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Review Article

# Role of anti vascular endothelial growth factor (VEGF) in ophthalmology

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## ABSTRACT

Vascular endothelial growth factor (VEGF), is a naturally occurring signal protein which is proinflammatory, stimulates angiogenesis and potent inducer of vascular permeability. Its role in normal physiology includes in embryonic development, wound healing and bone repair, neovascularization following MI and demonstrated in brain, kidney and GI mucosa. VEGF is responsible for many retinal diseases by causing new vessel growth and by increasing leakage and causing retinal swelling. Their use in ophthalmology includes in both anterior and posterior segment pathologies. This article explains the role of VEGFs, their mechanism of action, anti VEGFs, classification, their use, various studies and other aspects.

**Keywords:** VEGF, Bevacizumab, Lucentis, BRVO, CRVO

## INTRODUCTION

Vascular endothelial growth factor (VEGF), first identified, purified and cloned in 1989, is a signal protein occurs naturally within the body, stimulates vasculogenesis and angiogenesis. VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor. VEGF is responsible for many retinal diseases by causing new vessel growth and by increasing leakage and causing retinal swelling. Their use in ophthalmology includes in both anterior and posterior segment pathologies. This article explains the role of VEGF, their mechanism of action, anti VEGF, classification, their use, various studies and other aspects.

## HISTORICAL BACKGROUND

In 1948-58 Michaelson, Ashton and Wise contribute to factor X hypothesis. In 1971 Folkman publishes tumor angiogenesis factor hypothesis. In 1983 Dvorok demonstrates tumor secretion of vascular permeability factor (VPF). In 1989 Ferrara clones VEGF and identifies as an angiogenic factor. In 1997 first clinical trials of anti angiogenic therapy in cancer patients initiated. In 1999 aptamer blocking $VEGF_{165}$ first tested in humans (Macugen for AMD). In 2003 first anti VEGF therapy shown to be efficacious in AMD. In 2004 first FDA approved anti VEGF therapy for colorectal cancer came.

## VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. Serum concentration of VEGF is high in bronchial asthma and low in diabetes mellitus. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury,

muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels.

When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body. Drugs such as Bevacizumab can inhibit VEGF and control or slow those diseases.

VEGF is a sub-family of growth factors, to be specific, the platelet-derived growth factor family of cysteine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (the *de novo* formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature).

The broad term 'VEGF' covers a number of proteins from two families, that result from alternate splicing of mRNA from a single, 8-exon, *VEGF* gene. The two different families are referred to according to their terminal exon (exon 8) splice site - the proximal splice site (denoted $VEGF_{xxx}$) or distal splice site ($VEGF_{xxx}b$). In addition, alternate splicing of exon 6 and 7 alters their heparin - binding affinity, and amino acid number (in humans: $VEGF_{121}$, $VEGF_{121}b$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{165}b$, $VEGF_{189}$, $VEGF_{206}$;[4,6] the rodent orthologs of these proteins contain one fewer amino acid). These domains have important functional consequences for the VEGF splice variants, as the terminal (exon 8) splice site determines whether the proteins are pro-angiogenic (proximal splice site, expressed during angiogenesis) or anti-angiogenic (distal splice site, expressed in normal tissues). In addition, inclusion or exclusion of exons 6 and 7 mediate interactions with heparin sulfate proteoglycans (HSPGs) and neuropillin co-receptors on the cell surface, enhancing their ability to bind and activate the VEGF receptors (VEGFRs).[2] Recently, VEGF-C has been shown to be an important inducer of neurogenesis in the murine subventricular zone, without exerting angiogenic effects.[3]

The human VEGF isoforms are 121, 145, 165, 189, 206. The isoform numbers refer to the number of amino acids contained in the mature, secreted protein.

## MECHANISM

All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors (the VEGFRs) on the cell surface, causing them to dimerize and become activated through transphosphorylation, although to different sites, times and extents (Figure 1). The VEGF receptors have an extracellular portion consisting of 7 immunoglobulin-like domains, a single transmembrane spanning region, and an intracellular portion containing a split tyrosine kinase domain. VEGF-A binds to VEGFR-1 (Flt-1) and VEGFR-2[2] (KDR/Flf-1). VEGFR-2 appears to mediate almost all of the known cellular responses to VEGF.[5] The function of VEGFR-1 is less well-defined, although it is thought to modulate VEGFR-2 signaling. Another function of VEGFR-1 may be to act as a dummy/decoy receptor, sequestering VEGF from VEGFR-2 binding (this appears to be particularly important during vasculogenesis in the embryo). VEGF-C and VEGF-D, but not VEGF-A, are ligands for a third receptor (VEGFR-3), which mediates lymphangiogenesis.

**Table 1: Classification.**

| VEGF family members | Receptors | Functions |
|---|---|---|
| VEGF-A | VEGFR-1<br>VEGFR-2<br>Neuropilin-1 | Angiogenesis<br>Vasodilatation<br>Chemotactic (macrophages and granulocytes) |
| VEGF-B[1] | VEGFR-1 | Embryonic angiogenesis |
| VEGF-C[3] | VEGFR-2<br>VEGFR-3 | Lymphangiogenesis |
| VEGF-D | VEGFR-2<br>VEGFR-3 | Lymphangiogenesis |
| VEGF-E( viral factor) | VEGFR-2 | Angiogenesis |
| PIGF | VEGFR-1<br>Neuropilin | Vasculogenesis inflammation |

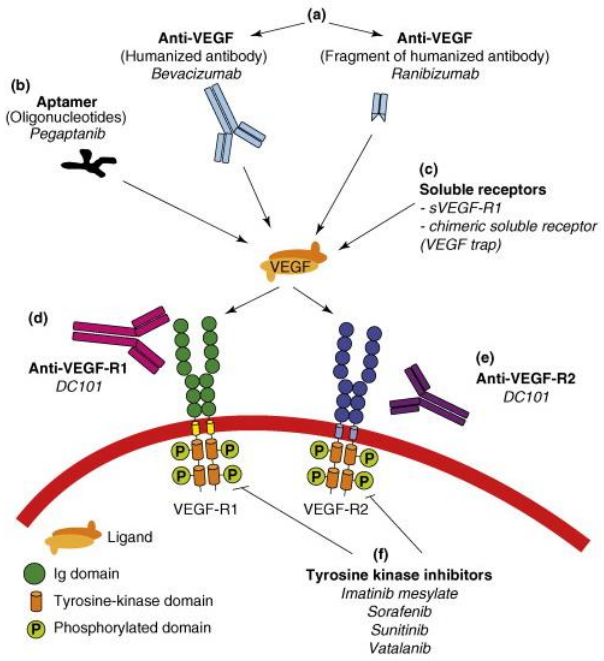


**Figure 1: Anti VEGFs.**

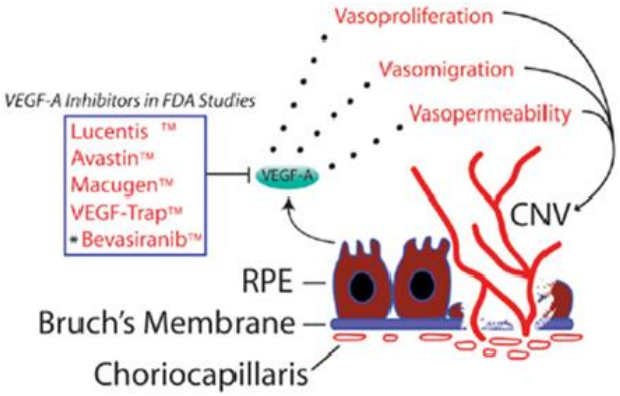


**Figure 2: Therapies that target VEGF-A through RNA interference.**

## PRODUCTION

$VEGF_{xxx}$ production can be induced in cells that are not receiving enough oxygen.[5] When a cell is deficient in oxygen, it produces HIF, hypoxia-inducible factor, a transcription factor. HIF stimulates the release of $VEGF_{xxx}$, among other functions (including modulation of erythropoiesis). Circulating $VEGF_{xxx}$ then binds to VEGF Receptors on endothelial cells, triggering a Tyrosine Kinase Pathway leading to angiogenesis.

HIF1 alpha and HIF1 beta are constantly being produced but HIF1 alpha is highly $O_2$ labile, so, in aerobic conditions, it is degraded. When the cell becomes hypoxic, HIF1 alpha persists and the HIF1alpha/beta complex stimulates VEGF release.

### Anti-VEGF therapies

- Monoclonal antibody- Bevacizumab (Avastin)
- Antibody derivative- Ranibizumab (Lucentis)
- Aptamer- Pegaptanib (Macugen)
- Oral small molecules- Lapatinib, Sunitinib, Sorafenib
- Fusion proteins- VEGF Trap eye (Aflibercept)
- Miscellaneous- si-RNA Bevasiranib, adPEDF

## INDICATIONS

*In posterior segment-*

- Wet age related macular degeneration
- CRVO/BRVO- neovascular complications and macular edema
- Proliferative diabetic retinopathy
- Diabetic macular edema
- Pre-op (5-7)[7,8] days in surgery for PDR and vitreous hemorrhage

*Expanding role-*

- Retinopathy of prematurity (zone 1) or posterior 2 (stage 3) [ BEAT-ROP study]
- Eales disease[9]
- Refractory post-surgical CME[10]
- Coats disease[11]

*In anterior segment*

- Neovascular glaucoma
- Iris neovascularization
- Before keratoplasty to reduce corneal vascularization[12,13]
- Pterygium
- Trabeculectomy ( to modulate wound healing)[14]

## CONTRAINDICATIONS

- Fibrovascular proliferation threatening the macula.
- With active ocular or periocular inflammation.
- Known hypersensitivity to Ranibizumab, Pegaptanib or Bevacizumab.
- Uncontrolled hypertension.
- Cardiovascular disease.

## GENERAL COMPLICATIONS

- Increase in IOP (13-17.6%, MARINA)
- Cataract (15.5% MARINA) (0.07%/injection VISION)
- Endophthalmitis (1% MARINA)[15], (0.16%/injection VISION)
- Potential risk of arterial thromboembolic events (4.6% MARINA)
- Rebound macular edema[16]
- Retinal detachment (0.08%, injection VISION)
- Immunoreactivity (4.4%-6.3%, MARINA)

VEGF-A is a major contributor to the progression of wet AMD. During this process, abnormal choroidal blood vessels migrate through Bruch's membrane into the RPE and neural retina. Multiple VEGF-A targeted therapies are currently in advanced clinical trials for CNV, with some already gaining FDA approval and widespread use. Asterisk denotes therapies that target VEGF-A through RNA interference (Figure 2).

## INDIVIDUAL DRUGS

***Pegaptanib***

- Macugen
- Pegylated aptamer
- 50 kDa
- Selectively binds $VEGF_{165}$ with high affinity, prevents activation of ocular VEGFR and inhibiting angiogenesis.

Absorption- slow systemic absorption
Vitreous t1/2- 94 hrs
Dosage- 0.3 mg (without PEG)/90 microlit
1/6 weeks, single dose in pre filled syringe

**Table 2: Clinical studies.**

| Compound | Disease | Study | |
|---|---|---|---|
| Macugen | Wet ARMD | VISION (VEGF Inhibition Study On Ocular Neovascularization | |
| | RVO | Macugen for RVO study group | |
| | DME | Macugen Diabetic Retinopathy study group | |
| Lucentis | Wet ARMD | MARINA<br>ANCHOR<br>PiER (Phase 3b sham injection controlled study of efficacy and safety of Ranibizumab in t/t of subfoveal CNVM) | |
| | CRVO | PRONTO (prospective OCT study with Lucentis for neovascular AMD) | |
| | BRVO<br>DME<br>Neovascular glaucoma | CRUISE<br>BRAVO<br>READ-2<br>RAVE (rubeosis anti VEGF trial) | |
| Avastin | Wet ARMD | SANA (systemic avastin therapy for neovascular age related macular degeneration study) | |
| Combination therapy | Wet ARMD | FOCUS | **AIM** |
| | | PROTECT | PDT vs PDT+Lucentis |
| | | DENALI | PDT and LUCENTIS on same day safety<br>LUCENTIS+PDT vs LUCENTIS |
| | | BRIDGE | |
| | | COBALT<br>CABERNATE/MERITAGE | LUCENTIS vs Anecortave<br>LUCENTIS+Bevasiranib<br>Ranibizumab+ Sr-90 |
| Comparison | Wet ARMD | CATT (US)<br>IVAN (UK) | Avastin vs Lucentis cost effectiveness |

***Ranibizumab***

- Lucentis
- RhuFab
- 48 KD
- Binds and inactivates all isoforms of VEGF-A thus preventing its interaction with its receptor on endothelial cell surface.[17]
- Unlike the full length antibody, it penetrates the ILM and can gain access to subretinal space.[18]
- Vitreous T1/2- 3 days (animals)
  - 9 days (humans)
- Reduction in Ranibizumab clearance in renal impairment is considered insignificant and dose adjustment is not need to be expected.
- Dose- 0.5 mg (0.05ml), 1/ month, single use glass vial (2cc)

***Bevacizumab***

Humanized monoclonal antibody that recognizes and blocks VEGF-A.
- 149 KDA
- Not yet approved by FDA-off label use in ophthalmology

- Rosenfield et al were the first ones to describe and publish the off label use of intravitreal use of Bevacizumab in 2005.[19]
- Vitreous T1/2- 5.6 days
- Systemic T1/2- 21 days
- Casky et al showed that Bevacizumab elimination follows first order kinetics with T1/2 in human vitreous of about approximately 10 days.[20]

*Non ocular uses of Bevacizumab:*

- Metastatic colorectal cancer (2004)
- Non small cell lung cancer (2006)
- Metastatic breast cancer (2008)
- Metastatic renal cell carcinoma (2009)
- Glioblastoma multiforme (2009)

***Eylea (VEGF Trap-Eye; aflibercept)***

Eylea is a protein that binds with the active forms of VEGF (VEGF-A) and placental growth factor (PIGF), another molecule involved in the formation of new blood vessels. Like other anti-VEGF agents this product is delivered by means of an intravitreal injection. In the CRVO trials the drug was administered once a month. In some of the other trials, not directed toward CRVO, Eylea was given once every other month.

## CONCLUSION

The approval of vascular endothelial growth factor (VEGF) has revolutionized the treatment of age related macular degeneration, retinal vein occlusions and diabetic macular edema.

The use of anti-VEGF therapy continues to be a mainstay of medical retina therapy. With further study and an increasing number of agents available, more information about the frequency of dosing, the selection of agents, and the long-term effects of these agents in patients with diabetic macular edema (DME), age related macular degeneration (AMD), and retinal vein occlusion (RVO) is becoming available. Increased use of these agents also has led to questions about their efficacy and safety, frequency of treatment, the possible application of "treat-and-extend" therapy. A better understanding of the VEGF pathways and ways that VEGF affects normal physiology also will increase our knowledge of these diseases.

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