

Comparing Anti-VEGF Therapies in Neovascular Age-Related Macular Degeneration

Some of the most promising new developments in the treatment of neovascular AMD have centered on targeting and inhibiting VEGF-A or its signaling pathways.

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Neovascular age-related macular degeneration (AMD) is the leading cause of blindness in people aged >65 years in the United States¹ and is characterized by choroidal neovascularization (CNV), that is, abnormal blood vessel growth from the choriocapillaris, through Bruch's membrane, into or under the retinal pigment epithelium (RPE), or into the subretinal space.² Because of increased vascular permeability, these abnormal vessels often leak or bleed into the subretinal macular region, causing serous or hemorrhagic detachment of the neurosensory retina or subpigment epithelial space and potentially scarring, all of which lead to vision loss.^{2,3}

Vascular endothelial cell growth factor A (VEGF-A, also referred to as VEGF) is a potent inducer of angiogenesis and vascular permeability,⁴ and has been implicated in the development of CNV secondary to AMD.^{5,6} High levels of VEGF-A mRNA and protein expression in CNV membranes surgically excised from patients with AMD have been described.^{7,8} Significantly increased expression of VEGF-A has been detected in the RPE cells and the choroidal blood vessels of patients with AMD.⁵

Some of the most promising new developments in the treatment of neovascular AMD have centered on targeting and inhibiting VEGF-A or its signaling pathways. Two VEGF-A-targeted agents, pegaptanib sodium (Macugen, OSI/Eyeteck and Pfizer, both in New York, NY) and ranibizumab (Lucentis; Genentech, San Francisco), have been approved by the US Food and Drug Administration (FDA) for the treatment of neovascular AMD.

Bevacizumab (Avastin; Genentech) is an anti-VEGF agent indicated for treatment of colorectal cancer but recently used off-label for treatment of AMD.

Although both pegaptanib and ranibizumab target VEGF-A, ranibizumab may provide greater clinical benefit than pegaptanib to patients with CNV secondary to AMD. Despite their similar mechanisms of action, pegaptanib and ranibizumab differ significantly in terms of their molecular composition and their VEGF-A target specificity.

VEGF-A

There are nine VEGF-A isoforms. Identifying which isoforms contribute to physiological and pathological vascular growth and permeability has been the focus of many studies. Alternative RNA splicing of the human VEGF-A gene gives rise to the VEGF-A isoforms; the four most prominent isoforms are VEGF_{121'}, VEGF_{165'}, VEGF_{189'}, and VEGF_{206'}, with VEGF_{165'} being the most abundantly expressed (Table 1). Other VEGF-A isoforms include VEGF_{145'}, VEGF_{148'}, VEGF_{162'}, VEGF_{165b'}, and VEGF_{183'}.^{9,10} The longer VEGF-A isoforms, including VEGF_{165'}, contain a heparin-binding domain, which results in nearly all of VEGF_{189'} and VEGF_{206'} and 50% to 70% of VEGF_{165'} being sequestered within the extracellular matrix (ECM). VEGF_{121'}, which lacks the heparin-binding domain, and the remaining 30% to 50% of VEGF_{165'} is diffusible.¹¹⁻¹³

Although some studies have suggested that the heparin-binding domain is important for mitogeni-

TABLE 1. SUMMARY OF THE FOUR MOST PROMINENT VEGF-A ISOFORMS AND BIOACTIVE CLEAVAGE PRODUCTS

VEGF-A	Activities	HBD	ECM-Bound	Diffusible	References
VEGF ₂₀₆	Vascular permeability activity	Yes	Yes	Released by heparin or plasmin	Houck, 1991 ⁹
VEGF ₁₈₉	Vascular permeability activity	Yes	Yes	Released by heparin or plasmin	Houck, 1991 ⁹
VEGF ₁₆₅	Most abundant isoform mitogenic and vascular permeability activities sufficient for normal retinal angiogenesis	Yes	Yes (partial)	Yes (partial)	Houck, 1991 ⁹ Ruhrberg, 2002 ¹⁰ Stalmans, 2002 ¹¹
VEGF ₁₂₁	Mitogenic and vascular permeability activities lack vascular branching and patterning activities	No	No	Yes	Zhang, 2000 ¹² Ruhrberg, 2002 ¹⁰ Stalmans, 2002 ¹¹
VEGF ₁₁₀	Plasmin cleavage product of VEGF-A isoforms, mitogenic, vascular permeability activity	No	No	Yes	Houck, 1992 ¹²

HBD, heparin-binding domain

ty,^{13,14} other studies have shown that VEGF₁₂₁, which lacks this domain, has significant mitogenic activity in vitro and in vivo.^{15,16} In cell culture assays, all four of the major isoforms (VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉, and VEGF₂₀₆) demonstrated mitogenic activity¹² as well as vascular permeability activity.¹⁵ In addition, plasmin cleavage of ECM-bound VEGF-A isoforms gives rise to a bioactive cleavage product, VEGF₁₁₀.^{11,13} VEGF₁₁₀ has been shown to have both endothelial cell mitogenic activity as well as vascular permeability activity in the Miles assay,¹¹ although its potency seems to be considerably reduced compared with VEGF₁₆₅.^{13,15}

Pegaptanib is an RNA aptamer approved for intravitreal use in all subtypes of CNV secondary to AMD.

TARGETING VEGF-A IN NEOVASCULAR AMD

Pegaptanib. Pegaptanib is an RNA aptamer approved for intravitreal use in all subtypes of CNV secondary to AMD. It is the first aptamer to be FDA approved as a therapeutic agent in humans, and it was the first anti-VEGF agent approved for treatment of neovascular AMD. Pegaptanib was designed using the systematic evolution of ligands by exponential enrichment (SELEX) process to specifically target VEGF₁₆₅, which has been suggested to contribute significantly to pathological

neovascularization.¹⁷ Because it binds to the heparin-binding domain of VEGF₁₆₅,¹⁸ however, pegaptanib is also likely to inhibit the VEGF-A isoforms longer than VEGF₁₆₅.

Efficacy. Pegaptanib offers several advantages over photodynamic therapy (PDT) with verteporfin, (Visudyne; Novartis, East Hanover, NJ), which was approved by the FDA in 2000 and has since been a standard treatment for neovascular AMD. Pegaptanib treatment resulted in lower rates of visual loss and an improvement of vision in a small percentage of cases in patients with all sizes and subtypes of CNV secondary to AMD.^{19,20} After 1 year of intravitreal pegaptanib injections (0.3 mg) every 6 weeks, 70% of pegaptanib-treated patients lost <15 letters of visual acuity (VA), compared with 55% of sham-treated patients. In addition, 6% of pegaptanib-treated patients gained ≥15 letters compared with 2% of sham-treated patients.²¹ Patients who were treated in the early stages of disease had slightly better responses (76%-80% lost <15 letters, 12%-20% gained ≥15 letters).²² Despite pegaptanib treatment, however, mean VA decreased over 1 year, although the decrease was significantly less than that reported for the sham-treated patients ($P<.002$).²¹

Safety. The most common adverse event associated with pegaptanib therapy was ocular inflammation.^{21,23} Reported serious adverse events related to injection procedure included endophthalmitis (1.3% patients), retinal detachment (0.7% of patients), and traumatic injury to the lens (0.6% of patients).²¹ No systemic adverse events were reported, although patients with known cardiovascular, cerebrovascular, or peripheral

**TABLE 2. VEGF-A THERAPEUTIC AGENTS USED TO TREAT NEOVASCULAR AMD: AN OVERVIEW
COMPARISON OF PEGAPTANIB, RANIBIZUMAB, AND BEVACIZUMAB**

	Pegaptanib	Ranibizumab	Bevacizumab
Size	50 kD	48 kD	149 kD
Immunogenicity (following ITV administration)	None	Slight	N/A
Half-life: vitreal (following ITV administration)	3.8 days (monkey) ³⁹	3 days (monkey) ⁴⁰ 9 days (human) (ranibizumab product insert 2006)	N/A
Half-life: serum (following ITV administration)	3.6-4.3 days (monkey) ³⁹ 10 days (human 3-mg dose) (pegaptanib product insert)	3.5 days (monkey) ⁴⁰ 0.08 days (human) (Xu L et al, unpublished data)	N/A
Half-life: Serum (following IV administration)	0.4 days (monkey) ⁴¹	0.6 days (monkey) ⁴⁰	20 days (bevacizumab product insert 2006)
Half-life: Serum (following SC administration)	0.5 days (monkey) ⁴¹	N/A	N/A
Retinal penetration	All retinal layers (personal communication from Adamis A)	All retinal layers including RPE and choroid (Gaudreault J, et al, unpublished data)	All retinal layers not in RPE or choroid ⁴²
Target specificity	VEGF ₁₆₅ (and larger isoforms)	All VEGF-A isoforms	All VEGF-A isoforms

ITV, intravitreal; SC, subcutaneous

vascular comorbidities were excluded from the pivotal trials.²¹

Ranibizumab. Ranibizumab is a humanized monoclonal antibody fragment (Fab) approved for intravitreal treatment of all CNV subtypes secondary to AMD. It targets all isoforms of VEGF-A as well as the bioactive cleavage product VEGF₁₁₀.²⁴

Efficacy. Ranibizumab is the first therapeutic agent to stabilize or improve vision significantly in most patients with neovascular AMD. In phase 3 trials comparing ranibizumab with verteporfin or sham treatment, monthly intravitreal injections of 0.5 mg of ranibizumab resulted in 96% and 95% of ranibizumab-treated patients losing <15 letters in VA after 1 year, compared with 64% of verteporfin-treated patients and 62% of sham-treated patients, respectively.^{25,26} Approximately 40% and 34% of 0.5 mg ranibizumab-treated patients gained ≥15 letters of VA, compared with 6% of verteporfin-treated patients and 5% of sham-treated patients, respectively.^{25,26} After 2 years, 90% of 0.5 mg ranibizumab-treated patients and 53% of sham-treated

patients lost <15 letters in VA.²⁷ Approximately 33% of 0.5 mg ranibizumab-treated patients gained ≥15 letters of VA, compared with 4% of sham-treated patients.²⁵ Ranibizumab treatment also resulted in an increase in mean VA of 7 letters at 2 years, compared with a loss in mean VA of 10 letters among sham-treated patients.²⁵

Safety. Ranibizumab is generally well tolerated. The most common reported adverse event was mild, reversible intraocular inflammation. Serious ocular adverse events associated with intravitreal ranibizumab were uncommon. In patients treated with 0.5 mg intravitreal ranibizumab (n=239), presumed endophthalmitis and uveitis were each reported in 1.3% of patients; retinal tear, vitreous hemorrhage, and lens damage were each reported in 0.4% of patients; and no cases of retinal detachment were reported.²⁵ A slight increase in non-ocular hemorrhage compared with sham treatment was also reported. There were no reported significant trends in systemic adverse events potentially related to VEGF inhibition, including proteinuria or hypertension,^{27,28} even in the phase 3 trials that did not

exclude patients who had histories of cardiovascular, cerebrovascular, or peripheral vascular comorbidities.^{25,26}

Bevacizumab. Bevacizumab is an anti-VEGF full-length humanized monoclonal antibody that received FDA approval in February 2004 to treat metastatic colorectal cancer. It was the first FDA-approved antiangiogenesis therapeutic agent. Off-label use of intravitreal bevacizumab for neovascular AMD has increased dramatically in the last year. As with ranibizumab, bevacizumab targets all VEGF-A isoforms as well as the bioactive cleavage product, VEGF₁₁₀.

Efficacy. One phase 1/2 study of IV bevacizumab treatment of patients with neovascular AMD (Systemic Avastin for Neovascular AMD [SANA] Study) provided promising preliminary evidence that bevacizumab can increase mean VA, decrease mean central retinal thickness, and reduce vascular leakage.²⁹ The use of intravitreal bevacizumab for the treatment of neovascular AMD has been reported in a number of case reports suggesting that intravitreal bevacizumab may be efficacious in neovascular AMD.³⁰⁻³⁴

Safety. In the SANA study of IV bevacizumab in AMD, seven of nine patients experienced transient hypertension, which was controlled through medication.²⁹ Concerns about potential systemic safety risks, such as cardiovascular and cerebrovascular adverse events, have discouraged further study of IV bevacizumab in neovascular AMD outside of controlled studies. An Internet-based, voluntary safety survey, established to collect information from multiple case studies on adverse events resulting from intravitreal bevacizumab treatment of ocular diseases, indicated no increase in drug-related ocular or systemic adverse events.³⁵ One incident of noninfectious uveitis resulting from intravitreal bevacizumab in a patient with CNV secondary to AMD has been reported.³⁶ Overall, currently available data suggest that intravitreal bevacizumab is generally safe, although its long-term safety is unknown.

PEGAPTANIB VERSUS RANIBIZUMAB

Although it is difficult to compare the efficacies of pegaptanib and ranibizumab in neovascular AMD directly, without data from a head-to-head study, the available data on each agent suggest that ranibizumab is associated with better visual outcomes than is pegaptanib in patients with neovascular AMD. Ranibizumab treatment resulted in considerably lower rates of vision loss and higher rates of vision gain than pegaptanib treatment over similar periods. In addition, ranibizumab treatment culminated in an increase of mean VA after 2 years, whereas pegaptanib only slowed the decline in

mean VA. The underlying causes of the difference in efficacy of pegaptanib and ranibizumab are unclear, although the different development approaches of these two agents highlight differences that could affect their efficacy, such as the class of molecule (or molecular composition) and target specificity. The physical and pharmacologic characteristics of pegaptanib and ranibizumab are compared in Table 2.

Size and retinal penetration. Pegaptanib and ranibizumab are similar in size. The pegaptanib aptamer is a modified 27-base ribonucleotide linked to a 40-kD polyethylene glycol (PEG) moiety, and it has a total molecular weight of approximately 50 kD. Ranibizumab is a Fab of a full-length monoclonal antibody with a molecular weight of approximately 48 kD.

Retinal penetration of anti-VEGF therapeutic agents delivered by intravitreal injection are potentially important factors in the efficacy of the agents in neovascular AMD.

The ability and extent of retinal penetration of anti-VEGF therapeutic agents delivered by intravitreal injection are potentially important factors in the efficacy of the agents in neovascular AMD. Although differences in retinal penetration could theoretically explain the different activities of pegaptanib and ranibizumab, the two agents are similar in size, and both diffuse across the retina. The extent of pegaptanib penetration, however, has not yet been well defined.

Immunogenicity. Aptamers are purportedly nonimmunogenic, even in large doses, because of their small size and their similarity to endogenous molecules.³⁷ Accordingly, no antibodies against pegaptanib and no local or systemic hypersensitivities were detected in response to pegaptanib treatment of patients with neovascular AMD in two phase 3 clinical trials.²¹

Reduced immunogenicity was also a proposed advantage of Fab molecules over full-length monoclonal antibodies. Fab molecules were predicted to have significantly reduced risk from innate immune responses (antibody-dependent, cell-mediated cytotoxicity, or complement-dependent cytotoxicity) owing to an absence of the constant antibody region (Fc). Early safety studies of ranibizumab reported no detection of antibodies to ranibizumab;^{27,28} however, an increased risk of inflammation^{28,38} led to a reformulation of ranibizumab. The full-length monoclonal antibody bevacizumab, by virtue of its Fc domain, is predicted to be more im-

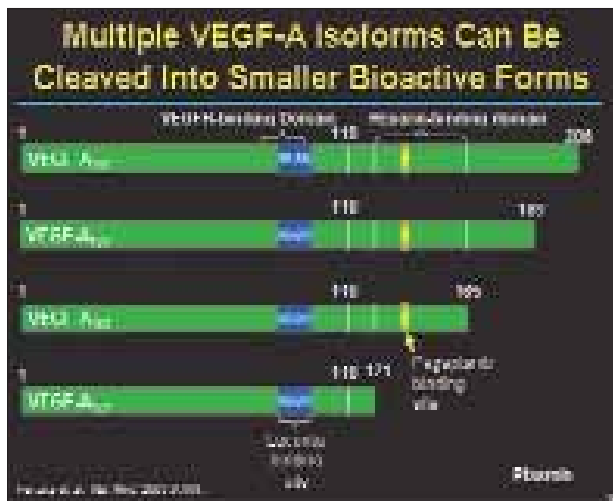


Figure 1. All VEGF-A isoforms have nearly identical amino acid sequences up to the plasmin cleavage site between arginine 110 and alanine 111. VEGF₁₆₅ and longer isoforms contain regions of basic amino acids encoded by exons 6 and 7 that constitute a HBD. Pegaptanib binds this domain in VEGF₁₆₅.²¹ Ranibizumab binds to the VEGF receptor binding domain,⁴⁸ which is present in all VEGF-A isoforms and the active plasmin-cleavage product, VEGF₁₁₀.

munogenic than ranibizumab, but the immunogenicity profile of bevacizumab has not yet been examined in a clinical trial.

Theoretically, differences in the immune response elicited by pegaptanib and ranibizumab could potentially account for differences in efficacy between the two agents; however, pegaptanib appears to have a slightly better immunogenicity profile than that of ranibizumab.

Pharmacokinetics. As nucleic acids, aptamers are prone to nuclease degradation, and they are subject to rapid clearance from the body because of their small size.¹⁷ Modification of the nucleotide backbone, however, can increase aptamer resistance to nuclease attack, and rates of renal clearance of aptamers can be reduced by site-specific addition of PEG moieties.³⁷ Pegaptanib was modified to contain 2'-O-methyl ribopurines and 2'-F ribopyrimidines in addition to the 40-kD PEG moiety.³⁹ In rhesus monkeys, the vitreous elimination half-life of intravitreal pegaptanib was approximately 3.8 days. The serum elimination half-life of pegaptanib was determined to be about 0.4 days for IV administration, 0.5 days for subcutaneous administration, and between 3.6 and 4.3 days for intravitreal administration.^{40,41} After a single 3-mg intravitreal dose of pegaptanib (10 times the recommended dose), the serum half-life in humans was estimated to be about 10 days.⁴²

The vitreous elimination half-life of intravitreal ranibizumab was 2.9 and 3 days in rabbits (Gaudreault J, et al, unpublished data) and monkeys,⁴³ respectively. Similarly, the vitreous elimination half-life of an intravitreal humanized Fab related to ranibizumab (rhuMAB VEGF Fab) was about 3.2 days in rhesus monkeys, compared with 5.6 days for the intravitreal full-length humanized HER2 monoclonal antibody.⁴⁴ The serum elimination half-life of ranibizumab in monkeys was 3.5 days for intravitreal administration and 0.6 days for IV administration.⁴³ In humans, the vitreous elimination half-life of intravitreal ranibizumab was estimated to be about 9 days, whereas the serum elimination half-life of intravitreal ranibizumab was estimated to be about 2 hours (Xu L, et al, unpublished data).²⁴ The estimated serum elimination half-life of bevacizumab after IV administration in humans is approximately 20 days.²⁴

Significant differences in the molecular composition of pegaptanib and ranibizumab raise the possibility of significant differences in pharmacokinetics between the two agents. The pharmacokinetic profile of pegaptanib, however, appears to be comparable to that of ranibizumab (Table 2).

Target specificity. As mentioned previously, pegaptanib was designed to target VEGF₁₆₅ using SELEX technology, which allows identification of highly specific aptamers that bind with high affinity to selected targets. Pegaptanib selectively binds to the heparin-binding domain of VEGF₁₆₅.^{18,39} Because VEGF-A isoforms larger than VEGF₁₆₅ contain the same heparin-binding domain, however, they are also probable targets of pegaptanib. Pegaptanib does not recognize VEGF-A isoforms smaller than VEGF₁₆₅, namely, VEGF₁₄₅ and VEGF₁₂₁, or the bioactive cleavage product of the larger VEGF-A isoform, VEGF₁₁₀.^{18,39} In contrast, ranibizumab targets and inhibits all VEGF-A isoforms through the VEGF receptor binding domain, including the bioactive cleavage product VEGF₁₁₀.⁴⁸ (Lowe J, et al, unpublished data).

Target specificity differences for VEGF-A between pegaptanib and ranibizumab therapeutic agents could uniquely affect the course of disease and the associated clinical outcomes. Differences in target specificity between pegaptanib and ranibizumab (Figure 1) appear to be the most distinguishing characteristic that might explain the observed difference in clinical efficacy in patients with neovascular AMD.

CONCLUSION

The factors leading to differences in the observed clinical efficacy of two currently approved anti-VEGF therapies, pegaptanib and ranibizumab, could be relat-

ed to differences in the class of the molecules and differences in the target specificities of the two agents. Despite one agent being an RNA aptamer and the other an antibody fragment, their differences in class do not appear to correlate to significant differences in the pharmacokinetics. The molecules are also almost identical in size, and both have been shown in preclinical studies to penetrate the retina. Pegaptanib appears to be less immunogenic than ranibizumab, although neither agent induces unmanageable immune responses or inflammation.

Whereas ranibizumab binds and neutralizes all isoforms of VEGF-A and the bioactive cleavage product, VEGF_{110'}, pegaptanib binds only VEGF₁₆₅ and potentially other larger VEGF-A isoforms that contain the heparin-binding domain. The strongest contributing factor to the observed difference in the clinical efficacy of the two approved anti-VEGF therapeutics for neovascular AMD appears to be differences in the VEGF-A target specificity of the two agents. ■

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