# Short and long term real-life outcomes in patients with diabetic macular oedema treated with intravitreal aflibercept injections

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# SHORT AND LONG TERM REAL-LIFE OUTCOMES IN PATIENTS WITH DIABETIC MACULAR OEDEMA TREATED WITH INTRAVITREAL AFLIBERCEPT INJECTIONS

**DOCTORAL DISERTATION**

**Split, November 2021**

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**DOCTORAL DISERTATION**

**Mentor:**
**Assoc Prof Kajo Bućan, PhD, MD, FEBO**

**Split, November 2021**

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- **LIST OF SYMBOLS AND ABBREVIATIONS**

ACCORDION - The Action to Control Cardiovascular Risk in Diabetes Follow-on

ADA - American Diabetes Association

AMD – Age-related Macular Degeneration

ANOVA – Analysis of Variance

anti-VEGF – Anti Vascular Growth Factor

CA – California

CA – Clinical Audit

CDC - Centre for Disease Control and Prevention

CFT – Central Foveal Thickness

Da Vinci - One-year outcomes of the da Vinci Study of VEGF Trap-Eye in eyes with diabetic macular edema

DCCT - Diabetes Control and Complications Trial

DM – Diabetes Mellitus

DME – Diabetic Macular Edema

DMO – Diabetic Macular Oedema

DR – Diabetic Retinopathy

DRCR - The Diabetic Retinopathy Clinical Research

DRCR.net - The Diabetic Retinopathy Clinical Research Network

EDICS - The Epidemiology of Diabetes Interventions and Complications Study

ETDRS - Early Treatment Diabetic Retinopathy Study

EU – European Union

EURETINA – European Society of Retina Specialists

FAZ – Foveal Avascular Zone

FDA – Food and Drug Adminstration

FFA – Fundus Fluorescein Angiography

FLT 1 - Fms-related tyrosine kinase 1

GLD – Greatest Linear Dimension

HbA1c – Haemoglobin A1c

IgG1 – Immunoglobulin G1

ICE - International Expert Committee

kDA – kilodaltons

KDR - Kinase Domain-containing Receptor

MEH – Moorfields Eye Hospital

mmol/L – millimole per litre

mmol/mol – millimole per mole

mRNA – messenger Ribonucleic Acid

MV – Macular Volume

NHS – National Health Service

NICE - National Institute of Care and Excellence

OCT – Optical Coherence Tomography

OCTA – Optical Coherence Tomography Angiography

PlGF – Placental Growth Factor

PKC – Protein Kinase C

RESOLVE - Safety and efficacy of ranibizumab in diabetic macular edema Study

RESTORE - Ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema

RETAIN - Ranibizumab 0.5 mg treat-and-extend regimen for diabetic macular oedema

RIDE - A Study of Ranibizumab Injections in Subjects with Clinically Significant Macular Edema (ME) with Centre Involvement Secondary to Diabetes Mellitus

RISE - A Study of Ranibizumab Injections in Subjects with Clinically Significant Macular Edema (ME) with Centre Involvement Secondary to Diabetes Mellitus

SD – Standard Deviation

T2DM – Type 2 Diabetes Mellitus

UK – United Kingdom

UKPDS - The United Kingdom Prospective Diabetes Study

US – United States

USA – United States of America

VA – Visual Acuity

VEGF – Vascular Endothelial Growth Factor

VEGFR-1 – Vascular Growth Factor Receptor 1

VEGFR-2 – Vascular Growth Factor Receptor 2

VIVID – Intravitreal Aflibercept Injection in Vision Impairment Due to DME

VISTA – Study of Intravitreal Aflibercept Injection in Patients with Diabetic Macular Edema

WHO - World Health Organization

# 1. INTRODUCTION

Health services globally face challenges to manage the significant increase in the number of patients with diabetes mellitus. Most of the costs of diabetes mellitus are related to the treatment and management of its complications. Ophthalmologists play a significant role in managing some of the most severe complications of diabetes which are diabetic retinopathy (DR) and diabetic macular oedema (DMO).

Diabetic macular oedema is the leading cause of blindness in the working-age population in developed countries. Therefore, the preservation of vision in those patients has a significant impact on the lives of those patients and overall society. At the end of the last millennium, the primary treatment modality for diabetic macular oedema was macular laser. Soon after the start of the new millennium, a new paradigm of the pathophysiology of diabetic macular oedema has been established. It relies on the evidence that the condition is provoked by local inflammation with the Vascular Endothelial Growth Factor (VEGF) as a central molecule. This doctoral dissertation will summarize the pathophysiology and subsequent development of the so-called anti-VEGF drugs, considering the pivotal role of the VEGF molecule.

The published evidence of landmark clinical trials revolutionized the approach in the treatment of diabetic macular oedema. The evidence has been strongly suggested that anti-VEGF is superior in visual acuity gain as compared to laser treatment, which was the standard golden treatment at that time. However, the retina specialists and primarily those on the European continent faced those proposed protocols that may not be implemented as suggested by the trials. Furthermore, the exclusion criteria would be considered discriminatory in real life. Due to poor diabetic control, many patients may lose vision as they are not eligible for the treatment. Such controversies between the landmark clinical trials' protocols and our real-life experience could cause under treatment of patients where patients might face worse vision prognosis if we strictly followed the suggested protocols. Such a fact can only cause frustration and feeling of helplessness among retina specialists. Therefore, the only rationale was to investigate whether the protocols that we established in our clinical services may be non-inferior compared to landmark clinical trials' results. When we started our investigations, the effects on intravitreal ranibizumab had a primate in publications in terms of real-life evidence. In contrast, the efficacy of intravitreal aflibercept in real-life conditions, which has been

Moorfields' first-line treatment agent, was not published by the time of initiation of our work nor publications of the articles which are pooled in this doctoral dissertation.

Due to its pharmacological performances, we will explain the rationale of listing intravitreal aflibercept on Moorfields Eye Hospital diabetic macular oedema protocol. Furthermore, we analysed outcomes of patients who were switched from intravitreal ranibizumab to intravitreal aflibercept, aiming to see whether the potentially more potent anti-VEGF agent may bring additional benefits or fasten the recovery of the vision. Eventually, this doctoral dissertation will show the importance of baseline visual acuity and previous macular laser or lens status effects on overall results.

## 2. EPIDEMIOLOGY AND SOCIO-ECONOMICS DATA ON DIABETES MELLITUS AND DIABETIC RETINOPATHY

The pandemic of diabetes mellitus represents a substantial global health challenge. According to the Centre for Disease Control and Prevention (CDC) more than 100 million adults are diagnosed with diabetes or prediabetes in the USA.[1,2] The International Diabetes Federation published the data which indicate that the prevalence of diabetes mellitus in Europe is 8.5% of people aged between 20 and 79 years, which means that 33 million people in the European Union are affected by this silent and severe condition.[3] The Croatian Institute for Public Health published that 315.298 of Croatian citizens diagnosed with diabetes mellitus were registered onto the National Diabetes register in 2019.[4] According to the International Diabetes Federation data, the prevalence of diabetes mellitus among the adult population in Croatia is 6.7%.[3] Furthermore, the number of patients diagnosed with diabetes mellitus has been significantly growing each year. There are around 100 000 and 1.4 million newly diagnosed patients with diabetes mellitus per year in the UK and the USA.[1,2] In Croatia, there were 96.349 of those newly diagnosed with diabetes in 2019 as per Croatian Institute for Public Health. [4] The total number of people diagnosed with diabetes mellitus worldwide is projected to rise to 366 million in 2030.[5] In addition, the International Diabetes Federation predicts there are going to be 68.9 million patients diagnosed with diabetes mellitus in Europe by 2035.[3]

The numbers mentioned above may indicate that the management of complications of diabetes mellitus affect significantly national health services as well as national economies worldwide. The current cost of direct patient care for those living with diabetes is estimated at 9.8 billion pounds per year in the UK and around 327 billion US dollars in the USA.[6,7] The Croatian Institute for Public Health published the approximate cost of complications of diabetes mellitus in the EU, which was estimated to 161 billion US dollars in 2019.[4] The published report from Saric at all revealed that cost of diabetes mellitus type 2 in 2016 took nearly 20% of budget of the Croatian Institute of Health Insurance.[8] Furthermore, eighty-eight percent of the overall costs were related to complications of diabetes mellitus.[8]

One of the most significant and common complications of diabetes mellitus are diabetic retinopathy and diabetic macular oedema (DMO), which is the leading cause of visual loss in the working-age population.[9] According to *The UK National Ophthalmology Database study*

clinically significant macular oedema was present in 15.8–18.1% of eyes, and in 8.7–10.0% of eyes this involved the central macula.[10] The report published by the European Society of Retina Specialists (EURETINA) in 2017 revealed the prevalence of diabetic retinopathy of 25% among diabetic patients in 5 EU countries; Germany, United Kingdom, France, Spain and Italy.[11] Furthermore, a meta analysis of 14 prospective studies showed that 1 in 4 with diabetes develop diabetic retinopathy within the mean follow-up period of 5.7 years, whilst another meta-analysis of 35 prospective studies revealed incidence of DR among diabetics of 34.6%.[12,13]

## 3. PATHOMECHANISM OF DIABETIC MACULAR OEDEMA

Diabetic macular oedema is one of the most common and significant complications of diabetes mellitus. It is the leading cause of vision loss among the working-age population in developed countries.[9] The pathophysiology of diabetic macular oedema is complex and multifactorial.[14] We focused on this doctoral dissertation only on the role of VEGF in the pathophysiology of diabetic retinopathy and diabetic macular oedema. The reason is that the VEGF is the target molecule in treating patients with diabetic macular oedema included in our publications. The complexity of the pathophysiology of diabetic retinopathy is out of the scope of this doctoral dissertation.

There are identified four major pathways triggered by hyperglycaemia-induced events, which end-results are diabetic microvascular dysfunction, neuronal apoptosis, and glial activity with component depositions.[15] Within the retina, those hyperglycaemia-induced events are causing a break of the inner blood-retinal barrier and leakage of plasma constituents into interstitial retinal tissue.[15,16] Those pathways include *1. Diacylglycerol (DAG)–protein kinase C (PKC) 2. advanced glycation endproducts/ receptor for advanced glycation endproducts 3. Polyol (sorbitol) and 4. Hexosamine pathways.* Some of them are directly related to the VEGF molecule, which is explained in further text.[15]

The internal retinal-blood barrier wall consists of tight junctions between endothelial cells, surrounding basal lamina, pericytes, astrocytes, and microglia.[14] The recent evidence confirms that diabetic macular oedema is a localized inflammatory disease with loss of pericytes and breakdown of tight endothelial junctions.[15] The inflammatory factors involved in the mechanisms of diabetic macular oedema include VEGF-A, PlGF, interleukins 8,6 and 1β, tumor necrosis factor α and metalloproteinases.[16-18]

The VEGF – A has a pivotal role in the pathogenesis of diabetic macular oedema. It can induce vascular permeability, which was first noted in guinea pigs, and as such, it has been called vascular permeability factor.[19,20] The VEGF-A is part of the VEGF family, which along with VEGF-A, includes Placental Growth Factor (PlGF), VEGF-B, VEGF-C, VEGF-D, and VEGF-E.[21] The VEGF-A has five different isoforms in humans, where VEGF -$A_{165}$ is the most

commonly investigated in clinical trials. VEGF-A binds to two out of three VEGF receptors (VEGF receptor one and VEGF receptor 2).[22]

It has been identified that VEGF-A has different roles in the pathophysiology of diabetic retinopathy. One of the essential roles is the upregulation of adhesion molecules that cause leukostasis (adherence of monocytes and neutrophils to endothelial cells), proven *in vitro and in vivo*.[23-26] Then, the VEGF-A mediates the effect of protein kinase C in the protein kinase C pathway.[27] One of the effects of PKC is phosphorylation of tight junctions of endothelial cells, which causes permeability of vessels.[28] The PKC is also involved in hyperglycaemia-induced retinal neuronal apoptosis.[29] VEGF is also related to advanced glycated end products where such products induce increased production of VEGF molecules.[30]

The sorbitol pathway has an essential role in the development of diabetic retinopathy. [31] The increased intracellular glucose reactivates enzyme aldose reductase, which leads to the accumulation of sorbitol and fructose. The over-accumulation of sorbitol directly leads to microvascular damage.[32] The additional effect on the breakdown of the blood-retinal barrier has a hexosamine pathway. The activation of this pathway causes over-production of mitochondrial superoxide, which induces oxidative stress and subsequently neuronal apoptosis, endothelial dysfunction, and breakdown of the blood-retinal barrier.[33-35]

## 4. THE IMPORTANCE OF THE HbA1C IN DIABETIC RETINOPATHY AND DIABETIC MACULAR OEDEMA

The role of glycated haemoglobin (HbA1c) in its correlation with the risk of development and progression of diabetic retinopathy has been well established. In this chapter, we will summarize published data and current knowledge on the role of the HbA1c and its relationship with the development and progression of diabetic retinopathy and diabetic macular oedema.

The HbA1c is an important indicator of long-term glycemic control to reflect the cumulative glycemic history of the preceding two to three months.[36] The HbA1c not only provides a reliable measure of chronic hyperglycemia but also correlates well with the risk of long-term diabetes complications.[36] The American Diabetes Association (ADA), International Expert Committee (IEC), and the World Health Organization (WHO) recommend the use of HbA1c to diagnose diabetes, using a threshold of 6.5%.[37] For people without diabetes, the normal range for the hemoglobin A1c level is between 4% and 5.6%. Hemoglobin A1c levels between 5.7% and 6.4% mean you have prediabetes and a higher chance of getting diabetes. [38] Levels of 6.5% or higher mean you have diabetes. The target A1c level for people with diabetes is usually less than 7%. The higher the hemoglobin A1c, the higher is the risk of having complications related to diabetes. In 2011, a new measurement of HbA1c had been introduced with the intention of internationalization the values of the HbA1c.[39] The new measures are presented in mmol/mol. The targets of diabetic patients under the old classification were between 6.5 and 7.5%, while the new measurement indicates 48-59 mmol/mol.[39]

The prevalence of DR was 48.4% in the population type 1 diabetes mellitus and 28.3% in the population with type 2 diabetes mellitus (T2DM) in one UK study.[40] In addition, a meta-analysis of 35 prospective studies revealed the incidence of DR among people with diabetes of 34.6% [13]

One of the first studies investigating the importance of HbA1c in the prevalence of diabetic retinopathy was the Diabetes Control and Complications Trial (DCCT) conducted from 1982-1993. The DCCT study was a controlled clinical trial with 1441 subjects with type 1 diabetes mellitus. The study compared conventional therapy approach versus intensive treatment.[41] The intensive treatment utilized three or more daily insulin injections or insulin pump therapy

guided by self-monitored glucose. The intensive control of blood glucose levels, where the mean HbA1c was 7.2%, achieved a decrease in the incidence of diabetic retinopathy by 76% and slowing the progression of diabetic retinopathy by 54%.[41] The Epidemiology of Diabetes Interventions and Complications Study (EDICS) observed the cohort of the DCCT study and confirmed the study results 30 years after the initiation of the DCCT trial.[42] The United Kingdom Prospective Diabetes Study (UKPDS) investigated the effect of glucose level on the development of diabetic retinopathy and other microvascular complications in patients with diabetes mellitus type 2. The study proved that tight glucose control minimizes the progression of diabetic retinopathy.[43] Patients with a median haemoglobin A1c (HbA1c) of 7.0% showed a substantial reduction in the risk for microvascular complications.[43,44] Furthermore, tight blood pressure control plays a role in mitigating the progression of diabetic retinopathy.[43] It has been concluded that the progression rate of retinopathy is 37% less for each 10mmol/l (1%) reduction in the HbA1C or 1mmol/l of blood sugar.[41,43] Some more recent publications confirm the conclusions of the first results on the importance of reasonable glycaemic control on the progression of diabetic retinopathy. The Action to Control Cardiovascular Risk in Diabetes Follow-on (ACCORDION) Research Group confirms the vital role of ophthalmologists in the monitoring of diabetic patients where the ACCORDION study results have shown that intensive glycemic control has long-lasting effects in reducing the risk of retinopathy progression.[45]

When we consider the role of baseline HbA1c in diabetic maculopathy and diabetic macular oedema, the publications give inconsistent conclusions.[47-51] One report from the Eye Journal, by Chou et al., concludes that HbA1c of 8 or above had an increase in macular thickness in type 2 diabetic eyes.[46] The posthoc analysis of VISTA and VIVID clinical trials, the landmark clinical trials for intravitreal aflibercept, concluded that the baseline HbA1c did not play a significant role in visual acuity (VA) gain at week 52.[47] In contrast, it may play a role at week 100.[47] Nonetheless, the level of HbA1c plays a role in central foveal thickness (CFT) reduction at week 52 and week 100.[47] Furthermore, the post-hoc analysis of RISE and RIDE studies, which are landmark clinical trials for intravitreal ranibizumab, revealed that the improvement in VA, anatomic reduction of macular oedema, and improvement in DR severity score with ranibizumab treatment seem to be independent of baseline HbA1c.[48] A recent publication from Shalchi et al. confirmed that HbA1c does not play a role in visual and anatomical outcomes in patients with DMO treated with intravitreal ranibizumab at year 1.[49]

The conflicting conclusions on the effect of baseline HbA1c in diabetic macular oedema could be related to different analysis methods and different diabetic status in cohorts of patients represented in various publications. We consider that it is essential to differentiate diabetic maculopathy from diabetic macular oedema. Diabetic maculopathy represents changes in the posterior pole, secondary to diabetes mellitus, which are part of diabetic retinopathy but do not manifest the macular thickening.[50] The evidence may suggest that the HbA1c at the stage of maculopathy with no macular thickening may play a role as an indicator of development of diabetic macular oedema. In contrast, the importance of HbA1c when the oedema is already present may not be of such significance.[51] However, due to undeniable evidence of HbA1c in the progression of diabetic retinopathy, its control is essential also in patients with diabetic macular oedema but primarily to prevent further progression of diabetic retinopathy and likely not necessary to gain better results in the treatment of the diabetic macular oedema with intravitreal anti-VEGF agents. Therefore, we didn't consider the level of HbA1c as a decisive factor in the initiation of therapy in our cohorts of patients presented in this doctoral dissertation.

## 5. DEVELOPMENT OF ANTI-VEGF DRUGS

The ischaemic retinal conditions manifest with new vessels growth. Prior recognition of VEGF as a critical molecule in the development mechanism of new vessels on the retina and iris was postulated that an agent must stimulate the growth of those vessels. One of the greatest authorities in retinal vascular conditions, George Wise, hypothesized that "*pure retinal neovascularization is directly related to a tissue state of relative retinal anoxia. Under such circumstances, an unknown factor x develops in this tissue and stimulates new vessel formation, primarily from the capillaries and veins."*[52]

A critical demonstration that hypoxic retina produces VEGF was published in *The American Journal of Pathology* in 1994.[19] In that study, nonhuman primate retinal veins were treated with laser photocoagulation, consequently causing retinal ischaemia. Subsequently, the new iris vessels (iris rubeosis) were formed, proving a diffusible molecule supportive of angiogenesis. Levels of VEGF mRNA and protein were shown to be elevated in a manner that was spatially and temporally consistent with a role for VEGF in the growth of new vessels.[19,53]

The understanding of the role of VEGF in ischaemic retinal conditions caused quite an enthusiasm in the retinal community. Scientists and clinicians started to develop anti-VEGF drugs with regards to the treatment of retinal conditions. A few molecules have been developed and used for the treatment of retinal conditions which have an anti-VEGF effect, and those are **bevacizumab, ranibizumab, and aflibercept.** The **pegaptanib** (*Macugen®, Pfizer, Belgium*), an anti-VEGF drug approved by the FDA for treatment of neovascular AMD, has not prevailed.[54] Therefore, it has no significance in further discussion of anti-VEGF agents. In addition, there is a recent FDA- and EMA-approved anti-VEGF drug, **brolucizumab**, which has been approved to treat wet age-related macular degeneration, and it is still under the research phase for its potential efficacy in the treatment of DMO.[55-57]

### 5.1 Bevacizumab (Avastin®, Genentech, San Francisco, CA, USA)

Bevacizumab is a recombinant humanised monoclonal immunoglobulin G1 (IgG1) antibody (***93% human, 7% murine sequences - molecular weight 149 kDa***) that selectively binds with high affinity to all isoforms of human vascular endothelial growth factor (VEGF) and neutralizes VEGF's biologic activity through a steric blocking of the binding of VEGF to its receptors Flt-1 (VEGFR-1) and KDR (VEGFR-2) on the surface of endothelial cells.[58] Binding bevacizumab to VEGF receptors causes phosphorylation of the receptor, which subsequently induces a series of events that eventually block survival signals for vascular cells. Thus, the angiogenesis or new vessels formation is directly blocked, which causes the stopping of tumor growth (i.e., colorectal cancer) or stops the development of new vessels in retinal tissue (i.e., choroidal neovascular membrane in AMD form or new vessels in proliferative ischaemic retinal conditions).[58] The drug was initially used to treat metastatic colorectal cancer along with chemotherapy and was approved by the US Food and Drug Administration in 2004.[59] During the research phase of the studies, it was accidentally noted that a subgroup of patients who had an exudative form of age-related macular degeneration improved vision. That resulted in the setting up of ophthalmic-focused studies, which summaries could be found in subsequent chapters of this dissertation. Bevacizumab is an off-labelled drug for ophthalmic use due to the below-mentioned reasons.

### 5.2 Ranibizumab (Lucentis®, Novartis, Basel, Switzerland)

As the bevacizumab molecule is a full-length antibody (with two heavy and two light chains), it was thought that it might not reach through the retina and enter the choroid sufficiently enough. Therefore, the same company which produces Avastin®, Genentech, did not start the approval process for the ophthalmic use of the drug in front of the US Food and Drug Administration. Instead, it began developing another drug whose characteristics should complement its purpose in treating retinal conditions better. The molecule is known under its generic name **ranibizumab.**

The ranibizumab is a recombinant humanized monoclonal immunoglobulin G1 (IgG1) antibody fragment that inhibits human vascular endothelial growth factors.[60,61] Complementarity-determining region mutation and monovalent phage display were used in the

process of recombination and eventually resulted in a variant with high potency to bind VEGF (some of them had improvement of 100-fold in potency for inhibition of VEGF).[60] It is a 45 kilodaltons fragment of an antibody, which rapidly penetrates through the retina to reach the choroid and binds all isoforms of VEGF-A.[61] FDA has approved it for treatment of neovascular age-related macular degeneration (2006), for treatment of macular oedema following retinal vein occlusion 2010), for treatment of diabetic macular oedema (2012), for myopic choroidal neovascularisation (2017), and all forms of diabetic retinopathy (2017).[62]

### 5.3 Aflibercept (Eylea®, Bayer, Basel, Switzerland)

The experience with the aforementioned anti-VEGF antibodies raised a need to develop anti-VEGF drugs with higher potency than anti-VEGF antibodies have as a frequent treatment with current medications were not sustainable in the long run. Therefore, other molecules entered the research phase, and aflibercept was the next agent approved for ophthalmic use. Aflibercept is a recombinant fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 formulated as an iso-osmotic solution for intravitreal administration.[63-65] Aflibercept is a dimeric glycoprotein with a molecular protein weight of 97 kilodaltons (kDa).[63-65] It contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. The aflibercept molecule mode of action is binding VEGF from both sides of the VEGF dimer. Therefore, sometimes term trap is used. In addition, the aflibercept molecule binds VEGF-A, VEGF-B, and Placental Growth Factor (PlGF) – 2. [64,65] All three molecules have their role in angiogenesis and vascular permeability. The aflibercept's action of combining all three molecules is thought to play a significant difference compared to its competitors. FDA has approved it for treatment of neovascular age-related macular degeneration (2011), for treatment of macular oedema following retinal vein occlusion 2014), for treatment of diabetic macular oedema (2014), for myopic choroidal neovascularisation (2014), and all forms of diabetic retinopathy (2019).[66]

# 6. CLINICAL PHARMACOLOGY OF ANTI-VEGF DRUGS

The pharmacodynamics and pharmacokinetics of anti-VEGF drugs discussed in this doctoral dissertation are affected by the structure of each molecule. Bevacizumab is a recombinant humanised monoclonal immunoglobulin G1 (IgG1) antibody that selectively binds with high affinity to all isoforms of human vascular endothelial growth factor (VEGF) A and neutralizes VEGF's biologic activity through a steric blocking of the binding of VEGF to its receptors Flt-1 (VEGFR-1) and KDR (VEGFR-2) on the surface of endothelial cells.[58] The ranibizumab is a recombinant humanized monoclonal immunoglobulin G1 (IgG1) antibody fragment that inhibits human vascular endothelial growth factors (all isoforms of VEGF-A).[60,61] At the same time, the aflibercept is a recombinant fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1, which can bind VEGF-A, VEGF-B, and PlGF-2.[63-65]

## 6.1 Pharmacodynamics

Each anti-VEGF drug discussed in this doctoral dissertation has different target selectivity, affinity, and potency. Drug affinity measures how strong a drug can bind to its receptor. At the same time, potency is the amount of medication needed to produce a pharmacological effect (the smaller the dosage required, the more potent the drug). Affinity is defined as the degree of attraction between the drug and the target, and it is expressed by the dissociation constant, $K_d$ (that is, the ratio between dissociation ($K_{off}$) and association ($K_{on}$) rates). $K_d$ is the inverse of the affinity to the binding site (i.e., the lower the $K_d$, the higher the affinity). Potency is the amount of drug needed to produce the pharmacological effect. For these drugs, potency is expressed by the half-maximal inhibitory concentration ($IC_{50}$), a measure of the effectiveness of a substance in inhibiting a specific biological function (the lower the $IC_{50}$, the higher the potency).

The significant variations in results on pharmacodynamics were noted depending on the method and assay used in the research. Fogli et al. did a great analysis of published results on the pharmacology of anti-VEGF drugs.[67-72] Despite the discrepancies in the studies, the highest affinity measured based on Fogli's research for bevacizumab, ranibizumab and aflibercept were as follows; 58 pM, 9.2 pM, and 0.49 pM, respectively.[68-72] According to the same analysis, the

highest measured potency for bevacizumab, ranibizumab, and aflibercept was as follows: 500 pM, 88 pM, and 16 pM, respectively.[68-72]

### 6.2 Pharmacokinetics

The pharmacokinetics of anti-VEGF show a significant interspecies variation, which may be expected due to their different eyeballs' different structures and volumes. In a rabbit model, the vitreous half-life ($t_{1/2}$) of bevacizumab, ranibizumab, and aflibercept was 6.99, 2.51, and 3.65 days.[74-76] According to Fogli's analysis, the vitreous half-life ($t_{1/2}$) in humans for bevacizumab and ranibizumab was 6.7 days and 7.2-9 days, respectively.[67] There is no data up to date on vitreous half-life for aflibercept. Some authors hypotheses that half-life time for aflibercept is nine days.[73]

It has been found that the blood-retinal barrier contains a neonatal FC receptor (FcRn) which has a significant role in the penetration of Fc-containing anti-VEGF drugs in the systemic circulation.[77,78] Therefore, the pharmacological studies found that Fc-containing anti-VEGF drugs, bevacizumab and aflibercept, are excreted in the systemic bloodstream, affecting the drug's recirculation and potential systemic side effects. The calculated plasma half-life for bevacizumab and aflibercept is 19 and 5-6 days, respectively.[66] As ranibizumab is not an Fc-containing drug, its concentration in plasma is very low, and it is estimated on 0.083 days only.[67]

## 7. THE SUMMARY OF LANDMARK CLINICAL TRIALS DATA

It has been proven that vascular endothelial growth factor (VEGF) has a pivotal role in diabetic macular oedema's pathogenesis.[16-18,79] The landmark clinical trials have demonstrated the functional and anatomical effectiveness of intravitreal anti-VEGF drugs, such as bevacizumab, ranibizumab, and aflibercept, which are nowadays modern approach to DMO treatment.[80-111] The intravitreal anti-VEGF injection is the primary treatment for center involving DMO and has some advantages over its two rivals, laser, and local steroid injection.[80,87,89] Laser is associated with poorer acuity outcomes due in part to damage to the retinal pigment epithelium but mostly due to its relatively low effect on reducing central macular oedema. The intravitreal steroids are effective at reducing central oedema.[112-122] However, it carries two potential side effects not generally associated with anti-VEGF treatment, namely the development of raised intraocular pressure and cataract formation.[112,115] Effort has focussed on comparing various anti-VEGF agents to determine which is most effective at treating centre – involving DMO. [90,82-92,104] Bevacizumab is a recombinant humanized monoclonal antibody 149kDa in size, which binds to intravitreal VEGF, while ranibizumab is a 48kDa fragment of this antibody, which includes only the binding portion of the antibody.[58,60,61] Aflibercept is a 115kDa recombinant fusion protein that binds VEGF.[63-65,95] A distinctive effect of aflibercept is its ability to bind Placental Growth Factor molecule and Vascular Endothelial Growth Factor.[63-65,123,125] It has been shown that the PlGF molecule is significantly increased in diabetic patients where this molecule participates in the condition's pathomechanism.[15-18,124] In addition, several studies investigated the binding affinity of different isoforms of VEGF among anti-VEGF competitors. Some of them indicate that aflibercept may have a 10-fold more significant affinity to bind VEGF in the eye than another licensed drug, ranibizumab.[126] On the other hand, some studies showed a similar affinity to bind all the aforementioned molecules' isoforms.[127] The different methodology of forenamed studies may explain the difference in results.[67]

The Diabetic Retinopathy Clinical Research (DRCR.net) network found ranibizumab with or without laser was significantly better than laser alone for visual acuity and anatomical outcomes. [80,87,89] Around 30% of eyes in ranibizumab plus deferred laser arm had improvement of ≥ 15 ETDRS (Early Treatment Diabetic Retinopathy Study) letters in year 1.[80] In addition,

nearly 50% of the same arm's eyes improved ≥ 10 ETDRS letters. The RISE and RIDE studies showed ranibizumab is significantly more effective than sham for center-involving DMO.[81,84,85] In the RISE and RIDE studies, patients received monthly injections of ranibizumab. The VIVID and VISTA studies compared the safety and efficacy of intravitreal aflibercept to macular laser, finding a mean 10.7-12.5 letter gain in the aflibercept groups compared to 0.2 letters in the laser group at year 1.[105] Both arms maintained similar and stable vision over year 2 and 3.[107,108] The efficacy of aflibercept was further highlighted in DRCR Protocol T, in which aflibercept showed an advantage over ranibizumab at year 1.[91] However, there was no significant difference between ranibizumab and aflibercept at year 2.[94] It demonstrated a 13.3 letter gain with aflibercept therapy, with the mean visual acuity (VA) at baseline being 64.8 ETDRS letters. The median number of intravitreal aflibercept injections in the aflibercept arm in Protocol T was 10 over year 1 and 5 over the second year of follow-up.[94] Overall, the major trials examining the anti-VEGF effect in DMO (VIVID, VISTA, RESOLVE, RESTORE, RISE, RIDE, RETAIN, Da VINCI) had a baseline VA that ranged from 56.9 letters to 64.8 ETDRS letters with VA gain ranging from 6.8 to 13.1 letters over the first year of the study.[81,83,84,85,86,101,105,107,108,128]

## 8. THE RATIONALE FOR CONDUCTING REAL-LIFE STUDIES

All preceding landmark clinical trials have been strictly driven by timely-given protocols and pre-defined inclusion and exclusion criteria. In VIVID and VISTA studies, the q4 arm was treated every 4 weeks with intravitreal injections of aflibercept over 2 years whilst patients from other arms needed at least to attend the trial units every month.[108] Otherwise, if patients did not participate in the trial visit within the accepted window, they were withdrawn from the studies. Furthermore, clinical trials strictly indicate inclusion and exclusion criteria, wherein RISE and RIDE trials those patients with HbA1c above 12% were excluded during the screening process.[81] Away from clinical trials, due to various reasons (i.e., diabetes patients' multi-system and multi-clinic appointments, working-age population, capacity issues, patients' missing appointments, patients' willingness to have treatment on the day of visit), the strict trial regimen is not possible to be replicated in the real world. Hence, we are aware that we do not follow the protocols suggested by clinical trials and undertreat our patients due to forenamed reasons. The published data on real-life results in treating patients with intravitreal ranibizumab injections showed worse visual outcomes than comparative randomized trials.[129-139] Regardless of this disappointing, although expected outcomes (as patients in real-life settings are treated with fewer injections), we wanted to investigate whether the practical and pragmatic protocols of treatment of DMO with intravitreal aflibercept injections may reach comparable and non-inferior results as compared to landmark clinical trials.

In addition to suggested data that protocols implemented in everyday clinical practices lead to worse clinical outcomes, it has been observed that some patients respond incompletely even to multiple injections of a given intravitreal anti-VEGF agent. Given the aflibercept molecule's differing properties with its increased binding affinity to the VEGF, retina specialists have attempted to switch anti-VEGF therapy instead. Several studies demonstrate improved visual acuity and anatomical outcomes in patients who have been switched from ranibizumab or bevacizumab to aflibercept.[140-145] Controversy has surrounded the exact reasons for the observed improvement as there is no head to head data available that would compare patients switched from ranibizumab or bevacizumab to aflibercept with those who initially started with aflibercept and then switched to another anti-VEGF agent for diabetic macular oedema treatment. Indeed, those studies have confirmed both anatomical and visual acuity improvement in patients who were initially treated with bevacizumab or ranibizumab and who

were eventually switched to aflibercept, with the pharmacological preferences of aflibercept in its increased binding affinity for VEGF, longer duration of action, and ability to bind placental growth factor all being cited as reasons for a switch of therapy.[140-145] Repeated intravitreal injections are associated with a financial and temporal commitment for patients, doctors, and healthcare systems, and they are not administered without risk. It is therefore essential that clinicians explore the most efficacious options available. The aim in real-life settings is to achieve the best possible results within the shortest time possible due to variant reasons: patients' need to have good vision to be able to conduct their daily duties, hospital capacities, decrease the risk of complications (fewer injections bring less chance of endophthalmitis, theoretically), reduce overall costs of treatment. Therefore, we wanted to define the protocols when it would be reasonable to switch patients from ranibizumab to aflibercept and investigate whether those patients may achieve some additional benefits.

## 9. AIM OF COMPILED RESEARCH PAPERS

Randomized controlled clinical trials are driven by time-given protocols defined by strict exclusion and inclusion criteria. As a result, clinical trials provide the best possible outcomes in terms of efficacy and safety of investigational medical products. Therefore, they represent the perfect results we endeavor to achieve in our everyday clinical practices. Nonetheless, the implementation of protocols suggested by clinical trials has been shown as non-pragmatic into standard treatment care. Following such protocols cause an overburden on clinics and increase the costs of treatment significantly. This means that our routine treatment care does not provide the benefits of therapy as clinical trials suggest. Therefore, we wanted to investigate whether our proposed treatment protocols with diabetic macular oedema, designed for standard treatment of care, may achieve non-inferior results compared to clinical trials where the non-inferiority was defined by a difference the vision of up to 10 ETDRS letters.

Furthermore, we wanted to investigate the mean number of injections needed to achieve non-inferior results, the effect of treatment based on baseline visual acuity, the impact of treatment in phakic and pseudophakic patients, and the effect of previous macular laser in treatment-naïve patients. Considering that there are two licensed anti-VEGF agents approved for the treatment of DMO in the United Kingdom, we investigated those who had started treatment with intravitreal aflibercept injections, which follows Moorfields DMO pathway. The rationale for choosing intravitreal aflibercept agent as our primary treatment of interest is its favorable pharmacokinetic data compared to licensed ranibizumab and off-licensed drug bevacizumab. In addition, as there is a group of patients who start treatment with another anti-VEGF agent (intravitreal ranibizumab injections) and among those is a subgroup of patients who do not respond on initial treatment, we wanted to investigate whether switching of agents (ranibizumab switched to aflibercept) may lead to improvements in visual acuity and anatomical outcomes. The rationale for that was a different mode of action between the two agents mentioned above. Eventually, we made a comparison of short-term results and analyzed the rationale to switch those who didn't respond to intravitreal ranibizumab and investigate the relevance of switching time between the agents.

# 10. COPIES OF RESEARCH PAPERS (ARTICLES) COMPILED IN THE DISSERTATION

## 10.1 The list of pooled articles proposed as a doctoral dissertation.

1. Lukic M, Williams G, Shalchi Z, Sim D, Patel PJ, Keane PA, Hykin PG, Sivaprasad S, Menon D, Bruynseels A, Hamilton RD., Rajendram R. Intravitreal aflibercept for diabetic macular oedema: Moorfields' real-world 12-month visual acuity and anatomical outcomes. European Journal of Ophthalmology. 2020 May;30(3):557-62.

2. Lukic M, Williams G, Shalchi Z, Patel PJ, Hykin PG, Hamilton RD, Rajendram R. Intravitreal aflibercept for diabetic macular oedema in real-world: 36-month visual acuity and anatomical outcomes. European Journal of Ophthalmology. 2019 Jul 1:1120672120925034.

3. Lukic M, Williams G, Shalchi Z, Patel PJ, Hykin PG, Hamilton RD, Rajendram R. One-year real-life results on effect of intravitreal aflibercept in patients with diabetic macular oedema switched from ranibizumab. European Journal of Ophthalmology. 2020 May 26:1120672120927275.

## 10.2 Intravitreal Aflibercept for Diabetic Macular Oedema; Moorfields' Real-World 12 Month Visual Acuity and Anatomical Outcomes

### *10.2.1. Introduction*

Diabetic macular oedema (DMO) is a leading cause of visual loss in the working age population. (1) It has been recognized that vascular endothelial growth factor (VEGF) is pivotal to the pathogenesis of DMO. (2,3) The modern approach to DMO treatment therefore relies on the proven safety and efficacy of intravitreal anti-VEGF drugs such as bevacizumab, ranibizumab and aflibercept, all of which have demonstrated functional and anatomical efficacy in clinical trials. (2-7)

Several large randomised controlled studies have established the efficacy of ranibizumab in diabetic macular oedema. The Diabetic Retinopathy Clinical Research (DRCR) network found ranibizumab with or without laser was significantly better than laser alone for visual acuity and anatomical outcomes. (8) Around 30% of eyes in ranibizumab plus deferred laser arm had improvement of ≥ 15 ETDRS (Early Treatment Diabetic Retinopathy Study) letters in year 1. In addition, nearly 50% of eyes of the same arm had improvement of ≥ 10 ETDRS letters. The RISE and RIDE studies showed ranibizumab is significantly more effective than sham for centre-involving DMO. (9) In the RISE and RIDE studies patients received monthly injections of ranibizumab. Our group recently published real-world outcomes of ranibizumab in DMO at our institution, showing comparable outcomes to these clinical trials. (10)

The VIVID and VISTA studies compared the safety and efficacy of intravitreal aflibercept to macular laser, finding a mean 10.7-12.5 letter gain in the aflibercept group compared to 0.2 letters in the laser group at 1 year. (11) The efficacy of aflibercept was further highlighted in DRCR Protocol T, in which aflibercept showed an advantage over ranibizumab at year 1, although there was no significant difference between ranibizumab and aflibercept at year 2. (12) However, clinical trials select the most motivated of patients and have personnel to ensure efficient attendance and timely trial completion. Trials also have strict exclusion criteria such as very poor glycaemic control. We wanted to investigate the efficacy of intravitreal aflibercept for the treatment of centre-involving DMO in a real-world setting where "all-comers" are seen.

### *10.2.2 Methods*

This retrospective cohort study included 102 eyes (of 92 diabetic patients) with centre-involving diabetic macular oedema (≥ 400 microns as per National Institute of Care and Excellence (NICE) criteria). This study entered only treatment naïve eyes which were funded for intravitreal aflibercept treatment for DMO between November 2015 and May 2016. Patients older than 18 years of age with either diabetes mellitus (DM) type 1 or type 2 were included. All grades of diabetic retinopathy (DR) were included. DMO and DR were graded by using the modified Early Treatment Diabetic Retinopathy Study (ETDRS) classification system based on clinical appearance. HBA1c was recorded but did not influence treatment decisions at onset of treatment.

Patients with hypertension, other comorbidities and patients with vitreoretinal conditions such as epiretinal membrane were not excluded. Each patient prior initiation of treatment had FFA and/or OCTA imaging done which is part of the Moorfields' guidelines. We definied severe macular ischaemia using ETDRS criteria by using FAZ size, FAZ outline and capillary loss in central subfield. Patients who had FAZ greater than 1500 microns GLD in size, capillary outline completely destroyed and who had severe capillary loss were considered as severe macular ischaemia. None of the patient had severe macular ischaemia or were excluded.

The study was approved prospectively by the Clinical Audit and Assessment Committee of Moorfields Eye Hospital and registered with the trust clinical audit department (reference no: CA17/MR/06). Patients who had consented to imaging and anonymised data collection and analysis of outcomes as part of their clinical care were included and the study followed the tenets of the Declaration of Helsinki. All patients were under the care of Moorfields Eye Hospital (MEH) National Health Service (NHS) Trust, London, United Kingdom.

All eyes included in the study were treatment naïve at baseline and were treated with intravitreal aflibercept injections. Patients were initiated on a loading phase of five one-monthly intravitreal aflibercept injections, followed by injections if needed as per clinicians' discretion. Clinical decision on further injections following the loading phase was on the basis of treating towards Visual Acuity and OCT scan stability i.e. if there was potential for further VA and/or OCT improvement (e.g. persistent fluid) after the loading phase, further injections were given. Visual acuity (VA) measurements expressed in Early Treatment Diabetic

Retinopathy Study (ETDRS) letters and Optical Coherence Tomography (OCT) (Topcon, Tokyo, Japan) scans were performed at each visit.

Primary outcomes were visual acuity (VA), central foveal thickness (CFT) and macular volume (MV) 12 months after commencing treatment. Secondary outcomes were percentage of eyes that achieved visual acuity gain of ≥ 10 and ≥ 15 ETDRS letters as well as percentage of eyes achieved reduction in CFT of 100 microns or more. Additionally, we carried out subgroup analysis according to the baseline VA (worse than 69 ETDRS letters or ≥ 69 ETDRS letters) and mean change in VA, CFT and MV at month 12.

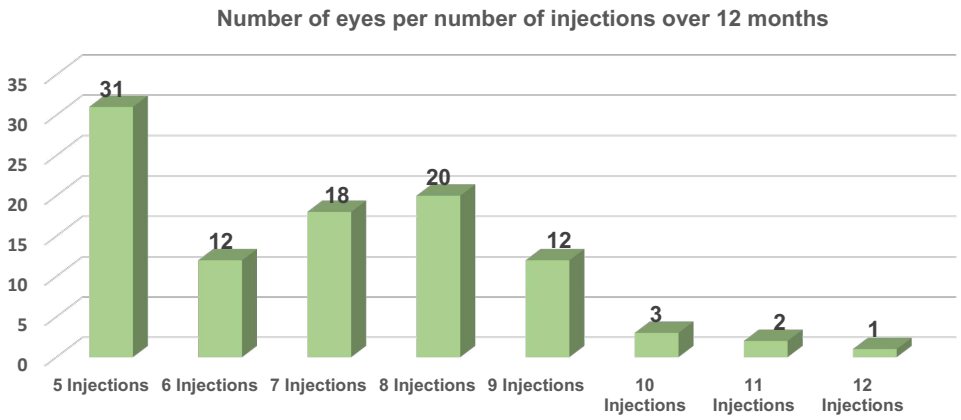
Key exclusion criteria included a history of an acute coronary event or cerebrovascular accident in the previous 3 months, pregnancy or lactation, active infection or intraocular inflammation in either eye, poor view of the fundus, severe macular ischaemia, other pathologies contributing towards macular oedema, anti-VEGF treatment received for any other condition and other macular diseases present at baseline that might confound the outcomes such as a coexistent retinal vein occlusion.

We assessed the primary and secondary outcomes at 12 months. The t-paired sample test was used to determine statistical significance (https://www.graphpad.com/quickcalcs/ttest1.cfm) . A P value of <0.05 was interpreted as statistically significant.

### *10.2.3 Results*

Ninety-nine aflibercept treated eyes (89 patients) entered the statistical analysis. Three eyes out of 102 were excluded as they were switched to other treatment over 12 - month follow up period. The mean number of aflibercept injections received was 6.92 **(Figure 1).** Fourteen percent of included eyes had less than 5 monthly loading doses (minimum 3) due to either clinicians' discretion or patients did not attend or cancelled their appointments. Thirty percent of included eyes did not have further injections after the loading phase. Two patients (two eyes from the cohort) did not complete the follow up of 12 months. 33% of patients were pseudophakic and 67% were phakic at baseline.

**Figure 1. Number of eyes per number of injections of the cohort in 12 months follow-up**




*A) Aflibercept cohort outcomes*

At baseline, the mean VA (SD) (Snellen) was 59.7 (16.1) (20/63) ETDRS letters, the mean CFT (SD) was 431 (129) µm whilst the mean MV (SD) was 9.53 (1.79) $mm^3$. **(Table 1)** At 12 months, the mean VA (SD) (Snellen) was 69.6 (15.2) (20/40) ETDRS letters ($p < .0001$). The mean CFT (SD) was 306 (122) µm ($p < .0001$) and the mean MV (SD) was 8.43 (1.58)

***Table 1 Aflibercept cohort: General data; CFT = Central Foveal Thickness, MV = Macular Volume, SD = Standard Deviation, VA = Visual Acuity***

| | **AFLIBERCEPT COHORT** |
|---|---|
| **TREATED EYES** | 99 |
| **MEAN VA BASELINE (SD) (Snellen)** ***ETDRS letters*** | 59.66 (16.11) (20/63) |
| **MEAN VA 5 MONTHS (SD) (Snellen)** ***ETDRS letters*** | 66.5 (13.65) (20/40) |
| **MEAN VA 12 MONTHS (SD) (Snellen)** ***ETDRS letters [p value]*** | 69.56 (15.24) (20/40) [p < .0001] |
| **MEAN VA CHANGE** ***ETDRS letters*** | + 9.9 |
| **MEAN CFT BASELINE (SD)** ***microns*** | 431 (129) |
| **MEAN CFT 5 MONTHS (SD)** ***microns*** | 298 (101) |
| **MEAN CFT 12 MONTHS (SD)** ***microns [ p value]*** | 306 (122) [p < .0001] |
| **MEAN CFT CHANGE** ***microns*** | -128 |
| **MEAN MV BASELINE (SD)** ***mm3*** | 9.53 (1.79) |
| **MEAN MV 5 MONTHS (SD)** ***mm3*** | 8.5 (2.03) |
| **MEAN MV 12 MONTHS (SD)** ***mm3*** **[p value]** | 8.43 (1.58) [p < .0001] |
| **MEAN MV CHANGE** ***mm3*** | -1.08 |
| **MEAN NUMBER OF INJECTIONS** | 6.92 |

$mm^3$ ($p < .0001$) at 12 months. Thirty-three (33.67 %) eyes gained ≥ 15 ETDRS letters at month 12, and 50 (55.55%) eyes had a decrease in CFT of ≥ 100 microns **(Table 2).** Three (3.06 %) eyes lost ≥ 15 ETDRS letters and 6 (6.66 %) eyes had an increase in CFT of ≥ 100 microns at the end of follow up period. Forty-seven (46.53%) eyes achieved 10 ETDRS letters or more gain at month 12, whilst 5 (4.95%) eyes lost 10 ETDRS letters or more at the end of follow up.

***Table 2 Changes in VA and CFT; CFT = Central Foveal Thickness, MV = Macular Volume, SD = Standard Deviation, VA = Visual Acuity***

| **VA** | **COHORT (eyes)** | % |
|---|---|---|
| **≥ 15 letters gain** | 33 | 33.67 % |
| **< 15 letters gain** | 47 | 47.95 % |
| **≥ 15 letters loss** | 3 | 3.06 % |
| **< 15 letters loss** | 15 | 15.30 % |
| **≥ 10 letters gain** | 46 | 46.66 % |
| **≥ 10 letters loss** | 5 | 5.05 % |
| **CFT** | | |
| **≥ - 100 microns** | 50 | 50.50 % |
| **< - 100 microns** | 21 | 21.21 % |
| **≥ 100 microns** | 6 | 6.06 % |
| **< 100 microns** | 13 | 13.13 % |

*B) Mean changes and sub-group analysis according to baseline VA and CFT*

We calculated the changes in VA, CFT and MV after 12 months. The mean change in VA was + 9.9 ETDRS. The mean change in MV was -1.08 $mm^3$ whilst the mean change in the CFT was -128 μm.

We sub-divided the included eyes into two subgroups according to the baseline visual acuity; < 69 ETDRS letters (< 20/50 Snellen) and ≥ 69 ETDRS letters (≥ 20/40 Snellen) and according to the baseline CFT; 400- 499 microns or ≥ 500 microns. Sixty-six percent of eyes had baseline visual acuity less than 69 ETDRS letters (< 20/50 Snellen). The mean change in visual acuity in the subgroup with baseline VA less than 69 letters (< 20/50 Snellen) was +13.8 ETDRS letters **(Figure 2).** Thirty-four percent of eyes had baseline visual acuity ≥ 69 ETDRS letters (≥ 20/40 Snellen) and the mean change in the visual acuity after 12 months in that subgroup was + 2.6 ETDRS letters. The subgroup of eyes with initially worse visual acuity (< 20/50 Snellen) had mean 7.4 intravitreal injections of aflibercept over 12 months whilst the subgroup with initial visual acuity of ≥ 20/40 Snellen had mean 6.6 injections over same follow up period (p = .07) Twenty-seven percent of included eyes had baseline CFT of 500 microns or more. The mean change in CFT in that subgroup was -265 microns. In the subgroup where the baseline CFT was between 500 and 400 microns the mean change in CFT was -86 microns.

**Figure 2. Visual acuity change over 12 months in the main cohort and two subgroups (< 69 ETDRS letters at baseline and > 69 ETDRS letters at baseline)**

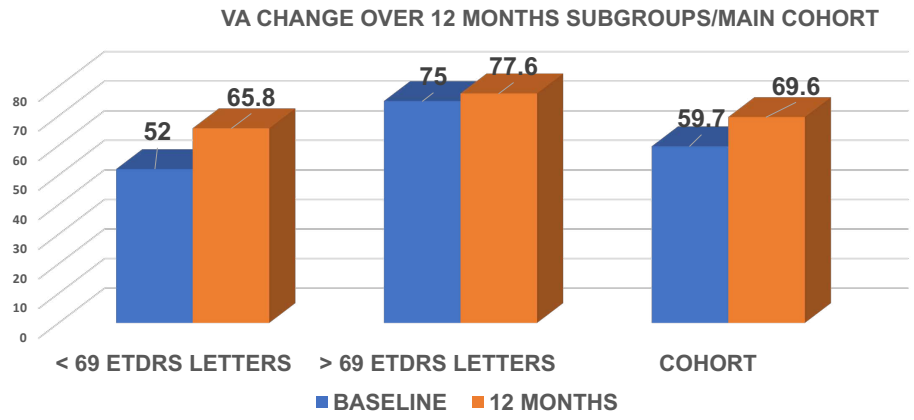



***Table 3 Macular oedema appearance at baseline; DMO = Diabetic Macular Oedema, CSMO = Clinically Significant Macular Oedema***

| **TYPE OF MACULAR OEDEMA** | **PERCENTAGE (%)** |
|---|---|
| FOCAL, CENTRE-INVOLVING DMO | 29 |
| DIFFUSE CSMO, INVOLVING FOVEA | 64 |
| FOCAL CSMO, INVOLVING FOVEA | 7 |
| PRESENCE OF SUBRETINAL FLUID | 18 |
| PRESENCE OF EPIRETINAL MEMBRANE | 13 |
| VITREO-MACULAR TRACTION | 2 |

### *10.2.4. Discussion*

Clinical trials generally produce results above what would be expected to occur in a normal patient population, with real world evidence rarely indicating equivalence. There are myriad reasons for this, including tight inclusion and exclusion criteria, a well-motivated patient population, more injections given and a mandated tight appointment schedule. The DRCR.net Protocol T study demonstrated a 13.3 letter gain with aflibercept therapy, with the mean VA at baseline being 64.8 ETDRS letters. (12) The major trials examining anti-VEGF effect in DMO (VIVID, VISTA, RESOLVE, RESTORE, RISE, RIDE, RETAIN, Da VINCI) had a baseline VA that ranged from 56.9 letters to 64.8 ETDRS letters with VA gain ranging from 6.8 to 13.1 letters over the first year of the study.(13-16) An inverse correlation was noted whereby patients with the higher baseline VA demonstrated the lower improvement in acuity.

Real-world results have not displayed the same amount of improvement in visual acuity with anti VEGF treatment in DMO, with the frequency of injections being the factor that tends to be cited in order to explain this finding. (17) There are no large-scale real-world data looking at aflibercept therapy for DMO. However, it was previously hypothesised based on diminished number of injections in a real world setting that the results would be inferior to the major trials. Our study is look at real world evidence of aflibercept use and with an average of 6.92 injections, significantly less than the 9-10 observed in DRCR.net protocol T, with around 10 ETDRS letters of improvement noted. In those eyes with VA of less than 69 letters, the improvement in acuity was markedly greater than in those with higher baseline visual acuity scores, thus confirming the ceiling effect seen when treating patients with good initial baseline acuity. The ceiling effect was noted when divided our cohort based on degree of foveal thickening. Our results indicate that despite a significantly lower number of injections over a 12-month period than those observed in the landmark trials, good visual and anatomical outcomes are attainable.

The number of injections was less than those used in the large clinical studies. We believe this is a significant collection of real-world outcomes that show very good results with aflibercept therapy for diabetic macular oedema in a real-world setting.

Regardless to the limitations of this study, which are number of patients included, and lack of more detailed analysis of macular perfusion, we believe that the reporting of real-world

outcomes is of benefit to clinicians who are treating patients in the real world, rather than a clinic trial setting and thus do not see this as a limitation.

Real world evidence is important in making decisions about how to treat patients with DMO in an efficient and cost-effective manner. Modern healthcare systems may not be able to provide injections at the same frequency for sustained periods of time as was observed in the major studies. This is the largest published dataset examining aflibercept therapy provided in a real world setting and our observed improvement could potentially be explained in theory by the pharmacokinetic advantages of aflibercept in its increased binding affinity for VEGF, its longer duration of action and ability to bind placental growth factor. Whatever the reason we have demonstrated that it is possible to deliver very good visual acuity and anatomical outcomes in a real-world setting using less injections than those used in the published literature. Diabetic maculopathy is a major cause of sight impairment amongst working age people and the prevalence of type 2 diabetes is rapidly increasing in both the developed and less well-developed world economies. We demonstrate that good outcomes can be achieved in the real world away from clinical trials and this should support doctors and patients together in managing diabetic macular oedema.

### *10.2.5. References*

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## 10.3 Intravitreal Aflibercept for Diabetic Macular Oedema in Real-world; 36- Month Visual Acuity and Anatomical Outcomes

### *10.3.1 Introduction*

The pandemic of diabetes mellitus represents a huge global health challenge. Complications of diabetes mellitus affect significantly national economies worldwide. One such complication is diabetic macular oedema (DMO) which is the leading cause of visual loss in the working age population.[1] According to *The UK National Ophthalmology Database study* clinically significant macular oedema was present in 15.8–18.1% of eyes, and in 8.7–10.0% of eyes this involved the central macula.[2] There are now over three million people in England with a diagnosis of diabetes mellitus and according to the Centres for Disease Control and Prevention (CDC) there are more than 100 million adults diagnosed with diabetes or prediabetes in the USA.[3,4] Over last decades, the number of patients diagnosed with diabetes mellitus in each year increased significantly and there are around 100 000 and 1.4 millions of new diagnosed patients with diabetes mellitus per year in the UK and in the USA, respectively.[3,4] The current cost of direct patient care for those living with diabetes is estimated at 9.8 billion pounds per year in the UK and around 327 billion US dollars in the USA.[5,6] The total number of people diagnosed with diabetes worldwide is projected to rise to 366 million in 2030.[7]

The vascular endothelial growth factor (VEGF) has essential role in the pathogenesis of diabetic macular oedema.[8,9] The landmark clinical trials have demonstrated the functional and anatomical effectiveness of intravitreal anti-VEGF drugs, such as bevacizumab, ranibizumab and aflibercept, which are nowadays modern approach to DMO treatment.[8-14]

Nevertheless, clinical trials are based on strictly defined protocols which precisely define exclusion criteria such as those with very poor glycaemic control or active proliferative diabetic retinopathy. In addition, the protocol strictly determines the time frame for follow ups. Away from clinical trials, due to various reasons (i.e. diabetes patients' multi-system and multi-clinic appointments, working age population, capacity issues, patients' missing appointments, patients' willingness to have treatment on the day of visit) the strict trial regimen is not possible to be replicated in real world. Nevertheless, we must be able to find a compromise which

delivers this effective therapy in a practical and pragmatic way for patients. We desired to look into the efficacy of intravitreal aflibercept for the treatment of centre-involving DMO in a real-world setting over three years and to see the proportion of injections needed between year 1 and 2 as well as between year 2 and 3 to maintain stable vision gained over year 1.

### *10.3.2 Methods*

This is a retrospective cohort study which included only treatment naïve eyes funded for intravitreal aflibercept treatment for DMO between November 2015 and November 2016. Overall, the study includes 64 eyes (of 57 diabetic patients) which met National Institute of Care and Excellence (NICE) criteria for treatment (centre-involving diabetic macular oedema ≥ 400 microns) and with baseline VA equal or less than 80 ETDRS letters. All patients included were older than 18 years of age and have been diagnosed with either diabetes mellitus (DM) type 1 or type 2. Both DMO and DR were graded by using the modified Early Treatment Diabetic Retinopathy Study (ETDRS) classification system based on clinical appearance. We recorded the HbA1c at baseline and discussed it with patients, however, this did not have effect on making treatment decisions.

Patients who had other systemic comorobidities, i.e. hypertension, or who had vitreoretinal conditions like epiretinal membrane were considered eligible for this study and we did not exclude them. Prior the treatment, each of patient included had FFA imaging done as part of the Moorfields' guidelines. To define severe macular ischaemia we were using the ETDRS criteria by using FAZ size, FAZ outline and capillary loss in central subfield. Reference for > 1500 microns being considered severe ischaemia, or where the capillary outline completely disrupted and who had severe capillary loss were considered as severe macular ischaemia. None of the patient had severe macular ischaemia nor were any patients excluded. One of the aims of doing FFA was to be able to enable discussion with patients about possible outcomes prior to initiation of therapy.

The Clinical Audit and Assessment Committee of Moorfields Eye Hospital approved the study and the study has been registered with the trust clinical audit department (reference no: CA17/MR/06). Patients who had consented to imaging and anonymised data collection and analysis of outcomes as part of their clinical care were included and the study followed the

tenets of the Declaration of Helsinki. All patients were under the care of Moorfields Eye Hospital (MEH) National Health Service (NHS) Trust, London, United Kingdom.

Patients who had prior macular laser were included and none of the included patients had macular laser during the follow up period. The treatment was initiated on a loading phase of five one-monthly intravitreal aflibercept injections, followed by injections if needed as per clinicians' discretion. The Moorfields' guidelines state to extend the intravitreal injections after the loading phase (up to 8 weeks). Clinical decision on further injections following the loading phase within year 1 or decision on re-starting treatment in year 2 and 3 was on the basis of treating towards visual acuity and OCT scan stability. In general, if there was potential for further VA and/or OCT improvement (e.g. persistent fluid or presence of new fluid) further injections towards this aim were offered. At each visit, we measured visual acuity (VA) in Early Treatment Diabetic Retinopathy Study (ETDRS) letters scale and each patient had retina imaging done by using the Optical Coherence Tomography (OCT) (Topcon, Tokyo, Japan).

We carried out the primary outcomes which were visual acuity (VA), central foveal thickness (CFT) and macular volume (MV) 12, 24 and 36 months after commencing treatment. Then, we calculated the percentage of eyes achieved visual acuity gain of ≥ 10 and ≥ 15 ETDRS letters as well as percentage of eyes achieved improvement in CFT for 100 microns or more at the end of follow up. As tertiary outcomes, we made a subgroup analysis according to the baseline VA (worse than 69 ETDRS letters or ≥ 69 ETDRS letters). Lastly, we did subgroup analyses of VA and CFT according to lens status and history on pre-treatment macular laser.

For the statistical analysis we used the t-paired sample test to ascertain the statistical significance of our results when we compared two samples (https://www.graphpad.com/quickcalcs/ttest1.cfm) . When we compared three samples we used the ANOVA analysis (https://goodcalculators.com/one-way-anova-calculator/) .A P value of < .05 was interpreted as statistically significant.

Key exclusion criteria included a history of an acute coronary event or cerebrovascular accident in the previous 3 months, pregnancy or lactation, active infection or intraocular inflammation in either eye, poor view of the fundus, severe macular ischaemia, other pathologies contributing towards macular oedema, anti-VEGF treatment received for any other

condition and other macular diseases present at baseline that might confound the outcomes such as a coexistent retinal vein occlusion.

### *10.3.3 Results*

Thirty one of included patients were males and 26 were females. The mean age of patients included in cohort was 53.2 [24-71]. The mean number of aflibercept injections received was 12.59 at month 36 **(Table 1).** Eleven percent of included eyes had less than 5 monthly loading doses due to either clinicians' discretion or patients' choice (they did not attend or they cancelled their appointments). Thirteen percent of included eyes did not require further injections at all after the loading phase of 5 injections. Around 30% of patients did not require injections in year 2 and nearly 40% of patients from our cohort did not require any injection in year 3.

Seven percent (9 eyes) of eyes included had active proliferative disease at baseline and had PRP treatment before baseline and between intravitreal injections. Twenty-eight percent of eyes included were pseudophakic at baseline. Nineteen percent of phakic eyes had cataract surgery during 36-month follow-up.

*A) Primary and secondary outcomes*

At baseline, the mean VA (SD) (Snellen) was 61.45 (16.30) (20/63) ETDRS letters, the mean CFT (SD) was 422 (138) μm whilst the mean MV (SD) was 9.51 (2.01) $mm^3$. (Table 1) At 36 months, the mean VA (SD) (Snellen) was 68.34 (13.66) (20/50) ETDRS letters ($p = .0003$). The mean CFT (SD) was 303 (106) μm ($p < .0001$) and the mean MV (SD) was 8.35 (1.62) $mm^3$ ($p = 0.0022$) at 36 months. The mean change in VA was + 6.89 ETDRS from baseline. The mean change in MV was -1.16 $mm^3$ whilst the mean change in the CFT was -119 μm at the end of 36-month follow up.

Sixteen (25 %) eyes gained ≥ 15 ETDRS letters at month 36, and 33 (52%) eyes had a decrease in CFT of ≥ 100 microns at the same time. Twenty-three (36 %) eyes achieved 10 ETDRS letters or more gain at month 36, whilst 5 (8 %) eyes lost 10 ETDRS letters or more at the end of follow up. Three (5 %) eyes lost ≥ 15 ETDRS letters and 4 (6 %) eyes had an increase in CFT of ≥ 100 microns at the end of follow up period.

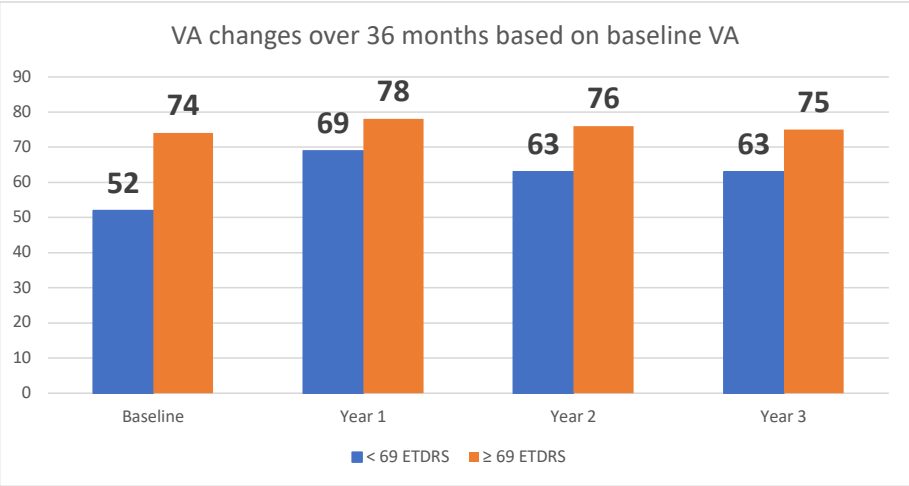
**Table 1. General data; VA = Visual acuity, CFT = Central Foveal Thickness, MV = Macular volume.**

| | **NAÏVE** |
|---|---|
| **TREATED EYES** | 64 |
| **VA MEAN baseline (SD) ETDRS letters** | 61.45 (16.30) |
| **VA MEAN 12 MONTHS (SD) ETDRS letters** | 72.66 (13.53) |
| **VA change from baseline** | 11.21 |
| **VA MEAN 24 MONTHS (SD) ETDRS letters** | 68.38 (13.72) |
| **VA CHANGE from baseline** | 6.24 |
| **VA CHANGE from year 1** | -4.28 |
| **VA MEAN 36 MONTHS (SD) ETDRS letters** | 68.34 (13.66) |
| **VA CHANGE from baseline** | 6.89 |
| **VA CHANGE from year 1** | -4.32 |
| **VA CHANGE from year 2** | -0.04 |
| **CFT MEAN baseline (SD) microns** | 421.81 (137.87) |
| **CFT MEAN 12 MONTHS (SD) microns** | 281.74 (100.04) |
| **CFT change from baseline** | -140.07 |
| **CFT MEAN 24 MONTHS (SD) microns** | 303.08 (93.43) |
| **CFT CHANGE microns from baseline** | -118.73 |
| **CFT CHANGE microns from year 1** | 21.34 |
| **CFT MEAN 36 MONTHS (SD) microns** | 302.63 (106.39) |
| **CFT CHANGE microns from baseline** | -119.18 |
| **CFT CHANGE microns from year 1** | 20.89 |
| **CFT CHANGE microns from year 2** | -0.45 |
| **MV baseline MONTHS (SD) mm3** | 9.51 (2.01) |
| **MV MEAN 12 MONTHS (SD) mm3** | 8.13 (1.23) |
| **MV change from baseline** | -1.38 |
| **MV MEAN 24 MONTHS (SD) mm3** | 8.27 (1.36) |
| **MV MEAN CHANGE mm3 from baseline** | -1.24 |
| **MV MEAN CHANGE mm3 from year 1** | 0.14 |
| **MV MEAN 36 MONTHS (SD) mm3** | 8.35 (1.62) |
| **MV MEAN CHANGE mm3 from baseline** | -1.16 |
| **MV MEAN CHANGE mm3 from year 1** | 0.22 |
| **MV MEAN CHANGE mm3 from year 2** | 0.08 |
| **MEAN NUMBER OF Eylea INJECTIONS at year 1 (range)** | 7.09 (5-10) |
| **MEAN NUMBER OF Eylea INJECTIONS at year 2 (range)** | 2.93 (0-7) |
| **MEAN NUMBER OF Eylea INJECTIONS at year 3 (range)** | 2.57 (0-9) |
| **MEAN NUMBER OF Eylea INJECTIONS over 3 years** | 12.59 |
| **Macular laser prior treatment** | 32.81% (21 eye) |

### *B) Sub-group analysis according to baseline VA and CFT*

We sub-divided the included eyes into two subgroups according to the baseline visual acuity; < 69 ETDRS letters (< 20/50 Snellen) and ≥ 69 ETDRS letters (≥ 20/40 Snellen) and according to the baseline CFT; < 500 microns or ≥ 500 microns. Fifty-eight percent of eyes had baseline visual acuity less than 69 ETDRS letters (< 20/50 Snellen). (Figure 1). The mean change in visual acuity in the subgroup with baseline VA less than 69 letters (< 20/50 Snellen) was +11.27 ETDRS letters ($p < 0.0001$). Forty-two percent of eyes had baseline visual acuity ≥ 69 ETDRS letters (≥ 20/40 Snellen) and the mean change in the visual acuity after 36 months in that subgroup was + 0.88 ETDRS letters ($p = 0.6041$). The subgroup of eyes with initially worse visual acuity (< 20/50 Snellen) had mean 11.89 intravitreal injections of aflibercept over 36 months whilst the subgroup with initial visual acuity of ≥ 20/40 Snellen had mean 13.59 injections over same follow up period ($p = 0.20$) Twenty-two percent of included eyes had baseline CFT of 500 microns or more. The mean change in CFT in that subgroup was -337 microns ($p < 0.0001$). In the subgroup where the baseline CFT was less than 500 microns the mean change in CFT was - 53 microns ($p = 0.0021$).

**Figure 1. Visual acuity changes over 36 months based on baseline visual acuity (< 69 ETDRS letters at baseline and > 69 ETDRS letters at baseline).**



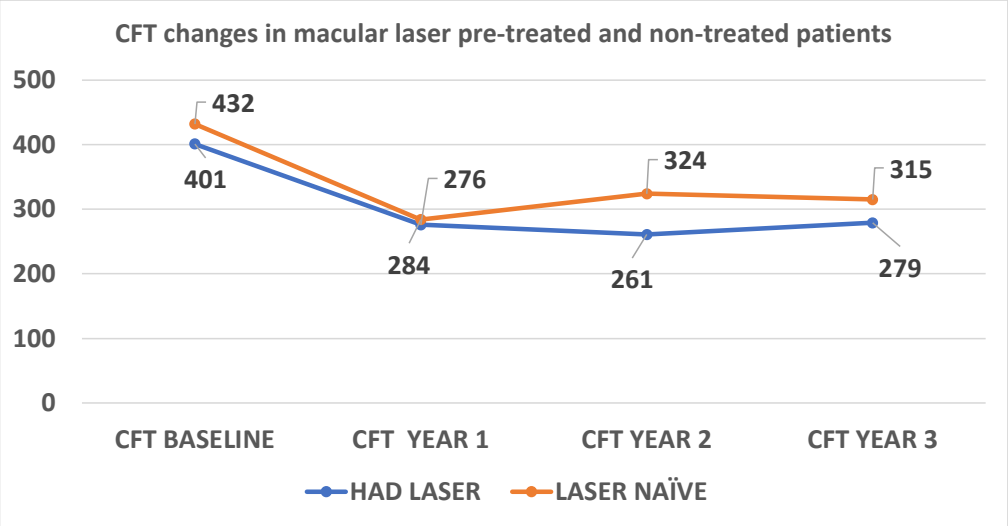
*C) Subgroup analyses according to lens status and history on pre-treatment with macular laser*

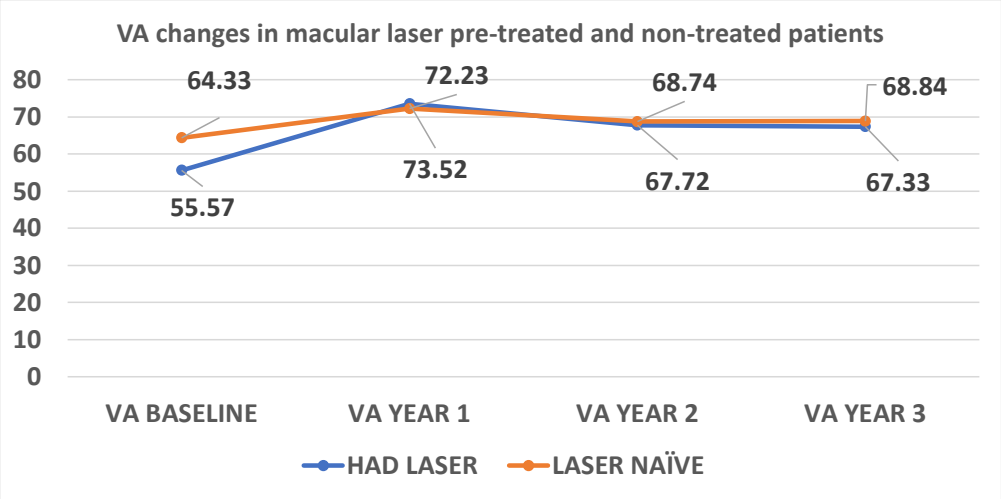
We calculated visual acuity changes in baseline pseudophakic patients (28% of eyes included). At baseline, the mean VA (SD) (Snellen) was 62.50 (15.19) (20/63) ETDRS letters whilst the mean VA (SD) (Snellen) was 64.78 (14.21) (20/40) ETDRS letters at month 36 (p = 0.24). At the end of year 3 there were no statistically significant changes in VA or CFT between subgroups according to their lens status (p value for VA among three sub-groups was 0.10779, p value for CFT among three sub-groups was 0.6196). The more detailed data on subgroup analysis according to baseline lens status has been shown in Table 2. Then, there were no statistically significant changes in either VA or CFT in those who had and those who had no macular laser prior treatment with intravitreal injections (p = 0.333) **(Figure 2).** The macular laser had been done in all patients 6 months or more prior initiation of treatment with intravitreal aflibercept. Visual acuity and central foveal thickness data according to lens status over follow up period.

**Table 2 Sub-group analysis according to lens status**

| | **Mean VA baseline** | **Mean VA year 1** | **Mean VA year 2** | **Mean VA year 3** |
|---|---|---|---|---|
| **Phakic** | 61 | 77 | 71 | 71 |
| **Pseudophakic at baseline** | 63 | 70 | 70 | 65 |
| **Those who had phaco** | 61 | 63 | 58 | 66 |
| | **Mean CFT baseline** | **Mean CFT year 1** | **Mean CFT year 2** | **Mean CFT year 3** |
| **Phakic** | 413 | 258 | 285 | 284 |
| **Pseudophakic at baseline** | 420 | 299 | 324 | 330 |
| **Those who had phaco** | 448 | 318 | 327 | 316 |

**Figure 2. Visual acuity and central foveal thickness changes in patients with and without macular laser prior the baseline over 36 months of follow up period.**



### 10.3.4. Discussion

This retrospective, real-life analysis shows results of visual acuity and anatomical outcomes in patients with sight-impairing, centre-involving diabetic macular oedema treated with intravitreal aflibercept over 3 years. The mean VA improved to 72.66 (+11.21) ETDRS letters over year 1 and maintained stable VA with mean VA of 68.38 and 68.34 ETDRS letters at year 2 and 3, respectively. Despite the loss of 4 ETDRS letters within second and third year of treatment, the cohort gained statistically significant improvement in visual acuity and anatomical outcomes over three years of treatment. In addition, we showed that the lens status (in case the lens is not significantly opacified vs pseudophakia) and history on previous macular laser do not make statistically significance in VA and CFT outcomes.

The VIVID and VISTA studies showed improvement in vision in both aflibercept arms (q4 and q8) for 10-11 ETDRS letters over year 1 and both arms maintained similar and stable vision over year 2 and 3.[12] The real -world results have not displayed the same amount of improvement in visual acuity with anti VEGF treatment in DME, with the frequency of injections being the factor that tends to be cited in order to explain this finding.[16,17] Our cohort had a mean of 12.59 injections in 3 years of follow-up which is significantly less than the VIVID and VISTA protocols suggest for both aflibercept arms.

Indeed, clinical trials generally produce results above what would be expected to occur in a normal patient population, with real world evidence rarely indicating equivalence. There are myriad reasons for this, including tight inclusion and exclusion criteria, a well-motivated patient population, more injections given and a mandated tight appointment schedule. For example, in the VIVID and VISTA studies visits were scheduled every 4 weeks.[12] However, this regimen is quite difficult to achieve in everyday clinical practice with a real-world patient population of working age who may have multiple appointments as diabetes may affect multiple systems.

According to our subgroup analysis on visual acuity outcomes as per baseline visual acuity, we showed there is a statistically significant improvement in vision in those eyes which had initially worse visual acuity and we maintained stable vision in the subgroup with initially better visual acuity. Recently published Protocol V showed that the eyes with good visual acuity could be observed only instead of starting the treatment with intravitreal injections which

is, indeed, reassuring for everyday clinical practices and may help in decrease of burden of injections clinics and initial costs of the treatment.[18] Nonetheless, our data showed that at the end of year 1, year 2 and year 3 the subgroup with initially better visual acuity had statistically significant better VA as compared to the one which had initially worse vision. Hence, start of the treatment when the baseline VA is good may maintain the vision stable and better over longer follow up period.

Regardless to the limitations of this study, which are number of patients included and lack of more detailed analysis of macular perfusion, we believe that the reporting of real-world outcomes is of benefit to clinicians who are treating patients in the real world, rather than a clinic trial setting and thus do not see this as a limitation as this therapeutic modality is shown to deliver visual improvements for patients in the real world.

There is no doubt that clinical trials are important. They give us information on efficacy and safety of investigated products as well as, very likely, the most ideal outcomes we may expect. Moreover, the clinical trials should lead us towards expectations on outcomes for patients in real life. Nonetheless, the real-world evidence gives more realistic picture on outcomes and allows us to discuss this therapy with patients who have DME in order to offer a realistic therapy schedule with realistic outcomes. Modern public healthcare systems may not be able to provide injections at the same frequency for sustained periods of time as was observed in the major studies. However, modern healthcare systems must have established protocols which lead to results in vision which allow people to work and live normal lives. Diabetic maculopathy is a major cause of sight impairment amongst working age people and the prevalence of type 2 diabetes is rapidly increasing in both the developed and less well-developed world economies. We demonstrate that good outcomes can be achieved in the real world away from clinical trials and this should support doctors and patients together in managing diabetic macular oedema. Patients should not be denied this valued therapy even if it is not possible to deliver 24 or 36 monthly injections, and we have demonstrated that there is a gain in vision with significantly less injections over 3 years.

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## 10.4. One Year Real-life Results on Effect of Intravitreal Aflibercept in Patients with Diabetic Macular Oedema Switched from Ranibizumab

### *10.4.1. Introduction*

Diabetic macular oedema (DMO) is one of the most important causes of vision loss amongst working age people.[1] It has been recognised that whilst VEGF is an important component of the inflammatory process that lies at the heart of the pathogenesis of DMO, there may also be a wider inflammatory cascade at play that is also responsive to intravitreal steroids.[2,3] The landmark clinical trials proved safety and effectiveness of intravitreal anti-VEGF drugs (aflibercept (Eylea®), ranibizumab (Lucentis®) and bevacizumab (Avastin®)) in which all of them the vision was significantly improved.[2-7] Intravitreal anti-VEGF injection is the primary treatment for centre involving DMO and has some advantages over its two rivals, laser and local steroid injection.[3] Laser is associated with poorer acuity outcomes due in part to damage to the retinal pigment epithelium but mostly due to its relatively poor effect on reducing central macular oedema. Intravitreal steroid is effective at reducing central oedema although carries with it two potential side effects not generally associated with anti-VEGF treatment; namely the development of raised intraocular pressure and cataract formation.[8]

Effort has focussed on comparing various anti-VEGF agents to determine which is most effective at treating centre involving DMO. [2-6] Bevacizumab is a recombinant humanised monoclonal antibody 149kDa in size which binds to intravitreal VEGF, whilst ranibizumab is a 48kDa fragment of this antibody which includes only the binding portion of the antibody. Aflibercept is a 115kDa recombinant fusion protein which binds VEGF.[9]

In everyday clinical practice, it has been observed that despite the outcomes of the large trials, a significant number of patients respond incompletely to multiple injections of a given intravitreal anti-VEGF agent. In view of the differing properties of the aflibercept molecule with its increased binding affinity to VEGF retina specialists have attempted to switch anti-VEGF therapy instead, with several studies demonstrating an improvement in both visual acuity and anatomical outcome in patients who have been switched from ranibizumab or bevacizumab to aflibercept.[10-15]

Controversy has surrounded the exact reasons for the observed improvement as there is no head to head data available which would compare patients switched from ranibizumab or bevacizumab to aflibercept with those who initially started with aflibercept and then switched to another anti-VEGF agent for diabetic macular oedema treatment. We here describe a retrospective analysis of real-world data using visual acuity and anatomical outcomes in patients who had been switched from ranibizumab therapy to aflibercept.

### 10.4.2. Methods

We investigated those patients with fovea-involving diabetic macular oedema who had been initially treated with intravitreal ranibizumab injections and were switched to intravitreal aflibercept injections between November 2015 and May 2016. This is a retrospective cohort study which analysis included 90 eyes of 67 patients older than 18 years of age and with either diabetes mellitus (DM) type 1 or type 2. All eyes included met National Institute of Care and Excellence (NICE) criteria for treatment of diabetic macular oedema (central foveal thickness ≥ 400 microns). The diabetic retinopathy grading was done by using the modified Early Treatment Diabetic Retinopathy Study (ETDRS) classification based on clinical appearance at initial visit.

This study is registered with the trust clinical audit department (reference no: CA17/MR/06) and was approved by the Clinical Audit and Assessment Committee of Moorfields Eye Hospital. Patients who had consented to imaging and anonymised data collection and analysis of outcomes as part of their clinical care were included and the study followed the tenets of the Declaration of Helsinki. All patients were under the care of Moorfields Eye Hospital (MEH) National Health Service (NHS) Trust, London, United Kingdom.

Moorfields's guidelines request that each patient prior start of treatment for DMO has fluorescein angiography (FFA) and/or OCT-angiography (OCT-A) imaging done to assess macular perfusion and exclude those with severe macular ischaemia which we defined by using FAZ size (> 1500 microns), FAZ outline (completely disrupted) and capillary loss (severe capillary loss) in central subfield as per ETDRS criteria. None of the patients included in this analysis had severe macular ischaemia using criteria above and therefore neither of patients were excluded. In addition, we did not exclude those with hypertension or other systemic comorbidities as well as those who had vitreoretinal conditions such as epiretinal membrane.

A history of an acute coronary event (i.e unstable angina or myocardial infarction) or cerebrovascular accident (trans-ischaemic attack or stroke) in the previous 3 months, pregnancy or lactation, active infection or intraocular inflammation in either eye, poor view of the fundus, other pathologies contributing towards macular oedema, anti-VEGF treatment received for any other condition and other macular diseases present at baseline that might confound the outcomes such as a coexistent retinal vein occlusion were all considered as exclusion criteria.

The cohort consisted of patients who had previously received ranibizumab and had subsequently been switched from ranibizumab to aflibercept. Visual acuity (VA) measurements expressed in ETDRS letters and Optical Coherent Tomography (OCT) (Topcon, Tokyo, Japan) scans were performed at each visit with the aim of achieving fluid resolution.

Patients were switched from ranibizumab to aflibercept when improved efficacy of treatment was sought. Switched patients had been treated with at least three one-monthly ranibizumab injections and subsequently offered a switch to aflibercept based on three clinical situations: 1. no anatomical response as determined by OCT measurement (patients whose response was decrease in central foveal thickness for less than 20 microns during loading phase (3-5 monthly intravitreal injections) where the decision on switch was done by clinician's discretion), 2. an inadequate response based on OCT and/or visual acuity benefit (patients who had some improvement after 5 loading intravitreal injections but still had presence of macular oedema and whose vision was improved for less than 5 ETDRS letters), or 3. a desire to extend the interval between injections in those who necessitated 4-weekly ranibizumab or developed new macular oedema after initial good response on intravitreal ranibizumab.

Our rationale for changing therapy was threefold; no response to intravitreal ranibizumab, inadequate response to intravitreal ranibizumab or a beneficial response although requiring ongoing 4-weekly ranibizumab or a desire to increase the intervals in between injections.

In situations 1 and 2; three one-monthly aflibercept injections were selected, whilst in situation 3, three one-monthly or three bi-monthly injections were offered based on the opinion of the treating ophthalmologist. Decision on further injections after initial phase was made by clinician's discretion.

The baseline visit was defined as when patients received the first aflibercept injection. The follow-up period was 12 months after the first injection of aflibercept was given. The switch-

time period between ranibizumab and aflibercept was a minimum of one month and a maximum of one year. We performed visual (visual acuity) and anatomical (central foveal thickness and macular volume) outcomes 1 year after starting aflibercept. In addition, we finalised subgroup analysis of the cohort based on the duration of time between the ranibizumab being discontinued and the aflibercept commencing, breaking this cohort down into a group switched after a period of less than 3 months and a group switched after an anti-VEGF free period of 3 months or more (max 12 months).

We used the t-paired sample test to calculate the statistical significance in assessing outcomes at 12 months where the P value of <0.05 was considered as statistically significant.

### 10.4.3. Results

Ninety switched eyes (67 patients) were included in this study. Twelve months follow up data was available for seventy-nine eyes with non-attendance determining the primary cause for incomplete data. In this instance, the last valid set of data was carried forward.

*A) Pre-switched data*

The mean VA (SD) at the time of first ranibizumab injection was 58.02 (17.53) ETDRS letters. The mean CFT (SD) and the mean MV(SD) were 494.67 μm (170.22) and 10.76 (2.72) $mm^{3,}$ respectively.

*B) Post-switched data*

In our cohort the mean VA (SD) at baseline (time of switching) was 63 (15.8) ETDRS letters. (Figure 1) The mean baseline CFT (SD) in the SW group was 418 (158.4) μm and the mean baseline MV (SD) in the same group was 9.96 (2.44) $mm^3$. After 1 year, the mean visual acuity (SD) in the group increased to 67 (15.8) ETDRS letters ($p = 0.0048$). The mean CFT (SD) and mean MV (SD) improved at 12 months and were 280.8 (110.1) μm ($p < 0.0001$) and MV 8.43 (1.81) $mm^3$ ($p < 0.0001$), respectively. Additionally, 13 (14.4%) eyes achieved improvement of ≥ 15 ETDRS letters at month 12 **(Table 1).** Forty-five (51.7 %) switched eyes had a decrease in CFT of ≥ 100 microns. However, 6 (6.7%) eyes lost ≥ 15 ETDRS letters and 6 (6.7%) switched eyes had an increase in CFT of ≥ 100 microns.

**Table 1. Subgroup analysis of visual acuity and central foveal thickness changes over follow up period of 12 months. VA = Visual acuity, CFT = Central foveal thickness.**

| **VA changes** | Switched patients (eyes) | % |
|---|---|---|
| ≥ 15 letters gain (%) | 13 | 14.4 |
| < 15 letters gain (%) | 51 | 56.7 |
| ≥ 15 letters loss (%) | 6 | 6.7 |
| < 15 letters loss (%) | 20 | 22.2 |
| **CFT changes** | | |
| ≥ -100 microns | 45 | 51.7 |
| < - 100 microns | 27 | 31 |
| ≥ 100 microns | 6 | 6.9 |
| < 100 microns | 9 | 10.3 |

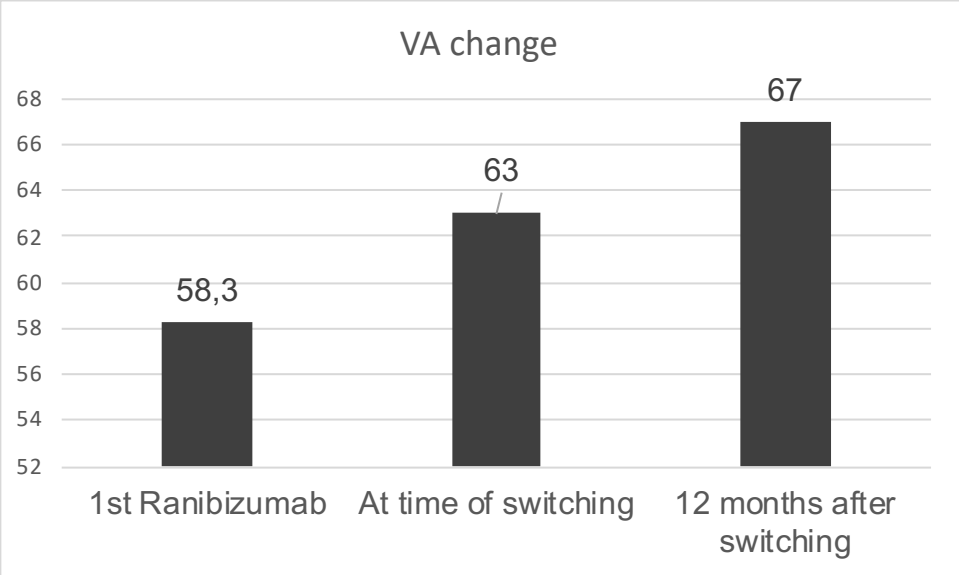



Figure 1. Change in visual acuity from 1st ranibizumab injection to end of 12-month follow up period post switching to aflibercept; VA = visual acuity

### *C) Subgroup analysis*

We sub-divided the switched eyes into two subgroups according to the duration of time between the ranibizumab being discontinued and the aflibercept commencing; 53 eyes were switched with an interval of less than 3 months (mean 7.14 weeks) after the last injection of ranibizumab and 37 eyes were switched after a duration of 3 months or more had elapsed. The mean (SD) VA, CFT and MV for eyes switched with interval less than 3 months had elapsed was 65.7 (15.7) ETDRS letters, 273.2 (122.7) µm, 8.63 (2.08) $mm^3$ at the end of follow up period whilst in the other subgroup (interval l≥ 3 months) the mean (SD) VA, CFT, MV were 69.4 (16) ETDRS letters, 291.4 (90.5) µm and 8.14 (1.31) $mm^3$ **(Table 2).**

In the combined cohort 25 (27.8%) eyes had a gain of 10 letters or more while 9 (10%) eyes had a loss of ten letters or more. Of those switched less than three months after the last ranibizumab injection 14 (26.4%) eyes had a gain of ten letters or more while 7 (13.2%) eyes had a loss of ten letters or more. Eleven (29.7%) of those switching after a three-month interval displayed a ten or more letter VA gain and 2 (5.4%) eyes a ten letter or more loss.

Twenty-five percent of overall switched patients had macular laser prior to baseline. Twenty-free percent and 24% of those who were switched in less than 3 months and those who were switched in 3 months or more had macular laser, respectively. Overall, the cohort had mean 8.8 (SD ± 5.20) intravitreal ranibizumab injections (prior switching) and mean 6.58 (SD ± 2.31) aflibercept injections over the 12-month follow up period.

**Table 2. Data on subgroups related to time of switching; VA = Visual acuity, CFT = Central foveal thickness, MV = Macular volume, ETDRS = Early Treatment Diabetic Retinopathy Study**

| | **LESS THAN 3 MONTHS** | **3 MONTHS OR MORE** |
|---|---|---|
| **TREATED EYES** | 53 | 37 |
| **VA MEAN BASELINE (SD)** ***ETDRS letters*** | 62.57 (14.37) | 63.65 (17.79) |
| **VA MEAN 12 MONTHS (SD)** ***ETDRS letters*** | 65.66 (15.66) | 69.38 (15.96) |
| **VA CHANGE** ***ETDRS letters*** | 3.09 | 5.73 |
| **CFT MEAN BASELINE (SD)** ***microns*** | 410.38 (166.79) | 427.92 (147.32) |
| **CFT MEAN 12 MONTHS (SD)** ***microns*** | 273.18 (122.72) | 291.41 (90.52) |
| **CFT CHANGE** ***microns*** | -137.2 | -136.51 |
| **MV MEAN BASELINE (SD)** ***mm3*** | 10.15 (2.60) | 9.69 (2.20) |
| **MV MEAN 12 MONTHS (SD)** ***mm3*** | 8.63 (2.08) | 8.14 (1.31) |
| **MV MEAN CHANGE** ***mm3*** | -1.52 | -1.55 |
| **MEAN NUMBER OF AFLIBERCEPT (SD) INJECTIONS** | 6.35 (±2.26) | 6.91 (±2.19) |
| **MEAN NUMBER (SD) OF RANIBIZUMAB INJECTIONS** | 9.42 (±5.89) | 7.92 (±3.93) |

### *10.4.4. Discussion*

Repeated intravitreal injections are associated with financial and temporal commitment for patients, doctors and healthcare systems and they are not administered without risk. It is therefore essential that clinicians explore the most efficacious options available. Should the patients' vision not respond as hoped to current treatment, consideration must be given to current treatment course length and as to whether a switch to an alternative anti-VEGF agent, an alternative modality i.e. steroid, laser or a combination approach might achieve better outcomes for the patient. Several real world analyses have described the results achieved from switching between the various anti-VEGF agents, switching from an anti-VEGF agent to a long acting intravitreal steroid and switching from an anti-VEGF agent to a short acting intravitreal steroid.[10-15] Indeed, these studies have confirmed both anatomical and visual acuity improvement in patients who were initially treated with bevacizumab or ranibizumab and who were eventually switched to aflibercept, with the pharmacological preferences of aflibercept in its increased binding affinity for VEGF, longer duration of action and ability to bind placental growth factor all being cited as reasons for a switch of therapy.[10-15]

One of the limits of this study is absence of control group of patients who continued with ranibizumab injections despite the fact of presence of persistent macular oedema. The secondary analysis of Protocol T suggested there was no significant drop in vision in patients with persistent macular oedema and who continued with the same treatment as they received over first 24 weeks over 2 years of follow up.[16] Consequently, the caution was warranted for switching as a general approach for treatment of those patients. In real-life setting, where the aim is to achieve the best vision possible at the shortest time possible, having control group with continuous treatment with slower effective drug is doubtful. Furthermore, majority of patients diagnosed with diabetes mellitus are part of working population, hence, achieving good vision as soon as possible should be our paramount goal. Secondly, the difference between aflibercept and ranibizumab arms in percentage of those who achieved more than 10 ETDRS improvement in visual acuity over 2 years was 18% in favour of aflibercept arm.

We agree that overall results in terms of final visual acuity gain may be similar or non-significantly different among those who continued with the same treatment or who were switched to another agent. However, as we argued above, the aim in real-life settings is to achieve the best possible results within the shortest time possible due to variant reasons:

patients' need to have good vision to be able to conduct their daily duties, hospital capacities, decrease risk of complications (less injections bring theoretically less chance of endophthalmitis), decrease overall costs of treatment. Lastly, the second analysis of Protocol T results in terms of significant loss in vision is based upon the criterion of loss of 10 ETDRS letters or 2 lines on VA chart.[16]

The role of macular ischaemia was not assessed formally in this study, nor was the morphology of the outer retina, both of which might have helped in assessing the validity of our conclusion. This is real world data in a real world setting that shows definitive benefit for patients in altering intravitreal injection therapy from ranibizumab to aflibercept. The length of follow up from baseline was 12 months, which is longer than other switch studies.[15]

One of goals of this manuscript is to show whether the time of switching plays role in overall results. We showed that within each subgroup there was a statistically significant improvement in visual acuity and macular OCT-derived outcomes. However, there was no statistically significant difference in results among the subgroups which means that time of switching in our cohort did not play role in overall results.

The observed results seen in this study adds to the evidence seen in other smaller studies examining this issue that there is an anatomical and functional benefit in switching Anti-VEGF agent in DMO in order to achieve better outcomes for the patient. [10-15] In the future, the role of steroids either as an alternative to anti-VEGF agents or as an adjunct to intravitreal therapy will be better known and real-world data particularly on switching and combination of therapies will be helpful in contributing to the evidence.

### *10.4.5. References*

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## 11. METHODS OF RESEARCH

Manuscripts amalgamated in the doctoral thesis involved patients with fovea-involving macular oedema who were eligible for treatment with intravitreal anti-VEGF injections, which is the golden-standard treatment for the condition mentioned above. All patients applied met the National Institute of Care and Excellence (NICE) criteria for treatment (center-involving diabetic macular oedema ≥ 400 microns) and with baseline visual acuity (VA) equal or less than 80 ETDRS letters. Participating subjects included were older than 18 years of age and diagnosed with either diabetes mellitus (DM) type 1 or type 2. Both DMO and diabetic retinopathy (DR) were graded using the modified Early Treatment Diabetic Retinopathy Study (ETDRS) classification system based on clinical appearance.

The primary cohort of interest (treatment-naive cohort) involved 89 patients (99 eyes) who were treatment-naive and funded for intravitreal aflibercept injections. We analysed primary, secondary and tertiary outcomes in short and long terms, explained in more detail below. In addition, we wanted to see whether those patients who had started treatment on a different anti-VEGF agent (ranibizumab as another licensed anti-VEGF product in the United Kingdom) and didn't respond well to the initiated treatment may benefit from switching to intravitreal aflibercept injections, which may have more potent effect as per available pharmacokinetic data.[119] Therefore, we formed a secondary cohort that involved 67 switched patients (90 eyes) who had started treatment with intravitreal ranibizumab and were switched to intravitreal aflibercept. We considered baseline point when those patients were switched to intravitreal aflibercept and compared outcomes with the treatment-naive cohort. Likewise, we analysed whether the time of switching plays a role in overall results.

The treatment was initiated on a loading phase of five one-monthly intravitreal aflibercept injections in the treatment-naive cohort, followed by injections if needed as per physicians' discretion. The clinical decision on further injections following the loading phase within year 1 or decision on re-starting treatment in years 2 and 3 was based on treating towards visual acuity and OCT scan stability. In general, if there was potential for further VA and/or OCT improvement (e.g., persistent fluid or presence of new fluid), further injections towards this aim were offered.

Patients were switched from ranibizumab to aflibercept when improved efficacy of treatment was sought. Switched patients had been treated with at least three one-monthly ranibizumab injections and subsequently offered a switch to aflibercept based on three clinical situations: 1. no anatomical response as determined by OCT measurement (patients whose response was decrease in central foveal thickness for less than 20 microns during loading phase (3-5 monthly intravitreal injections) where the decision on switch was done by clinician's discretion, 2. an inadequate response based on OCT and/or visual acuity benefit (patients who had some improvement after 5 loading intravitreal injections but still had presence of macular oedema and whose vision was improved for less than 5 ETDRS letters), or 3. a desire to extend the interval between injections in those who necessitated 4-weekly ranibizumab or developed new macular oedema after initial good response on intravitreal ranibizumab.

In both cohorts, the primary outcomes were visual acuity (VA), central foveal thickness (CFT), and macular volume (MV) 12 months after treatment was commenced/after switching off a drug. The analysis of the same primary outcomes was performed for the treatment-naive cohort 36 months after initiating treatment. Then, we calculated the percentage of eyes that achieved visual acuity gain of ≥ 15 ETDRS letters and the percentage of eyes that achieved improvement in CFT for 100 microns or more at the end of year 1 and year 3. As tertiary outcomes, we made a subgroup analysis according to the baseline VA (worse than 69 ETDRS letters or ≥ 69 ETDRS letters). We did subgroup analyses of VA and CFT according to lens status and history on pre-treatment macular laser for the treatment-naive group only. For the switched group only, we performed the analysis of outcomes based on the time of switching.

Critical exclusion criteria for both cohorts included a history of an acute coronary event or cerebrovascular accident in the previous 3 months, pregnancy or lactation, active infection or intraocular inflammation in either eye, poor view of the fundus, severe macular ischaemia, other pathologies contributing towards macular oedema, anti-VEGF treatment received for any other condition and other macular diseases present at baseline that might confound the outcomes such as coexistent retinal vein occlusion.

For the statistical analysis, we used the t-paired sample test to ascertain our results' statistical significance when we compared two samples (https://www.graphpad.com/quickcalcs/ttest1.cfm). When we compared three samples, we

used the mixed and repeated ANOVA analysis (https://goodcalculators.com/one-way-anova-calculator/ and ). To analyse the effect of switching time in the switched cohort, we used two methods; the t-paired sample test and Pearson's coefficient calculation. A P value of $< .05$ was interpreted as statistically significant within all measures.

# 12. SUMMARY OF POOLED RESULTS

## 12.1 Primary outcomes of the main and switched cohorts

The main cohort was formed of eighty-nine treatment-naïve patients (99 eyes) who started to receive intravitreal aflibercept at baseline and finished the short-term follow up of 12 months. Eventually, fifty-four patients (64 treated eyes) of those who entered the study finished the long-term follow up of 3 years. The reasons of those who were excluded from the analysis after the end of year 1 were two-fold; cessation or lost of follow up during 2$^{nd}$ and 3$^{rd}$ year of treatment. The comparison (unpaired Welch's t-test) of baseline variables and the 12-month outcomes between the patients who were followed short-termed only (12 months) and those who finished 36-month follow up was done and the results showed there was no statistically significant difference in outcomes which may be potentially caused by change of the sample ($P > 0.05$ in all comparisons). The mean age of patients included in the main cohort was 54.45 [range 25-80]. The mean number of aflibercept injections received in year 1, 2 and 3 was 7.08, 2.93 and 2.57, respectively. Eleven percent of included eyes had less than 5 monthly loading doses due to either clinicians' discretion or patients' choice (they did not attend or they cancelled their appointments). Thirteen percent of included eyes did not require further injections at all after the loading phase of 5 injections. Around 30% of patients did not require injections in year 2 and nearly 40% of patients from our cohort did not require any injection in year 3.

The cohort of switched patients was formed of 90 eyes switched from intravitreal ranibizumab to intravitreal aflibercept. They were treated with mean 6.58 [2-10] of intravitreal aflibercept injections after switching and had received mean 8.8 ranibizumab injections [3-26] prior switching. The mean VA (SD) at the time of first ranibizumab injection was 58.02 (17.53) ETDRS letters. The mean CFT (SD) and the mean MV(SD) were 494.67 μm (170.22) and 10.76 (2.72) $mm^{3,}$ respectively. The post-switched data is presented in Table 1 along with the data from the main cohort (both short and long term). The mixed ANOVA analysis showed there was no statistically significant difference in baseline variables and 12-month outcomes between short- and long-term followed treatment naïve patients and switched patients.

## 12.2 Secondary and Tertiary outcomes of the main and switched cohorts

*A) Visual acuity gain outcomes*

Within all three cohorts, long and short term treatment naïve ones and the switched one, we calculated the percentage of those who improved vision for 15 ETDRS letters or more and those who had decrease in central foveal thickness for 100 microns and more. The more detailed results are shown in Tables 1 and 2.

**Table 1. Comparison of Visual acuity and Central foveal thickness gains/losses of three investigated cohorts**

| **VA changes** | **Switched patients** | **Treatment-naïve 12 months** | **Treatment-naïve 36 months** |
|---|---|---|---|
| ≥ 15 letters gain (%) | 14.4 | 33.67 | 25 |
| < 15 letters gain (%) | 56.7 | 47.95 | 50 |
| ≥ 15 letters loss (%) | 6.7 | 3.06 | 5% |
| < 15 letters loss (%) | 22.2 | 15.30 | 20 |
| **CFT changes** | | | |
| ≥ 100 microns decrease | 51.7 | 50.50 | 52 |
| < 100 microns decrease | 31 | 21.21 | 19 |
| ≥ 100 microns increase | 6.9 | 6.06 | 6% |
| < 100 microns increase | 10.3 | 13.13 | 19 |

*B) Sub-group analysis according to baseline VA*

The Protocol T indicated the importance of initiating treatment when the baseline vision is better (≥ 69 ETDRS letters). We wanted to investigate whether those findings are consistent in our real-life based cohort. We sub-divided the included eyes into two subgroups according to

the baseline visual acuity; < 69 ETDRS letters and ≥ 69 ETDRS letters as per Protocol T methodology. Fifty-eight percent of eyes of the main cohort had baseline visual acuity less than 69 ETDRS letters. The mean change in visual acuity in the subgroup with baseline VA less than 69 letters was +11.27 ETDRS letters ($p < 0.0001$). Figure 1. Forty-two percent of eyes from the main cohort had baseline visual acuity ≥ 69 ETDRS letters and the mean change in the visual acuity after 36 months in that subgroup was + 0.88 ETDRS letters ($p = 0.6041$). The subgroup of eyes with initially worse visual acuity had mean 11.89 intravitreal injections of aflibercept over 36 months whilst the subgroup with initial visual acuity of ≥ 69 ETDRS letters had mean 13.59 injections over same follow up period ($p = 0.20$). The subgroup analysis based on baseline VA in switched group, using the repeated ANOVAs analysis, showed there was significant change in VA from first ranibizumab injection until the end of 12-month period post switching whilst the subgroup which had better VA at baseline showed no statistically signifcant change in vision.

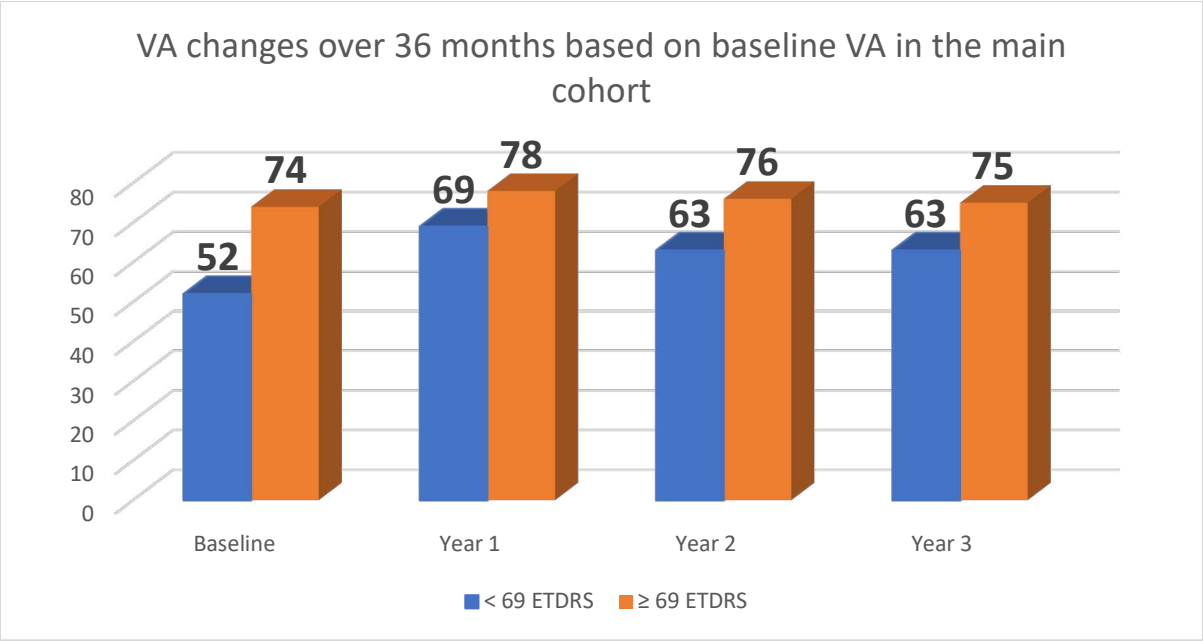



**Figure 1. Visual acuity changes over 36 months based on baseline visual acuity (< 69 ETDRS letters at baseline and > 69 ETDRS letters at baseline).**

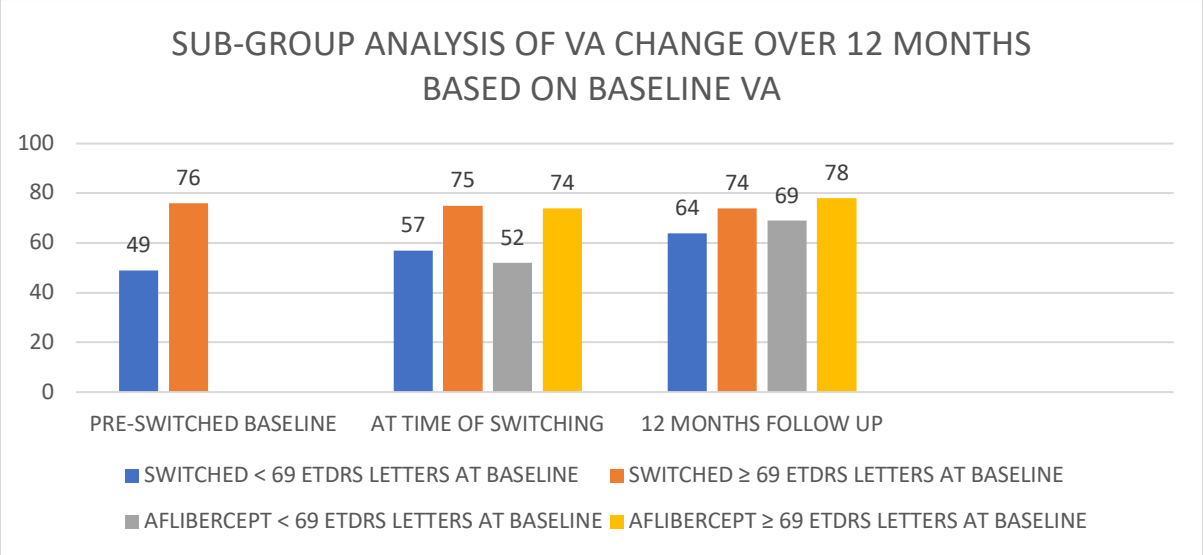



**Figure 2. Visual acuity changes over 12 months based on baseline visual acuity (< 69 ETDRS letters at baseline and > 69 ETDRS letters at baseline). The results contain data from both cohorts, treatment-naïve and switched.**

| | **SHORT TERM TREATMENT NAÏVE OUTCOMES** | **LONG TERM TREATMENT NAÏVE OUTCOMES** | **SWITCHED PATIENTS** |
|---|---|---|---|
| **TREATED EYES** | 99 | 64 | 90 |
| **VA MEAN baseline (SD) ETDRS letters** | 59.7 (16.1) | 61.45 (16.30) | 63 (15.8) |
| **VA MEAN 12 MONTHS (SD) ETDRS letters** | 69.6 (15.2) | 72.66 (13.53) | 67 (15.8) |
| **VA change from baseline** | 9.9 | 11.21 | 4 |
| **VA MEAN 24 MONTHS (SD) ETDRS letters** | - | 68.38 (13.72) | - |
| **VA CHANGE from baseline** | - | 6.24 | - |
| **VA CHANGE from year 1** | - | -4.28 | - |
| **VA MEAN 36 MONTHS (SD) ETDRS letters** | - | 68.34 (13.66) | - |
| **VA CHANGE from baseline** | - | 6.89 | - |
| **VA CHANGE from year 1** | - | -4.32 | - |

| | | | |
|---|---|---|---|
| **VA CHANGE from year 2** | - | -0.04 | - |
| **CFT MEAN baseline (SD) microns** | 431 (129) | 422 (138) | 418 (158) |
| **CFT MEAN 12 MONTHS (SD) microns** | 306 (122) | 282 (100) | 281 (110) |
| **CFT change from baseline** | -128 | -140 | -137 |
| **CFT MEAN 24 MONTHS (SD) microns** | - | 303 (93) | - |
| **CFT CHANGE microns from baseline** | - | -119 | - |
| **CFT CHANGE microns from year 1** | - | 21 | - |
| **CFT MEAN 36 MONTHS (SD) microns** | - | 303 (106) | - |
| **CFT CHANGE microns from baseline** | - | -119 | - |
| **CFT CHANGE microns from year 1** | - | 21 | - |
| **CFT CHANGE microns from year 2** | - | -0.45 | - |
| **MV baseline MONTHS (SD) mm3** | 9.53 (1.79) | 9.51 (2.01) | 9.96 (2.44) |
| **MV MEAN 12 MONTHS (SD) mm3** | 8.43 (1.58) | 8.13 (1.23) | 8.43 (1.81 |
| **MV change from baseline** | -1.08 | -1.38 | -1.53 |
| **MV MEAN 24 MONTHS (SD) mm3** | - | 8.27 (1.36) | - |

| | | | |
|---|---|---|---|
| **MV MEAN CHANGE mm3 from baseline** | - | -1.24 | - |
| **MV MEAN CHANGE mm3 from year 1** | - | 0.14 | - |
| **MV MEAN 36 MONTHS (SD) mm3** | - | 8.35 (1.62) | - |
| **MV MEAN CHANGE mm3 from baseline** | - | -1.16 | - |
| **MV MEAN CHANGE mm3 from year 1** | - | 0.22 | - |
| **MV MEAN CHANGE mm3 from year 2** | - | 0.08 | - |
| **MEAN NUMBER OF Eylea INJECTIONS at year 1 (range)** | 6.92 (5-10) | 7.09 (5-10) | 6.58 (2-11) |
| **MEAN NUMBER OF Eylea INJECTIONS at year 2 (range)** | - | 2.93 (0-7) | - |
| **MEAN NUMBER OF Eylea INJECTIONS at year 3 (range)** | - | 2.57 (0-9) | - |
| **MEAN NUMBER OF Eylea INJECTIONS over 3 years** | - | 12.59 | - |
| **Macular laser prior treatment** | 25% (25 eyes) | 32.81% (21 eyes) | 25% (22 eyes) |

**Table 2. Comparison of general data between all three cohorts**

## 12.3 Subgroup analyses according to lens status and history on pre-treatment with macular laser in the treatment-naïve cohort

We calculated visual acuity changes in baseline pseudophakic patients (28% of eyes included). At baseline, the mean VA (SD) (Snellen) was 62.50 (15.19) (20/63) ETDRS letters whilst the mean VA (SD) (Snellen) was 64.78 (14.21) (20/40) ETDRS letters at month 36 ($p = 0.24$). At the end of year 3 there were no statistically significant changes in VA or CFT between subgroups according to their lens status (p value for VA among three sub-groups was 0.10779, p value for CFT among three sub-groups was 0.6196). The more detailed data on subgroup analysis according to baseline lens status has been shown in Table 2. Then, there were no statistically significant changes in either VA or CFT in those who had and those who had no macular laser prior treatment with intravitreal injections ($p = 0.333$) (Figure 2). The macular laser had been done in all patients 6 months or more prior initiation of treatment with intravitreal aflibercept.

## 12.4 Subgroup analysis based on time of switching in the switched cohort

We wanted to investigate whether the time of switching plays a role in both visual acuity and anatomical outcomes. Therfore, we sub-divided the switched eyes into two subgroups according to the duration of time between the ranibizumab being discontinued and the aflibercept commencing; 53 eyes were switched with an interval of less than 3 months after the last injection of ranibizumab and 37 eyes were switched after a duration of 3 months or more had elapsed. There was no statistically significant difference in outcomes ( $p > 0.05$) between those subgroups and the results are shown in Table 3. The Pearson's correlation coefficient was 0.0086 and the p value based on that was 0.41702.

**Table 3 – Primary outcomes between subgroups based on time of switching**

| | **LESS THAN 3 MONTHS** | **3 MONTHS OR MORE** |
|---|---|---|
| **TREATED EYES** | 53 | 37 |
| **VA MEAN BASELINE (SD)** | | |
| ***ETDRS letters*** | 62.57 (14.37) | 63.65 (17.79) |
| **VA MEAN 12 MONTHS (SD)** | | |
| ***ETDRS letters*** | 65.66 (15.66) | 69.38 (15.96) |
| **VA CHANGE *ETDRS letters*** | 3.09 | 5.73 |
| **CFT MEAN BASELINE (SD) *microns*** | 410 (167) | 428 (147) |
| **CFT MEAN 12 MONTHS (SD) *microns*** | 273 (123) | 291 (91) |
| **CFT CHANGE *microns*** | -137 | -137 |
| **MV MEAN BASELINE (SD) *mm3*** | 10.15 (2.60) | 9.69 (2.20) |
| **MV MEAN 12 MONTHS (SD) *mm3*** | 8.63 (2.08) | 8.14 (1.31) |
| **MV MEAN CHANGE *mm3*** | -1.52 | -1.55 |
| **MEAN NUMBER OF AFLIBERCEPT (SD) INJECTIONS** | 6.35 (±2.26) | 6.91 (±2.19) |
| **MEAN NUMBER (SD) OF RANIBIZUMAB INJECTIONS** | 9.42 (±5.89) | 7.92(±3.93) |

## 13. SCIENTIFIC CONTRIBUTION OF POOLED ARTICLES

Our both short and long term results of visual acuity and anatomical outcomes in patients with sight-impairing, centre-involving diabetic macular oedema treated with intravitreal aflibercept shown a significant improvement in vision, comparable with results suggested by landmark clinical trials. The mean VA improved to 72.66 (+11.21) ETDRS letters over year 1 and maintained stable VA with mean VA of 68.38 and 68.34 ETDRS letters at year 2 and 3, respectively. Despite the loss of 4 ETDRS letters within second and third year of treatment, the cohort gained statistically significant improvement in visual acuity and anatomical outcomes over three years of treatment. Furthermore, our results were non-inferior when compared to landmark clinical trials related to safety and efficacy of intravitreal aflibercept in treatment of DMO (VIVID, VISTA and Protocol T).[90,94,105,107] In our subgroup analysis based on baseline visual acuity we proved the importance of starting treatment when visual acuity is initially better. In those whose baseline visual acuity was 69 ETRDS letters or better (up to 80 ETDRS letters) we achieved stable vision over three years of follow up. Those patients kept their good vision which meets driving criteria. Despite the fact that those who had baseline visual acuity worse than 69 ETDRS letters achieved a significant improvement after 3 years of treatment, their final mean visual acuity was significantly worse as compared to other subgroup. This correlation is also shown in the baseline VA subgroup analysis in the switched cohort. Our results are very first published results from the real-world which show the importance of planning the treatment guided by baseline vision which had been firstly indicated in Protocol T study. Furthermore, the Protocol V suggests that those who have visual acuity 20/25 or better do not require treatment and may be only monitored.[146] Integrating the data from protocol V and our real-life results may suggest that in real-life practices we may monitor those patients who have visual acuity 20/25 or better and aim to start treatment when their vision drops to 6/9. Following this, we have a great chance to keep stable vision in diabetic patients. Regarding the results in our switched cohort, we showed that switching from intravitreal ranibizumab to intravitreal aflibercept has rationale at least for speeding up the visual recovery and improving the anatomical outcomes which in everyday life offer patients sooner improvement and better functioning by using their vision. We proved that time of switching does not play role in primary outcomes (VA, CFT, MV) which may indicate that indeed the reason of clinical improvement in our switched cohort rely on mechanism and pharmacokinetics of aflibercept molecule.

## 14. ABSTRACT

**Purpose:**

Randomized controlled clinical trials are driven by time-given protocols defined by strict exclusion and inclusion criteria. As a result, clinical trials provide the best possible outcomes regarding the efficacy and safety of investigational medical products. Nonetheless, the implementation of protocols suggested by clinical trials has been shown as non-pragmatic into standard treatment care. Not following the protocols recommended by clinical trials in our standard routine treatment care does not provide the benefits of therapy as clinical trials suggest. Therefore, we wanted to investigate whether our proposed treatment protocols with diabetic macular oedema, designed for standard treatment of care, may achieve non-inferior results compared to clinical trials where the non-inferiority was defined by a difference in the vision of up to 10 ETDRS letters.

**Methods:**

Manuscripts amalgamated in this doctoral thesis involved patients with fovea-involving macular oedema who were eligible for treatment with intravitreal anti-VEGF injections. All patients applied met the National Institute of Care and Excellence (NICE) criteria for treatment (center-involving diabetic macular oedema ≥ 400 microns) and with baseline visual acuity (VA) equal to or less than 80 ETDRS letters. All patients were treated in Moorfields Eye Hospital NHS Foundation Trust, in London, UK.

The primary cohort of interest (treatment-naive cohort) involved 89 patients (99 eyes) who were treatment-naive and funded for intravitreal aflibercept injections. We analyzed primary, secondary and tertiary outcomes in short and long terms. In addition, we wanted to see whether those patients who had started treatment on a different anti-VEGF agent (ranibizumab as another licensed anti-VEGF product in the United Kingdom) and didn't respond well to the initiated treatment may benefit from switching to intravitreal aflibercept injections, which may have more potent effect as per available pharmacokinetic data. Therefore, we formed a second cohort that involved 67 switched patients (90 eyes) who had started treatment with intravitreal ranibizumab and were switched to intravitreal aflibercept.

In both cohorts, the primary outcomes were visual acuity (VA), central foveal thickness (CFT), and macular volume (MV) 12 months after treatment was commenced/after switching off a drug. The analysis of the same primary outcomes was performed for the treatment-naive cohort 36 months after initiating treatment. Then, we calculated the percentage of eyes that achieved visual acuity gain of ≥ 15 ETDRS letters and the percentage of eyes that achieved improvement in CFT for 100 microns or more at the end of year 1 and year 3. As tertiary outcomes, we made a subgroup analysis according to the baseline VA (< 69 ETDRS letters or ≥ 69 ETDRS letters). For switched cohorts, we analysed whether the time of switching plays a role in overall results.

**Results**

The mixed ANOVA analysis showed no statistically significant difference in baseline variables and 12-month outcomes between short- and long-term followed treatment naïve patients and switched patients. The mean change in vision after three years of initiation of treatment with intravitreal aflibercept injections was +6.89 EDTRS letters, while improvement in CFT and MV during the same time was -140 microns and – 1.38 $mm^3$, respectively.

At the end of follow-up of 36 months, 25% of patients improved vision for three or more ETDRS lines (15 letters or more). Just more than 50% of patients had improvement in central foveal thickness for 100 microns or more.

Fifty-eight percent of eyes of the primary cohort had baseline visual acuity less than 69 ETDRS letters. The mean change in visual acuity in the subgroup with baseline VA less than 69 letters was +11.27 ETDRS letters ($p < 0.0001$). Forty-two percent of eyes from the primary cohort had baseline visual acuity ≥ 69 ETDRS letters). The mean change in the visual acuity after 36 months in that subgroup was + 0.88 ETDRS letters ($p = 0.6041$).

We sub-divided the switched eyes into two subgroups according to the duration of time between the ranibizumab being discontinued and the aflibercept commencing; 53 eyes were switched with an interval of fewer than three months after the last injection of ranibizumab,

and 37 eyes were switched after three months or more had elapsed. There was no statistically significant difference in outcomes ( $p > 0.05$) between those subgroups.

## Conclusion

Our short and long-term results of visual acuity and anatomical outcomes in patients with sight-impairing, center-involving diabetic macular oedema treated with intravitreal aflibercept showed a significant improvement in vision, comparable with results suggested by landmark clinical trials.

Furthermore, our results were non-inferior when compared to landmark clinical trials related to the safety and efficacy of intravitreal aflibercept in the treatment of DMO (VIVID, VISTA, and Protocol T). Our subgroup analysis based on baseline visual acuity proved the importance of starting treatment when visual acuity is initially better. We achieved stable vision over three years of follow-up in those whose baseline visual acuity was 69 ETRDS letters or better (up to 80 ETDRS letters). Those patients kept their good vision which meets driving criteria.

Our results are the first published results from the real world, which show the importance of planning the treatment guided by baseline vision, which was first indicated in the Protocol T study.

Regarding the results in our switched cohort, we showed that switching from intravitreal ranibizumab to intravitreal aflibercept has a rationale for speeding up the visual recovery and improving the anatomical outcomes in everyday life offer patients sooner improvement and better functioning by using their vision. We proved that time of switching does not play a role in primary outcomes (VA, CFT, MV), which may indicate that indeed the reason for clinical improvement in our switched cohort relies on the mechanism and pharmacokinetics of the aflibercept molecule.

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