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REVIEW

Resistance to anti-VEGF therapy in neovascular age-related macular degeneration: a comprehensive review

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Abstract: As a progressive chronic disease, age-related macular degeneration (AMD) is the leading cause of irreversible vision impairment worldwide. Experimental and clinical evidence has demonstrated that vascular endothelial growth factor (VEGF) plays a vital role in the formation of choroidal neovascularization. Intravitreal injections of anti-VEGF agents have been recommended as a first-line treatment for neovascular AMD. However, persistent fluid or recurrent exudation still occurs despite standardized anti-VEGF therapy. Patients suffering from refractory or recurrent neovascular AMD may develop mechanisms of resistance to anti-VEGF therapy, which results in a diminished therapeutic effect. Until now, there has been no consensus on the definitions of refractory neovascular AMD and recurrent neovascular AMD. This article aims at clarifying these concepts to evaluate the efficacy of switching drugs, which contributes to making clinical decision more scientifically. Furthermore, insight into the causes of resistance to anti-VEGF therapy would be helpful for developing possible therapeutic approaches, such as combination therapy and multi-target treatment that can overcome this resistance.

Keywords: age-related macular degeneration, vascular endothelial growth factor, choroidal neovascularization, resistance

Introduction

Age-related macular degeneration (AMD) is a progressive chronic disease. The World Health Organization has indicated that AMD ranks as one of the leading causes of blindness globally due to the aging populations in many countries.[1] Neovascular AMD is characterized by pathologic choroidal neovascularization (CNV) that breaks through Bruch's membrane into the subretinal pigment epithelium space and/or the subretinal space, leading to exudation, hemorrhage, retinal edema, pigment epithelial detachment, and fibrous scarring,[2] which may produce serious impairments in visual acuity.

CNV is a process that involves both angiogenesis and inflammation.[3] Experimental and clinical evidence has shown that vascular endothelial growth factor (VEGF) is a key component in promoting neovascularization.[4–6] Intravitreal anti-VEGF agents have greatly improved visual outcomes.[7–13]

There are five anti-VEGF agents approved for the treatment of neovascular AMD. Pegaptanib became the first one to be approved by the US Food and Drug Administration (FDA), which selectively binds $VEGF_{165}$.[14–16] The VISION study demonstrated that pegaptanib 0.3 mg given intravitreally every 6 weeks resulted in 70% of patients losing fewer than 15 letters of visual acuity.[8] However, pegaptanib has been gradually

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Table 1 Comparison of current anti-VEGF agents for neovascular AMD

Anti-VEGF agents	Structure	Biological target	K_D for $VEGF_{165}$ (pM)	Molecular weight (kDa)	Approvals
Pegaptanib	Pegylated RNA aptamer	$VEGF-A_{165}$ only[15]	50[14]	50[16]	FDA (2004) EMA (2005)
Bevacizumab	Recombinant humanized monoclonal IgG1 antibody	All isoforms of VEGF-A[18]	58[19]	149[19]	FDA (2004) EMA (2005) CFDA (2010)
Ranibizumab	Recombinant humanized IgG1-κ isotype monoclonal antibody fragment	All isoforms of VEGF-A[17]	46[19]	48[17]	FDA (2006) EMA (2007) CFDA (2012)
Aflibercept	Fusion protein: domain 2 of VEGFR-1 and domain 3 of VEGFR-2 fused with IgG1 Fc	All isoforms of VEGF-A, VEGF-B, and PIGF[23]	0.5[23]	115[19]	FDA (2011) EMA (2012)
Conbercept	Fusion protein: domain 2 of VEGFR-1 and domains 3 and 4 of VEGFR-2 fused with IgG1 Fc	All isoforms of VEGF-A, VEGF-B, VEGF-C, and PIGF[25]	0.5[24]	143[24]	CFDA (2013)

Abbreviations: AMD, age-related macular degeneration; CFDA, China Food and Drug Administration; EMA, European medicines agency; FDA, US Food and Drug Administration; IgG, immunoglobulin G; K_D, binding affinity to $VEGF-A_{165}$; PIGF, placental growth factor; RNA, ribonucleic acid; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

replaced by the pan-VEGF-A inhibitors. Ranibizumab is a humanized monoclonal Fab fragment, while bevacizumab is a full-length humanized monoclonal antibody. These drugs could neutralize all the active isoforms of VEGF-A.[17–19] Ranibizumab was demonstrated to be effective in the MARINA and ANCHOR trials, based on the observation that ~90% of patients receiving monthly intravitreal treatment with ranibizumab lost fewer than 15 letters after 2 years.[7,10] Bevacizumab presented a similar efficacy to ranibizumab in the CATT trials and IVAN study.[9,11,20] Aflibercept and conbercept are recombinant fusion proteins that act as soluble decoy receptors for VEGF family members.[21–25] In the Phase III VIEW 1 and 2 trials, the administration of an intravitreal aflibercept injection monthly or every 2 months after three initial monthly doses achieved similar visual outcomes comparable to monthly intravitreal ranibizumab.[12] Conbercept was tested in the AURORA study, and most patients reported improved functional and morphologic parameters.[13] A comparison of current anti-VEGF agents for neovascular AMD is shown in Table 1. As the incidence of severe vision loss and blindness has been greatly reduced by 46%–51% in many countries,[26–28] anti-VEGF therapy is now considered a first-line treatment for neovascular AMD.

Although anti-VEGF agents have shown a dramatic breakthrough in neovascular AMD treatment recently, some patients have poor or nonresponse to anti-VEGF agents with standardized treatment or experience a slow loss of efficacy of anti-VEGF agents after repeated administration over time. Persistent fluid is still common after regular therapy. The CATT revealed that, despite monthly treatment with anti-VEGF agents for 2 years, 51.5% of patients receiving intravitreal ranibizumab and 67.4% of patients treated with bevacizumab had evidence of persistent fluid on time-domain

optical coherence tomography (OCT).[11] There are still 19.7%–36.6% of patients with active exudation on either angiography or OCT after 1 year of regular 2.0 mg aflibercept treatments (q4wk or q8wk).[12]

For these phenomena, researchers have offered various descriptions and explanations about the loss of the drug's effectiveness, such as "incomplete response",[29] "poor response",[30] "nonresponse",[30,31] "unresponsive",[32] "tolerance",[33–35] "tachyphylaxis",[34–40] "treatment resistant",[41–43] "resistance to anti-VEGF",[44] "refractory to anti-VEGF",[45] and "resistance to anti-VEGF treatment".[46] When describing and classifying patients with persistent fluid or recurrent exudation, researchers frequently use the terms "refractory neovascular AMD",[47–50] "recalcitrant neovascular AMD",[51–54] "recurrent neovascular AMD",[44,47,55–57] and "treatment-resistant neovascular AMD".[41–43] However, no present agreement exists on the definition of these terms, and this point has been highlighted and marked as needing further action. Clarifying and consolidating these concepts are of great importance for an effective evaluation of switching to other anti-VEGF drugs, combination therapy, and multi-target treatment. Furthermore, gaining an insight into the causes of resistance to anti-VEGF therapy would be helpful for developing novel strategies to improve the efficacy of anti-angiogenic therapies.

Definition of refractory neovascular AMD and recurrent neovascular AMD
Refractory neovascular AMD
In many clinical trials and scientific papers, researchers frequently use the terms "refractory neovascular AMD" and "recalcitrant neovascular AMD", but there is still debate

regarding what can be defined as "refractory neovascular AMD" or "recalcitrant neovascular AMD". Some researchers consider patients who show stationary or increased intraretinal or subretinal exudation despite more than three consecutive injections, even if an initial partial response could be observed temporarily, to be suffering from refractory neovascular AMD or recalcitrant neovascular AMD.[45,47,48,58,59] Arcinue et al[44] concluded that eyes with persistent fluid collection despite at least five monthly consecutive ranibizumab/bevacizumab injections might qualify as refractory neovascular AMD as well.

Previously, many researchers considered that patients with persistent fluid after three initial injections suffer from refractory or recalcitrant AMD, which is mainly based on remarkable vision improvement after three monthly injections. However, as the responses of >30% of patients were delayed after 4 months of treatment in the MARINA and ANCHOR trials,[7,10] a response to only three initial injections should not be considered an indicator of visual prognosis. Therefore, some researchers considered to redefine the threshold for refractory or recalcitrant AMD. Broadhead et al[41] considered persistent exudation after at least 6-month regular anti-VEGF therapy, which could be defined as "treatment resistance". Fung et al[55] defined "refractory CNV" as persistent fluid on spectral-domain OCT (SD-OCT) at <30 days after the last of six intravitreal injections of an anti-VEGF agent at monthly intervals. Grewal et al[52] put forward the concept of "recalcitrant exudative AMD" after 6 months of monthly anti-VEGF treatment.

Since "recalcitrant neovascular AMD" and "refractory neovascular AMD" are synonyms, we recommend the uniform use of "refractory neovascular AMD". We consider that "refractory neovascular AMD" should be defined in those patients who have a persistence of exudation as evident on clinical examination and also on imaging studies (leakage on fluorescein angiography, or fibrovascular pigment epithelial detachment with intraretinal fluid [IRF] or subretinal fluid [SRF] on SD-OCT), or even increasing hemorrhage compared to the baseline after six consecutive injections at monthly intervals. Nevertheless, structural lesions that can mimic leakage on SD-OCT, such as outer retinal tubulations[60] and chronic intraretinal cysts,[61] are considered chronic markers of atrophy and do not require anti-VEGF treatment, which should not be considered as evidence of refractory neovascular AMD.

Our understanding of "refractory neovascular AMD" is consistent with the definition of "recalcitrant exudative AMD" by Grewal et al[52] and has some characteristics in common with several experts' ideas, such as Broadhead et al[41]

and Fung et al.[55] Broadhead et al used the term "treatment-resistant neovascular AMD" and agreed with the idea of receiving standard anti-VEGF therapy for at least 6 months to evaluate the therapeutic response. "Treatment resistance" was another description of "refractory", but Broadhead et al failed to point out whether the 6-month anti-VEGF therapy was maintained at monthly intervals or at unfixed intervals. Meanwhile, Fung et al offered a definition of "refractory CNV", which was very similar to our concept of "refractory neovascular AMD".

These experts consider the persistence of exudation after 6 months of monthly anti-VEGF therapy as an indicator of "refractory neovascular AMD" based on abundant clinical experience in practice and scientific summary from clinical trials. "Refractory AMD" is a really important concept, which contributes to finding the right time of switching treatments and making clinical decision more scientifically. Further multicentric clinical trials are needed to demonstrate that six consecutive, monthly anti-VEGF injections are a turning point of anatomical changes and/or functional changes.

Recurrent neovascular AMD

Apart from persistence of exudation, there are still patients who suffer from the appearance of new retinal hemorrhage or SRF/IRF accumulation after the initial resolution of exudative changes. Kuroda et al[56] found that 65.7% of patients experienced a recurrence of retinal exudative change within 12 months and 74.8% reported the same within 24 months.

Yonekawa et al[47] considered that "recurrent" means exudation suppressed but requiring frequent injections. In our point of view, eyes have shown complete resolution of retinal exudative change after regular anti-VEGF treatment; once the treatment is withdrawn, multiple recurrences (a minimum of two) of new or increased IRF or SRF with or without vision changes or symptoms are defined as "recurrent neovascular AMD". Our understanding of "recurrent neovascular AMD" is consistent with Arcinue et al.[44] Furthermore, only one recurrence of exudation could be diagnosed as the recurrence of neovascular AMD, instead of "recurrent neovascular AMD". Recurrent retinal exudation in patients receiving uninterrupted treatment is preferable to experiencing "refractory neovascular AMD" after an initial response.

Despite multiple recurrences of exudation, some patients with recurrent neovascular AMD respond well to frequent retreatment and eventually become dry macular. However, other patients slowly become less responsive over time and maintain persistent exudation. These patients could be qualified as "refractory neovascular AMD".

Resistance to anti-VEGF therapy resulting in a diminished therapeutic effect

Regardless of whether the diagnosis is refractory neovascular AMD or recurrent neovascular AMD, various clinical manifestations are caused by significant interindividual differences in response to an anti-VEGF agent. There is no authoritative consensus as to how to classify "responder status". Recently, Amoaku et al[30] categorized the response to anti-VEGF therapies in neovascular AMD. It is divided into optimal (good) response, poor response, and nonresponse based on both functional and morphological outcomes. We consider it an appropriate definition/categorization of the response of neovascular AMD to anti-VEGF therapies. Patients who have poor response or nonresponse to anti-VEGF under the standardized treatment may gradually develop mechanisms of resistance to anti-VEGF therapy.

There is currently no consensus on the definition of "resistance to anti-VEGF therapy". Tranos et al[46] considered that half of the patients who did not improve and ~10% of the patients who had no response at all despite ongoing therapies with the current standard anti-VEGF approach were resistant to anti-VEGF therapy. Bakall et al[62] reported that some patients, however, had a good initial response with a resolution of fluid but then developed recurrent exudation and became resistant to further treatment. We consider patients who showed poor response or nonresponse to the initial therapy, or who had a successful initial response to anti-VEGF therapy but experienced a slow loss of response as "resistant to anti-VEGF therapy".

Some ophthalmologists make no distinction between "resistant", "refractory", and "recurrent". The term "resistant" is aimed at describing the status of a diminished therapeutic effect despite continuous treatment, while "refractory" or "recurrent" focuses on describing the characteristics of AMD itself, as previously explained. Therefore, phrases such as "resistance to anti-VEGF therapy", "refractory neovascular AMD", and "recurrent neovascular AMD" may be more useful and effective. In addition, it is also essential to distinguish "resistance to anti-VEGF therapy" and "resistance to anti-VEGF agents". The former is a broader concept that encompasses "resistance to anti-VEGF agents".

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

Resistance can occur at any time during the course of therapy.[41] Anti-VEGF therapy may fail from the beginning or following an initial successful treatment period. An incomplete effect of the initial therapy may be caused by several clinical factors, including misdiagnosis and genetic predisposition. Resistance to anti-VEGF agents and sustained activation of other pathogenic pathways result in the development of persistent or recurrent exudation after an initial successful treatment period. We draw on these facets to provide a framework to show why the phenomenon of resistance to anti-VEGF therapy occurs and how to deal with it (Figure 1).

An incomplete initial effect caused by clinical factors
Misdiagnosis
Misdiagnosis appears to be one common clinical factor that results in poor response or nonresponse to anti-VEGF therapy.
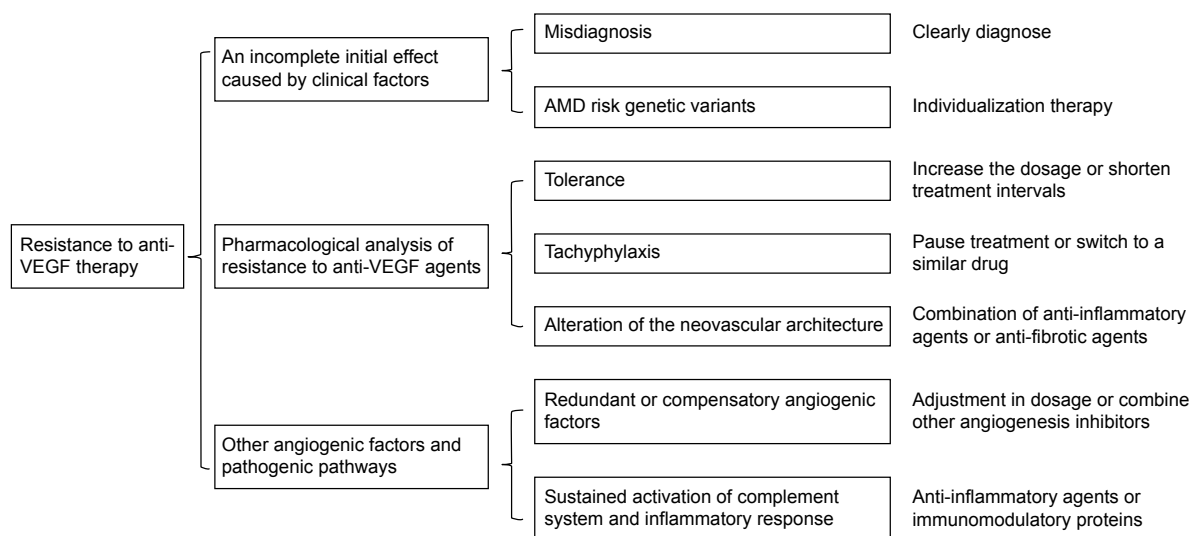

Figure 1 A framework to show the causes of resistance to anti-VEGF therapy and possible therapeutic approaches.
Abbreviations: AMD, age-related macular degeneration; VEGF, vascular endothelial growth factor.

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 at length in several papers.[40,45]

In contrast to CNV, which is secondary to AMD (CNV–AMD) in Western populations, PCV, an important variant of AMD, appears to be the predominant subtype of neovascular AMD in Asian populations.[63] PCV may account for as high as 22.3%–61.6%[64–72] of cases in Asians and 8%–13%[73] of Caucasian patients who present with presumed neovascular AMD. PCV may mimic CNV on fundus photography and fluorescence fundus angiography, further confusing the diagnosis. Focal hyperfluorescent polyps on early-phase indocyanine green angiography are still the gold standard for diagnosis.[74] Considering the lower prevalence of PCV in Caucasian patients, Western ophthalmologists are relatively less experienced in its diagnosis and treatment than Asian experts. Therefore, there might be higher rates of misdiagnosis of patients with PCV in Western countries than in Asia. On the other hand, as indocyanine green angiography is not a routine examination, misdiagnosis is still common worldwide.

Because the role of VEGF in the pathogenesis of PCV is believed to be substantially less important than in CNV, patients with PCV who are misdiagnosed for CNV may be resistant to anti-VEGF agents (ranibizumab and bevacizumab). Therefore, the diagnosis must be reevaluated, and more attention should be paid toward avoiding this misdiagnosis. If a patient has received a diagnosis of PCV, the treatment options should be changed. The optimal treatment for PCV requires further clarification.[75] PCV is usually treated with anti-VEGF monotherapy, photodynamic therapy (PDT) monotherapy, or a combination of anti-VEGF/PDT therapy, but ranibizumab and bevacizumab have limited effect on polypoidal lesions. Aflibercept, a new anti-VEGF drug, has been demonstrated to improve both visual acuity and macular morphology in a large number of treatment-naive eyes with PCV.[76]

RAP, which is also known as a variant of neovascular AMD, represents an estimated 10%–12%[77,78] of newly diagnosed neovascular AMD lesions. Freund et al[79] considered RAP to be a type 3 neovascularization in order to distinguish it from the type 1 and 2 CNV anatomic classifications. However, RAP may mimic type 1 and 2 CNV on fluorescence fundus angiography. There is a characteristic hyperfluorescent "hot spot" in early RAP lesions on indocyanine green angiography, which has previously been considered the best approach to diagnose RAP.[80] OCT angiography is a new noninvasive, motion contrast imaging modality for retinal microvasculature.

OCT angiography will play an important role in the early diagnosis of RAP to reduce the rate of misdiagnosis.[81]

RAP differs from typical neovascular AMD in its natural course and has previously been reported to have poor visual gain in response to anti-VEGF monotherapy.[82–86] However, a subanalysis of CATT found that RAP had an optimal response to anti-VEGF therapy.[87–89] Applying PDT simultaneously with intravitreal anti-VEGF agents effectively maintained or improved patients' visual acuity and reduced or eliminated edema in the short term.[90]

AMD risk genetic variants

AMD is influenced by both environmental and genetic factors. Numerous genetic variants, such as *CFH*, *HTRA1/ARMS2*, *C3*, *CFB/C2*, and *APOE* genes, confer significant risk for the development of AMD.[91] However, genetic testing is not considered to be included in the standard AMD diagnosis or treatment at present. Some ophthalmologists have speculated that a genetic predisposition may also contribute to resistance to anti-VEGF therapy.

Polymorphism rs1061170 (T1277C, Y402H) has been found to be strongly associated with exudative AMD[92] and AMD progression.[93] When investigating the association between polymorphism rs1061170 and the treatment response of neovascular AMD, patients harboring homozygous for the variant risk C-allele (CC genotype) are consistent with a decreased response to treatment by ~1.6-fold when compared to patients carrying homozygous for the ancestral T-allele (TT genotype).[94] Lee et al[95] found that patients harboring homozygous for the CFH Y402H risk allele had a significantly higher risk (37%) of requiring additional ranibizumab injections. In other words, the response to treatment of AMD with ranibizumab differed according to the patient's specific CFH genotype.

As for *ARMS2* gene, Abedi et al[96] found single nucleotide polymorphism rs10490924 (A69S) in the *LOC387715/ARMS2* gene with poor outcome of intravitreal anti-VEGF injections in neovascular AMD. A literature-based meta-analysis was performed of studies relevant to A69S polymorphism in the *ARMS2* gene and the response to anti-angiogenesis treatment by Hu et al.[97] They also found A69S could be considered predictive of the anti-angiogenic effects, especially in Asian populations.[97]

These patients with AMD risk genetic variants might have higher background levels of inflammation, which may continue to affect the disease progression and probably lead to a more rapid recurrence of neovascularization, which produces a diminished therapeutic effect.[95] It is conceivable that future AMD treatments may depend on the patient's individual genetic risk profile to develop individualized

therapy.[98] For example, intravitreal exogenous CFH or CFH-related complement inhibitors may be a beneficial therapy for patients with polymorphism rs1061170.

Pharmacological analysis of resistance to anti-VEGF agents

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.[99] However, efficacy is not restored even when the treatment is halted temporarily.[100] Drug tolerance could be divided into several different types, including pharmacodynamic tolerance, pharmacokinetic (metabolic) tolerance, and behavioral tolerance (for certain psychoactive drugs).

During anti-VEGF therapies, pharmacodynamic tolerance may be caused by the increased expression of VEGF (especially derived from those macrophages that locate within the choroidal neovascular tissue and respond to VEGF inhibition by upregulating the production of VEGF itself), increased expression of VEGF receptors, changes in signal transduction, or a shift of the stimulus for CNV growth toward other growth factors.[34] Pharmacokinetic tolerance occurs because a decreased quantity of the substance reaches the site it affects. A systemic immune response, the development of neutralizing antibodies,[34] increased clearance from the eye, or reflux of the drug following injection may all result in pharmacokinetic tolerance. The Biologics License Application states that the baseline incidence of immunoreactivity to ranibizumab is 0%–3%, which rises to ~1%–6% after monthly dosing with ranibizumab for 12–24 months based on 1-year clinical efficacy and safety data from two pivotal Phase III trials, ANCHOR and MARINA, and the Phase I–II FOCUS trial.[36] Theoretically, it is therefore necessary to increase the dosage or shorten treatment intervals if tolerance has developed.

Several studies have investigated the relationship between increasing the dose and further anatomical and visual outcomes. The HARBOR trial[101] and Forooghian et al's[36] study demonstrated that high-dose ranibizumab/bevacizumab given monthly did not restore therapeutic responses in eyes that had developed a tolerance, while the evaluation of high-dose ranibizumab (2.0 mg) in the management of AMD in patients with persistent/recurrent macular fluid (LAST) study[55] and Brown et al's[51] trial found that 2.0 mg of ranibizumab could maintain anatomical results and preserve or improve best-corrected visual acuity in patients with

persistent or recurrent SRF or IRF despite previous standard anti-VEGF therapy. Compared to Forooghian et al's study, the LAST study, and Brown et al's trial, the conclusion of the HARBOR trial may be more persuasive because of that study's relatively larger sample. The study indicated that intravitreal high-dose anti-VEGF agents may not be readily effective at restoring a complete therapeutic response in all patients. Apart from unclear efficacy, the treatment is also an economic burden for patients when the dosage is increased, which makes it difficult to apply in clinical practice.

Few large trials have evaluated the effect of increasing the frequency of treatment to more than once a month. Stewart et al[102] found that dosing a drug (ranibizumab, bevacizumab, and aflibercept) every 2 weeks resulted in markedly improved trough binding activity, so the short-term use of biweekly dosing may be an attractive treatment option for those eyes that respond within 2 weeks of an injection but then rebound with increased macular fluid after a month. Treatment every 2 weeks may present a challenge for patients with poor compliance and also carries a significant cost implication. Moreover, shorter dosing time intervals of every 2 weeks have not yet been approved by the FDA for neovascular AMD.

Tachyphylaxis

Tachyphylaxis is a medical term describing an acute (sudden) decrease in the response to a drug after its administration.[103] It can occur after an initial dose or following a series of small doses. Keane et al[104] was the first to suggest that possible tachyphylaxis had appeared after treatment with ranibizumab, while other researchers have considered that tachyphylaxis may occur as early as after two injections.[37–39,105] 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. However, the mechanism of tachyphylaxis during anti-VEGF therapies for exudative AMD is still not clear.

If tachyphylaxis occurs, clinicians should stop the treatment for a while or switch to a similar drug with different properties.[34] The majority of these therapies involve switching patients from bevacizumab to ranibizumab,[37,106–109] from ranibizumab to bevacizumab,[37,105,107–110] and from bevacizumab/ranibizumab to aflibercept.[42,43,47,52,62,111–119]

The proposed mechanism of switching between two anti-VEGF drugs, 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. Ranibizumab was found diffusely across the retina after intravitreal injection because of its smaller size. Bevacizumab may also reach the subretinal space with a different distribution in

the retina after intravitreal injection.[120] Aflibercept is a novel VEGF inhibitor with a higher binding efficacy and a wider spectrum of action than both bevacizumab and ranibizumab.[21] Aflibercept may help patients with persistent fluid despite standard treatment with ranibizumab and bevacizumab. Fourteen trials have all demonstrated that patients who are resistant to ranibizumab or bevacizumab have a therapeutic, anatomical structure response when switched to aflibercept, but only five of them[43,111–113,118] experienced improved visual outcomes.

Conbercept has a similar molecular structure to that of aflibercept, which is also a recombinant fusion protein of the ligand-binding elements of VEGF receptors.[22] Conbercept was approved by the China FDA in December 2013 and has not yet reached the market in other countries. Therefore, there was no evidence to verify the efficacy of switching to conbercept when tachyphylaxis occurs. Given its similar structure to aflibercept, excellent safety and efficacy profile, conbercept is expected to be effective for such patients, but further investigation is needed.

Alteration of the neovascular architecture

Vascular endothelial cells (ECs) play a crucial role in vascular formation. EC mutations may potentially lead to conformational changes in receptors and affect the expression profile and the resultant sensitivity to available antiangiogenic agents.[121] In addition, 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,[122] which might be one of the reasons for the regression of CNV.

Oncologists have demonstrated that tumor vessels have enhanced vessel diameter, mature pericytes, immunoreactivity for desmin, platelet-derived growth factor receptor-β, and late-stage maturity marker α smooth muscle actin to enhance vascular maturity during antiangiogenic blockade. Prolonged antiangiogenesis significantly alters the expression of angiogenic factors implicated in vascular mural cell recruitment, causing extensive morphological changes in the vessels.[123] We could speculate that there may be similar changes in CNV architecture during prolonged antiangiogenic blockade, which forms a complicated barrier to current therapy.

Chronic inflammation may cause permanent structural damage to the vascular walls of the CNV complex, which could conceivably result in permanent abnormal vascular permeability and persistent exudation that is no longer amenable to anti-VEGF therapy.[101] Inflammatory stimulation could also increase fibrosis of the CNV, which acts as a resorption barrier and decreases patients' sensitivity to anti-VEGF drugs.

Mutations in ECs, maintenance of vascular sleeves, vascular remodeling, and chronic inflammatory changes of CNV can be influential in their therapeutic effects. There is currently still no effective therapy for EC mutations and apoptosis during antiangiogenic therapy. However, the combination of anti-inflammatory agents or anti-fibrotic agents might play a role in delaying the process of chronic inflammatory changes of CNV.

Various redundant proangiogenic factors and other pathogenic pathways
Redundant or compensatory angiogenic factors

Although VEGF is a key driver of the formation of CNV, many other proangiogenic factors 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. VEGF signaling might be closely linked to other pathways, such as PDGF[124,125] and fibroblast growth factor[126,127] signaling. 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.

Treatment may require dynamic adjustment in the dosage of the therapy or a combination with other angiogenesis inhibitors, such as anti-PDGF agents. PDGF participates in the recruitment of pericytes; thus, anti-PDGF therapy could prevent the pericytes from protecting the vessels, possibly increasing neovascular sensitivity to anti-VEGF therapy.[128] Fovista is an anti-PDGF agent. A Phase IIb clinical trial has demonstrated that patients who received ranibizumab combined with Fovista obtained a significantly higher final visual acuity than those administered ranibizumab monotherapy. A Phase III randomized, double-blind, controlled trial is underway.

Sustained activation of the complement system and inflammatory response

In addition to angiogenesis, complement activation and inflammation have also been implicated in the pathogenesis of AMD. Anti-VEGF therapy can only inhibit VEGF-induced neovascularization, but sustained activation of the complement system and inflammatory response may reduce the sensibility to anti-VEGF agents.

Neovascular AMD, with its various pathogens and multiple pathogenic mechanisms, is a complicated disease that requires multi-targeted and comprehensive treatment, such as

a combination of anti-inflammatory agents or immunomodu-latory proteins. Triamcinolone is a long-acting synthetic corticosteroid that has been used intravitreally to reduce macular edema. Schaal et al[39] have found that the combination of triamcinolone acetate and anti-VEGF therapy may lessen the effect of decreased bioefficacy after repeated intravitreal injections. Tandospirone, a serotonin receptor agonist, has a local neuroprotective, anti-inflammatory effect and is being investigated at present. Complement system-modulating substances, such as antibodies (LFG316, FCFD4514S, eculizumab), peptides (POT-4), aptamers (ARC1905), and antibody fragments (lampalizumab), show promising prospects in AMD therapy.[98]

Conclusion

Five anti-VEGF agents have been introduced in the field of ophthalmology since 2004. These agents have brought dramatic changes in the treatment of neovascular AMD, with fewer patients losing their vision and a reasonable proportion showing vision improvement. Despite the outstanding advances made by anti-VEGF therapy, most patients require repeated injections frequently and long-term follow-up regularly. The SEVEN-UP study[129] showed that the mean visual acuity gradually decreased during long-term follow-up with retreatment using a pro re nata regimen when patients exited from the MARINA or ANCHOR trial. These findings indicated that anti-VEGF therapy is a long and arduous process. Emerging terms such as "refractory neovascular AMD" and "recurrent neovascular AMD" are widely used today. As novel anti-VEGF agents, aflibercept and conbercept have a higher binding efficacy and a wider spectrum of action than both bevacizumab and ranibizumab.[21,22] Switching to aflibercept or conbercept may be effective for patients resistant to treatment with bevacizumab or ranibizumab. To consolidate and define these concepts is of great importance in clinical decision making with regard to the switching opportunity and also an evaluation of its effects.

We have to realize that beyond VEGF, there are still abundant angiogenic signaling cascade and other pathways that are related to the pathophysiology of neovascular AMD altogether. Many investigational drugs have the potential to not only reduce patient visits and injections but also improve outcomes by targeting additional pathways, increasing the target's affinity, and lengthening treatment durability.[128] Insight into the mechanisms of resistance to anti-VEGF therapy would be helpful to guiding treatment decisions regarding when to switch to other anti-VEGF drugs or choose a combination therapy or multi-target treatment, which will be a real breakthrough in the treatment of neovascular AMD.

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