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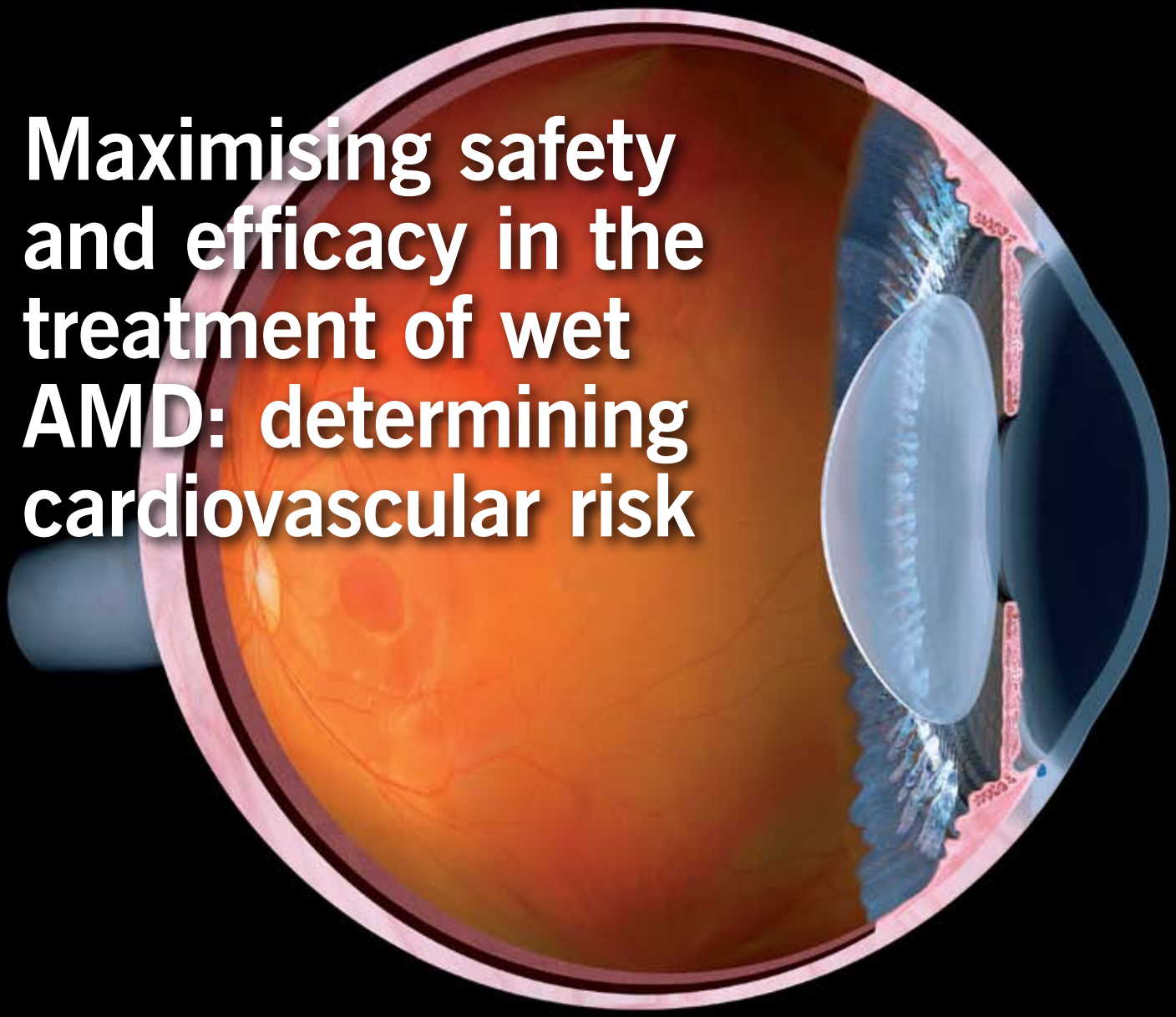
Maximising safety and efficacy in the treatment of wet AMD: determining cardiovascular risk



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Maximising safety and efficacy in the treatment of wet AMD

Introduction

The prevalence of neovascular age-related macular degeneration (wet AMD) is predicted to rise to more than 300,000 patients in the UK alone by the year 2025. The personal and economic costs are considerable. It leads to worsening of vision-related function and overall wellbeing, with one third developing clinical depression. The majority of patients progress to legal blindness in the affected eye within two years of diagnosis, and healthcare utilisation costs are seven times higher in affected patients compared to age-matched controls. Thus, the development of new treatments for wet AMD, and of access to such treatments, is clearly important.

Vascular endothelial growth factor (VEGF) plays a critical role in stimulating abnormal neovascularisation, inflammation and vascular permeability, all factors involved in the pathogenesis of wet AMD. Inhibition of VEGF with intravitreal ranibizumab, pegaptanib and (off-licence) bevacizumab is currently first-line therapy for this condition.

However, VEGF plays a pivotal role in maintaining vascular integrity, particularly under conditions of ischaemia and hypoxia. This is particularly significant since most (but not all) studies have suggested that patients with wet AMD have a higher incidence of coronary heart disease and stroke, and because treatment with VEGF inhibitors may be required for some years in an elderly cohort of patients.

This supplement provides clinicians with detailed information about some of the current issues in this field. It discusses the determination of cardiovascular risk related to current treatment of wet AMD; the properties and functions of the VEGF system, including its complex roles in neuroprotection and inflammation; the effects of VEGF and anti-VEGF treatments on the vasculature, indicating that VEGF inhibition could have unwanted effects such as prothrombotic and vasoconstrictive effects; and, finally, findings on efficacy and safety of intravitreal pegaptanib in the treatment of wet AMD. This agent binds with high specificity to isoform 165 of VEGF, the isoform preferentially involved in choroidal neovascularisation.

Rachel Arthur
Supplement Editor

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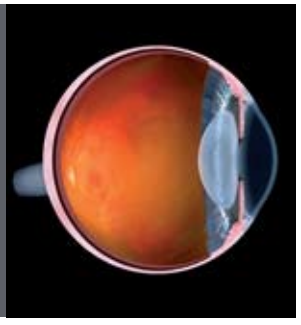

Cover image shows age-related macular degeneration. Credit: Eyeland Design Network

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Current treatment of wet age-related macular degeneration: determining the cardiovascular risk

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Introduction

Age-related macular degeneration (AMD) is a common ocular condition that may destroy central vision and has a devastating effect on the patient's quality of life. More than eight million Americans, particularly those over the age of 55 years, suffer from age-related macular degeneration, and the overall prevalence of advanced AMD is projected to increase by more than 50% by the year 2030.[1] In the UK, the annual incidence of neovascular AMD was calculated to be around 24,000 in 2005, with a prevalence of 243,000; this is predicted to rise to over 300,000 by 2025.[2] The majority of patients with neovascular AMD progress to legal blindness in the affected eye within two years of diagnosis, and there is a 43% probability of progression to neovascular AMD in the other eye within five years.[1] Until recently, the only pharmacological-based therapy for treatment of patients with neovascular degeneration has been photodynamic therapy with verteporfin.

Although the pathophysiology is still poorly understood, it is increasingly clear that vascular endothelial growth factor (VEGF) plays an important role in promotion of the neovascularisation and vessel leakage that lead to loss of central vision. Therefore, intravitreal antiangiogenic therapy (injection of antiangiogenic agents directly into the vitreous) is currently the primary therapy for neovascular AMD. Currently, the most common therapeutic agents are ranibizumab, pegaptanib and bevacizumab (used off-label). Anti-VEGF agents administered systemically for other indications in oncology have been associated with serious systemic adverse events and death.[3] Since breakdown of the blood-ocular barrier is common in wet AMD, repeated intravitreal anti-VEGF therapy may lead to a small amount of systemic VEGF inhibition, possibly resulting in serious long-term adverse events, though these have not yet been shown in clinical studies.[4] We here review the pathogenesis of the disease, the therapeutic options currently used in clinical practice and the possible safety concerns about anti-VEGF therapy in patients with neovascular AMD.

Methods

The leading journals that publish basic science and clinical research in the area of cardiovascular and ophthalmological diseases, and MEDLINE using PubMed, were scanned. The main terms used were "age-related macular degeneration", "cardiovascular disease", "ranibizumab", "bevacizumab", "pegaptanib" and "VEGF". The publications were largely selected from the past five years, but older publications which are commonly referenced or highly regarded were not excluded. The reference lists of articles identified by this search strategy were screened and relevant articles were selected. Review articles are cited to provide the reader with information and references beyond the scope of this review.

Age-related macular degeneration

Early age-related macular degeneration is characterised by the presence of a few (< 20) medium-size drusen or retinal pigmentary abnormalities. Intermediate age-related macular degeneration is characterised by at least one large druse, numerous medium-size drusen, or by geographic atrophy that does not extend to the centre of the macula. As reviewed recently by de Jong,[5] damage to the retinal pigment epithelium and a chronic aberrant inflammatory response can lead to large areas of retinal atrophy (called geographic atrophy), the expression of angiogenic cytokines such as vascular endothelial growth factor, or both. These processes may manifest as advanced AMD, which can either be non-neovascular (dry, atrophic or non-exudative) or neovascular (wet or exudative). Advanced non-neovascular AMD is characterised by drusen and

Figure 1: Age-related macular degeneration (AMD): the right eye (left image) shows a large fibrosis subsequent to untreated neovascular AMD. Visual acuity is counting fingers. The left eye (right image) has characteristic signs of a newly developed neovascular AMD. The patient complains about recent loss of vision.

University Hospital Zurich

geographic atrophy extending to the centre of the macula. Advanced neovascular AMD is characterised by choroidal neovascularisation and its sequelae.[6] **Figure 1** shows the fundus image of a patient with a large fibrosis, the end stage of neovascular AMD, in one eye and recent onset of neovascular AMD in the second eye.

Risk factors for AMD

Several risk factors for the development and progression of AMD have been established in recent years, including advanced age, white race, heredity and a history of smoking. The prevalence of early AMD has been reported to increase from 8% among people 43-54 years of age to 30% among people 75 years or older. Similarly, the prevalence of advanced AMD increases from 0.1% among people 43-54 years of age to 7.1% among people 75 years or older. AMD is more common in whites than Hispanics or Asian persons, whereas blacks have the lowest prevalence of the disease.[7] Data from the Human Genome Project revealed that polymorphisms in the complement factor H (CFH), complement factor B (CFB) and the complement component C2 genes may account for 75% of AMD cases.[8] A polymorphism (Ala69Sr) on the age-related maculopathy susceptibility 2 gene (ARMS2 or LOC387715) has also been strongly associated with development of AMD.[9]

Patients with a history of more than 10 pack-years of smoking have an increased risk for the development of AMD and even passive smokers also appear to have a doubled risk of AMD.[10] Other modifiable risk factors for advanced AMD include arterial hypertension,[11] obesity,[12] high dietary fat intake[13] and low plasma concentrations of antioxidants and zinc.[14,15]

Cardiovascular risk factors

Coronary heart disease (CHD) is the leading cause of mortality in the US and Europe for both men and women. Although in England and Wales mortality rates of CHD continue to fall among older age groups, the actual burden of coronary heart disease is increasing due to the ageing of the population.[16] The rate of improvement in CHD mortality appears to be declining, and may even be reversing among younger women.[16] Atherosclerosis is the underlying cause of most ischaemic events and can result in angina, myocardial infarction, congestive heart failure, cardiac arrhythmias or sudden cardiac death. Risk factors for cardiovascular disease (CVD) have been extensively reviewed in numerous publications.[17-19] Data from the INTERHEART study revealed that the major cardiovascular risk factors smoking, hypertension, hypercholesterolaemia, diabetes mellitus, abdominal obesity, sedentary lifestyle and several psychosocial factors account for >92% of cardiovascular events worldwide.[19] In the same study, daily consumption of fruits and vegetables, regular alcohol consumption and regular physical activity were associated with a reduced cardiovascular mortality. Most important, these findings were noted in men and women, in old and young people, and all over the world.

Risk factors associated with CVD and AMD

As early as the 1970s, researchers wondered whether AMD might be part of an underlying systemic vascular process or a result of factors that also influence the development of CVD.[20,21] The Framingham Eye Study found an association between AMD and systemic blood pressure and its sequel left ventricular hypertrophy.[21] The NHANES-I study reported a positive association between AMD and systemic hypertension, and between AMD and cerebrovascular disease.[22] In a large population-based study, the odds ratio of carotid artery plaques was 4.7 times higher in patients with wet AMD, while peripheral arterial disease was associated with a 2.5 times increased risk for AMD.[23]

The potential link between AMD and CVD was highlighted further in two recent large US studies. The Atherosclerosis Risk in Communities (ARIC) study in more than 10,000 patients demonstrated that subjects with late AMD were significantly more likely to be diagnosed with incident coronary heart disease over 10 years than patients without late AMD (30.9% *vs.* 10.0%, respectively),[24] and also showed a higher incidence of stroke (4.1% *vs.* 2.1%).[25] The US Medicare Study, a population-based cross-sectional and cohort study involving more than 1.5 million Medicare enrolees ≥ 65 years, found

a 20% increased risk of incidental myocardial infarction in patients with neovascular AMD.[26] Importantly, the Blue Mountains Eye Study, which included more than 3,600 baseline participants in a population-based cohort study of common eye diseases in an Australian population aged ≥ 49 years of age, demonstrated that early AMD predicted a doubling of cardiovascular mortality (relative risk [RR] 2.3, 95% confidence intervals [CI] 1.03 – 5.19) over the next decade after controlling for traditional risk factors.[27] Late AMD predicted five-fold higher cardiovascular mortality (RR 5.57, 95% CI 1.35 – 22.99) and ten-fold higher stroke mortality (RR 10.21, 95% CI 2.39 – 43.6) after adjusting for age and gender only.[27] Other studies, however, have found no relationship between AMD and CVD.[28-34]

A potential link between AMD and cardiovascular disease would have important therapeutic implications given current concern that some intravitreal anti-VEGF treatments for wet AMD could increase cardiovascular, and particularly cerebrovascular, risk.[35]

Indeed, VEGF may be regarded as a double-edged sword. Although it is key in the pathogenesis of wet AMD, it at the same time plays a pivotal role in maintaining vascular integrity, particularly under conditions of ischaemia and hypoxia. Thus, any beneficial effects of anti-VEGF therapies in the eye must be weighed against potential long-term systemic effects of these agents, particularly when potent "pan"-anti-VEGF therapies such as ranibizumab and bevacizumab may exert unwanted systemic extra-ocular effects due to the blocking of the cardioprotective functions of VEGF.

The endothelium is increasingly recognised not only as a target (with vascular remodelling occurring in response to an injury and resulting in atherosclerosis), but as a mediator in the pathogenesis of vascular damage.[36] Indeed, endothelial cells play an important homeostatic role in the cardiovascular system through the expression of numerous molecules and release of mediators such as nitric oxide (NO), superoxide and endothelin-1 (ET-1). Studies demonstrating dysfunction of these mediators in patients at risk or with fully developed forms of cardiovascular disease strongly suggest involvement of endothelium-derived factors in the pathogenesis of atherosclerosis and its sequelae.

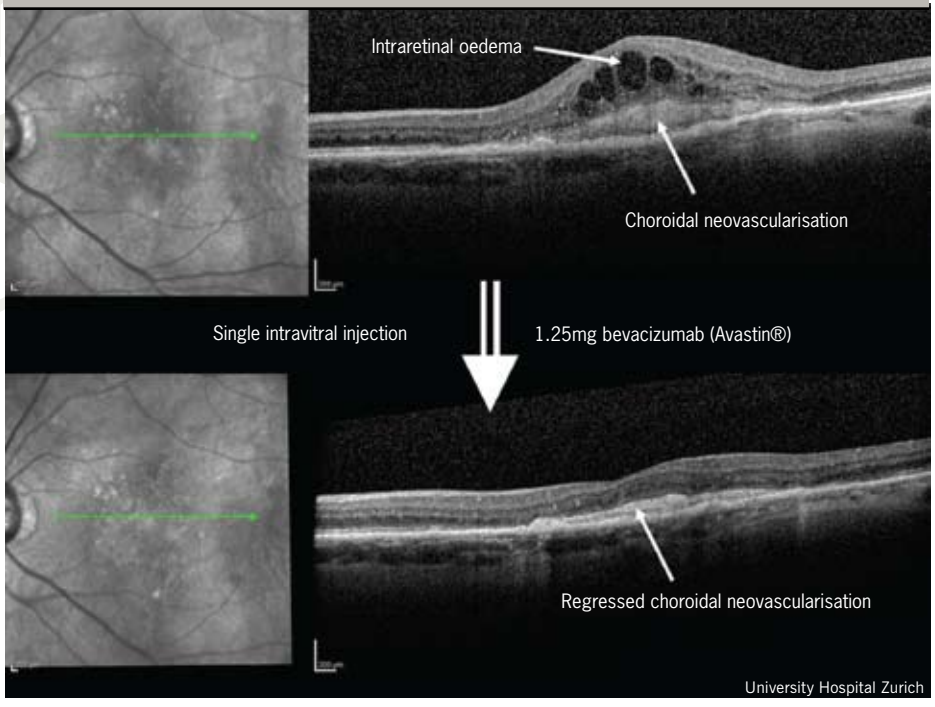
Functional alterations of the endothelial L-arginine / NO pathway may be important in cardiovascular disease since NO can inhibit substantially several components of the atherogenic process such as vascular smooth muscle cell contraction, proliferation and migration; platelet aggregation and adhesion; monocyte adhesion and oxidative modification of low-density lipoprotein (LDL). Hence, reduced endothelial NO release may accelerate the progression of atherosclerotic lesions. Most importantly, NO is the downstream mediator of VEGF and is considered an important defence system in maintaining vascular integrity.[37]

VEGF was first described as a tumour-derived factor with potent ability to induce endothelial cell permeability,[38] proliferation and angiogenesis.[39,40] VEGF induces angiogenesis, and increases vascular permeability and inflammation: all of these are thought to contribute to the progression of the neovascular form of AMD. VEGF levels are raised in the retinal pigment epithelium and choroidal blood vessels of the macula and in the ocular fluid of most patients with proliferative diabetic retinopathy and retinal vein occlusion.

While VEGF occurs in several biologically active forms, a recombinant, humanised monoclonal antibody fragment (Fab), ranibizumab, neutralises all forms of the growth factor. In 2006, two trials with ranibizumab showed that monthly intravitreal injections prevented vision loss and, in many cases, significantly improved the visual acuity of patients with neovascular AMD.[41,42] Bevacizumab is a full-length monoclonal antibody that – like ranibizumab – binds and inhibits all isoforms of VEGF, but with a lower affinity, and has a longer half-life compared to the fragment form.

The Food and Drugs Administration (FDA) approved intravenous bevacizumab for patients with metastatic colorectal cancer in

Figure 2: Optical coherence tomography (OCT): High resolution OCT allows an optical biopsy of the retina in cross section. The images shown are of the same patient as in figure 1. Before intravitreal injection of 1.25mg bevacizumab (Avastin®) the upper images clearly indicates intraretinal oedema and a choroidal neovascularisation (CNV). One month following treatment intraretinal oedema has completely resolved and the CNV has regressed.



February 2004. The same year the first use of systemic bevacizumab for mostly bilateral neovascular AMD was reported.[43] Rapid regression of choroidal neovascularisation was associated with a visual acuity improvement of 1-2 lines. A common adverse effect was an increase in systolic blood pressure.[44,45] The studies were too small, however, to exclude other serious systemic complications, including increased risk of thromboembolic events, haemorrhage, proteinuria, wound healing complications, and gastro-intestinal perforation.[46] In 2005 the first report on the intravitreal use of bevacizumab in neovascular AMD was reported.[47] The driving force for the "off-label" use of bevacizumab was, in addition to its obvious clinical effectiveness (**figure 2**), its low price of less than 50 USD per intravitreal injection. In the meantime, several retrospective and prospective studies indicate good functional outcomes.[48-52]

So far there has been no evidence for an increased risk for systemic adverse events, but studies are overall too small and follow-up is too short to fully evaluate potential systemic adverse events. Recent meta-analyses indicated comparable functional outcomes and safety with bevacizumab and ranibizumab.[53,54] Several large prospective randomised clinical trials comparing bevacizumab and ranibizumab are currently ongoing. Intravenous use of bevacizumab in cancer patients may have serious systemic complications, including increased risk of thromboembolic events, hypertension, haemorrhage, proteinuria, wound healing complications and gastro-intestinal perforation.[55] Whether these systemic complications are relevant to wet AMD patients receiving very low doses by intravitreal injection is unknown. The absence of systemic and ocular adverse events in prospective studies is reasssuring, but the long-term safety of intravitreal bevacizumab remains to be established.[48,50,52]

Pegaptanib was granted marketing authorisation by the European Medicines Agency on 31 January 2006 for the treatment of neovascular AMD. Pegaptanib is a pegylated modified oligonucleotide that binds with high specificity and affinity to extracellular vascular endothelial growth factor ($VEGF_{165}$), inhibiting its activity. $VEGF_{165}$ is the VEGF isoform preferentially involved in pathological ocular neovascularisation. Pegaptanib blocks mainly $VEGF_{165}$, reducing the growth of pathological blood vessels and associated bleeding and leakage.

The main prospective clinical trials in patients with AMD have been conducted using pegaptanib and ranibizumab.[41,42,56,57] Indirect comparison of pegaptanib and ranibizumab, indicates about a 3-line difference in visual acuity outcomes in favour of ranibizumab. Pegaptanib appears to be associated with fewer adverse effects although a direct comparison has never been studied. The three pivotal studies on ranibizumab reported a dose-related increased frequency of cardiovascular events (including stroke) and bleeding relative to the placebo group, although these increases were not statistically significant.

Indeed, whilst these new treatments represent an important breakthrough in wet AMD management, the overall safety of intravitreal anti-VEGF drugs remains unclear. First, although the drug is administered by injection through the sclera into the vitreous cavity, systemic absorption does occur, with potential for systemic adverse effects. In particular, human data are scant. Most importantly, since anti-VEGF treatment is potentially required for years, chronic treatment, particularly with non-selective VEGF inhibitors, may cause adverse effects that may only become clinically apparent over time. Second, because these trials were not designed to detect small differences in risk, much larger cohorts would be necessary to allow the evaluation of systemic adverse effects. In the reported clinical studies, the overall mortality rates were low given the advanced mean age of these populations (nearly 80 years); this could be mainly due to the exclusion of patients with a history of, or with risk factors for, cardiovascular disease.

Potential implications in neovascular AMD

It is of note that VEGF, mostly through its downstream mediator NO, has many essential physiological functions in maintaining vascular integrity, including the potential formation of collateral vessels crucial for the maintenance of perfusion to ischaemic tissues, as in acute myocardial infarction, in particular.[58] Intriguingly, while VEGF is crucial in maintaining vascular homeostasis, particular in clinical conditions associated with ischaemia and hypoxia, its role in maintaining plaque stability is currently a matter of debate.[59] Conceptually, in view of the cardioprotective role of VEGF, non-selective "pan"-anti-VEGF antagonism with ranibizumab or bevacizumab could be of even greater concern than blocking VEGF with selective antagonists such as pegaptanib. However, whether and to what degree more selective VEGF inhibition translates into fewer unwanted systemic effects and thus results in better cardiovascular outcomes remains unproven.

It is of note that cardiovascular safety of anti-VEGF drugs has not yet been addressed in randomised clinical trials. Unfortunately, the numbers of cardiovascular events in these AMD trials without pre-specified cardiovascular safety end points are too small to provide any clinically relevant evidence of safety. Indeed, the absence of evidence does not show evidence of absence. While trials for ranibizumab reported a marginally higher rate of arterial thromboembolic events in the higher dose treatment arm, this trend did not reach statistical significance.[60] (These trials were not powered to detect small differences in risk.) The issue is complicated further by a recent retrospective reanalysis of systemic safety outcomes with ranibizumab, which did not use the full dataset but nevertheless showed a significant increase in non-ocular haemorrhage in treated patients compared with controls ($p=0.01$), suggesting some impairment of systemic VEGF function.[61] Although the doses involved in intravitreal injections of anti-VEGF agents are smaller than intravenous doses, intravitreal injection leads to peak serum concentrations several orders of magnitude greater than physiological levels of VEGF (11–27 ng/ml *vs.* 100 pg/ml in healthy adults).[62,63] The potential capacity of both drugs to saturate circulating VEGF hints at the possibility of adverse systemic effects.

Trials with the only selective inhibitor, pegaptanib, have not as yet shown any cardiovascular safety signals.[56] Importantly, however, all trials both with less selective or "pan"-anti-VEGF agents

were underpowered and thus it is not possible to rule out existing cardiovascular safety concerns. Uncertainty about the cardiovascular risk of intravitreal anti-VEGF treatment will remain until additional systemic safety data become available. These adverse events may be explained by endothelial dysfunction induced by anti-VEGF drugs.

Pan anti-VEGF therapy has shown to stabilise vision in about 95% of patients with neovascular macular degeneration compared to 70% with selective VEGF inhibition. The results are most impressive compared to laser led photocoagulation 15 years ago, which led to an unselective destruction of the retina and a primary loss of vision in order to stop progression of neovascular AMD. This obvious benefit from an ophthalmological perspective stands in contrast to potential cardiovascular risks. Only adequately powered randomised clinical trials that prospectively address cardiovascular safety will provide the evidence to show whether the proven benefits of VEGF antagonism in the eye may come at the cost of potential systemic adverse effects, particularly increasing atherosclerosis and its clinical sequelae. Until this trial evidence becomes available, ophthalmologists should avoid continuous VEGF suppression by using individualised treatment protocols[64] and should synchronise their efforts with cardiologists to reduce the cardiovascular burden of patients with wet AMD

Conflict of interest
None declared.

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VEGF function in ocular health and disease: implications for therapeutic intervention in wet AMD

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Introduction

Vascular endothelial growth factor (VEGF) plays a pivotal role in stimulating abnormal neovascularisation, a key characteristic of neovascular age-related macular degeneration (so-called wet AMD).[1] VEGF is a secreted protein that is able to diffuse and trigger mitogenic activity in endothelial cells.[2] It is produced by multiple retinal cell types, and blood vessels in the retina have several receptors for VEGF. It is known that VEGF inhibition can both prevent and reverse breakdown of the blood–retinal barrier.[3] Indeed, elevated VEGF levels have been linked to neovascularisation and vascular permeability.[4-8] Consequently, it is proposed that VEGF inhibition could block the underlying pathogenic process of wet AMD.

However, VEGF is an intercellular signalling factor with numerous functions throughout the body. These functions can be both physiological and pathological: examples of these functions are provided in **table 1**.

The VEGF system

VEGF is not a single protein but rather exists in a number of different isoforms (**figure 1**). These isoforms differ in the number of amino acids contained in the mature secreted protein and, most importantly, in their solubility and heparin-binding properties.[9,10] The solubility of the isoform influences its ability to diffuse in the extracellular space and heparin-binding properties influence the extracellular matrix interactions of the individual isoform. The balance of solubility and heparin binding provides the spatial cues to initiate a precisely branched vessel network.[11] A further level of complexity is added by the existence of proximal and distal splice forms: proximal splice forms are pro-angiogenic whereas distal splice forms are anti-angiogenic.[12] A switch in splicing from anti-angiogenic to pro-angiogenic isoforms of VEGF may be associated with diabetic retinopathy.[12]

In the eye, $VEGF_{121}$ and $VEGF_{165}$ are the major isoforms: $VEGF_{145}$ and $VEGF_{206}$ are not detected in the eye. High-affinity receptors for VEGF are expressed by endothelial cells. VEGF receptor 1 (VEGFR-1) and VEGF receptor 2 (VEGFR-2) bind to all isoforms of VEGF. In contrast, neuropilin-1 binds specifically to $VEGF_{165}$ via the exon-7-encoded domain of VEGF, which $VEGF_{121}$ lacks. Neuropilin-1 is thus recognised as a $VEGF_{165}$-specific receptor.[9]

VEGF and neuroprotection

The complexity of the VEGF system, utilising different isoforms and receptors, permits many functions for VEGF, some of which are beneficial and others detrimental. Recently, new roles in motor neuron development have been elucidated for VEGF. Studies in mice indicate that VEGF is involved in the coalescence of motor nuclei: disruption of the VEGF system results in a delay in migration of these cells.[13] These findings could have important implications for neuron preservation in the eye.

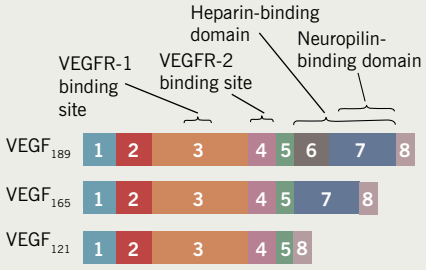
When the eye is subjected to an ischaemic insult, retinal neurons become apoptotic; in the presence of VEGF, apoptosis is greatly reduced.[14] However, if a long period of ischaemia (60 minutes) is preceded by a short period of ischaemia (five minutes), there appears to be a level of protection afforded, known as ischaemic preconditioning. VEGF is thought to

Table 1. Properties and functions of VEGF

- Angiogenic
- Permeability factor
- Pro-inflammatory
- Vasodilator
- Maintains non-thrombogenicity
- Fenestrae induction*
- Vessel survival factor
- Neuron migration
- Neuroprotection

*Fenestrae are specialised plasma membrane microdomains in endothelial cells that are involved in vascular permeability

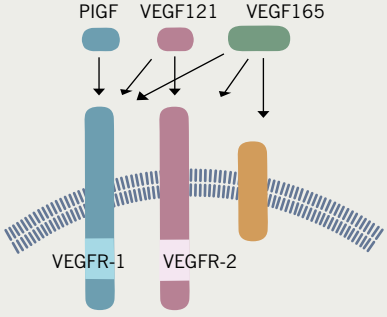
Figure 1. Vascular endothelial growth factor (VEGF) exists in multiple isoforms that differ in their solubility and heparin-binding characteristics



Data from: i. Robinson CJ *et al. J Cell Sci.* 2001; **114**: 853–865 ii. Neufeld G *et al. FASEB J.* 1999;**13**:9–22

Figure 2. There are a number of different receptors for VEGF in the eye. Different isoforms of VEGF have varying affinities for different receptor types

- $VEGF_{121}$ and $VEGF_{165}$: two major VEDF isoforms in the retina
- VEGF receptors VEGFR-1, VEGFR-2, and neuropilin-1



play a very important neuroprotective role in this ischaemic preconditioning. Indeed, if VEGF is injected into the eye following ischaemic insult, the majority of neuronal cell death can be prevented. It has been found that VEGFR-2 is present not only in the blood vessels but also on neurons and glia within the retina, providing a potential mechanism for this neuroprotective effect. By contrast, chronic suppression of the VEGF system leads to retinal ganglion cell death, which could have important implications for the use of anti-VEGF therapy in wet AMD.

VEGF and inflammation

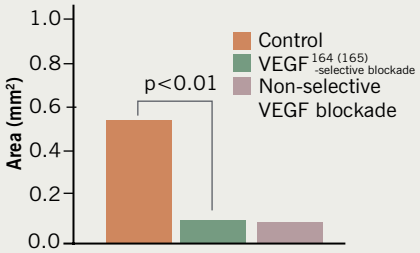
Evidence suggests that the $VEGF_{165}$ isoform has pro-inflammatory properties.[15] $VEGF_{165}$ blockade preferentially inhibits pathologic retinal neovascularisation. Indeed, $VEGF_{165}$-selective blockade and non-selective VEGF blockade inhibit pathologic neovascularisation to a similar extent (**figure 3**).[8] In $VEGF_{164}$-deficient mice ($VEGF_{164}$ in mice is equivalent to $VEGF_{165}$ in humans), no neovascularisation in the flat mount retina is observed, in contrast to wild-type mice where abnormal angiogenesis and vascular tuft formation are present.[15]

$VEGF_{165}$ has been shown to be the most potent of the VEGF isoforms at creating leukocyte-based inflammation.[15] It is likely that the pro-inflammatory activity of $VEGF_{165}$ contributes to the development of wet AMD. Inflammation plays an important role in AMD.[16]

Conclusion

VEGF has numerous physiological roles that must be considered when developing treatments for chronic conditions that may affect VEGF functionality in the body. More research is required to develop understanding of the different roles of VEGF isoforms in normal physiological functioning and the pathogenesis of disease.

Figure 3. $VEGF_{164}$ blockade preferentially inhibits pathologic retinal neovascularisation



$VEGF_{164}$ in mice is equivalent to $VEGF_{165}$ in humans. Adapted from Ishida S *et al. J Exp Med* 2003;**198**:483–9

At present, pan-VEGF inhibitors are used in both oncological and ophthalmological settings. There is a growing list of safety concerns as experience with these agents increases, although at present the benefits are considered to outweigh the risks. The risk/benefit will need to be continuously monitored as these agents are used longer-term, as preventive agents and for the potential treatment of diabetic retinopathy which is currently being investigated in the clinic. More selective VEGF inhibitors may provide an attractive option for treating patients with a higher risk profile

Conflict of interest

Professor Shima: none declared.

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Wet AMD: anti-VEGF treatments in the elderly population

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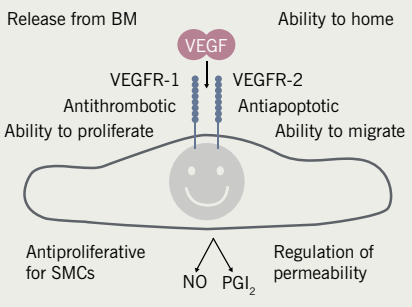
Background

In 1971 Folkman proposed that tumour growth was dependent upon angiogenesis, and consequently suggested that preventing angiogenesis might prevent tumour growth.[1] This concept led to research into manipulating angiogenesis in order to influence tumour progression, and subsequently other therapeutic areas, including cardiology and ophthalmology. In 1983 vascular permeability factor (VPF) was discovered, followed by vascular endothelial growth factor (VEGF) in 1989. It later transpired that they were in fact the same molecule.

Effect of VEGF on the vasculature

VEGF plays a primarily protective role in the vasculature (**figure 1**).[2] It is known that VEGF stimulation of the endothelium has an antithrombotic effect. It stimulates endothelial cells to proliferate and migrate, which is crucial for the renewal of the endothelium. VEGF also has an anti-apoptotic effect, allowing endothelial cells to survive for longer periods of time. VEGF stimulation of endothelial cells is involved in the induction and release of nitric oxide and prostacyclin, enabling the endothelial cells to interact with other cells.[3,4] Furthermore, VEGF has an antiproliferative effect on smooth muscle cells and regulates vessel wall permeability.[5] It is therefore clear that inhibition of VEGF is likely to have unwanted effects on the vasculature: it would be prothrombotic, pro-apoptotic and vasoconstrictive.

Figure 1. Vascular endothelial growth factor (VEGF) stimulation of endothelial cells has a number of effects, primarily protective



Key: BM=bone marrow; SMCs=smooth muscle cells; NO=nitric oxide; PGI_2=prostacyclin

Endothelial cells have a major role in angiogenesis. However, circulating monocytes also carry VEGF receptors and contribute to the formation of collateral vessels. In the heart and peripheral circulation, collateral vessels are protective, for example by providing additional tissue perfusion in the presence of blood vessel blockage. Indeed, VEGF stimulation can be used therapeutically to promote the growth of collateral vessels.[6] If the femoral artery of a mouse is ligated (to induce claudication), perfusion can be significantly increased following the intravenous application of VEGF for seven days.[7] Furthermore, this elevated perfusion appears to be largely mediated via VEGF receptor 1 (VEGFR-1), which is present on the surface of both endothelial cells as well as circulating monocytes.

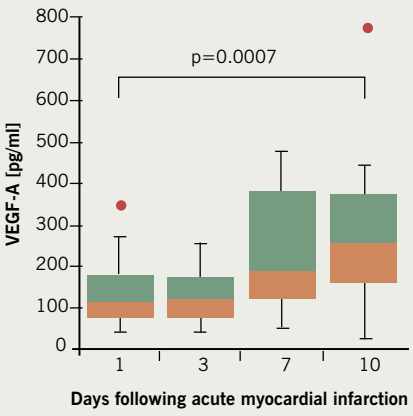
A number of phase II and III trials of therapeutic angiogenesis have been conducted, many using VEGF receptor-stimulating agents, but with mixed results (**table 1**).[8-17] Consequently, there is no currently accepted pro-VEGF therapy with proven efficacy in the clinical situation. It is thought that a reason for treatment failure may have been the relatively short timeframe of VEGF application: in order for VEGF to exert a biological effect on collateral vessel growth, it needs to be present for at least one week in sufficient concentrations. All

Table 1. Summary of phase II and III trials of therapeutic angiogenesis

Trial	Therapeutic agent	n	Endpoint	Result
Coronary heart disease (CHD)				
VIVA trial[8]	r-VEGF-A protein	178	ETT at 60d	negative
FIRST trial[9]	r-FGF-2 protein	337	ETT at 90d	negative
GM-CSF trial[10]	r-GM-CSF protein	21	Invasive collateral flow at 2 weeks	positive
AGENT trial[11]	Adenovirus-FGF-4	79	ETT at 4 weeks pos.	positive
KAT trial[12]	Adenovirus-VEGF$_{165}$ Plasmid/liposomeVEGF	103	Improved myocardial perfusion at 6 months	positive
REVASC trial[13]	Adenovirus-VEGF$_{121}$	67	Time to 1mm ST segm. depression on ETT 26wk	positive
Euroinject One trial[14]	Plasmid-VEGF$_{165}$	74	Myocardial perfusion at 3 months	negative
Peripheral arterial occlusive disease (PAOD)				
TRAFFIC trial[15]	r-FGF-2 protein	190	ETT at 90d	positive
VEGF-FAOD trial[16]	Adenovirus-VEGF$_{165}$ Plasmid/liposomeVEGF	54	Increased vascularity in angiography at 3 months	positive
RAVE trial[17]	Adenovirus-VEGF$_{121}$	105	PWT at 12 weeks	negative

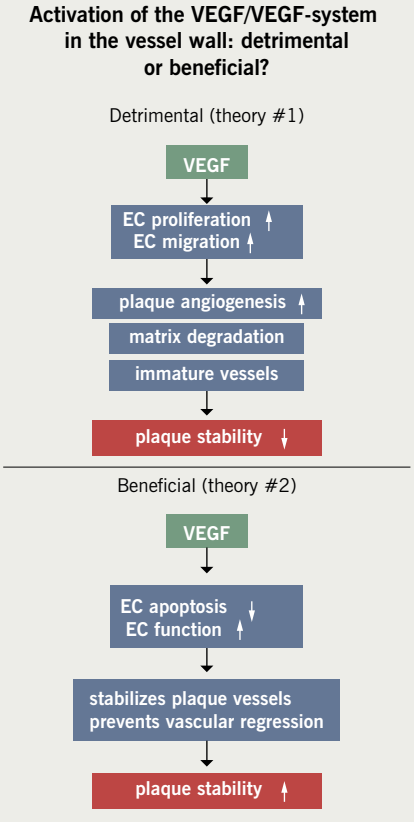
Key: ETT= exercise tolerance test: PWT = peak walking time

Figure 2. Serum VEGF levels rise in the presence of ischaemia, as demonstrated following myocardial infarction[18]



Data from: Kranz *et al. J Mol Cell Cardiol* 2000;**32**:65-72

Figure 3. Possible mechanisms for effects of VEGF on atherosclerotic plaque stability



Key: VEGF=vascular endothelial growth factor; EC=endothelial cells

the therapies trialled so far have resulted in only a short spike in VEGF concentration. With improvement of the duration of VEGF receptor stimulation, better results might be seen.

In the presence of ischaemia, VEGF levels rise, suggesting that hypoxia stimulates the VEGF system (**figure 2**).[18] However, VEGF also appears to be present in non-ischaemic tissue, suggesting that a baseline level of VEGF is required to maintain normal vascular function. Reduction in this baseline level of VEGF is likely to result in adverse effects on the circulatory system.

There are a number of different strategies that can be utilised to inhibit VEGF signalling. These include: anti-VEGF antibodies, soluble VEGF receptors, aptamers, VEGF receptor antibodies and inhibitors of the VEGF receptor signalling pathway (tyrosine kinase inhibitors).[19,20]

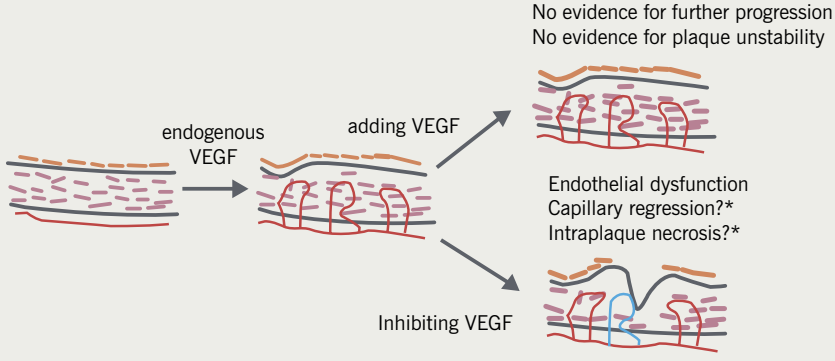
Effects of VEGF on the lung

VEGF inhibition has been used for the treatment of various cancers.[21] An interesting finding from the treatment of lung cancer is that a baseline level of VEGF activity appears to be required for the maintenance of normal lung function. Following the introduction of a VEGF receptor-inhibiting agent, lung cell apoptosis has been seen to occur.[22] Thus, inhibition of VEGF could lead to emphysema and lung destruction.

Effects of VEGF on atherosclerosis

A number of factors are known to be present within atherosclerotic plaques that stimulate

Figure 4. Impact of VEGF on atherosclerotic plaque development and stability



Key: VEGF=vascular endothelial growth factor; *Predicted from oncology

the development and growth of plaques. Among these is VEGF. It has been proposed that, in the atherosclerotic plaque, VEGF stimulates endothelial proliferation and migration, leading to an increase in plaque angiogenesis, matrix degradation and immature vessels and resulting in plaque instability. In turn, plaque instability can lead to thrombotic events, including myocardial infarction and ischaemic stroke. However, it is also possible that VEGF could provide an increase in plaque stability by decreasing endothelial cell apoptosis and improving endothelial cell function, resulting in the prevention of vascular regression (**figure 3**).

In trials where VEGF was added to atherosclerotic plaques, no evidence for further progression of the plaque or evidence of increased instability has been observed.[23-25] However, extrapolation from oncological findings predicts that inhibiting VEGF would result in capillary regression, capillary thrombosis and intraplaque necrosis (**figure 4**). This would lead to an increase in thrombotic events in those receiving VEGF-inhibiting therapy.

The age-related macular degeneration population

Coronary atherosclerosis is a frequent finding in the elderly population, and the risk of coronary atherosclerosis rises with age. The one-day mortality of acute myocardial infarction is around 35%.[26] The one-year mortality of patients above 75 years of age with ST-elevation myocardial infarction (STEMI) is 52.4% for conservative treatment and 19.2% for primary percutaneous intervention.[27]

At the time of diagnosis of age-related macular degeneration (AMD), the average patient is 75 years of age, with a life expectancy of 11.8 years.[28,29] Neovascular AMD (wet AMD) is often associated with significant co-morbidities, such as diabetes and cardiovascular disease.[30] Wet AMD may be treated with VEGF inhibitors, which, although administered intravitreally, are absorbed systemically and thus could potentially have systemic effects.

Summary

Angiogenic growth factors are important functional stimuli for vascular cells. As well as stimulating angiogenesis, they are involved in maintenance of the integrity of the vasculature. Inhibition of angiogenic growth factors efficiently inhibits tumour angiogenesis and reduces tumour growth. However, inhibition of angiogenic growth factors may lead to vascular dysfunction and vascular complications such as atherosclerotic plaque rupture and acute ischaemic syndromes.

This should be an important consideration when administering VEGF-inhibiting treatment for wet AMD, in a population that is already at high risk for cardiovascular events

Conflict of interest

Professor Waltenberger is an advisor to Pfizer.

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Efficacy and safety of intravitreal pegaptanib sodium in the treatment of neovascular age-related macular degeneration

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Introduction

Pegaptanib sodium (Macugen®) was approved for the treatment of neovascular AMD (wet AMD) in Europe in 2006. It is administered by intravitreal injection into the affected eye once every six weeks at a dose of 0.3 mg.[1] Pegaptanib is a pegylated modified oligonucleotide that binds with high specificity and affinity to extracellular vascular endothelial growth factor (VEGF) isoform 165, inhibiting its activity. VEGF is a secreted protein that induces angiogenesis, vascular permeability and inflammation, all of which are thought to contribute to the progression of wet AMD. $VEGF_{165}$ is the VEGF isoform preferentially involved in pathological ocular neovascularisation. In animals, this selective inhibition with pegaptanib proved as effective at suppressing pathological neovascularisation as pan-VEGF inhibition; however, pegaptanib spared the normal vasculature whereas pan-VEGF inhibition did not.[1]

Efficacy

The Vascular Endothelial Growth Factor Inhibition Study in Ocular Neovascularization (VISION) trial showed that 70% of patients with wet AMD treated for 12 months with six-weekly pegaptanib sodium responded to treatment (lost <15 letters). This compared with only 55% of control (sham) subjects. Furthermore, 6% of patients receiving pegaptanib experienced an improvement in their vision (gained ≥15 letters).[2]

In a retrospective analysis performed to acquire data on 'real-life' experience with pegaptanib, data were collected from 164 patients with any angiographic subtype of sub-foveal choroidal neovascularisation secondary to AMD from five European countries.[3] Patients were recruited consecutively with best-corrected visual acuities (BCVA) in the study eye of 20/40 to 20/800. All patients received 0.3 mg pegaptanib as first-line treatment and had a follow-up duration of at least six months. At 24 weeks, 90.2% of patients had met the response criterion of a loss of fewer than 15 letters (last observation carried forward [LOCF] analysis). The mean change in visual acuity at 24 weeks was a loss of 1.7 letters, with 63.4% not losing any letters (maintaining their vision) and 38.4% gaining at least five letters (improving their vision). Only 3% of patients experienced a severe visual loss.

The addition of another 92 patients from further centres to the analysis increased the proportion of patients losing fewer than 15 letters to 93.75% at 24 weeks. The proportion at least maintaining vision was similarly increased to 73% and the proportion improving vision (gain ≥5 letters) increased to 35.5%. At 54 weeks, 91.4% of patients had lost fewer than 15 letters, 71.4% had at least maintained their vision and 27.14% had improved their vision (gained five or more letters).

These real-life visual acuity findings with investigator-determined pegaptanib use suggest that better outcomes than those observed in the VISION study could be achievable.

Safety

There are potential safety concerns regarding the long-term use of anti-VEGF therapy. While, in the treatment of wet AMD, the anti-VEGF agent is administered directly into the eye, there is inevitably some systemic absorption.[4] Ranibizumab (Lucentis) is a humanised recombinant monoclonal antibody fragment with high affinity for the VEGF-A isoforms, which is licensed for the treatment of wet AMD.[5,6] In clinical trials, this pan-VEGF inhibitor has been associated with a higher incidence of arterial thromboembolic events (2.5%) compared

with control treatment (1.1%) at one year.[5] It is thought that the relative selectivity of pegaptanib for $VEGF_{165}$ should reduce the likelihood of such adverse events.

Pegaptanib safety data from the VISION trial indicate that at one and two years there is no evidence of an increase in events associated with systemic VEGF inhibition, such as thromboembolic events and hypertension.[2,7] Indeed, four-year safety results with pegaptanib have shown no changes in the previously reported safety profile (personal communication, JJ Wroblewski).

Conclusion

Pegaptanib has been shown to be safe and effective in the treatment of wet AMD. Despite the absence of a control group and limited number of patients continuing to four years' treatment in the VISION trial, analysis of the safety data is consistent with previous reports: the injection procedure was well tolerated by the patients and there is no evidence of an increased risk of ocular or systemic adverse events. In particular, there is no evidence that pegaptanib is associated with the major systemic adverse events that could accompany pan-VEGF inhibition

Conflict of interest

Dr Sivaprasad has received research and travel grants from Novartis and Pfizer.

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