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Review Article

Targeted Inhibition of Tumour Vascularisation Using Anti-PDGF/VEGF Aptamers

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Abstract

Platelet-derived growth factor (PDGF) is one of the numerous protein growth factors that plays a significant role in embryonic development, cell proliferation, cell migration and particularly, in blood vessel formation (angiogenesis). Its sub-family, vascular endothelial growth factor (VEGF) is another important growth factor that functions in creating new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels to bypass blocked vessels. Over expression of PDGF/VEGF can contribute to diseases such as vascular disease in the retina of the eye and other parts of the body, eventually leading to cancer. Thus, PDGF and VEGF antagonists for therapy of neovascular disorders and cancer have become important research topics for scientists during the last decade. Recently it has been reported that aptamers or chemical antibodies can bind to and inhibit the activity of PDGF/VEGF with highly affinity and specificity.

The effect of anti-PDGF and anti-VEGF therapy to treat tumour vascularisation is discussed in this review. Besides that, the benefits and drawbacks of three commercialized drugs, namely Avastin, Macugen and Lucentis, and examples of anti-VEGF/PDGF aptamers, such as E10030 have also been elaborately discussed.

Introduction

Platelet-derived growth factor (PDGF) is one of the critical proteins growth factors that stimulates the division and proliferation of cells by binding to its receptors on cell surfaces [1-3]. It is also defined as a potent mitogen and chemotactic factor for many connective tissue cells, such as fibroblasts, smooth muscle cells, and in-glial cells in culture [1-5]. Vascular endothelial growth factor (VEGF) is another signal protein from the sub-family of PDGF [5, 6]. It is a secreted disulfide-linked homodimer and considered as an important signalling protein that involved in vasculogenesis (the formation of new blood vessels by de novo production of endothelial cells without pre-existing vasculature) and angiogenesis (the growth of blood vessels from pre-existing vasculature) [6-9]. VEGF's normal function is to create new blood vessels during embryonic development [6], new blood vessels after injury [6,7], muscle following exercise [7,9], and new vessels (collateral circulation) to bypass blocked vessels [6-9]. On the other hand, PDGF plays similarly role in embryonic development [5], cell proliferation [1-3], cell migration [2,3] and particularly, in blood vessel formation (angiogenesis) [1-5]. When PDGF/VEGF is over expressed, it can contribute to disease such as vascular disease in the retina of the eye and other parts of the body [1-9,14,15].

Due to alternative the splicing of the VEGF gene, VEGF may occur in four isoforms including VEGF-121, VEGF-165, VEGF-189, and VEGF-206, all of which show different heparin-binding affinities [6-9]. VEGF-121 and VEGF-165 are diffusible whereas VEGF-189 and VEGF-206 remain predominantly localized to the cell membrane as a consequence of their high affinity for heparin [6,7]. In addition, VEGF-165 can also bind to heparin and it is the predominant isoforms in the body [7-9]. Only VEGF-121 is incapable of binding with heparin that appears to have a lower affinity for VEGF receptors as well as lower mitogenic potency [6-8]. All members of the VEGF family stimulate cellular responses by binding to receptors VEGFR-1(Flt-1) and VEGFR-2 (Flk-1/KDR) on the cell surface, allowing them to dimerize and get activated via trans-phosphorylation [6-9]. These receptors are classified as a receptor tyrosine kinase (RTK), a type of high affinity cell surface receptors for polypeptide growth factor [5]. The PDGF dimer on the other hand, stimulates responsive cells by cross linking two RTK receptor subunits [1-4]. In both mouse and human, the PDGF signalling network consists of four ligands, included A (PDGF-A), B (PDGF-B), C (PDGF-C), and D (PDGF-D), and two receptors, alpha (PDGFR α) and beta (PDGFR β) [1-5] (Table1).

The active PDGF molecule is composed of two disulfide-linked polypeptide chains and thus can exist in the homo dimer forms AA, BB, CC, DD or the hetero dimer form AB [1-5]. The different forms of PDGF exhibit different affinities for binding to the two forms of the PDGF receptor. For examples, PDGFR α can interact with PDGF-AA, BB, CC, and AB; PDGFR β binds only to PDGF-BB, DD with high affinity, and PDGF-AB with low affinity; whereas PDGFR α β binds PDGF-BB, CC, DD, AB notably, both α and β receptors transduce

Table 1: Cellular Effects Mediated by PDGF-α, β Receptors.

Effect	α -Receptor	β -Receptor	References
Cell growth	Stimulation	Stimulation	[1-4]
Apoptosis	N/A	Inhibition	[1-4]
Chemotaxis	Stimulation	Stimulation	[1-5]
Increased *IFP	N/A	Stimulation	[1-4]
Angiogenesis	Stimulation	Stimulation	[1-4]

***IFP:** Interstitial Pressure

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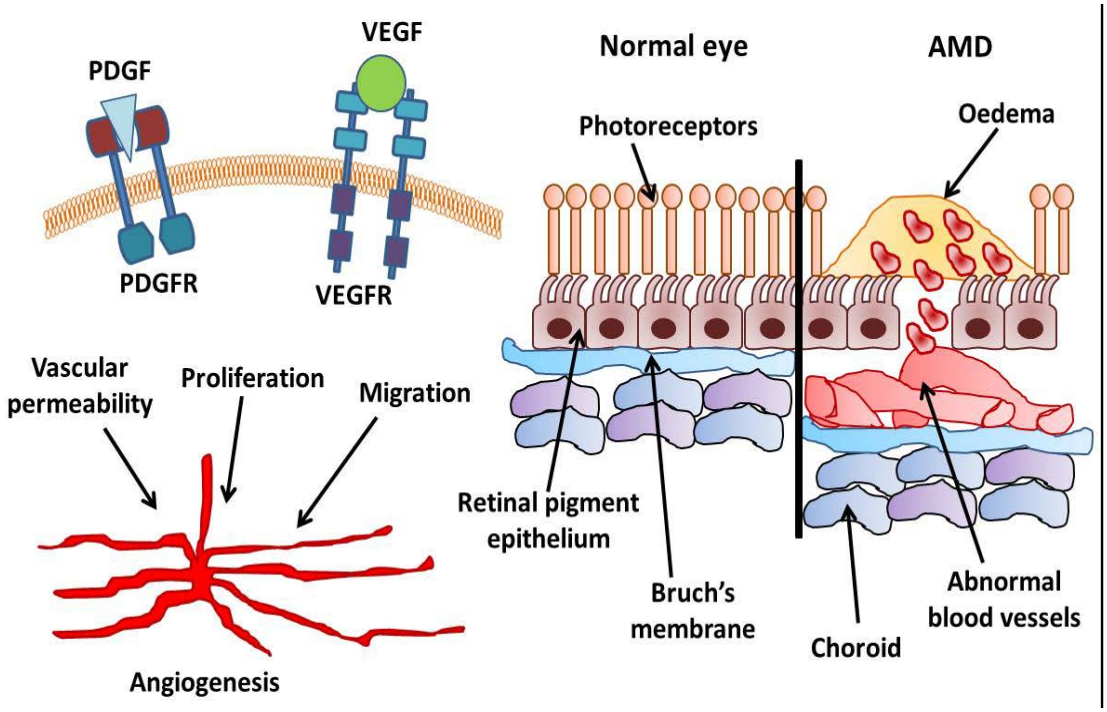

Figure 1: Role of PDGF and VEGF in angiogenesis and AMD. **Legend:** Diagrammatic representation showing role of VEGF and PDGF in angiogenesis. Uncontrolled angiogenesis further leads to cancer progression and abnormal angiogenesis can lead to ocular neovascular disorders such as age-related macular degeneration.

potent mitogenic signals [1-4]. Thus, the different combinations allow for different modulation of cell signalling pathways by different ligands-receptor pairs [1-4].

Elevation of interstitial fluid pressure (IFP) is a physiologically distinctive property of solid tumours that differs from healthy connective tissue, increases as a function of tumour size and malignancy [10-13]. This is considered to be a major obstacle in treatment of tumours by chemotherapy. High IFP in cancer patients perturbs the transcapillary transport which can be correlated with poor prognosis and poor drug uptake. Infact many solid tumours which are currently being treated with chemotherapy regimens, have high IFP and display paracrine signalling of PDGF and VEGF in their stromal compartments [10-13].

Angiogenesis (also called neovascularisation) is a normal but vital physiological process involving the growth of new blood vessels from pre-existing vessels [14-15]. Uncontrolled angiogenesis can lead to cancer [14]. *In vivo* studies [1-9,14,15] revealed that expression of PDGF and VEGF in normal cells occurs at undetectable or very low levels, while in cells with malignancies or developmental abnormalities, it is over expressed. In addition, both VEGF and PDGF-B have been reported [1-9] for their important role as a positive regulator of the abnormal angiogenesis accompanying tumour growth. There are several neovascular disorder involve alterations in angiogenesis, including psoriasis [4], rheumatoid arthritis [15], and ocular neovascular disorders such as age-related macular degeneration (AMD) and retinal vein occlusions (RVO) [16-30]. These two ocular diseases are the leading cause of blindness in Australia, affecting one person in three over the age of 80 [22-30]. There are also one in seven people aged over 50 have some evidence of these diseases in many developing countries [16-30].

AMD is accompanied by distortion of central vision with objects appearing larger or smaller or straight lines appearing distorted, bent, or without a central segment [16-30]. Both the two forms, dry and wet AMD can lead to severe vision loss but wet-AMD occur rapidly and are more visually devastating with a higher risk of blindness. Notably, wet-AMD occurs when abnormal blood vessels behind the retina starts to grow under the macula [20] (Figure 1).

On the other hand, RVO occurs due to the clotting of blood in a retinal vein, i.e. blood returning from the retina is blocked, causing the occluded retina to swell [35]. The two forms of RVO are central RVO (CRVO) and Branched RVO (BRVO); both types have differences and similarities in pathogenesis as well as clinical presentation [35]. CRVO is the obstruction of the central retinal vein at the optic nerve, and the obstruction at a branch of the retinal vein is known as BRVO [29].

During the last decade, using antagonists (or inhibitors) of PDGF and VEGF in therapy for neovascular disorders has been reported frequently. Generally, inhibition of PDGF and VEGF can be accomplished in a variety of ways. For example, nucleic acid molecules such as antisense RNA, ribozymes, and RNAi that inhibit PDGF and VEGF expression at the nucleic acid level are commonly used as antagonists [1-9]. Besides that, the potential of antibodies and aptamers have also been reported in inhibiting the activity or production of PDGF/VEGF. Aptamers (also known as nucleic acid ligands) are non-naturally occurring nucleic acids that can bind to and inhibit a pre-selected target, including small molecules (cocaine, adenosine, and potassium), biological entities such as proteins and

Table 2: Research trend in anti-PDGF/VEGF therapy.

Drug	Target	Approval Year	Disease	References
Lapatinib	Small molecule tyrosin kinase inhibitor (VEGF)	FDA, 2007	Breast cancer	[36,37]
Sunitinib (SU11248)	Small molecule tyrosin kinase inhibitor (VEGF, PDGFR-B)	FDA, 2006	Renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumour	[38]
Sorafenib	Small molecule tyrosin kinase inhibitor (VEGF, PDGFR-B)	FDA, 2005, 2007 and 2013	Renal cell carcinoma (2005), hepatocellular carcinoma (2007) and differentiated thyroid carcinoma (2013)	[39]
Axitinib	Small molecule tyrosin kinase inhibitor (VEGF)	FDA, 2012	Renal cell carcinoma	[40]
Pazopanib	Small molecule tyrosin kinase inhibitor (VEGF)	FDA, 2009, 2012	Advanced/metastatic renal cell carcinoma (2009) and soft tissues sarcoma (2012)	[41]
Bevacizumab (Avastin)	VEGF-A	FDA, 2004, 2006, 2008, 2009,	Metastatic colorectal cancer, Metastatic HER2 negative breast cancer (2008), metastatic renal cancer (2009), second-line treatment for glioblastoma (2009), first line treatment for non-small cell lung cancer (2006), second line treatment for metastatic colorectal cancer (2006) and first line treatment for metastatic colorectal cancer (2004).	[42]
Macugen (Pegaptanib)	VEGF-165	FDA, 2004	Age related macular degeneration	[43]
Lucentis (Ranibizuman)	VEGF-A	FDA, 2010, 2012	Macular edema following renal vein occlusion (2010) and diabetic macular edema (2012)	[44]
Verteporfin (visudyne)	Abnormal blood vessels (photodynamic therapy)	FDA, 2000	Wet age related macular degeneration	[45]
Anti-PDGF aptamer E10030 (Fovista)	PDGF-B	Phase III trials 2014	Subfovealchoroidal neovascularization secondary to age-related macular degeneration (AMD)	[46]

cells with high affinity and specificity [31-36]. Both antibodies [16-21] and aptamers [22-28] have been described to have similar therapeutic functions with binding affinities in the low nanomolar range. However, some unique properties of aptamers make them attractive therapeutic agents compared with antibodies [31-36]. Firstly, aptamers can be heated to 80°C or stored in various solvents/harsh environments, offer ease of screening, synthesis and stability [16-19]. By providing longer shelf-life, it gives molecular engineers the advantage of constructing aptamers in a wide range of bioassays as well as in the development of protein arrays [17-20]. Another important feature of aptamers is that they are essentially non-immunogenic even when administered in excess of therapeutic doses [31-33]. By contrast, antibodies are significantly immunogenic precluding repeat dosing unless they are "humanized" or produced fully in humans [31-35].

Anti-PDGF/ VEGF Treatment Decreases Interstitial Fluid Pressure

Both *in vitro* and *in vivo* studies demonstrate that treatment with inhibitors of PDGF or VEGF signalling lowers the IFP of tumours and improve chemotherapy, leading to tumour growth inhibition [1-9]. Studies conducted [10-13] by Pietras et al. have revealed that anti-PDGF-B aptamer can effectively inhibit PDGF-B–dependent IFP and increase the uptake of chemotherapeutics in tumour cells. As mentioned above, PDGF is known to up-regulate synthesis of collagen and mediate interactions of anchor proteins with extracellular matrix components. In normal tissues, PDGF regulates IFP by acting on stromal fibroblasts and causing an integrin-mediated contraction of the cells that affects the extracellular matrix [10,11]. In another study, Agnieszka [13] et al. investigated if the combination of PDGFR and VEGFR inhibitors could further reduce the IFP of KAT-4 human carcinoma tumours. Besides that, the effects of inhibitors on the therapeutic efficiency of chemotherapy were also investigated. In their study, STI571 was used as a PDGF receptor tyrosine kinase inhibitor and the inhibitor PTK/ZK was used for the VEGF receptor kinase. They concluded that simultaneous targeting of VEGFR and PDGFR kinase activity may be a useful strategy for decreasing tumour IFP.

Anti-PDGF/ VEGF Therapy Inhibits Angiogenesis

A summary of the research trend in anti-PDGF/VEGF therapy over the years with all the therapeutic involved has been represented in Table 2.

Some of the synthetic and aptamers based drugs achieved enormous success and gained much patient compliance over other drugs and therefore have been discussed in detail below.

Bevacizumab

Bevacizumab (Avastin) is a very popular angiogenesis inhibitor, and was one of the first commercialized drugs (approved by FDA) in the world [16-21]. As mentioned earlier, VEGF is a chemical signal that stimulates angiogenesis in a variety of diseases, especially in cancer, retinal proliferation of diabetes in the eye. Bevacizumab stops tumour growth by preventing the formation of new blood vessels by targeting and inhibiting the function of VEGF that stimulates new blood vessel formation [16-19]. Hence Bevacizumab therapy has also been applied in many diseases, including breast cancer, ovarian cancer, prostate cancer, liver cancer, pancreatic cancer and renal cell carcinoma [16-20]. Some clinical studies have been looking at the anti-angiogenesis effects of Bevacizumab [19,20].

Phase I clinical trial

Willett et al. [18] conducted the phase I clinical trial of Bevacizumab to prove that this VEGF-specific drug has anti-vascular effects in the human tumours. Six patients with primary and locally advanced adenocarcinoma of the rectum were enrolled in a preoperative treatment protocol of Bevacizumab administration (5 mg/kg intravenously) for 2 weeks, followed by concurrent administration of Bevacizumab with 5-fluorouracil (5-FU) and external beam radiation therapy to the pelvis, and surgery, 7 weeks after treatment completion. 12 days after Bevacizumab infusion, the

sigmoidoscopy showed that Bevacizumab induced tumour regression in a patient, but no significant change in tumour size in the remaining five patients. Besides that, functional computed tomography (CT) scans at this time point indicated significant decreases in tumour blood perfusion, blood volume, which led to the reduction of tumour micro vascular density (MVD). The study also revealed that high doses of Bevacizumab are more effective when used as monotherapy for highly VEGF-dependent tumours such as renal-cell carcinoma. In addition, the results also showed that 12 days after Bevacizumab treatment, interstitial fluid pressure (IFP) was decreased in a few patients.

Phase II Clinical trial

Kabbinavar et al. [20] conducted the phase II trial on efficacy and safety of Bevacizumab. This Phase II colorectal cancer trial was a randomized, open-label study that combined with 5-FU/leucovorin (LV)/ leucovorin (LV) chemotherapy in 104 subjects with previously untreated metastatic colorectal cancer. The Bevacizumab treatment group took longer time for disease progression and had longer duration of survival when compared with control group. Notably, the lower-dose Bevacizumab group (5 mg per kg every two weeks) fared better than the higher dose (10 mg per kg every two weeks). It is possible that the lower dose of Bevacizumab may have resulted in improved delivery of chemotherapy as well as an antitumor effect, whereas the higher dose could have led to more advanced regression of blood vessels. This phase II studies showed the ability to improve delivery of chemotherapy by reducing intra-tumour pressure which can result in greater efficacy than the ability to cause vascular collapse inside the tumour. Kabbinavar et al. [20] explained that diarrhoea, leukopenia, and stomatitis are known to be associated with 5-FU/ LV. As a result, the incidence and severity of these adverse events was as expected when Bevacizumab was added to the regimen. The most clinically significant of these events was thrombosis. Despite being a known complication of cancer therapy, thrombosis occurred more frequently with Bevacizumab than with chemotherapy alone. Kabbinavar et al. concluded that Bevacizumab should be used in combination with chemotherapy, which can be a new standard option for the treatment various tumour types (including renal-cell cancer, metastatic breast cancer and colorectal cancer) which is also currently under investigation.

Besides Bevacizumab, many other VEGF inhibitors have also been clinically pursued, such as Macugen and Lucentis.

Macugen (Pegaptanib)

Macugen (formerly known as NX1838) is the first aptamer approved by Food and Drug Administration USA (FDA) to treat all types of wet AMD [21-33]. It is administered locally into the eye, with sterile, aqueous solution aspegaptanib sodium via intravitreous injection [28-30]. Macugen is specific for the isoforms VEGF-165, which is proposed to make it safer and more specific than providing a complete VEGF blockade [22-26]. Since VEGF-165 is the most abundant isoforms, it is largely responsible for promoting leukocyte recruitment and enhanced expression of intracellular adhesion molecules that are associated with the development of ocular neovascularisation [21-27].

Prior to being called pegaptanib, Macugen was also referred to as EYE001. Susumu et al. have reported the anti-VEGF effect of EYE001 on another ocular disorder [22,23], known as diabetic retinopathy. This disease is frequently complicated by macular edema, a pathologic condition that is a direct consequence of blood–retinal barrier (BRB) breakdown [23]. Notably, macular edema is one of the greatest sources of vision loss in diabetes that can appear at any time during the course of diabetic retinopathy [23]. In their study [22,23], Susumu et al. found that treatment with the anti-VEGF165 aptamer EYE001 resulted in 72.4% blockade of early diabetic retinal leukostasis as compared with control groups. Besides that, treatment with the EYE001 also resulted in 82.6% blockade of early diabetic BRB breakdown as compared with three controls.

In the clinical studies by VISION (VEGF Inhibition Study in Ocular Neovascularisation) [26], Macugen (Pegaptanib sodium 0.3, 1 or 3 mg by intravitreous injection or sham injection) for 240 patients with wet macular degeneration, was administered every 4-6 weeks for 48 weeks. After two years, Macugen treatment significantly reduced the risk of moderate vision loss in patients with wet AMD. On the other hand, patients receiving usual care (who either received sham or no treatment) had the poorest visual outcomes throughout the two years. The clinicians concluded that Macugen cannot fully stop the disease, but it can slow the growth of abnormal blood vessels, thus slowing the damage to the sight. The most frequently reported side effects in patients treated with Macugen includes anterior chamber inflammation, conjunctivalhemorrhage, corneal edema, hypertension, increased intraocular pressure (IOP), ocular discomfort, punctate keratitis, vitreous floaters, vitreous opacities, burning sensation, eye pain, eye irritation, redness, light sensitivity, vision loss, blurred vision, visual disturbances, high blood pressure, and cataract.

Lucentis (Ranibizumab)

Lucentis (also known as ranibizumab) is the latest in a group of anti-angiogenic drugs that approved by the FDA on 2006, particularly for the treatment of neovascular (wet) AMD and RVO [28,29]. It is administered via injection into the back of the eye (intra vitreal injection). It binds with high affinity to the VEGF-A isoforms (e.g. VEGF110, EGF121 and VEGF165), thereby preventing binding of VEGF-A to its receptors VEGFR-1 and VEGFR-2 [28,29].

A phase III clinical study [29] called minimally classic/occult trial of the anti-VEGF antibody ranibizumab in the treatment of neovascular AMD (MARINA) reported that approximately 95% of patients treated with Lucentis maintained or even improved their vision after treatment. Vision improved by at least four lines (or 21.5 letters) on the study eye chart within two years. Alfredo et al. [28] have been looking at the efficacy and safety of anti-VEGF therapy with Lucentis for naive RVO. In their 1-year follow-up period, they injected 0.5 mg of ranibizumab to a 76-year old woman with CRVO at baseline and six months after the first intra vitreal injection, they found significant reduction in the patient's intraretinal bleeding, optic disc swelling and vascular leakage.

Anti-PDGF Aptamer- E10030

E10030 is a pegylated aptamer that binds to PDGF-B with high specificity and affinity and inhibits the functions of PDGF-B both *in vitro* and *in vivo* [47-49] As mentioned previously, PDGF plays

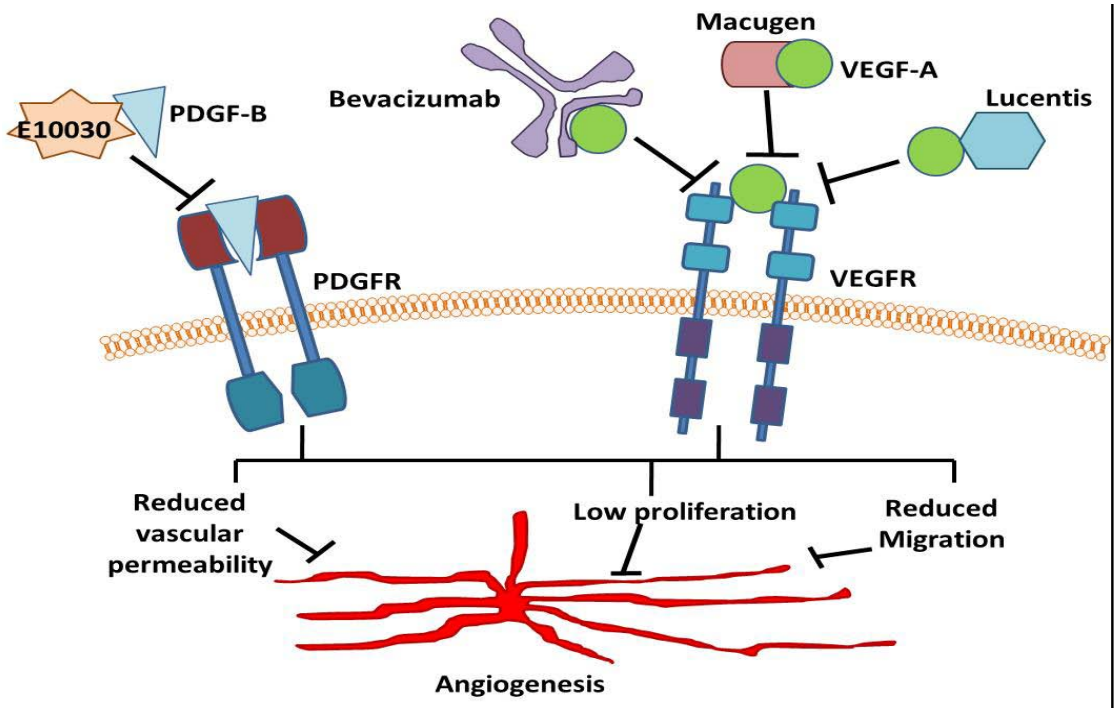

Figure 2: Currently used PDGF and VEGF antagonists. **Legend:** Diagrammatic representation showing the commercially available and most frequently used PDGF and VEGF antagonists that inhibit PDGF-B and VEGF-A respectively and suppress the uncontrolled and abnormal angiogenesis.

Table 3: Advantages and shortcomings of anti-PDGF/ VEGF therapy.

Therapeutic	Drawbacks	Benefits	References
Bevacizumab (Avastin)	Side effects such as: Gastrointestinal perforation, serious bleeding, delayed wound healing, abnormal passage in body, stroke or heart problems, kidney problems. Multiple dosages are required.	Is approved by FDA for: metastatic colorectal cancer, glioblastoma, advanced nonsquamous non-small cell lung cancer, metastatic kidney cancer, metastatic breast cancer, Can be used in combination with 5-fluorouracil (5-FU), carboplatin and paclitaxel. It is a targeted therapy against VEGF	[53-55]
Macugen (Pegaptanib)	Showed high affinity to VEGF-165 over other forms such as VEGF-121, Addition of PEG moiety reduced the affinity by ~4 fold. Side effects include: Respiratory disorder, gastrointestinal disorders, nervous system related disorders and cardiovascular disorders.	High intravitreal residence time. Is approved by FDA for age-related macular degeneration.	[56-57]
Lucentis (Ranibizumab)	Side effects include: eye pain, redness, bleeding, headache, impaired vision. Has to be administered with local anaesthetic.	Low dosage frequency. FDA approved for diabetic macular edema, neovascular (wet) age-related macular degeneration.	[44-58]
Anti-PDGF aptamer E10030 (Fovista)	Addition of PEG moiety reduces the binding affinity. Side effects include eye pain, irritation, blurred vision and headache.	Inhibits both PDGF as well as VEGF for patients with wet age-related macular degeneration. High intravitreal residence time.	[59]
Verteporfin (Visudyne)	Photodynamic therapy (drug is activated by light), not much of drug reaches eyes (systemic delivery), temporarily slow the wet age-related macular degeneration but can't reverse the disorder. Side effects include eye pain, irritation, blurred vision and photosensitivity.	Painless, specifically eliminates the abnormal blood vessels in eye. FDA approved for wet-age related macular degeneration	[60]

an important role in regulating the recruitment and maturation of pericytes as mentioned above. As a result, E10030 strongly binds to PDGF resulting in pericyte stripping in ocular and oncological models of pathologic neovascularisation [48]. In preclinical studies, E10030 also showed the potential to regress neovascularisation when used in combination with other VEGF inhibitor [48-51]. As mentioned above, monotherapy with an anti-VEGF agent is the current standard of care for wet AMD. However, there is a significant limitation of this monotherapy anti-VEGF approach, which is lack of regression of the new vessels that are responsible for the visual loss in wet AMD [49-51]. Therefore, by combining of E10030 and other anti-VEGF agent (such as Lucentis), thereby targeting pericytes and endothelial cells respectively, can induce significant neovascular regression in multiple preclinical ocular and tumour models of angiogenesis [47-51].

Ophthotech [52] reported that concurrent inhibition of the

binding of PDGF to its receptor, PDGFR-β plus an anti-VEGF agent was superior to inhibition of the VEGF-A pathway alone. In their Phase I clinical study for the treatment of wet AMD, E10030 was well tolerated with no significant drug-related adverse events. 12 weeks after the start of therapy, 59% of patients treated with E10030 and Lucentis gained significant vision. Besides that, there was a significant regression of neovascular lesions as shown in the mean decrease of 86% in the area of choroidal neovascularisation (CNV) at 12 weeks.

In addition, Jo et al. [52] also reported similar experiment as shown in Figure 1. In their experiment, the combination of anti-VEGF (Aptamer) with anti-PDGF (APB5) showed significant decrease in the area of CNV. They concluded that the combination therapy is more effective intervention for advanced ocular neovascular disease.

A comparative analysis of the advantages and shortcomings of the above mentioned therapeutics is provided in Table 3.

Future Prospect & Conclusion

In conclusion, there are many antagonists of PDGF and VEGF in therapy for neovascular disorders are currently being developed (Figure 2).

These included antisense RNA, ribozymes, antibodies, aptamers and etc. However, due to the small size, ease of synthesis, and low cost, aptamers provided versatile tools for validation of intracellular and extracellular targets. In the case of extracellular targets, such as VEGF and PDGF discussed here, aptamer-based validation affords a direct path to therapeutic development. Therapeutic aptamer leads can be readily stabilized or shielded from renal filtration by chemical or compositional modification for evaluation *in vivo* preclinical discovery programs. With a number of additional aptamers expected to enter into clinical trials over the next year, aptamers appear poised to make a significant contribution to the treatment of chronic diseases, included age-related macular degeneration (AMD).

Lucentis, Avastin and Macugen are the most common anti-VEGF drugs for wet AMD. Lucentis or ranibizumab is the latest in a group of anti-angiogenic drugs that acts against the growth of the new blood vessels of many eyes diseases. Nearly all patients (95 %) treated with Lucentis maintained and improved their vision in the clinical trials [29]. Avastin or Bevacizumab is a drug that blocks angiogenesis, and was used to treat various cancers, including colorectal, lung, and kidney cancer, and eye disease [16-20]. However, it is also one of the most expensive drugs widely marketed. There are many physicians and the media have criticized the high cost of a drug that has only limited usage in cancer treatment because it merely extends life rather than providing a cure [19]. In the USA, many insurance companies have refused to pay for all or part of the costs of Bevacizumab because of the reasons above. Macugen or Pegaptanib or EYE001 was the first anti-angiogenic aptamer to be approved by the FDA. It was shown to be quite effective in the therapy, but it did not restore vision in any statistically significant amount [39]. Because Lucentis is more effective in maintaining and restoring vision, Macugen is not used very often now.

In addition to these trials with single agents, the combined administration of different aptamers is a potentially promising avenue of investigation. In the studies by Agnieszka et al [13], the combination of two tyrosine kinase inhibitors resulted in the blockade of signalling by both VEGF and PDGF-B, effecting a more potent inhibition of tumour growth than either agent individually. Besides that, this improved anti-angiogenic effect has been leading to the vessel regression. By comparing with inhibition of VEGF signalling alone, it reflected the importance of PDGF for pericyte recruitment and the provision of endothelial cell survival signals. Consequently, a combination therapy approach, using both VEGF and PDGF inhibitors, can be a more effective intervention for the future of advanced ocular neovascular disease.

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