Characterisation of Poor Visual Outcomes of Neovascular Age-related Macular Degeneration Treated with Anti-Vascular Endothelial Growth Factor Agents

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Abstract

Purpose: To investigate the incidence, characteristics and baseline predictors of poor visual outcomes in eyes with neovascular age-related macular degeneration (nAMD) receiving intravitreal anti-vascular endothelial growth factor (anti-VEGF) agents in daily clinical practice.

Design: Observational study.

Participants: Treatment-naïve eyes starting anti-VEGF therapy for nAMD between 2007 and 2012 tracked in the Fight Retinal Blindness! registry. Cases had sustained ≥15 letters of loss from baseline without recovery of visual acuity (VA) at final endpoint. A subgroup analysis included eyes that sustained ≥30 letters of loss. Controls had not sustained ≥15 letters of loss.

Methods: Kaplan-Meier curves estimated time to first development of loss of ≥15 letters. Cox-proportional hazards models evaluated predictors of loss of ≥15 letters.

Main Outcome Measures: The proportion of eyes with sustained VA loss within 5 years, the time to development of sustained VA loss and baseline predictors of sustained VA loss.

Results: There were 1760 eyes in total and 856 eyes that completed 5 years follow-up. The proportion of eyes with sustained VA loss of ≥15 letters at 5 years was 22.9% (95%CI, 20.7-25.1) and VA loss of ≥30 letters was 10.8% (95%CI, 9.1-12.5). Factors independently associated with higher incidence of sustained ≥15 letter loss included age >80 years (odds ratio [OR], 1.33 for patients >80 years vs. ≤80 years; 95%CI, 1.05-1.69; P =.02), fewer injections (OR, 0.97 per injection; 95%CI, 0.96-0.98; P=.0005) and more visits at which the choroidal neovascularisation was graded as active (OR, 1.97 for eyes in upper quartile of active visits vs. eyes in lowest quartile of active visits; 95%CI, 1.39-2.79; P=.0001). Baseline VA≥70 letters was associated with reduced risk of sustained ≥30 letter loss (OR, 0.61; 95%CI, 0.38-0.98; P=.04). Baseline angiographic lesion criteria were not significantly associated with sustained VA loss.

Conclusions: Twenty-three percent of eyes with nAMD developed sustained VA loss of ≥15 letters over 5 years of anti-VEGF therapy. Baseline predictors of poor outcomes provide more accurate assessment of the potential benefit from anti-VEGF therapy.

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Chapter One - Literature Review

Introduction

Age-related macular degeneration (AMD) is a major cause of visual impairment and irreversible blindness in people aged over 50 years in the developed world.[1, 2] It affects the macula, the central area of the retina which is responsible for central vision, resulting in progressive loss of central vision (Figure 1).[3, 4] Early-stage AMD is characterized by clinical signs of drusen, accumulation of material between the retinal pigment epithelium (RPE) and Bruch's membrane, and RPE abnormalities at the macula. Late-stage AMD can be either non-neovascular (also referred to as dry, non-exudative, or atrophic) or neovascular (also referred to as wet or exudative). Late-stage AMD causes loss of vision which can result in irreversible visual impairment and reduced quality of life and ability to carry out activities of daily living. Late-stage AMD in the form of macular atrophy (MA) is associated with progressive atrophy of the RPE, photoreceptors and underlying choroidal capillaries. Neovascular AMD (nAMD) causes more rapid vision loss due to choroidal neovascularisation (CNV) which refers to growth of new blood vessels that originate from the choroid and break through Bruch's membrane into the sub-retinal pigment epithelial or subretinal space, leaking lipids, fluid and blood. This can eventually result in fibrous scarring and irreversible vision loss.[5, 6]

Age-related macular degeneration is a multifactorial disease involving the complement pathways, and angiogenic and inflammatory processes. Significant risk factors include age and family history, while significant environmental risk factors include smoking and low dietary intake of antioxidants such as carotenoids and zinc.[7, 8] Age-related macular degeneration is a potential major public health issue with significant socioeconomic implications since the number of patients with AMD globally is estimated to be 200 million by 2020 and expected to continue to increase.[4, 9, 10]

There are currently no available proven therapies for MA but agents are being investigated in clinical trials. High-dose dietary supplements consisting of zinc and antioxidants are currently used to slow progression from early-stage to late-stage disease.[3, 4]

Age-related macular degeneration remains a major cause of visual impairment globally but visual impairment has reduced in incidence since the introduction of anti-vascular endothelial growth factor (anti-VEGF) therapy. Vascular endothelial growth factor, which is upregulated in nAMD, is an endothelial cell-specific mitogen that promotes vascular permeability and retinal neovascularization. The management of nAMD was revolutionized with the development of anti-VEGF agents, which include ranibizumab (Lucentis, Genentech Inc.), bevacizumab (Avastin, Genentech Inc.) and aflibercept (Eylea, Regeneron Pharmaceuticals, Inc.).[11, 12] These therapies are effective in preserving vision in most patients with nAMD, at least in the short to medium term.[12-18]

Despite the effectiveness of anti-VEGF therapy for nAMD, significant loss of vision can still occur. The major clinical trials of ranibizumab, Minimally Classic/Occult Trial of the Anti-VEGF Antibody

Ranibizumab in the Treatment of Neovascular AMD (MARINA) and Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in AMD (ANCHOR), reported a loss of 15 or more letters of visual acuity (VA) in 8% to 10% of eyes at 2 years.[19, 20] In The Comparison of Age-Related Macular Degeneration Treatments Trials (CATT), 9.2% of patients treated with ranibizumab or bevacizumab lost 15 or more letters at 2 years while 75.1% of patients had the same or improved VA compared with baseline acuity.[16] Understanding the factors that contribute to vision loss in patients receiving therapy may lead to improved treatment and preventive strategies. Identification of baseline predictors associated with VA outcomes may provide a more accurate assessment of the potential benefit from anti-VEGF agent therapy.
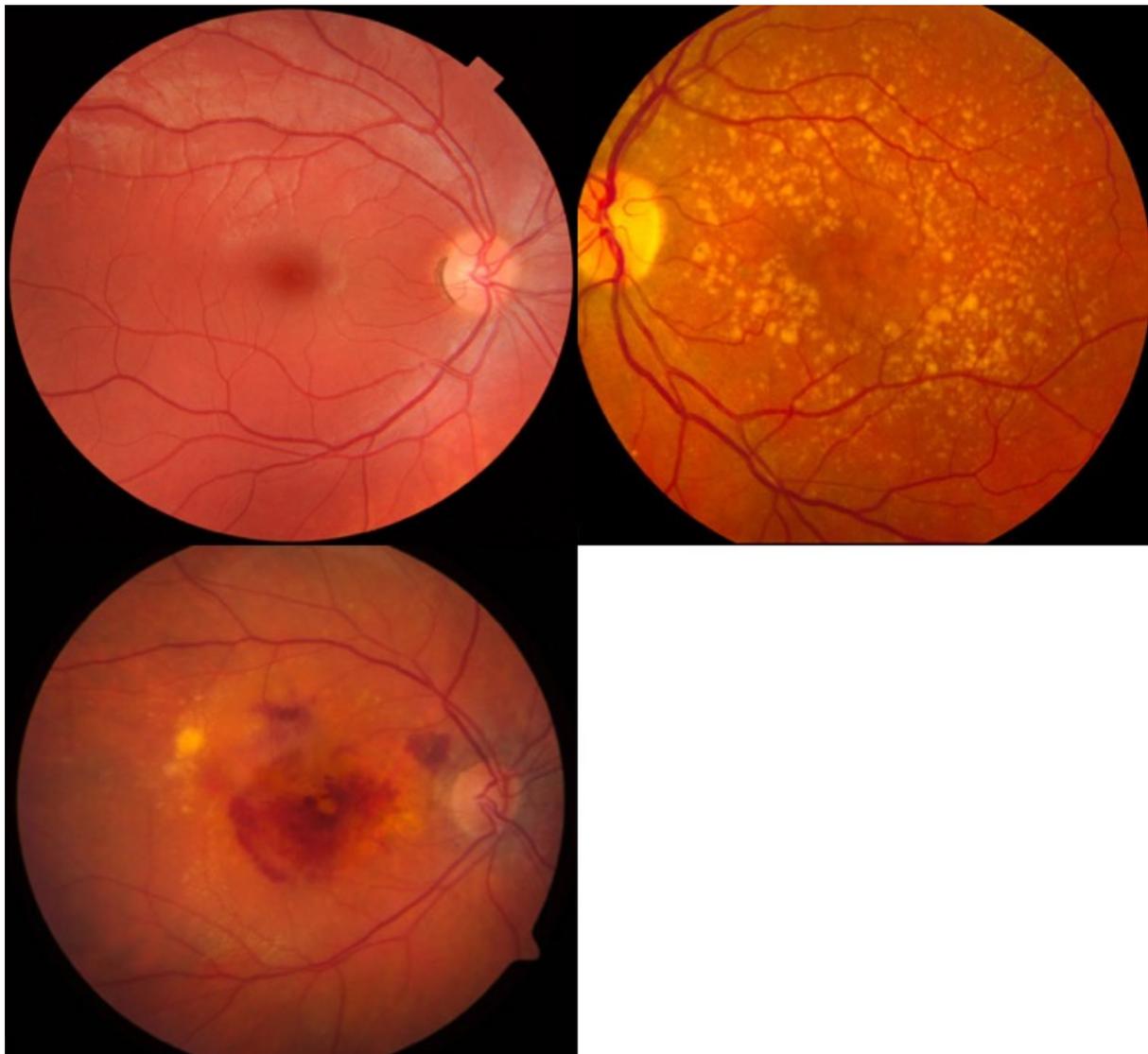

Figure 1. Normal and abnormal retina. Top left: Normal retina with normal macular, blood vessels and optic disc. Top right: Abnormal retina with macular drusen. Bottom left: Abnormal retina with macular haemorrhage.
National Eye Institute, National Institutes of Health [Internet]. NIH [updated 2019; cited 2019 Sep 9]. Available from: https://nei.nih.gov/photo

Epidemiology of AMD

Age-related macular degeneration is a leading cause of irreversible blindness among the elderly population in developed countries including Australia. The pooled prevalence of early-stage, late-stage and any AMD was reported by a recent meta-analysis of 12,727 cases (age range, 45-85 years) from 39 population-based studies to be 8.0%, 0.4% and 8.7%, respectively.[9] The incidence of AMD is predicted to increase with the aging of the population.[21-25] Three population-based cohort studies, the Rotterdam Study (RS), Beaver Dam Eye Study (BDES) and Blue Mountains Eye Study (BMES), have reported individual and pooled data on the prevalence of AMD in white populations.[26-29] The prevalence of late-stage AMD in these three studies was 0.2% (10 of 4797 patients) for people aged 55-64 years and increased to 13.1% (68 of 521) for people aged over 85 years.[30] The BMES reported that the 15-year incidence for early-stage AMD and late-stage AMD in people older than 40 years was 22.7% (462 of 2036) and 6.8% (165 of 2421), respectively.[31]

Although the prevalence of AMD is relatively higher in developed countries, there is increasing prevalence in Asian countries associated with the changing demographics and westernization of their diet and lifestyle.[32, 33] The estimated overall prevalence of late-stage AMD in people aged 40 years and older in the United States is 1.5%. The number of people in the United States with AMD is estimated to increase more than 50% from 1.75 million in 2000 to 2.95 million in 2020.[2, 33] The prevalence of late-stage AMD in India has been reported to be 1.4% with the prevalence increasing from 0.4% in those aged 50-59 years to 4.6% in those aged 70 years or older.[32] The 5-year incidence of early-stage and late-stage AMD in Singapore has been reported to be 5.6% and 1.0%, respectively.[34] The prevalence of any stage of AMD is higher in European white people than Asian (12.3% vs 7.4%) and African people (12.3% vs 7.5%) as reported in a global meta-analysis. There were no significant differences in prevalence found between Asian and African populations.[9]

Genetics and pathogenesis of AMD
Genetics

Age-related macular degeneration is a multifactorial disease with a significant genetic component. Genetic loci are known to be independently associated with late-stage AMD. The complement pathway and the age-related maculopathy susceptibility 2 (ARMS2) locus have been implicated.[35-37] Twin studies involving monozygotic twins have reported that twins with more advanced AMD had greater smoking exposure compared to twins with less advanced AMD.[38]

Pathogenesis

The macula contains the densest concentration of photoreceptors and is responsible for VA, allowing a patient to see fine detail, read and recognise faces. Posterior to the photoreceptors is the RPE which is part of the blood-ocular barrier and has important functions including photoreceptor phagocytosis, cytokine secretion and nutrient transport. Posterior to the RPE is Bruch's membrane which is a semipermeable exchange barrier that separates the RPE from the choroid and supplies blood to the outer layers of the retina.[39] Focal deposition of debris between the RPE and Bruch's membrane, known as drusen, can occur as the eye ages. Drusen are characteristic for AMD and are

clinically visible in the macula.[5] Drusen can be present in the periphery of the retinal but they do not generally affect VA.[40, 41]

Drusen correspond to basal linear deposits comprised of membranous material located between the basement membrane of the RPE and the inner collagenous layer of Bruch's membrane as seen on histology and electron microscopy.[42] Drusen are comprised of lipid, amyloid, complement factors and other cellular components.[43] The appearance of drusen is preceded by or concomitant with thickening of the Bruch's membrane collagenous layers, degeneration of elastin and collagen within Bruch's membrane, increased levels of glycation end products and accumulation of lipids and proteins. These changes have been proposed by some to act as a hydrophobic barrier impeding passage of nutrients and fluid between the choroid and outer retina resulting in focal ischemia. Subsequent ingrowth of neovascularization from the underlying choroidal capillaries may then occur through breaks in Bruch's membrane.[44] Retinal pigment epithelial abnormalities in AMD represent areas of RPE decompensation. Retinal pigment epithelial responses to cellular stress result in the main manifestations of early-stage AMD lesions which are drusen and RPE abnormalities.[41, 45] Macular atrophy is a late manifestation of AMD which is characterised by loss of RPE cells, overlying photoreceptors and underlying choroidal capillaries. Histological studies suggest that atrophy of the RPE occurs first in MA followed by degeneration of the underlying choroidal capillaries.[46]

The neovascular component of nAMD has been the focus of research that has led to the identification of proangiogenic molecules including VEGF, basic fibroblast growth factor and placental growth factor (PGF). The activation of endothelial cells by VEGF and other proangiogenic factors causes the release of proteases that degrade basement membrane. The activated endothelial cell proliferates and migrates toward the angiogenic stimulus using integrins for cell adhesion and results in neovascularisaton.[47, 48] Subtypes of CNV in nAMD are classified according to the extent of invasion into the retina. Type 1 neovascularisation ("occult" CNV) refers to CNV proliferation that occurs below the RPE and demonstrates an ill-defined pattern of leakage on fundus fluorescein angiography. Type 2 neovascularisation ("classic" CNV) refers to CNV proliferation above the RPE in the subretinal space and demonstrates intense fluorescein leakage on fundus fluorescein angiography. Type 3 neovascularisation ("retinal angiomatous proliferation" [RAP]) occurs when the retinal circulation is involved, with choroidal and retinal circulation anastomoses. A particular type of type 1 CNV is polypoidal choroidal vasculopathy, characterised by a large aneurysmal component and is more common in African and Asian patients than in European patients.[49, 50]

Classification of AMD

Broadly there are two clinical forms of AMD. Non-neovascular AMD is the most common form, accounting for 90% of the disease, is characterized by a slow and progressive degeneration of the RPE leading to photoreceptor cell death. The most severe manifestation of non-neovascular AMD is MA.[6] Neovascular AMD is less common than non-neovascular AMD, but is associated with more

rapid progression to severe vision loss.[1, 39] The hallmark of nAMD is the development of CNV along with the leakage of fluid, haemorrhage, scarring and severe vision loss if not treated.[3, 5, 39]

Although the classification of AMD into non-neovascular and neovascular forms is useful, this classification does not correlate with the risk of vision loss or aid in assessing the risk of disease progression. A useful classification in this regard was proposed by the Age-Related Eye Disease Study (AREDS) which consisted of a nine-level classification but was difficult to implement in clinical practice.[51, 52] A simplified version is better applied in clinical practice and consists of three levels of severity: "Early-stage AMD" is defined by the presence of a few medium-sized drusen and no RPE abnormalities; "Intermediate-stage AMD" is characterized by at least one large druse, various medium-sized drusen or any RPE abnormalities; "Late-stage AMD" can be either non-neovascular or neovascular and is characterized by the presence of MA or by CNV and its sequelae.[52-54]

Risk factors for AMD

Many risk factors for AMD have been identified including genetic, demographic, medical, ocular, nutritional and lifestyle factors.[5, 39] Increasing age, current cigarette smoking, previous cataract surgery and a family history of AMD have been reported to have strong and consistent associations with late-stage AMD by a meta-analysis of 18 prospective and cross-sectional studies and 6 case control studies involving 113,780 patients with 17,236 cases of late-stage AMD.[7]

Age is the strongest risk factor with almost all late-stage AMD cases occurring in patients aged over 60 years.[30] Smoking and diet are the most significant environmental factors associated with AMD.[55] Smoking, the strongest modifiable risk factor for AMD, has been consistently associated with at least a two-times increased risk for developing late-stage AMD and approximately a 10-year younger age at onset.[30, 56] Larger amount smoked was reported in the AREDS Report 19 to be significantly associated with the incidence of nAMD (odds ratio [OR] >10 vs. ≤ 10 pack-years, 1.55, 95% confidence interval [CI], 1.15-2.09).[8, 57, 58] The mechanism through which smoking harms the retina is unknown, but it has been associated with increased oxidative stress, platelet aggregation and reduced plasma high-density lipoproteins and antioxidant levels.

Family history is an established risk factor for AMD demonstrated in twin studies. It is now accepted that dysregulation of the innate complement pathway leads to aberrant inflammatory responses resulting in the accumulation of debris within Bruch's membrane. The identification of these genes provides evidence that AMD is a genetic disease and inflammation plays an important role.[59-61]

Risk factors with moderate associations include history of cardiovascular disease, hypertension and higher body mass index. Risk factors with weaker and inconsistent associations include gender, ethnicity, diabetes, history of cerebrovascular disease, and serum cholesterol and triglyceride levels.[7]

The possible risks of cataract surgery in eyes with early-stage AMD are inconclusive. The AREDS 1 study, Report 25, was not able to identify a clear effect of cataract surgery on the risk of progression to late-stage AMD in its examination of 4,577 patients (8,050 eyes).[62] Insufficient evidence to support cataract surgery as a risk factor for late-stage AMD has been reported by a systematic review of randomized controlled trials (RCTs).[63]

Implications of AMD

It is thought that the impact of AMD will not manifest equally in all countries. The more developed countries, with longer life expectancy, may suffer more significant economic burden.[2, 33] Age-related macular degeneration can have significant implications on quality of life with patients with AMD reporting lower satisfaction and activity levels and increased prevalence of depression, compared with similarly aged patients without AMD.[64, 65] The BMES reported that patients with AMD of any stage had two-times higher risk of negative effects on activities of daily living compared with patients without AMD.[66] Late-stage AMD was associated with elevated rates of all-cause (hazard ratio [HR] 1.20, 95%CI 1.02-1.41) and cardiovascular mortality (HR 1.46, 95%CI 1.13-1.98).[67]

Clinical features of AMD
Symptoms and diagnosis

Early symptoms of AMD include distorted vision when reading, watching television or driving, difficulty recognising faces and a dark or grey patch in the central vision. Early-stage AMD is often asymptomatic although patients may notice mild distorted vision, especially when reading and difficulty reading in low light conditions. Late-stage AMD can cause severe vision loss and can progress rapidly over weeks or months in the neovascular form, or more slowly over years or decades with MA.[3, 39]

Age-related macular degeneration is diagnosed on the basis of clinical examination and various imaging modalities. Spectral-domain optical coherence tomography (OCT) and fundus fluorescein angiography (FFA) are important imaging modalities used to assist in diagnosis and monitoring of AMD and its treatment. Fundus autofluorescence (FAF) and indocyanine green angiography (ICG) are less commonly used in clinical practice.[5] Optical coherence tomography angiography is a non-invasive approach that detects the presence of choroidal vascular networks seen in CNV, but does not detect leakage. Fundus fluorescein angiography is more invasive as it requires venous cannulation and injection of fluorescein dye. It allows for detection of CNV and its location and activity by assessing the nature and extent of dye leakage.[68, 69]

Non-neovascular AMD

Non-neovascular AMD is characterised by the presence of drusen, RPE abnormalities and MA in late-stage disease. Drusen clinically appear as focal, yellow-white subretinal lesions that usually cluster in the posterior pole but can appear anywhere in the retina.[5] Drusen are located between the RPE and Bruch's membrane and vary in number, size, shape and pattern of distribution. Most drusen are characterized as "hard" or "soft" as well as small (<63 μm), intermediate (between 63 μm and 125 μm)

or large (≥125 μm).[53] Hard drusen, which appear as round, discrete yellow-white spots, are about 63 μm in size. Hard drusen are commonly present in many populations. They are not necessarily age-related and do not carry an increased risk for development of neovascularization. Soft drusen, which have non-discrete borders, are 63 μm or greater in size. Population based studies and clinical trials have reported that large, soft, confluent drusen are age-related and associated with risk for progression to late-stage AMD and neovascularization.[53, 70] Retinal pigment epithelium abnormalities are another important clinical feature of non-neovascular AMD. The risk of developing soft drusen and MA significantly increases in their presence.[71]

Macular atrophy clinically appears as well delineated areas of hypopigmentation due to absence or severe attenuation of the underlying RPE. The larger, deep choroidal vessels are more easily visualized through the atrophic patches that also lack photoreceptors and the underlying choroidal capillaries. Macular atrophy can be unifocal or multifocal and can surround but spare the central macula initially. If the foveal center is spared, good VA may be preserved, although reading vision may be poor because of a constricted central visual field. Many eyes that have MA also exhibit drusen and most cases of MA occur in a pattern corresponding to the regression of prior drusen. Vision loss from non-neovascular AMD is generally due to MA involving the foveal region.[72, 73] Multimodal imaging is useful to detect and monitor the progression of MA because lesion borders and extent can be quantified accurately using OCT and FAF.[74]

Neovascular AMD

Neovascular AMD is characterised by the presence of neovascularization within the macula. Choroidal neovascularisation results from the ingrowth of neovascularization from the choroidal capillaries under neural retina. Neovascularisation may also arise predominantly within the retina and is known as RAP. There can be retinal-choroidal anastomoses which are communications between the retinal and the choroidal circulations in the advanced forms of nAMD. The CNV complex includes lesions such as presence of fluid or retinal haemorrhage, RPE detachments, hard exudate, or subretinal fibrous scar tissue. Imaging, particularly with OCT, demonstrates these manifestations clearly and provides information on the size, location and extent of drusen, and the presence and activity of CNV.[68] A retinal pigment epithelial detachment (PED) can be caused by serous fluid, fibrovascular tissue, haemorrhage or the coalescence of drusen beneath the RPE. The neovascularization in end-stage nAMD results in a fibrovascular or atrophic scar at the macula and subsequent severe and permanent loss of central vision.[75]

Natural history and prognosis

Early-stage AMD can progress to late-stage AMD resulting in severe vision loss. The risk of progression depends on the severity and extent of the features of early-stage AMD. The drusen may progress following different growth patterns. They can increase or decrease in volume and area, develop MA or CNV or remain stable.[76] The AREDS, Report 18, reported that patients with small drusen in both eyes have a very low risk of progression to late-stage AMD of 0.4% over five years. Intermediate size drusen in both eyes confer a risk of progression to late-stage AMD of 2.1% over five

years. Large drusen in both eyes confer a risk of progression to late-stage AMD of 13% over five years. If large drusen and RPE abnormalities are present in both eyes this risk increases substantially to 47%.[53]

Macular atrophy initially develops as focal areas of hypopigmentation which eventually coalesce or expand to involve the central macula causing progressive loss of vision. The mean time from the onset of MA until legal blindness occurs is 5 to 9 years.[77, 78] Neovascular complications have a more acute onset with sudden development of central vision loss. If untreated, the area of neovascularization expands rapidly and a large fibrous scar develops in the macula. The MARINA trial reported that a small number of patients receiving ranibizumab had severe vision loss (30 letters or more) from baseline (0.8% of the 0.3-mg group and 1.2% of the 0.5-mg group), compared with 14.3% of the control group at 12 months ($P < 0.001$). At 24 months follow-up, 3.4% of the 0.3-mg group and 2.5% of the 0.5-mg group had severe vision loss, compared with 22.7% of the control group ($P < 0.001$). A significant proportion of eyes with nAMD that are untreated over two years will have severe vision loss. More than 90% of patients with nAMD treated with anti-VEGF therapy will maintain stable vision and one-third will improve.[19, 20]

Treatment and prevention

The treatment of nAMD significantly changed with the introduction of anti-VEGF drugs and resulted in improved outcomes for patients with nAMD.[79] Timely treatment given at the onset of CNV leads to better visual outcomes which highlights the importance of early identification of patients at risk of progression to the late stages. Choroidal neovascular lesions can occasionally be detected outside the fovea and when they are relatively small if patients are monitored closely.[80]

Prevention of AMD progression

Treatment with a supplement containing high doses of zinc and antioxidants (vitamin C, vitamin E and β carotene) reduced the risk of progression to late-stage AMD by 25% after a six-years follow-up in the AREDS clinical trial.[81] Age-Related Eye Disease Study 2 (AREDS2) investigated the effect of adding lutein and zeaxanthin to the AREDS formulation. It was reported that patients in the lowest quintile in terms of dietary lutein and zeaxanthin intake benefited most from the addition of these carotenoids. Addition of lutein and zeaxanthin to the AREDS formulation did not change the risk of progression to late-stage AMD, but due to the potential increased incidence of lung cancer in ex-smokers, lutein and zeaxanthin was suggested to be an appropriate carotenoid substitute in the AREDS formulation.[82, 83]

There is evidence for a linear association between fish consumption and risk of AMD as reported in a meta-analysis of 4,202 cases with 128,988 patients from eight cohort studies (relative risk (RR), 0.76, 95%CI, 0.65-0.90 for any AMD). Subgroup analyses by AMD stages showed that fish consumption reduced the risk of both early-stage (RR 0.83, 95%CI, 0.72-0.96) and late-stage (RR 0.76, 95%CI, 0.60-0.97) AMD.[84] There is no evidence that increasing dietary omega-3 fatty acids prevents or slows

AMD progression. A systematic review of two RCTs including 2,343 patients with AMD who were randomised to receive either omega-3 fatty acid supplements or a placebo, and the AREDS2 study reported no benefit.[82, 85]

Treatment of non-neovascular AMD

Currently there is no proven therapy that halts the progression of MA. Vitamin supplementation, cessation of smoking and dietary modification are the current recommendations to slow the progression of the disease.[3] Various strategies are being studied to treat non-neovascular AMD including drugs that modulate the visual cycle, anti-inflammatory agents and neuroprotective agents. Complement inhibition has been identified as a potential therapy for non-neovascular AMD with drugs that inhibit the complement pathway such as Lampalizumab. Lampalizumab is an antigen-binding fragment of a humanized monoclonal antibody that binds specifically to complement factor D. In the MAHALO phase II trial, monthly intravitreal lampalizumab therapy demonstrated a 20% reduction in MA lesion area expansion versus control.[86] However, findings from the phase III trials Chroma and Spectri reported that lampalizumab did not reduce MA lesion expansion compared with a control during 48 weeks of treatment.[87] Eculizumab, another drug that inhibits the complement pathway, was investigated in the COMPLETE trial. It specifically binds to the terminal complement component 5 which plays a key role in the late stage of the complement cascade. Systemic complement inhibition with eculizumab did not significantly reduce the growth rate of MA over six months.[88] The ultimate goal of treating non-neovascular AMD is to target the underlying cause of the disease and prevent or slow vision loss but this is yet to be adequately determined.[89]

Treatment of neovascular AMD

The current standard of care for the treatment of nAMD involves intravitreal injection of anti-VEGF agents. Other treatment modalities that are now rarely used include transpupillary thermotherapy, submacular surgery and macular translocation.[3, 5, 18, 90, 91]

Verteporfin photodynamic therapy (PDT) is used by some for polypoidal choroidal vasculopathy, a type of CNV thought by some to be a separate entity to nAMD. Verteporfin photodynamic therapy uses a photosensitizing drug which accumulates preferentially in CNV and generates reactive oxygen species after it has been activated that causes changes to local endothelial cells. This triggers platelet binding and aggregation resulting in neovascular thrombosis formation and CNV closure. It has been reported to be effective and safe in nAMD. Photodynamic therapy combined with corticosteroid or anti-VEGF agent has been reported to reduce the need for re-treatment and produces VA outcomes better than PDT monotherapy, but these results have been eclipsed by the VA and anatomic benefits reported with anti-VEGF monotherapy.[92-94]

Anti-VEGF therapy for neovascular AMD

The RPE plays a critical role in the control of vascular supply, permeability, growth, immunologic responses and repair in the retina. Factors produced by the RPE include VEGF which can stimulate

normal or pathologic neovascular growth. Maintenance of the normal retinal vasculature requires proangiogenic and antiangiogenic factors to be in balance. Ischemia, inflammation or neoplasia can disrupt this balance. Increased expression of VEGF in the retina and vitreous stimulates vascular permeability and neovascularisation. Hypoxia-inducible factor (HIF) is a promoter for VEGF. Hypoxia suppresses HIF, which results in increased levels of VEGF.[5,18, 90, 91]

Ranibizumab was Food and Drug Administration (FDA) approved for the treatment of nAMD in 2006. It is a humanized antibody fragment that binds to VEGF to prevent it from binding to its receptor thereby inhibiting angiogenic activity. Its efficacy and safety were demonstrated in two major trials, MARINA and ANCHOR.[13, 14, 95] Bevacizumab is used off-label to treat nAMD. It is a humanized monoclonal antibody against VEGF. The off-label use of bevacizumab is an alternative to ranibizumab due to its efficacy, safety, availability and low cost. Multiple trials have demonstrated comparable efficacy and safety between ranibizumab and bevacizumab.[12, 16, 17, 79, 90, 96-106] Aflibercept is a newer agent, with FDA approval in 2011, for the treatment of nAMD. Aflibercept acts as a decoy receptor for VEGF. It binds to VEGF and prevents it from activating VEGF receptors. It also binds to PGF and prevents it from activating VEGF receptors. It was reported to have comparable efficacy and safety compared to ranibizumab in the VIEW 1 and VIEW 2 trials.[18, 107, 108, 109] The first FDA approved anti-VEGF agent, pegaptanib, is not currently used because of the better visual outcomes from ranibizumab, bevacizumab and aflibercept.[12, 18, 110, 111]

The ocular and systemic safety of anti-VEGF agents has been investigated. In regards to ocular safety, post-injection endophthalmitis was rare occurring in 0.06% (11 of 18,509 injections) of injections in the CATT study.[112] Infectious endophthalmitis was reported to occur in 0.02% (18 of 88,150 injections) of injections in a large observational study in real-world clinical practice.[113] A meta-analysis of randomised controlled trials reported no relationship between ranibizumab and mortality but a possible relationship between more intensive therapy, which involved higher dosages and more frequent injections, and risk of systemic vascular events.[114]

Anti-VEGF therapy regimens

Treatment of nAMD with anti-VEGF therapy involves a loading or induction phase followed by a maintenance phase. The best approach during the loading phase of treatment has been established to be monthly injections until inactivity occurs. It is during the maintenance phase of treatment when management decisions are needed and there are variations in the approach that clinicians use.[115] It is generally accepted that clinicians now mostly use some type of variable dosing regimen of anti-VEGF therapy after a loading phase.[116-118] A common approach is to begin with three injections at monthly intervals followed by a variable regimen because clinical trials have reported progressive improvement of VA mainly over the first three months of treatment before plateauing.[116, 117, 119, 120] The pivotal phase III trials have reported good outcomes with monthly injections of ranibizumab, or two-monthly in the first year with aflibercept after the three monthly loading doses.[19, 20, 95, 108] These protcocol-based regimens, particularly monthly treatment, may be unnecessary and are difficult to

accomplish in real-world practice.

Another treatment regimen, *pro re nata* (PRN), involves using three loading doses at monthly intervals followed by as needed dosing based on monthly monitoring with VA and OCT guided re-treatment criteria to achieve functional and morphological changes similar to those achieved after the three loading doses.[121, 122] Another regimen that is used is "treat-and-extend" which aims for a persistently "dry" (inactive) retina by extending treatment intervals after the neovascular lesion has been rendered inactive until it reactivates. This is the "break point" at which point the interval is reduced by a week or two. PRN generally treats only when lesions are graded as active, whereas treat-and-extend essentially increases the intervals between treatments after CNV has been stabilized to keep the lesion inactive with the fewest possible treatments.[117, 123] Outcomes of treat-and-extend management of nAMD has been reported to be a good alternative to monthly and PRN approaches, with comparable visual outcomes and fewer patient visits, even in a real-world setting.[124, 125]

Alternative anti-VEGF therapies

Other anti-VEGF therapies that are being investigated for treatment of nAMD include conbercept and brolucizumab.[126, 127] Conbercept is similar to aflibercept and is a recombinant fusion protein that inhibits all VEGF isoforms and PGF. It has been reported in phase II trials to have similar or better VA improvement and similar frequency of injection compared to ranibizumab therapy in the CATT.[126] Brolucizumab is a humanized single-chain antibody fragment that inhibits all isoforms of VEGF. It has been reported in phase II trials to be non-inferior to ranibizumab, and VA outcomes and safety profile with brolucizumab were comparable to those with aflibercept over 56 weeks of treatment.[127, 128]

Future directions

Future therapy for AMD could involve improved pharmacological interventions to slow the progression of MA which are currently under research, in addition to a combination of diet and lifestyle changes already established.[129] The use of statins has been proposed to be associated with regression of drusen but there is conflicting evidence regarding its effectiveness in AMD.[130, 131] Gene therapies in nAMD involving gene delivery to increase proliferation of anti-angiogenic proteins have been investigated in animal models. The proposed routes of administration are intravitreal injection or subretinal injections given in the operating theatre. Preliminary data from human studies of nAMD suggested efficacy and safety of subretinal injection of a recombinant adeno-associated viral vector that encodes soluble VEGF receptor 1 called "rAAV.sFLT-1". Stem cell-based therapies for AMD with MA to potentially replenish abnormal RPE with healthy RPE are currently being investigated.[132, 133]

Visual rehabilitation with low-vision aids, including spectacles, hand-held or stand magnifiers and closed-circuit television, have been the main method for supporting patients with late-stage AMD function. Although low-vision aids are effective for improving visual functioning they can be awkward to use.[134] Intraocular implants have been proposed to be an alternative to these low-vision aids but a review of several types of intraocular lenses for AMD patients found significant disadvantages in

intraocular lenses such as monocular vision or a limited range of intraocular lens powers. Further clinical studies with long-term follow-up are required before use of intraocular implants can be established.[135] Retinal prostheses such as the subretinal visual implant Alpha-IMS (Retina Implant AG, Germany) have been tested in human clinical trials. These prostheses have the potential to allow for improved visual function with light and dark discrimination, and recognition of large objects. The disadvantage of these prostheses is that they are costly which currently limits their use in routine clinical practice.[136]

Visual outcomes of neovascular AMD with anti-VEGF therapy
Incidence of vision loss

Some patients who underwent anti-VEGF therapy in RCTs had loss of VA compared to their baseline VA. A major visual outcome in most studies was avoidance of loss of more than 15 letters at 1 or 2 years with some studies evaluating avoidance of loss of more than 30 letters of VA.[12, 16, 17, 90, 96-107] In the MARINA and ANCHOR trials, 8% and 10% of patients respectively treated with ranibizumab had lost 15 letters of VA by 2 years.[19] In the CATT study, 9.2% of patients treated with ranibizumab or bevacizumab lost 15 or more letters at 2 years.[16, 95] In the VIEW 1 and VIEW 2 studies, 4.9% to 6.3% of patients treated with aflibercept or ranibizumab had lost 15 letters of VA by 2 years.[107]

Characteristics of eyes with neovascular AMD with poor response to anti-VEGF therapy

Although short- to medium-term efficacy is undisputed, there are few data on long-term outcomes of anti-VEGF therapy for nAMD, despite over 10 years of access to approved anti-VEGF agents in many countries. Long-term outcomes of eyes with nAMD commencing treatment with anti-VEGF therapy in routine clinical practice have been reported. The mean follow-up time of all 1,212 eyes was 53.5 months and 549 (45%) continued followup after 60 months. Mean VA improved from 55 to 61 letters after 6 months and remained above the mean baseline VA for 6 years. After 7 years, mean VA was 2.6 letters lower than baseline for the 131 eyes still being followed. Forty percent had VA of 70 (Snellen equivalent of 20/40) letters or more and 18% had VA of 35 letters (Snellen equivalent of 20/200) or less. Of those with 20/40 VA before treatment, 40% had lost it after 7 years. Macular atrophy affecting the fovea was thought to be the cause of a 10-letter or more loss after 6.5 years in 37% of a subset of such eyes that were retrospectively analyzed. Overall, good long-term outcomes of anti-VEGF therapy for nAMD were found in routine clinical practice but a proportion of patients had poor outcomes.[137]

The two pivotal phase III studies of ranibizumab, MARINA and ANCHOR, reported a loss of 15 letters or more in 8% to 10% of eyes at 2 years. There is limited evidence of the correlations between baseline characteristics and the identification of predictive factors for poor visual outcomes with anti-VEGF therapy.[138-140] This information would allow for more accurate prediction of prognosis. The study by Rosenfeld et al.[140] evaluated the characteristics of eyes that lost vision when receiving monthly ranibizumab as part of these trials. The study compared eyes that lost 15 or more letters (71 patients) with those that gained 15 or more letters (271 patients) after monthly treatment with

ranibizumab during the 2-year study to identify baseline characteristics that were associated with the risk of VA loss. In both ANCHOR and MARINA, a significant association existed between VA loss and RPE abnormalities observed in the colour fundus photographs.[140]

The CATT study reported that bevacizumab and ranibizumab were equally effective during a 2-year period to improve VA of eyes with nAMD. Although most patients in CATT (75.1%) had the same or improved VA at 2 years compared to baseline acuity, 9.2% had lost 15 or more letters. Some predictors of VA improvement identified in the CATT, including younger age, better baseline VA and smaller CNV area, were consistent with predictors identified from the MARINA and ANCHOR studies.[140-142] A retrospective analysis of a cohort of 61 patients from CATT who suffered "sustained VA loss" of 15 or more letters after receiving monthly or PRN treatment with ranibizumab or bevacizumab for 2 years has been reported. Sustained VA loss was defined as vision loss present at both weeks 88 and 104. The study reported that 5.9% of eyes of CATT patients developed sustained VA loss of 15 or more letters over 2 years of treatment with ranibizumab or bevacizumab. Foveal scar, RPE abnormalities and MA contributed to most cases (83.7%) of sustained VA loss. In addition, presence of baseline MA, larger CNV baseline area and bevacizumab treatment were independently associated with higher risk of sustained VA loss. Other risk factors such as age, baseline VA, RPE elevation, lesion type, RAP lesion and retinal thickness on OCT, were not significant predictors of sustained VA loss. It was thought that the gradual VA deterioration during 2 years in eyes that developed sustained VA loss was likely due to progressive scarring or RPE changes, some of which developed into MA.[138]

Limitations of current research

Despite the effectiveness of anti-VEGF therapy for nAMD, significant loss of vision can still occur as reported in the pivotal clinical trials. Visual outcomes from such clinical trials may not readily translate into routine clinical practice for many reasons, such as differences in patient selection and treatment protocols. Knowledge of the incidence of poor outcomes in eyes receiving therapy in routine clinical practice will complement data from clinical trials that may be more relevant to practitioners and patients. Understanding the factors that contribute to loss of vision in patients receiving therapy for nAMD may lead to better outcomes and provide a more accurate prediction of the potential benefit of anti-VEGF therapy.

Thesis aims

This thesis describes the incidence, characteristics and baseline predictors of poor visual outcomes in eyes with nAMD receiving intravitreal anti-VEGF therapy in daily clinical practice over 5 years.

Chapter Two - Methods

FRB! studies

The Save Sight Institute, a centre of the University of Sydney, established The Fight Retinal Blindness! (FRB) Project in 2009. It has developed an international prospective audit system that tracks anonymously the outcomes of nAMD treated with anti-VEGF agents in routine clinical practice. The FRB! registry collects data when eyes start treatment and from each subsequent clinical visit, including patient demographics, VA, whether the eye had received prior treatment for nAMD, lesion type, activity of the CNV lesion, type of treatment given and adverse events. Data has been collected from over 30 clinical practices located in Australia, New Zealand and Switzerland since January 2007.[143]

The benefit of data from observational studies from the FRB! registry, compared to clinical trials, is that they provide an indication of what is occurring in routine clinical practice. Although clinical trials determine whether a treatment is effective under ideal and standardized conditions, observational studies can produce longer-term results which reflect what actually happens in the real-world.[144]

The FRB! Registry has allowed for long-term outcomes of anti-VEGF therapy in large numbers of patients to be analysed. This has produced a number of clinically relevant observations on the treatment of nAMD with VEGF inhibitors, particularly that good outcomes can be obtained using a treat and extend regimen in real-world clinical practice.[115, 124, 137, 145-148]

Design and setting

This thesis analyzed anonymized data from the FRB! registry which were captured during routine clinical practice in Australia, New Zealand and Switzerland. Treatment decisions and visit schedules were entirely at the discretion of the treating clinician and patient. At the baseline visit, patient demographic and clinical information were obtained, including gender, year of birth, prior treatments for nAMD, and angiographic lesion size (greatest linear dimension, GLD) and type. Data were collected at each visit on VA letters read on a logarithm of the minimum angle of resolution (logMAR) chart (on which Early Treatment of Diabetic Retinopathy Study charts are based), type of treatment given, adverse events and CNV lesion activity. Activity of the CNV lesion was judged by the treating clinician according to a pre-specified definition of activity: fluid, haemorrhage or loss of vision thought to be due to activity of the lesion as seen on any of biomicroscopy, FFA or OCT. The best reading of uncorrected, corrected, or pinhole VA was used. The presence and location of MA, subretinal fibrosis (SRFi) and PED were also recorded since April 2016.[149]

Study population

Treatment-naive patients starting treatment with anti-VEGF therapy for nAMD between January 2007 and March 2012 were included in this present study. Cases were those who had "sustained VA loss", defined as at least two consecutive visits where there was loss of ≥15 letters from baseline without

recovery of VA, either at 5 years or at their last visit if they did not complete 5 years, after starting treatment irrespective of when the loss of vision occurred. A subgroup analysis included eyes that sustained ≥30 letters of loss. Controls were eyes that had not sustained ≥15 letters of loss from baseline throughout the duration of observation. Eyes with baseline VA <35 letters were excluded due to the lower likelihood of such eyes suffering a 15 or 30 letter loss even if they had suffered a significant adverse event might have biased the control group. Patients who were treated by clinicians that ceased participating in the project before they could have been followed for 5 years were also excluded.

Study outcomes
The main outcomes of the present study were the proportion of eyes with sustained VA loss within 5 years, the time to development of sustained VA loss and baseline predictors of sustained VA loss.

Statistical analysis
The proportion of eyes with sustained loss of ≥15 or ≥30 letter loss and time to first development of a ≥15 or ≥30 letter loss was estimated using Kaplan-Meier curves. The baseline predictors of ≥15 or ≥30 letters of loss were evaluated by multivariate analysis using Cox-proportional hazards models. The variables included baseline age, VA, angiography lesion criteria and lesion size, total number of injections and CNV activity.

Adjusted hazard ratios and associated 95% CIs were calculated from a multivariate Cox-proportional hazards model. Nesting effects of the variables were analysed to correlate outcomes between eyes within the same patient and same practice. Descriptive statistics included mean, standard deviation (SD), 95% CIs, median, range, first and third quartiles and percentages where appropriate. Characteristics at baseline were compared between eyes with and without sustained VA loss ≥15 letters, using the t-test and Pearson's chi-squared test where appropriate. Locally weighted regression smoothing (LOESS) curves were used to visualise longitudinal observations of VA throughout the follow-up period. All data analyses were performed using R version 3.4.1. with the survival package (version 2.41-3) for Kaplan-Meier survival analysis and the coxme package (version 2.2-7) for mixed-effect Cox-proportional hazards models.[150]

Chapter Three - Poor Outcomes

Study participants

In this present study there were 1760 treatment-naïve eyes from 1586 patients with nAMD that began anti-VEGF therapy between January 2007 and March 2012 with baseline VA >35 letters (Figure 2). Of these, 856 eyes (48.6%) of 774 patients completed 5 years of follow-up. Sixty-five percent of patients were females at baseline. The mean baseline VA of the eyes that developed sustained VA loss of ≥15 letters was similar to the group without sustained VA loss (59.6 vs. 59.3 letters, P = .72). The distribution of angiographic lesion gradings was also similar between the two groups with most lesions being occult (P = .36). The mean baseline age and median CNV lesion GLD of eyes that developed sustained VA loss of ≥15 letters were greater compared to the group without sustained VA loss (80.7 vs. 79.0 years, P = .001; and 2685 vs. 2200 μm, P = .03, respectively). Table 1 summarizes the baseline characteristics of the eyes observed.

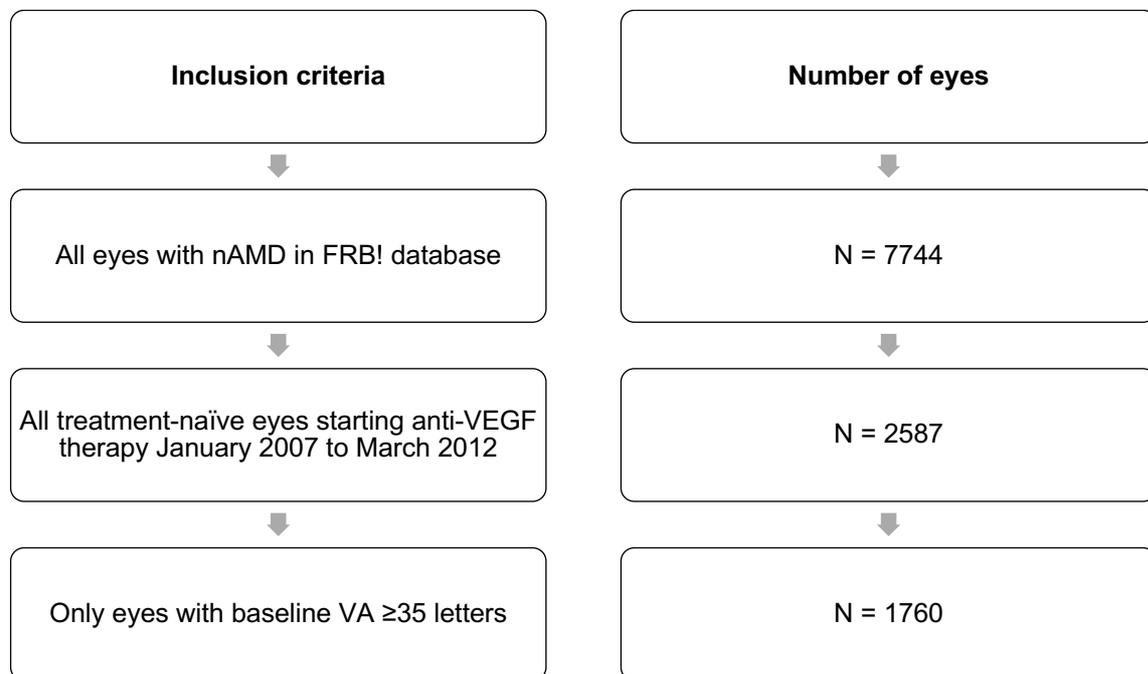| Inclusion criteria	Number of eyes
All eyes with nAMD in FRB! database	N = 7744
All treatment-naïve eyes starting anti-VEGF therapy January 2007 to March 2012	N = 2587
Only eyes with baseline VA ≥35 letters	N = 1760

Figure 2. Consort-style diagram showing the number of eyes in the study, the number excluded, and the reasons for exclusion. nAMD = Neovascular age-related macular degeneration; anti-VEGF = anti-vascular endothelial growth factor; FRB! = Fight Retinal Blindness!

Table 1. Demographics and Lesion Characteristics at Baseline

	≥15-Letter Losers	≥30-Letter Losers	Rest of the Cohort	P value[++]
No. of Eyes (%)[*]	326 (18.5)	150 (8.5)	1434	
No. of Patients	310	146	1276	
Mean Age, y (SD)	80.7 (7.4)	81.2 (7.0)	79.0 (8.1)	**.001**
Mean Baseline VA, letters (SD)	59.6 (13.5)	57.6 (12.6)	59.3 (13.3)	.72
Angiography lesion criteria, n (%)				
Occult	168 (51.5)	80 (53.3)	793 (55.3)	.36
Predominantly classic	67 (20.6)	30 (20.0)	245 (17.1)	
Minimally classic	38 (11.7)	20 (13.3)	183 (12.8)	
Other[+]	20 (6.1)	8 (5.3)	98 (6.8)	
Not recorded	33 (10.1)	12 (8.0)	115 (8.0)	
Median GLD, µm (Q_1, Q_3)	2685 (1500, 3700)	2500 (1534, 3600)	2200 (1484, 3200)	**.03**

SD = standard deviation; VA = Visual acuity; GLD = greatest linear dimension; Q_1, Q_3 = first quartile, third quartile

*Eyes with baseline VA <35 letters were excluded

[+]Includes disciform scar, idiopathic polypoidal choroidal vasculopathy (IPCV), juxtapapillary, retinal angiomatous proliferation (RAP)

[++]t test and Pearson's chi-squared test comparing ≥15-Letter Losers with the Rest of the Cohort

Incidence of sustained vision loss

The proportion of eyes with sustained VA loss of ≥15 letters was estimated to be 11.0% (95%CI, 9.4-12.5) at 2 years and 22.9% (95%CI, 20.7-25.1) at 5 years. The proportion of eyes with sustained VA loss of ≥30 letters was estimated to be 3.6% (95%CI, 2.7-4.6) at 2 years and 10.8% (95%CI, 9.1-12.5) at 5 years (Figure 3).

The mean VA of eyes with sustained ≥15 letter loss decreased gradually over time (Figure 4). There were 856 eyes that completed 5 years of follow-up. There was a higher rate of dropout of eyes with sustained VA loss of ≥15 letters (56%) than eyes without sustained VA loss (50%). There was a mean decrease of 31 letters from baseline at 5 years compared with a mean gain of 7 letters in eyes without sustained VA loss. The group of eyes that sustained VA loss of ≥30 letters had a mean decrease of 44 letters from baseline at 5 years (Table 2). The mean VA of the 145 eyes with sustained VA loss of ≥15 letters at year 5 was 33 letters and 18 letters for the 63 eyes that lost ≥30 letters from baseline (Table 2). Kaplan-Meier curves for time to develop a sustained loss of ≥15 letters in VA showed that the onset of VA loss occurred at a steady rate throughout the 5-year follow-up period (Figure 3).

Recovery of ≥15 letters occurred in 25% of eyes that completed one-year follow-up after the sustained VA loss occurred, with mean VA recovery of 24.7 (SD 10.4) letters.

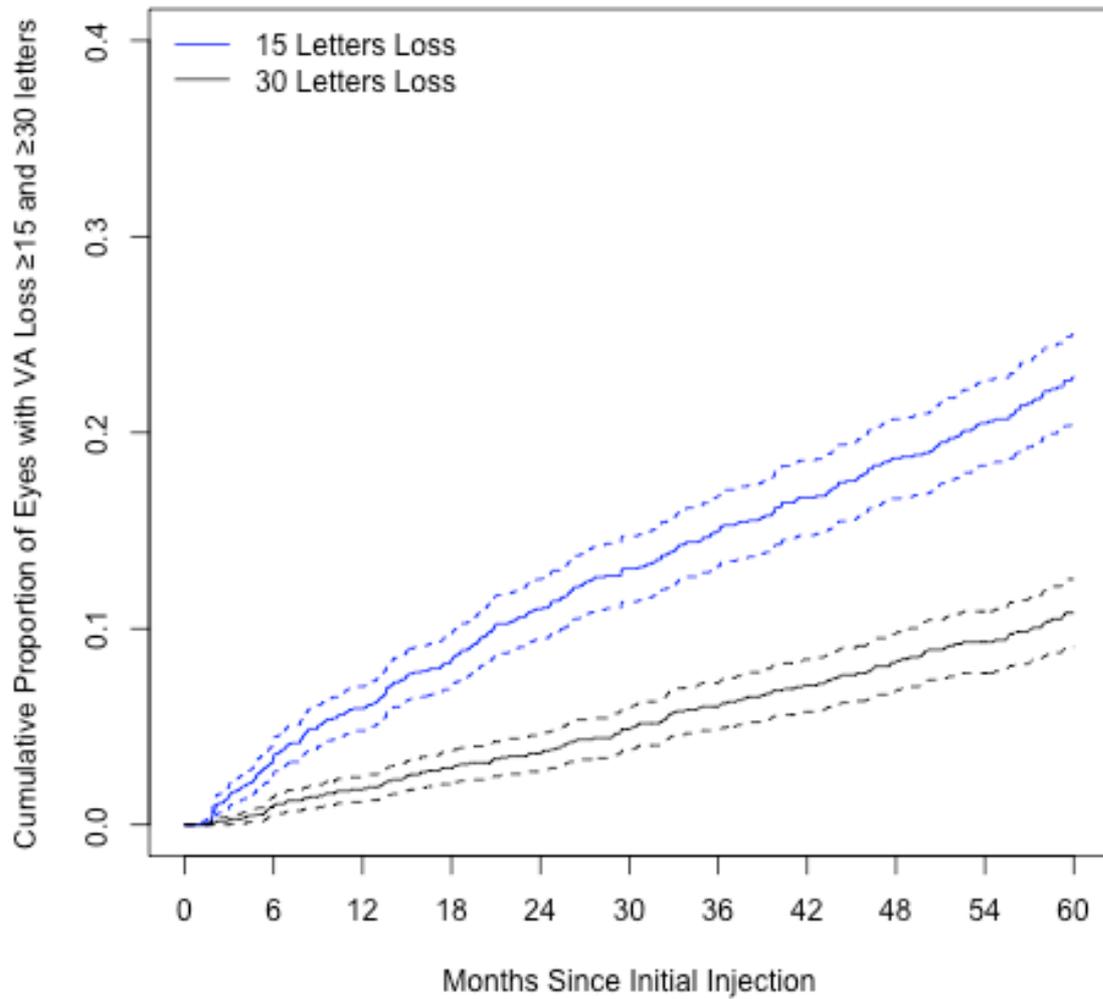
Figure 3. Kaplan-Meier Curve for Time to First Loss of ≥15- and ≥30-letters of Visual Acuity over 5 Years. The solid line is the point estimate of the proportion of eyes with VA loss. The dashed lines are the 95% confidence intervals. VA = Visual acuity
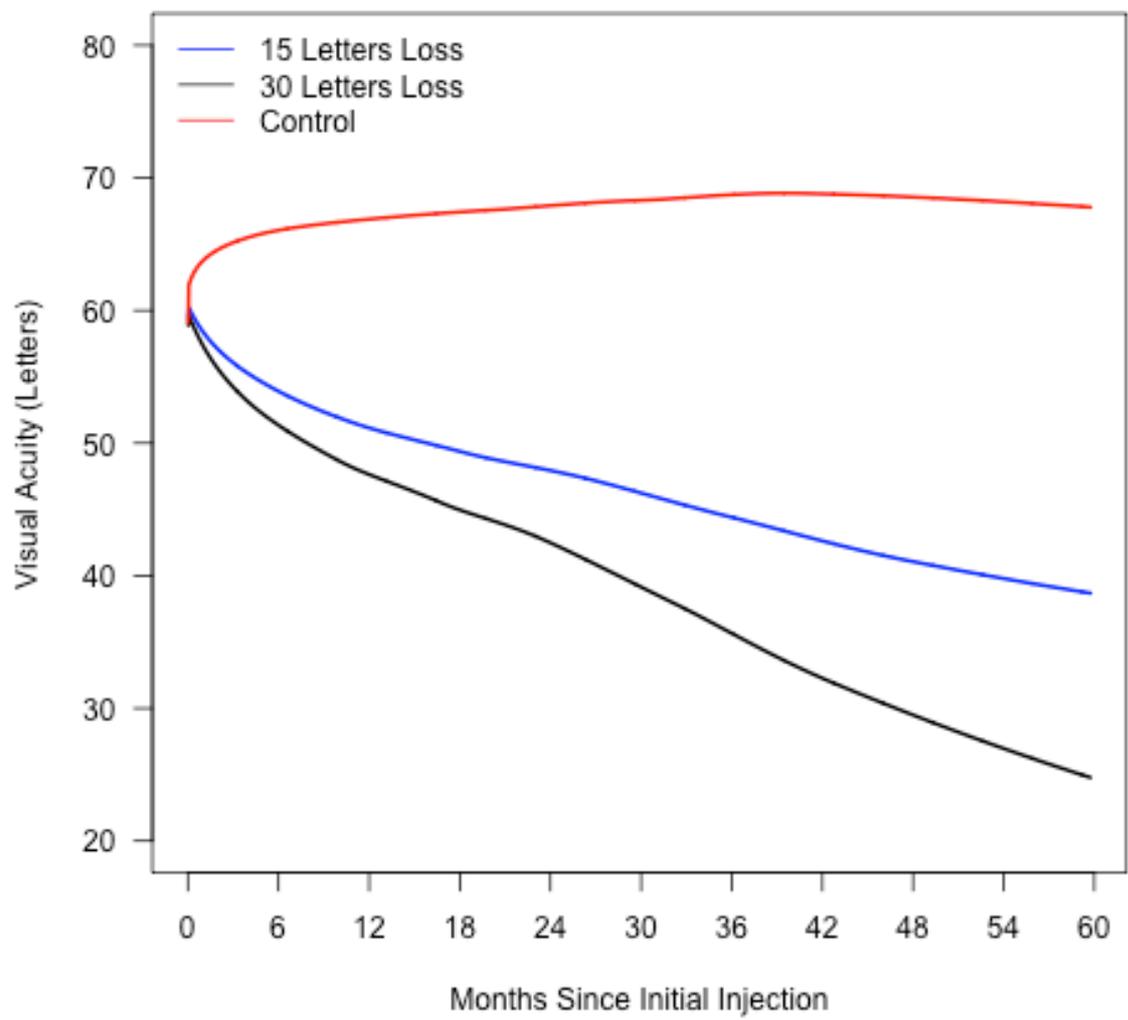
Figure 4. Loess Regression Curves for Mean Visual Acuity over Time up to 5 Years.

Table 2. Outcomes of ≥15- and ≥30-Letter Losers at 5 Years of Follow-up

	≥15-Letter Losers	≥30-Letter Losers	Rest of the Cohort	P value*
No. of Eyes (%)	145 (16.9)	63 (7.4)	711	
No. of Patients	139	62	635	
Mean Baseline Age, y (SD)	79.8 (7.0)	81.1 (6.8)	77.9 (7.8)	**.007**
Mean Baseline VA, letters (SD)	63.8 (13.3)	62.1 (12.4)	61.8 (12.9)	.09
Mean Final VA, letters (SD)	32.9 (19.9)	18.17 (15.6)	68.4 (12.6)	**<.0001**
Mean ΔVA from Baseline, letters (SD)	-31.0 (15.3)	-43.9 (13.3)	6.7 (12.3)	**<.0001**
CNV activity (proportion of visits active, %)	53.7	50.9	46.8	**.02**
Mean No. of visits completed (SD)	39.8 (15.8)	40.3 (15.5)	38.3 (17.1)	.34
Mean No. of anti-VEGF treatments (SD)	25.5 (14.1)	22.4 (12.7)	28.0 (14.0)	**.04**
Adverse events (proportion of visits, %)	0.54	0.63	0.33	**.004**

SD = standard deviation; VA = visual acuity; ΔVA = change in VA; CNV = choroidal neovascularisation.

**t test and Pearson's chi-squared test comparing ≥15-Letter Losers with the Rest of the Cohort

Characteristics associated with sustained vision loss

Eyes with sustained VA loss of ≥15 letters at 5 years were observed in older patients compared with eyes without sustained VA loss (79.8 vs 77.9, P = 0.07). Eyes that developed sustained VA loss were more likely to have had an adverse event than eyes without sustained VA loss (0.5% vs. 0.3% of visits, P = .004), had a higher mean proportion of visits with CNV graded as active (53.7% vs. 46.8%, P = .02) and received fewer anti-VEGF injections on average (25.5 vs. 28.0, P = .04) (Figure 5). The eyes that developed sustained VA loss completed a similar number of visits over 5 years as eyes without sustained VA loss (39.8 vs. 38.3, P = .34) (Table 2). The injection interval at the time of sustained VA loss was 4-weekly for 44% of eyes with loss of ≥15 letters and for 35% of eyes with VA loss of ≥30 letters.

Baseline Age

Total number of Injections

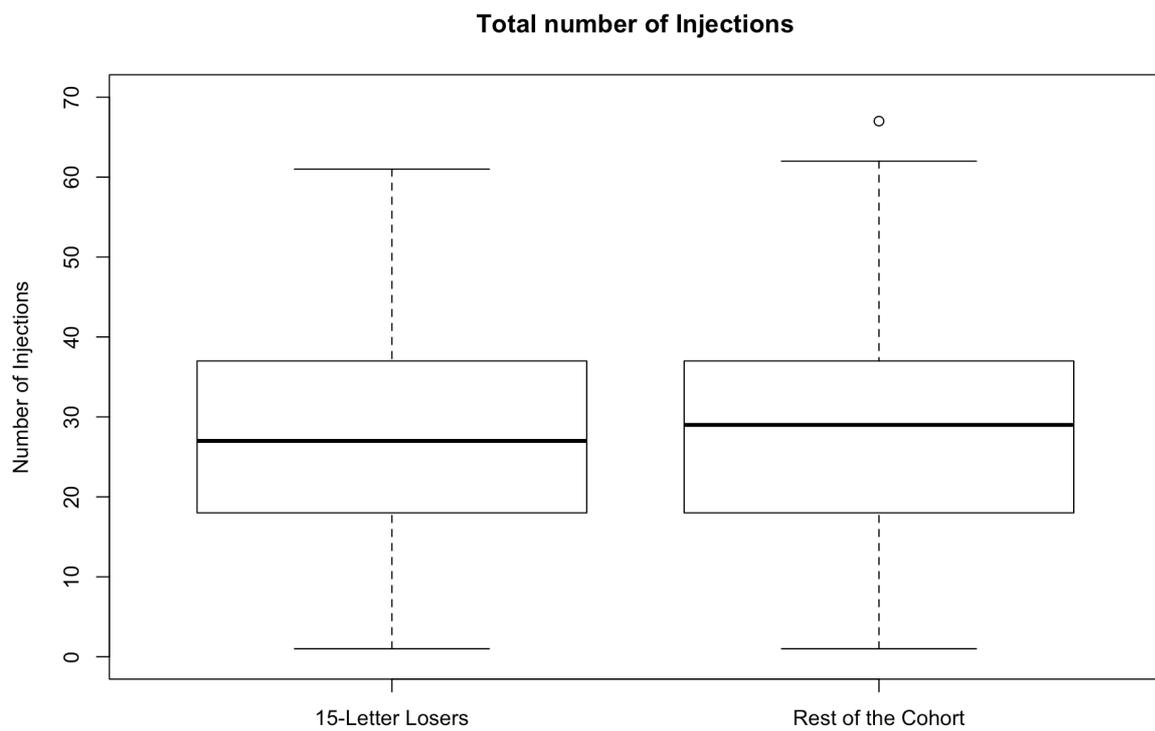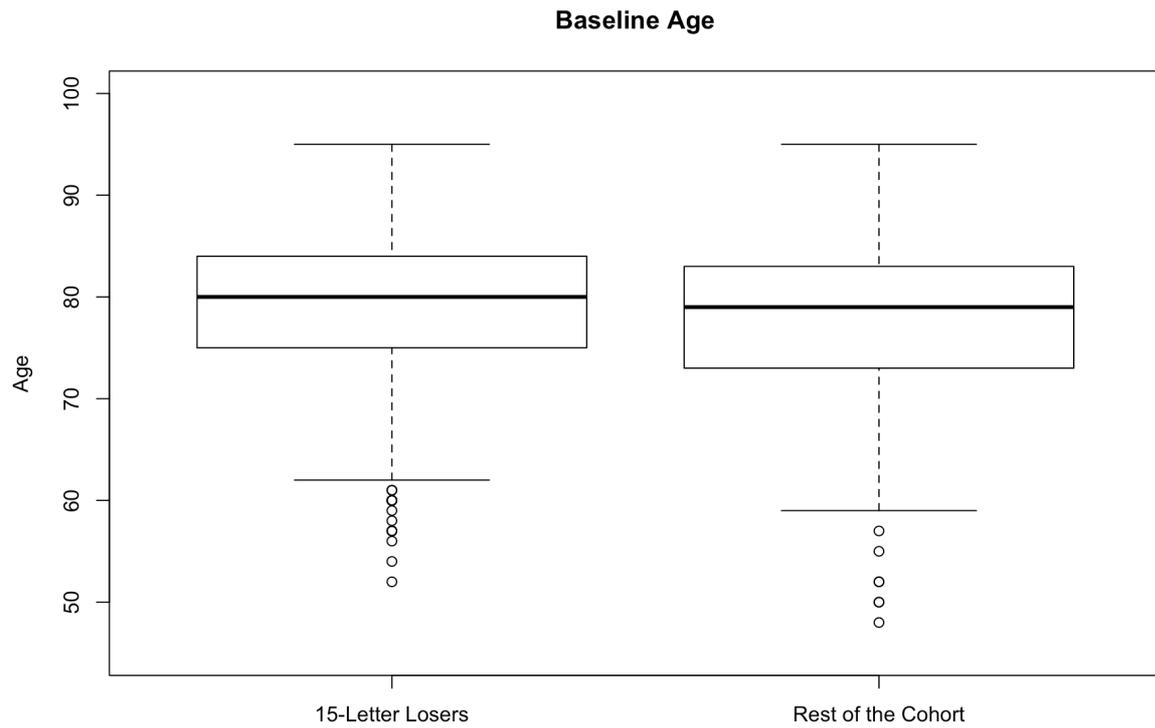Figure 5. Boxplot of baseline age and total number of injections in 15-letter losers and the rest of the cohort at 5 years.

Predictors of sustained vision loss

Factors independently associated with higher incidence of sustained ≥15 letter loss included age >80 years (OR, 1.33 for patients >80 years vs. ≤80 years; 95%CI, 1.05-1.69; *P* = .02), lower total number of injections (OR, 0.97 per injection; 95%CI, 0.96-0.98; *P* = .0005) and higher proportion of visits at which the CNV lesion was graded as active (OR, 1.97 for eyes in upper quartile of active visits vs. eyes in lowest quartile of active visits; 95%CI, 1.39-2.79; *P* = .0001) in multivariate analysis (Table 3). The same factors were associated with increased risk of sustained ≥30 letter loss. Baseline CNV lesion GLD showed a trend towards association with sustained ≥15 letter loss of vision (OR, 1.27 for patients >2500 μm vs. ≤2500 μm; 95%CI, 0.99-1.62; *P* = .06). In eyes with ≥30 letter loss, baseline VA >70 letters were associated with reduced risk of loss of vision (OR, 0.61; 95%CI, 0.38-0.98; *P* = .04) (Table 4). Baseline angiographic lesion criteria were not significantly associated with sustained VA loss.

Table 3. Multivariate Analysis of Predictors Associated with ≥15-Letter Visual Acuity Loss

	Hazards ratio (95% CI)	P value
Baseline Age, y		
≤80	1.00	**.02**
>80	1.33 (1.05 -1.69)	
Baseline VA, letters		
≤70	1.00	.23
>70	1.18 (0.89-1.56)	
Baseline Angiography lesion criteria		
Minimally classic	1.00	.18
Other*	0.92 (0.53-1.61)	
Occult	0.96 (0.67-1.37)	
Predominantly classic	1.32 (0.88-1.98)	
Baseline Greatest linear dimension, μm		
≤2500	1.00	.06
>2500	1.27 (0.99-1.62)	
Total number of injections	0.97 (0.96-0.98)	**.0005**
CNV activity (Quartiles)		
Low	1.00	**.0001**
Medium	1.24 (0.87-1.77)	
High	1.27 (0.88-1.82)	
Very High	1.97 (1.39-2.79)	

CI = confidence interval; VA = visual acuity.

*Includes disciform scar, idiopathic polypoidal choroidal vasculopathy (IPCV), juxtapapillary, retinal angiomatous proliferation (RAP)

Table 4. Multivariate Analysis of Predictors Associated with ≥30-Letter Visual Acuity Loss

	Hazards ratio (95% CI)	P value
Baseline Age, y		
≤80	1.00	**.006**
>80	1.64 (1.15-2.34)	
Baseline VA, letters		
≤70	1.00	**.04**
>70	0.61 (0.38-0.98)	
Baseline Angiography lesion criteria		
Minimally classic	1.00	.29
Other*	0.63 (0.26-1.49)	
Occult	0.87 (0.53-1.43)	
Predominantly classic	1.01 (0.57-1.80)	
Baseline Greatest linear dimension, μm		
≤2500	1.00	.71
>2500	1.07 (0.75-1.53)	
Total number of injections	0.96 (0.94-0.97)	**.0004**
CNV activity (Quartiles)		
Low	1.00	**.002**
Medium	1.23 (0.74-2.06)	
High	1.18 (0.70-2.01)	
Very High	2.22 (1.35-3.66)	

CI = confidence interval; VA = visual acuity.

*Includes disciform scar, idiopathic polypoidal choroidal vasculopathy (IPCV), juxtapapillary, retinal angiomatous proliferation (RAP)

Causes of sustained vision loss

Eyes with sustained VA loss of ≥15 letters at 5 years had more cases of haemorrhage reducing VA ≥15 letters or RPE tears than eyes without sustained VA loss (0.27% vs. 0.07% of visits; and 0.05% vs. 0.02% of visits, respectively). There were 510 eyes that completed 5 years follow-up that had information available on MA and SRFi. The group of eyes with sustained VA loss of ≥30 letters had more MA and SRFi than those with VA loss of ≥15 letters, which in turn had more than the group of eyes without sustained VA loss. Most cases of MA and SRFi were graded as subfoveal (Table 5).

Table 5. Frequency of Macular Atrophy and Subretinal Fibrosis at 5 years

	Number of eyes (%)*			P value+
	≥15-Letter Losers, n = 63	≥30-Letter Losers, n = 22	Rest of the Cohort, n = 447	
Macular Atrophy				
Not present	19 (30.2)	5 (22.7)	233 (52.1)	.01
Present	44 (69.8)	17 (77.3)	214 (47.9)	
Subfoveal	32	15	98	
Extrafoveal	12	2	116	
Subretinal Fibrosis				
Not present	27 (42.9)	6 (27.3)	303 (67.8)	.01
Present	36 (57.1)	16 (72.7)	144 (32.2)	
Subfoveal	32	14	104	
Extrafoveal	4	2	40	

*Macular atrophy and subretinal fibrosis data were not obligatory in the Fight Retinal Blindness data entry system until after April 2016

+Pearson's chi-squared test comparing ≥15-Letter Losers with the Rest of the Cohort

Discussion and key implications of results

The present study evaluated the incidence, characteristics and predictors of sustained VA loss among 1760 treatment-naïve eyes that began anti-VEGF therapy for nAMD in daily clinical practice. The proportion of eyes with sustained VA loss of ≥15 letters within 5 years was 22.9% and 10.8% in the subgroup with sustained VA loss of ≥30 letters. Factors independently associated with higher incidence of sustained ≥15 letter loss included age >80 years, fewer injections and higher proportion of visits at which the CNV lesion was graded active. Baseline lesion size was somewhat associated with sustained ≥15 letter loss of vision. Baseline VA >70 letters was associated with reduced risk of sustained ≥30 letter loss. Baseline angiographic lesion criteria were not significantly associated with sustained VA loss.

The primary efficacy end point in the pivotal phase III RCTs was the proportion of patients who had lost fewer than 15 letters of VA. In the MARINA and ANCHOR trials, 8% and 10% of patients respectively treated with ranibizumab had lost 15 letters of VA by 2 years.[19] In the CATT study, 9.2% of patients treated with ranibizumab or bevacizumab lost 15 or more letters at 2 years.[16, 95] In the VIEW 1 and VIEW 2 studies, 4.9% to 6.3% of patients treated with aflibercept or ranibizumab had lost 15 letters of VA by 2 years.[107] In the present study the proportion of eyes with sustained VA loss of ≥15 letters was estimated to be 11.0% at 2 years, higher than the pivotal clinical trials. The present study may differ from these RCTs as this study defined sustained VA loss as loss of ≥15 letters from baseline at two consecutive visits without recovery of VA either at 5 years or at their last visit if they

did not complete 5 years. This definition of "sustained" VA loss excluded random VA fluctuations over time or episodes of sporadic loss of vision due to, for example, CNV reactivation or keratitis related to the antiseptic agent used prior to the injection. The current study had an inclusion criterion of eyes with baseline VA ≥35 letters (Snellen equivalent of 20/200) while the pivotal trials had a lower threshold with inclusion criteria of VA of 70 to 25 letters (Snellen equivalent of 20/40 to 20/320). Eyes with poor baseline VA may be less likely to lose ≥15 letters which could have contributed to the higher incidence of vision loss in this present study. The protocol-based regimens used in RCTs, particularly monthly treatment which is seldom performed in routine clinical practice, may also explain the lower incidence of loss of VA in RCTs than found in the present study.

Several cohort studies and clinical trials have highlighted the risk of vision loss in patients with nAMD treated over more than 2 years with anti-VEGF agents. The FRB! Study Group[137] have previously reported outcomes of 1212 eyes treated with anti-VEGF agents. Loss of ≥10 letters occurred in 32% (42 of 131) of eyes that continued treatment for over 6.5 years. The CATT study reported 24% (153 of 647) of eyes losing ≥15 letters after 5 years.[151] A retrospective case series reported that 20% (42 of 208) of patients had lost ≥15 letters after 5 years of ranibizumab treatment on an "as-needed" regimen.[152] In the present study the proportion of eyes with sustained VA loss of ≥15 letters was estimated to be 22.9% at 5 years. This is comparable to prior cohort studies and clinical trials even though this current study included a wider range of patients from routine clinical practice who may have had a tendency to worse outcomes than those who meet the inclusion criteria of the clinical trials.

The present study found that eyes that had sustained VA loss had poor final VA outcomes. Of the 145 eyes with sustained ≥15 letter VA loss, the mean final VA was 33 letters at 5 years and worse for the 63 that had sustained ≥30 letter VA loss with mean final VA of 18 letters. Eyes with sustained VA loss were more likely to have dropped out, have had an adverse event, have had a higher proportion of visits at which the CNV lesion was graded as active and have received fewer anti-VEGF injections than eyes without sustained VA loss. Factors identified that were independently associated with sustained ≥15 letter loss included age >80 years, fewer injections and a higher proportion of visits at which the CNV lesion was active. Some of the differences in groups that were labelled significant statistically were small, but they are likely to reflect underlying influences of undertreatment and general infirmity (age) that could have contributed to poor outcomes in some but not all cases. Baseline GLD >2500 µm was somewhat associated with an increased risk of sustained ≥15 letter loss. Baseline angiographic lesion type was not significantly associated with sustained VA loss.

There have been retrospective analyses of predictors of VA loss in some phase III RCTs. Rosenfeld et al.[140] evaluated the characteristics of eyes that lost vision when receiving monthly ranibizumab in the MARINA and ANCHOR trials. They compared eyes receiving monthly treatment with ranibizumab that lost ≥15 letters (71 patients) with those that gained ≥15 letters (271 patients) during the 2-year studies. The baseline characteristics found to be associated with the risk of VA loss included

increased patient age, larger size of the CNV lesion and better VA at baseline. Ying et al.[138] performed a retrospective analysis of a cohort of 61 patients from the CATT study who suffered "sustained VA loss" of ≥15 letters during monthly or PRN treatment with ranibizumab or bevacizumab for 2 years. As in the present study, Ying et al.[138] compared morphologic features between eyes with sustained VA loss to all other eyes without sustained VA loss, rather than only eyes that gained ≥15 letters as Rosenfeld et al.[140] did. They reported that 5.9% of eyes of CATT participants developed sustained VA loss of ≥15 letters over 2 years of treatment with ranibizumab or bevacizumab, with their VA decreasing gradually over time. Foveal scar, pigmentary abnormalities and MA were reported to have contributed to most cases (83.7%) of sustained VA loss. The presence of baseline MA, larger CNV area at baseline and bevacizumab treatment were independently associated with higher risk of sustained VA loss. Other risk factors, such as age and baseline VA, were not significant predictors of sustained VA loss.[139] Ying et al.[151] recently reported baseline predictors of VA outcomes at 5 years after initiating treatment with ranibizumab or bevacizumab in the cohort of patients (647) enrolled in CATT. Worse baseline VA, larger CNV area at baseline and presence of baseline RPE elevation (which this present study did not measure) remained independently associated with worse VA at 5 years. This present study found that increased age (>80 years) was associated with sustained ≥15 letter VA loss while better baseline VA (>70 letters) reduced the risk of ≥30 letter VA loss. This difference could be related to the larger cohort and longer follow-up in this present study. Better baseline VA was reported to be associated with reduced risk of VA loss in the study by Westborg et al.[153] of patients with nAMD treated with ranibizumab or bevacizumab in routine clinical practice. These data came from 3912 patients tracked by the Swedish Macula Register from 2011 to 2014. For patients with VA more than 60 letters at baseline, the risk of having a VA lower than 60 letters after 1 or 2 years of treatment was 20%. For patients with lower VA at diagnosis (<60 letters) this risk was 60%.[153]

This present study found that eyes with sustained VA loss had a higher proportion of visits with an adverse event compared to those without sustained VA loss. The adverse events captured in this study were unlikely to be a significant contributor to VA loss given their low incidence. Seventy percent of eyes that sustained VA loss of ≥15 letters had MA and 57% had SRFi at 5 years. In comparison, 48% of eyes without sustained VA loss of ≥15 letters had MA and 32% had SRFi at 5 years. This significant difference could partly explain the major causes of vision loss however the present study was unable to analyse baseline MA and SRFi as risk factors for vision loss because this data for the FRB! data entry system was not obligatory until April 2016.

Study limitations and strengths

Data collected in observational studies such as the present study have strengths and weaknesses.[154] Data completeness was high for all variables (>99.5% VA, treatment given, activity grading fields and adverse event completed) due to the quality assurance features of the FRB! web-based data entry system with the exception of CNV lesion size (GLD; 80% completed) and lesion type (88% completed). It is likely that adverse events are under-reported in the FRB! database. Lack of

consistent MA and SRFi grading for a period of time is another limitation. The measurement of logMAR VA is reasonably objective. Case selection and treatment regimens in observational studies may be different than in clinical trials and among different clinicians. In contrast to phase III trials, clinicians made treatment decisions in routine practice without reference to reading center adjudications and study protocols. Subjective criteria, such as lesion activity or lesion type, may not be graded uniformly in observational studies because clinicians may have different opinions of whether a lesion is active. This would result in lower internal validity compared with RCTs, however results of this present study are more generalizable to actual clinical practice because this better reflects how treatment decisions are made in routine clinical practice.[143, 145] More than half of the eyes in this analysis did not complete 5 years of follow-up which could have biased the results. Patients that lost 15 or 30 letters prior to dropping out would still be included in the estimated proportions of poor outcomes. However, the proportion of eyes losing ≥15 or ≥30 letters may have been under-estimated if, for example, patients experienced poor outcomes but dropped out of the study before loss of 15 or 30 letters was observed. It was noted that many patients were also discontinued due to reasons unrelated to treatment outcomes, including patient going to another doctor and patient death.

Future directions for research

The findings in this thesis suggest that future improvements in VA among patients undergoing anti-VEGF therapy for nAMD might include identifying the reasons for fewer injections and investigating different treatment regimens to reduce the activity of CNV lesions in patients. Future investigations into the reasons for less frequent injections of anti-VEGF agents could lead to improved outcomes for such patients. Research into factors contributing to poor response or non-response to anti-VEGF therapy could focus on suboptimal dosing, genetic variation, tachyphylaxis, delayed diagnosis or poor access to treatment.[155]

It is not clearly understood why some lesions do not respond effectively to anti-VEGF therapy although VEGF is identified as playing a crucial role in the pathogenesis of nAMD. This study has identified that 23% of eyes with nAMD suffer significant loss of vision despite over 5 years of anti-VEGF therapy. The complement system, inflammatory processes and genetics may play a more prominent role in some lesions that contribute to the development of nAMD. Vascular endothelial growth factor has a neuroprotective role and suppression of VEGF may result in vision loss in patients who are more susceptible to the loss of VEGF.[140] This may not be true for all patients as the pivotal clinical trials did not show initial improvement in VA followed by progressive VA loss in all patients with repeated monthly injections of ranibizumab over 2 years.[19] There may be a subset of patients who are more susceptible to long-term VEGF inhibition and may gradually lose VA over 2 years. Further research into therapies such as neuroprotective, anti-inflammatory and visual cycle modulating agents that could protect photoreceptor and RPE, could be beneficial to prevent progression and treat nAMD. The findings in this thesis of the incidence and predictors of poor outcomes may allow refinement of inclusion criteria for clinical trials evaluating such future novel therapies for nAMD.[155, 156]

Conclusion

Despite the effectiveness of anti-VEGF therapy for nAMD, significant loss of vision can still occur in daily clinical practice. Twenty-three percent of eyes with nAMD managed with anti-VEGF therapy developed sustained VA loss of ≥15 letters over 5 years of treatment in daily clinical practice. Older age, fewer injections and higher proportion of visits at which the CNV lesion was graded as active were independently associated with less improvement in VA. Identification of the incidence and predictors of poor outcomes provides more accurate assessment of the potential benefit from anti-VEGF therapy. This may help clinicians manage patients' expectations and guide treatment decisions from the beginning of treatment and in this way provide a more personalized approach of treatment.

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