VEGF and anti-VEGF in Eye Disease

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Financial Disclosures

• None
SUMMARY

- Anti-VEGFs (aVEGFs) provide benefit by temporarily reducing the level of VEGF
- Patients’ response to VEGF is variable
- aVEGF’s therapeutic benefit is due to overcoming leakage (DR and wAMD)
- New approaches to overcome leakage: activating Tie2
Overview and concepts in Angiogenesis

VEGF/VPF

(vascular endothelial cell growth factor/vascular permeability factor)
VEGF: growth factor that triggers two vascular responses

angiogenesis

Permeability/Leakage
Leakage

• Leakage is different from hemorrhage, which results from ruptured vessels and allows tissue to be exposed to the entire contents of the vasculature.

• Leakage is also different from extravasation, which allows immune cells to exit from the circulation. Inflammation is an example of both leakage and extravasation.

• Leakage results in accumulation of fluid and proteins from the circulation into tissue.
There are 4 anti-VEGFs used to manage patients with angiogenesis-related ocular diseases

Table 3 Structural, pharmacodynamic, and pharmacokinetic properties of different anti-VEGF agents used in the clinical management of neovascular AMD

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*Note: Intravitreal half-life estimates were derived from experimental animal studies and not human eyes.

**Abbreviations:** VEGF, vascular endothelial growth factor; AMD, age-related macular degeneration; FDA, US Food and Drug Administration; PEG, polyethylene glycol; PIGF, placental growth factor.
Site of action
Dominant features of aVEGF therapy

It works!

Patient’s response is variable

Ranibizumab versus verteporfin for neovascular age-related macular degeneration.

The effects of a flexible visual acuity-driven ranibizumab treatment regimen in age-related macular degeneration: outcomes of a drug and disease model.
Holz FG … Weichselberger A.
aVEGF reduces the level of VEGF in AH (vitreous)

- $t_{1/2}$ of ranibizumab (lucentis) in vitreous is 2.9 days
- VEGF in AH of untreated patients is 70 pg/ml
- A 0.5 mg dose should neutralize all of the VEGF for approx 30 days
- aVEGF reduces the level of VEGF


VEGF in patients with exudative AMD treated with ranibizumab.
Muether PS1, Hermann MM, Viebahn U, Kirchhof B, Fauser S.
aVEGF functions by suppressing the level of VEGF

- Both morphological, and functional outcomes were good when VEGF was low, and deteriorated once the level of VEGF rebounded
- VEGF is a biomarker of efficacy

VEGF returns to the pre-therapy level
Because current approaches only transiently reduce the level of VEGF, second-generation approaches seek to enduringly suppress VEGF. Long-term suppression of VEGF is expected to result in long-term clinical benefit.

VEGF returns to the pre-therapy level.
Is there a downside of a more durable aVEGF?

Preclinical studies unequivocally demonstrate that VEGF is neuroprotective

Am J Pathol. 2013 Apr;182(4):1379-90. VEGF-A is necessary and sufficient for retinal neuroprotection in models of experimental glaucoma. Foxton RH ... Ng YS.


Is there a downside of a longer-acting aVEGF?

Clinical studies report that GA progresses in patients treated w aVEGF
  • Natural history versus side effect of aVEGF?!


Retinal pigment epithelial atrophy in patients with exudative age-related macular degeneration undergoing anti-vascular endothelial growth factor therapy.

Lois N1, McBain V, Abdelkader E, Scott NW, Kumari R.
Why does the eye produce VEGF under adverse conditions?
Reasons why the response to aVEGF is variable remains an open and clinically relevant question

- DME
- wAMD
- PDR
Anti-VEGF is effective in eye diseases because it

• A) causes regression of pathological blood vessels
• B) reduces retinal edema associated with leaking blood vessels
• C) reduces fibrosis, which compromises vision and can cause retinal detachment
• D) reduces intraocular pressure
A plausible reason that the response to aVEGF is variable is because VEGF is not the only agent elevated in vitreous of patients with leaking blood vessels

• Cytokines
• Members of the bradykinin pathway
Triamcilonone is efficacious in DME, but does not change the level of VEGF; do cytokines promote permeability of retinal vessels?

**TABLE 3. Changes in Aqueous Concentrations (pg/mL) of Inflammatory and Angiogenic Cytokines After Intravitreal Injection (Triamcinolone vs Bevacizumab) in Diabetic Macular Edema Group**

<table>
<thead>
<tr>
<th>Variable</th>
<th>IVTA Group (n=11)</th>
<th></th>
<th></th>
<th>IVBe Group (n=11)</th>
<th></th>
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</tr>
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<tr>
<td></td>
<td>Preinjection</td>
<td>Postinjection</td>
<td>P Value</td>
<td>Preinjection</td>
<td>Postinjection</td>
<td>P Value</td>
</tr>
<tr>
<td>IL-6</td>
<td>29.9 (10.1-82.5)</td>
<td>13.8 (2.8-36.3)</td>
<td>&lt;.01</td>
<td>26.7 (13.8-107.0)</td>
<td>24.0 (6.5-147.0)</td>
<td>.477</td>
</tr>
<tr>
<td>IL-8</td>
<td>28.2 (6.23-77.5)</td>
<td>25.3 (12.4-95.8)</td>
<td>.597</td>
<td>23.9 (11.1-39.7)</td>
<td>23.6 (11.0-74.2)</td>
<td>.374</td>
</tr>
<tr>
<td>IP-10</td>
<td>366.0 (171.0-1380)</td>
<td>249.0 (28.7-717.0)</td>
<td>.013</td>
<td>401.0 (126.0-1990)</td>
<td>433.0 (268.0-4570)</td>
<td>.110</td>
</tr>
<tr>
<td>MCP-1</td>
<td>3850 (2060-4380)</td>
<td>1090 (351-4150)</td>
<td>.010</td>
<td>3770 (2660-4490)</td>
<td>3840 (1790-4490)</td>
<td>.594</td>
</tr>
<tr>
<td>PDGF-AA</td>
<td>68.7 (31.4-141.0)</td>
<td>37.1 (10.9-89.7)</td>
<td>.016</td>
<td>81.0 (14.3-140.0)</td>
<td>72.7 (23.8-117.0)</td>
<td>.722</td>
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<tr>
<td>VEGF</td>
<td>55.0 (36.0-262.0)</td>
<td>10.5 (0.1-372.0)</td>
<td>.050</td>
<td>61.5 (31.8-200.1)</td>
<td>0.1 (0.1-28.3)</td>
<td>&lt;.01</td>
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Ophthalmol 2011;152:686–694
Vitreous contains multiple agents that induce permeability

Vitreous contains multiple agents that induce permeability


Extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through prekallikrein activation.

Gao BB, ... Aiello LP, Feener EP.
Is aVEGFs’ therapeutic benefit all about permeability?

- DME
- NPDR

RETINA 30:1012–1016, 2010

FIBROUS MEMBRANES IN DIABETIC RETINOPATHY AND BEVACIZUMAB

DAVID M. PATTWELL ... PAUL HISCOTT
Unlike DR and wAMD, aVEGF’s clinical benefit in ROP involves regression of blood vessels


Anti-Vascular Endothelial Growth Factor and the Evolving Management Paradigm for Retinopathy of Prematurity

Darwish D, Chee RI, Patel SN, Jonas K, Ostmo S, Campbell JP, Chiang MF, Chan RVP
Attempts to improve aVEGF

Increased durability
- smaller molecular weight compounds (single chain Abs)
- Longer half life

Combo therapy
Attempts to improve aVEGF therapy: aVEGF/aPDGF (aPDGFR) combo therapy

Strategy:

• Prevent maturation of blood vessels in order to enforce their dependence on VEGF
• Neutralizing PDGF prevents recruitment of pericytes
• Combo therapy should work better (durability, uniform response) than aVEGF monotherapy
Combo therapy to improve aVEGF therapy: aVEGF + enforcement of barrier function
Anti-VEGF-based therapy is effective for most patients afflicted with

- A) glaucoma
- B) dry AMD
- C) dry eye
- D) wet AMD
VEGF basics
The VEGF family

7 members

VEGF-A is the therapeutically relevant member of the VEGF family

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RNA splicing results in 8 isoforms of VEGF-A

Isoforms differ in their ability to bind HS and/or Nrp1/2

VEGF 165b has a distinct c-terminus and is anti-angiogenic
VEGF-C and VEGF-D promote lymphangiogenesis
Lymphedema is caused by lymphatic dysfunction

VEGF-C, which promotes formation of lymphatics, is being considered as a potential therapeutic option.
Recently appreciated functions of VEGF-B/VEGFR1

**VEGF-B promotes fatty acid uptake in the endothelium**

**VEGF-B promotes nerve regeneration in the cornea**

Proc Natl Acad Sci U S A. 2014 Dec 2;111(48):17272-7

**VEGF-B selectively regenerates injured peripheral neurons and restores sensory and trophic functions.**
Guaiquil VH, Pan Z, Karagianni N, Fukuoka S, Alegre G, Rosenblatt MI.


**VEGF-B promotes recovery of corneal innervations and trophic functions in diabetic mice.**
VEGF-A therapy to overcome ischemia

**Eur Heart J.** 2017; 38(18):1365-1371

Angiogenic gene therapy in cardiovascular diseases: dream or vision?

Ylä-Herttuala S, Bridges C, Katz MG, Korpisalo P
VEGF is a dimer and it assembles homo and heterodimeric receptors

**VEGFR**
- XC domain specific for one more VEGF family members
- TM to anchor it in the membrane
- The intracellular portion includes a tyrosine kinase that is activated upon ligand-induced dimerization of receptor monomers
Dimerization/phosphorylation changes the conformation of the enzyme from low to high catalytic activity.

Phosphorylation causes the lip to move out of the kinase catalytic site, thus increasing the ability of ATP and the protein substrate to bind.
The activated receptor triggers signaling events and changes in gene expression, which direct cellular responses

- PLCγ
- PI3K/Akt
- SFKs
- MEK/Erk
Cellular processes intrinsic to angiogenesis

1. Angiogenic factors (FGF, VEGF) bind to EC receptors
2. Basement membrane degradation by MMPs, uPAR
3. Endothelial cell proliferation and migration
4. Tube formation, elongation, and remodeling (integrins)
5. Vessel stabilization (pericytes and smooth muscle cells by Ang-1, TGF-β)
VEGF is a growth factor that

- A) acts via G protein-coupled receptors to promote cell survival
- B) reduces fluctuations in blood glucose
- C) promotes permeability of blood vessels
- D) is a major driver of pathology with no known role in physiology
Dysfunction of either of the two BRBs can contribute to retinal edema
There are several ways to get across the inner BRB

- Between endothelial cells
- Through endothelial cells
Governors of endothelial barrier function: Pericytes

Trafficking of Endogenous Immunoglobulins by Endothelial Cells at the Blood-Brain Barrier. Villaseñor R...Collin L.
Governors of inner BRB: Components of the endothelial cell junction
VEGF relaxes the inner BRB by causing phosphorylation of component of the AJ
Components of the endothelial cell junction

- Parts of the junction
- Scaffold on which these junctions stand
Activating Tie2 promotes the formation of a scaffold on which junctions stand
Multiple approaches to activate Tie2

Neutralizing Ang2
Multiple approaches to activate Tie2

Neutralizing Ang2

Inhibiting VE-PTP
Activating Tie2 appears to potentiate therapeutic benefit of aVEGF

**Neutralizing Ang2**
- Boulevard; Phase II; Roche
- aVEGF/aAng2 resulted in a greater improvement in BCVA, retinal thickness and ETDRS score as compared with aVEGF monotherapy

**Inhibiting VE-PTP**
- TIME-2; Phase Ila; Aerpio
- aVEGF + AK-9778 resulted a greater decline in retinal thickness as compared with aVEGF monotherapy
Attempts to improve aVEGF

Increased durability
• smaller molecular weight compounds (single chain Abs)
• Longer half life

Combo therapy
• aVEGF/activation of Tie2
  ✓ aAng2
  ✓ AKB-9778
Mechanisms to enforce the inner BRB

- Prevent opening of the barrier
  - Antagonize VEGF
  - Suppress the signaling enzymes that mediate VEGF-dependent phosphorylation of AJ components

- Enforce barrier closure
  - Activate Tie2 to generate the cytoskeletal scaffold on which junctions stand
Summary

• aVEGFs provide benefit by temporarily reducing the level of VEGF
• Patients’ response to VEGF is variable
• aVEGF’s therapeutic benefit is due to overcoming leakage (DR and wAMD)
• New approaches to overcome leakage: activating Tie2