased retinal vascular permeability many patients with DME. A Phase II clinical trial using an inhibitor of PKal (Kalvista) has been completed in DME patients. A Phase II trial using the same inhibitor in DME patients is in progress.[49]

Integrin inhibitors

Integrins are cell surface receptors to the extracellular matrix immunoglobulin molecules. The early step of inflammation, leukostasis, or adherence of leukocytes to the endothelium is dependent on specific integrins on the endothelium. A Phase II trial using an integrin antagonist, Luminate, ALG-1001 (Allegro Ophthalmics, LLC, USA), is in progress in DME patients for comparison with BVZ and focal laser therapy.[50]

Conclusions

The advent of anti-VEGF agents in the last decade has revolutionized the treatment of diabetic retinopathy. Anti-VEGF drugs are the first line of treatment in center-involving DME. Once the focal/grid laser therapy as recommended by the ETDRS was considered to be the standard treatment for DME, it is now being reserved only for noncenter-involving macular edema. The effect of anti-VEGF therapy is now shown to be noninferior to the PRP laser in PDR patients. However, the effect is much less robust in DME. Multiple, frequent monthly anti-VEGF injections are needed in DME patients, and a significant number of patients poorly respond to these anti-VEGF drugs. DME appears to be a heterogeneous disease with good responders to anti-VEGF drugs on one end of the spectrum, poor responders on the other end of the spectrum, and many intermediate responders in between. In the latter two groups, probably factors other than VEGF are responsible for alteration of the BRB. Multiple chemokines and cytokines are being targeted as alternative therapies for DME and are being tested in several clinical trials. Currently, there are no tests or biomarkers to predict which DME patients may be good, poor, or intermediate responders. The genetic factors may play an important role in this anti-VEGF responsiveness. New drug delivery systems using nanotechnology, sustained-release delivery implants, and stem cell therapy are in development. A combination therapy of novel inhibitors targeting the molecules beyond VEGF with laser or standard anti-VEGF agents may be more effective in treating DME in the coming years.

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Conflicts of interest

There are no conflicts of interest.

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