Research Article

Resistance to anti-angiogenic therapy in patients with neovascular age-related macular degeneration

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Abstract
Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in developed countries among individuals aged 50 years and older. The neovascular form of late AMD affects only 10% of patients but is responsible for most cases of severe vision loss due to the disease. Despite their good results in treatment of neovascular AMD, anti-angiogenic agents can be ineffective in a subset of patients considered resistant to therapy. Several mechanisms have been proposed to explain his phenomenon and, subsequently, different management strategies are currently under investigation to improve patient outcomes with anti-angiogenic therapy.
Keywords: anti-angiogenic, neovascular, macular degeneration

Introduction
Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in developed countries among individuals aged 50 years and older.(Klein, Klein et al., 1992) The neovascular form of late AMD affects only 10% of patients, but is responsible for most cases of severe vision loss due to the disease. (Ferris, Fine et al., 1984).

The introduction of vascular endothelial growth factor (VEGF) inhibitors has been a breakthrough in the management of neovascular AMD with many patients achieving meaningful gains in visual acuity or at least maintaining a stable vision. However, some patients exhibit suboptimal or nonresponse to anti-VEGF injections, while others experience a slowly diminishing effect of treatment after chronic injections for extended periods.(Broadhead, Hong et al., 2014) Various explanations have been proposed for this phenomenon; including tachyphylaxis, upregulation of angiogenic factors, chronic structural changes in choroidal neovascular membranes (CNVM) or even carrying certain genetic risk alleles.(Binder 2012).

Pathophysiology of neovascular AMD
Neovascular AMD includes choroidal neovascularization (CNV) and associated manifesttations such as retinal pigment epithelial detachment (PED), retinal pigment epithelial tears, fibrovascular disciform scarring, and vitreous hemorrhage.(Bressler, Bressler et al., 1988). In the absence of anti-vascular endothelial growth factor (anti-VEGF) therapy, the vast majority of people with severe vision loss (20/200 or worse in either eye) from AMD have the neovascular form.(Ferris, Fine et al., 1984)

Angiogenesis refers to the creation of new blood vessels from existing blood vessels, and therefore is contrasted from the process of vasculogenesis seen characteristically in utero in which vessels are created de novo. The process of angiogenesis has been characterized as occurring in a series of orderly events: (Leung L. 2018)

(1) Hypoxia induces the release of signaling factors to promote the growth of new blood capillaries from pre-existing vessels.
(2) Pericytes detach from the vessel, and endothelial cells are activated and lose their close contact as the vessel dilates.
(3) In sprout formation, a tip cell is selected which releases matrix metalloproteases to degrade the basement membrane and remodel the extracellular matrix.
(4) Stalk cells follow the tip cell and proliferate, extending the sprout. Proliferating stalk cells establish junctions with neighboring endothelial cells and release signals that bind to extracellular membrane components and regulate vascular lumen formation.
(5) Once blood flow is established, the perfusion of oxygen and nutrient reduces

angiogenic stimuli and inactivates endothelial cell oxygen sensors, reestablishing the quiescent state of the blood vessel.

Vascular endothelial growth factor (VEGF)

It is now well established that VEGF plays a principal role in pathologic neovascularization, including not only neovascular AMD, but also diabetic retinopathy, retinal vein occlusion and retinopathy of prematurity. (Ambati, Ambati et al., 2003)

The chemical structure of VEGF is that of a heparin-binding homodimeric glycoprotein of 45 kilodaltons. The human VEGF gene is organized into 8 exons separated by 7 introns and is localized in chromosome 6p21.3. Although there is only a single gene for VEGF-A, alternate posttranslational exon splicing results in the generation of between 4-6 different isoforms having 121, 145, 165, 183, 189, and 206 amino acids respectively. VEGF165 is thought to be predominantly responsible for pathologic neovascularization. (Ferrara 2004)

Anti-VEGF agents currently in use

Monoclonal antibody: Bevacizumab

Bevacizumab (Avastin; Genentech/Roche, South San Francisco, CA) is a humanized monoclonal antibody (IgG1) against human VEGF-A that selectively inhibits all isoforms and bioactive proteolytic breakdown products of VEGF-A. (Ferrara 2004)

Antigen-binding fragment: Ranibizumab

Ranibizumab (Lucentis; Genentech/Roche, South San Francisco, CA) is a humanized anti-VEGF-A recombinant Fab fragment that has been affinity-matured to increase its binding affinity for VEGF-A. Ranibizumab binds within the VEGFR binding domain of all biologically active isoforms of VEGF-A. Two randomized, double-masked, pivotal phase 3 clinical trials have demonstrated that monthly intravitreal injections of ranibizumab are an effective and safe treatment for subfoveal CNV in AMD patients. (Brown, Kaiser et al., 2006, Rosenfeld, Brown et al., 2006)

Soluble receptor: Aflibercept

Aflibercept (VEGF-TRAP EYE, Eylea, Regeneron Pharmaceuticals, Tarrytown, NY) is a soluble fusion protein that combines ligand-binding elements taken from the extracellular components of VEGF receptors 1 and 2 fused to the Fc portion of IgG1.(Holash, Davis et al., 2002) As with ranibizumab, it has a high affinity for VEGF, (Chappelow and Kaiser 2008) and aflibercept is thought penetrate all layers of the retina. Unlike bevacizumab and ranibizumab that inhibit just VEGF-A, aflibercept also binds VEGF-B and placental growth factor (PlGF).(Rudge, Thurston et al., 2005)

Resistance to anti-VEGF therapy

Defining resistance

Previously, many researchers considered that patients with persistent IRF or SRF after 3 initial injections suffer from refractory or recalcitrant AMD.(Moon, Lee et al., 2015). However, as the responses of >30% of patients were delayed after 4 months of treatment in the MARINA and ANCHOR trials, (Brown, Kaiser et al., 2006, Rosenfeld, Brown et al., 2006) some researchers considered to redefine the threshold for refractory or recalcitrant AMD as persistent exudation after at least 6 months of monthly anti-VEGF treatment.(Broadhead, Hong et al., 2014)

Mechanisms of resistance to anti-VEGF therapy and possible therapeutic approaches

A. An incomplete initial effect caused by clinical factors

1. Misdiagnosis

Previous research has shown that 46.2% of patients with a poor response to treatment require revision of the primary diagnosis. For example, the misdiagnosis of polypoidal choroidal vasculopathy (PCV) as CNV and a lack of distinction between retinal angiomatous proliferation (RAP) and typical CNV have been described in several papers. (Eghoj and Sorensen 2012)

2. AMD risk genetic variants

Some researchers have speculated that a genetic predisposition may also contribute to resistance to anti-VEGF therapy. When investigating the association between polymorphism rs1061170 (T1277C, Y402H) in the CFH gene and the treatment response of neovascular AMD, patients homozygous for the variant risk C-allele (CC genotype) displayed a decreased response to treatment by 1.6-fold when compared to patients homozygous for the ancestral T-allele (TT genotype).(Chen, Yu et al., 2012) Lee et al., found that patients homozygous for this risk allele had a significantly

higher risk (37%) of requiring additional ranibizumab injections.(Lee, Raya et al., 2009). As for ARMS2 gene, polymorphism rs10490924 (A69S) in the LOC387715/ARMS2 gene was found to be associated with poor treatment outcome.(Abedi, Wickremasinghe et al., 2013)

B. Various redundant proangiogenic factors and other pathogenic pathways

Several proangiogenic factors, other than VEGF, could also promote angiogenesis, such as fibroblast growth factor, transforming growth factor, tumor necrosis factor, interleukins, platelet-derived growth factor (PDGF), and placenta growth factor. An increase in the expression of these factors may possibly fuel alternate signaling pathways for angiogenesis, which could trigger VEGF-independent neovascularization and cause resistance to mono anti-VEGF drugs. Several drugs inhibiting some of these alternative pathways are currently under investigation. (Wu, Palmer et al., 2008)

C. Pharmacologic analysis of resistance to anti-VEGF therapy

1. Alteration of neovascular structure

Vascular endothelial cells (ECs) play a crucial role in vascular formation. Anti-VEGF therapy may promote apoptosis of ECs, leading to empty vascular sleeves formed by the persistence of pericytes and the vascular basement membrane. These empty vascular sleeves serve as channels for EC proliferation when anti-VEGF therapy is halted, which might be one of the reasons for the regression of CNV. (Abdullah and Perez-Soler 2012)

2. Tolerance

Drug tolerance is a pharmacology concept, where a subject's reaction to a specific drug and the physiological concentration of the drug are reduced followed by repeated use, subsequently requiring an increased dosage or shorter dosing time intervals to achieve the desired effect. However, efficacy is not restored even when the treatment is halted temporarily. Drug tolerance could be divided into several different types, including pharmacodynamic tolerance, pharmacokinetic (metabolic) tolerance, and behavioral tolerance (for certain psychoactive drugs). (Rogers 1963)

Pharmacodynamic tolerance may be caused by increased VEGF expression, increased expression of VEGF receptors, changes in signal transduction, or a shift of the stimulus for CNV growth toward other growth factors.(Binder 2012) Pharmacokinetic tolerance occurs because a decreased quantity of the substance reaches the site it affects. A systemic immune response, the development of neutralizing antibodies, increased clearance from the eye, or reflux of the drug following injection may all result in pharmacokinetic tolerance.(Binder 2012)

3. Tachyphylaxis

Tachyphylaxis is a medical term describing an acute decrease in the response to a drug after its administration. Tachyphylaxis cannot be overcome by increasing the dosage. However, efficacy can be restored if the medication is stopped for a short while or if the interval between doses is increased.(Mc 1951) Some investigators suggested that tachyphylaxis may occur as early as after 2 injections of ranibizumab. (Gasperini, Fawzi et al., 2012)

If tachyphylaxis occurs, clinicians should stop the treatment for a while or switch to a similar drug with different properties.(Binder 2012) The proposed mechanism of switching between bevacizumab and ranibizumab could be due to the different molecular sizes and associated transport of these molecules through the retina and into the subretinal space.(Kent, Iordanous et al., 2012)

Aflibercept is a novel VEGF inhibitor with a higher binding efficacy and a wider spectrum of action than both bevacizumab and ranibizumab. Aflibercept may help patients with persistent fluid despite standard treatment with ranibizumab and bevacizumab. Several studies have consistently demonstrated that patients who are resistant to ranibizumab or bevacizumab have a therapeutic, anatomical response when switched to aflibercept, but only a few of them have shown improved visual outcomes.(Singh, Srivastava et al., 2015)

Conclusion

Even though the current literature is in favor of switching anti-VEGF therapies as a valid option

for patients who begin to experience diminished treatment effects, larger randomized controlled trials need to be conducted to accurately define criteria for switching and validate drug choice.

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