

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

Lukić, Marko

Doctoral thesis / Disertacija

2021

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Split, School of Medicine / Sveučilište u Splitu, Medicinski fakultet**

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:171:576485>

Rights / Prava: [In copyright](#) / [Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2024-11-02**



Repository / Repozitorij:

[MEFST Repository](#)



**UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE**

Dr Marko Lukić, FEBO

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

DOCTORAL DISERTATION

Split, November 2021

**UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE**

Dr Marko Lukić, FEBO

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

DOCTORAL DISERTATION

Mentor:

Assoc Prof Kajo Bućan, PhD, MD, FEBO

Split, November 2021

Acknowledgments

I would like to thank my esteemed supervisor, Assoc Prof Kajo Bućan, Ph.D., MD, FEBO, for his invaluable supervision, support, and tutelage during my Ph.D. degree. My gratitude extends to NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology for the opportunity to undertake my research at the Medical Retina Service. Additionally, I would like to express gratitude to Mr. Robin Hamilton, FRCOphth, Medical Retina Service Director, and Mr. Ranjam Rajendram, FRCOphth, for their supervision and treasured support, which was influential in shaping my experiment methods and critiquing my results. Then, I also appreciate all the support I received from my co-authors of the manuscripts compiled in this doctoral dissertation. I would like to extend my sincere thanks to all senior consultants and colleagues at Moorfields Eye Hospital NHS Trust, especially to Ms. Dawn Sim, FRCOphth, Mr. Pearse Keane, FRCOphth, Mr Praveen Patel, FRCOphth and Mr. Mark Westcott, FRCOphth, for their unwavering support and belief in me. Their immense knowledge and plentiful experience have encouraged me in all the time of my academic research and daily life.

My tremendous appreciation goes to family and friends whose support has been significant during my studies, especially through the food and beverages. The mother-in-law's apple strudel, the crab and home-made pasta prepared by aunties Bojana and Nena, the pints of beers, well-selected wines and glasses of whiskey having with Nikica, Mance, Luka, Hilarije, Barbara, Cica, Stipe, and Jasmina continuously remind me that the small things or memories are the most valuable that a person can have. Finally, and above all, there is someone whose never-failing support, love, partnership, and tolerance have always been above and beyond. My beloved fiancé Mirja by sharing her cheerfulness, positivity, and honest advice, brings a joy sparkle in my life and inspires me to be a better doctor, clinician, partner, and person. Despite my grumpiness, especially when I have something to work on, she has always been here as my pillar. Thank you for all the marvellous travel journeys, delicious dining, and nagging me to try food I would probably never dare to do without you. I am entirely aware that I can never surpass your beef bourgignon or many delicious meals you prepared for me on a Masterchef-Australia level. However, the doctoral dissertation is finished, and I am ready to become your chef. Are you?

TABLE OF CONTENTS

- **LIST OF SYMBOLS AND ABBREVIATIONS.....8**

- 1. INTRODUCTION.....12**

- 2. EPIDEMIOLOGY AND SOCIO-ECONOMIC DATA ON DIABETES MELLITUS AND DIABETIC RETINOPATHY14**

- 3. PATHOMECHANISM OF DIABETIC MACULAR OEDEMA.....16**

- 4. THE IMPORTANCE OF HbA1C IN DIABETIC RETINOPATHY AND DIABETIC MACULAR OEDEMA.....18**

- 5. DEVELOPMENT OF ANTI-VEGF DRUGS.....21**
 - 5.1 Bevacizumab (Avastin®, Genentech, San Francisco, CA, USA)22**
 - 5.2 Ranibizumab (Lucentis®, Novartis, Basel, Switzerland)22**
 - 5.3 Aflibercept (Eylea®, Bayer, Basel, Switzerland)23**

- 6. CLINICAL PHARMACOLOGY OF ANTI-VEGF DRUGS24**
 - 6.1. Pharmacodynamics.....24**
 - 6.2. Pharmacokinetics.....25**

- 7. SUMMARY OF LANDMARK CLINICAL TRIALS.....26**

- 8. THE RATIONALE OF CONDUCTING REAL-LIFE STUDIES.....28**

- 9. AIM OF COMPILED RESEARCH PAPERS.....30**

10. COPIES OF RESEARCH PAPERS (ARTICLES) COMPILED IN THIS DOCTORAL DISSERTATION.....	31
10.1 The list of pooled articles proposed as a doctoral dissertation.....	31
10.2 Intravitreal aflibercept for diabetic macular oedema: Moorfields’ real-world 12-month visual acuity and anatomical outcomes.....	32
10.2.1. Introduction.....	32
10.2.2. Methods.....	33
10.2.3. Results.....	34
A) <i>Aflibercept cohort outcomes.....</i>	<i>35</i>
B) <i>Mean changes and sub-group analysis according to baseline VA and CFT.....</i>	<i>38</i>
10.2.4 Discussion.....	40
10.2.5. References.....	42
10.3 Intravitreal aflibercept for diabetic macular oedema in real-world: 36-month visual acuity and anatomical outcomes.....	45
10.3.1. Introduction.....	45
10.3.2. Methods.....	46
10.3.3. Results.....	48
A) <i>Primary and secondary outcomes.....</i>	<i>48</i>
B) <i>Sub-group analysis according to baseline VA and CFT.....</i>	<i>50</i>
C) <i>Subgroup analyses according to lens status and history on pre-treatment with macular laser.....</i>	<i>52</i>
10.3.4 Discussion.....	54
10.3.5. References.....	56
10.4 One-year real-life results on effect of intravitreal aflibercept in patients with diabetic macular oedema switched from ranibizumab.....	59

10.4.1. Introduction	59
10.4.2. Methods	60
10.4.3. Results	62
<i>A) Pre-switched data</i>	62
<i>B) Post-switched data</i>	62
<i>C) Subgroup analyses</i>	64
10.4.4 Discussion	66
10.4.5. References	68
11. METHODS OF RESEARCH	71
12. SUMMARY OF POOLED RESULTS	74
12.2 Primary outcomes of the main and switched cohorts	74
12.2 Secondary and Tertiary outcomes of the main and switched cohorts	75
<i>A) Visual acuity gain outcomes</i>	75
<i>B) Sub-group analysis according to baseline VA</i>	75
12.3 Subgroup analyses according to lens status and history on pre-treatment with macular laser in the treatment-naïve cohort	81
12.4 Subgroup analysis based on time of switching in the switched cohort	81
13. SCIENTIFIC CONTRIBUTION OF THE POOLED ARTICLES	83
14. ABSTRACT	84
15. REFERENCES	87

• **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 Administration

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 1 β , 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 -A₁₆₅ 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 glycosylated 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 glycaemic 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, **brlucizumab**, 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 hypothesises 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 defined 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.

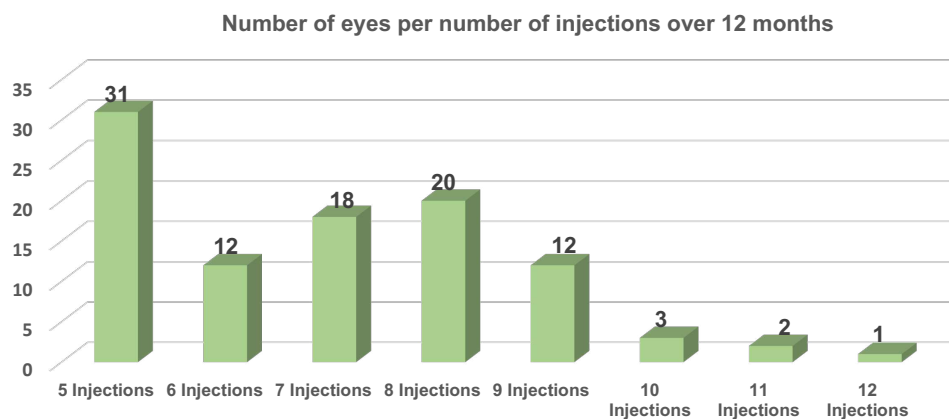
Key exclusion criteria included a history of an acute coronary event or cerebrovascular accident in the previous 3 months, pregnancy or lactation, active infection or intraocular inflammation in either eye, poor view of the fundus, severe macular ischaemia, other pathologies contributing towards macular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

mm³ ($p < .0001$) 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		
$\geq - 100$ 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 mm³ 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)

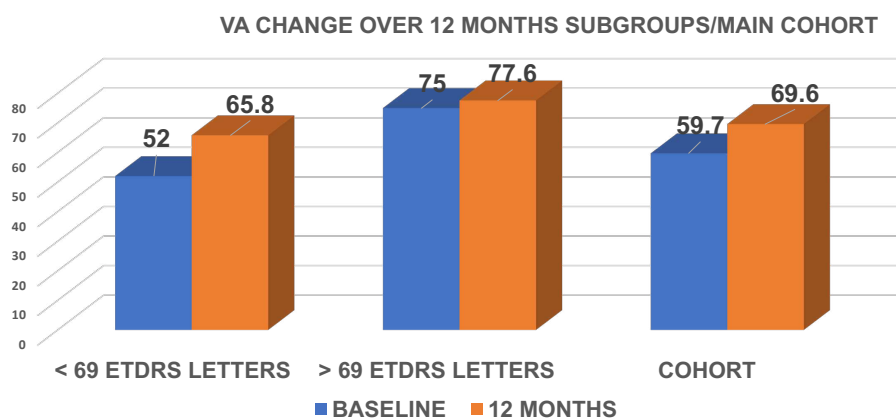


Table 3 Macular oedema appearance at baseline; DMO = Diabetic Macular Oedema, CSMO = Clinically Significant Macular Oedema

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

1. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes care*. 2012;35(3):556-64.
2. Wells JA, Glassman AR, Ayala AR, Jampol LM, Bressler NM, Bressler SB, et al. Aflibercept, Bevacizumab, or Ranibizumab for Diabetic Macular Edema: Two-Year Results from a Comparative Effectiveness Randomized Clinical Trial. *Ophthalmology*. 2016;123(6):1351-9.
3. Bressler SB, Glassman AR, Almkhatar T, Bressler NM, Ferris FL, Googe JM, Jr., et al. Five-Year Outcomes of Ranibizumab With Prompt or Deferred Laser Versus Laser or Triamcinolone Plus Deferred Ranibizumab for Diabetic Macular Edema. *American journal of ophthalmology*. 2016;164:57-68.
4. Heier JS, Korobelnik JF, Brown DM, Schmidt-Erfurth U, Do DV, Midena E, et al. Intravitreal Aflibercept for Diabetic Macular Edema: 148-Week Results from the VISTA and VIVID Studies. *Ophthalmology*. 2016;123(11):2376-85.
5. Boyer DS, Nguyen QD, Brown DM, Basu K, Ehrlich JS. Outcomes with As-Needed Ranibizumab after Initial Monthly Therapy: Long-Term Outcomes of the Phase III RIDE and RISE Trials. *Ophthalmology*. 2015;122(12):2504-13.e1.
6. Rajendram R, Fraser-Bell S, Kaines A, Michaelides M, Hamilton RD, Esposti SD, et al. A 2-year prospective randomized controlled trial of intravitreal bevacizumab or laser therapy (BOLT) in the management of diabetic macular edema: 24-month data: report 3. *Arch Ophthalmol*. 2012;130(8):972-9.
7. Virgili G, Parravano M, Evans JR, Gordon I, Lucenteforte E. Anti-vascular endothelial growth factor for diabetic macular oedema: a network meta-analysis. *Cochrane Database of Systematic Reviews*. 2017(6).

8. Elman MJ, Aiello LP, Beck RW, Bressler NM, Bressler SB, Edwards AR, et al. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology*. 2010;117(6):1064-77.e35.
9. Brown DM, Nguyen QD, Marcus DM, Boyer DS, Patel S, Feiner L, et al. Long-term outcomes of ranibizumab therapy for diabetic macular edema: the 36-month results from two phase III trials: RISE and RIDE. *Ophthalmology*. 2013;120(10):2013-22.
10. Patrao NV, Antao S, Egan C, Omar A, Hamilton R, Hykin PG, Sivaprasad S, Rajendram R, Moorfields Diabetic Macular Oedema Study Group. Real-World Outcomes of Ranibizumab Treatment for Diabetic Macular Edema in a United Kingdom National Health Service Setting. *Am J Ophthalmol*. 2016; 172:51-57.
11. Korobelnik JF, Do DV, Schmidt-Erfurth U, Boyer DS, Holz FG, Heier JS, et al. Intravitreal aflibercept for diabetic macular edema. *Ophthalmology*. 2014;121(11):2247-54.
12. Wells JA, Glassman AR, Ayala AR, Jampol LM, Aiello LP, Antoszyk AN, et al. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *The New England journal of medicine*. 2015;372(13):1193-203.
13. Massin P, Bandello F, Garweg JG, Hansen LL, Harding SP, Larsen M, et al. Safety and efficacy of ranibizumab in diabetic macular edema (RESOLVE Study): a 12-month, randomized, controlled, double-masked, multicenter phase II study. *Diabetes care*. 2010;33(11):2399-405.
14. Nguyen QD, Brown DM, Marcus DM, Boyer DS, Patel S, Feiner L, et al. Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. *Ophthalmology*. 2012;119(4):789-801.
15. Do DV, Nguyen QD, Boyer D, Schmidt-Erfurth U, Brown DM, Vitti R, et al. One-year outcomes of the da Vinci Study of VEGF Trap-Eye in eyes with diabetic macular edema. *Ophthalmology*. 2012;119(8):1658-65.

16. Dugel PU, Hillenkamp J, Sivaprasad S, Vögeler J, Mousseau M-C, Wenzel A, et al. Baseline visual acuity strongly predicts visual acuity gain in patients with diabetic macular edema following anti-vascular endothelial growth factor treatment across trials. *Clinical Ophthalmology (Auckland, NZ)*. 2016;10:1103.

17. Kiss S, Liu Y, Brown J, Holekamp NM, Almony A, Campbell J, et al. Clinical utilization of anti-vascular endothelial growth-factor agents and patient monitoring in retinal vein occlusion and diabetic macular edema. *Clinical ophthalmology (Auckland, NZ)*. 2014;8:1611.

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 comorbidities, 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.

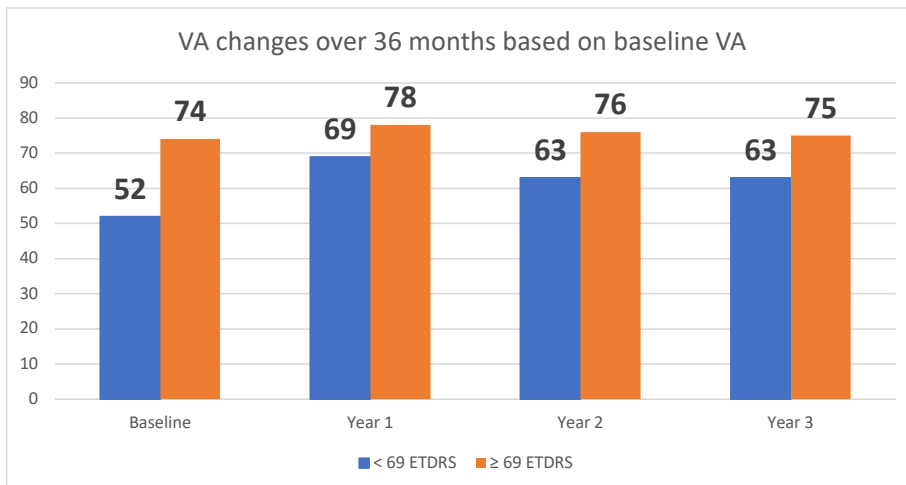
Table 1. General data; VA = Visual acuity, CFT = Central Foveal Thickness, MV = Macular volume.

	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 \geq 69 ETDRS letters (\geq 20/40 Snellen) and according to the baseline CFT; < 500 microns or \geq 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 \geq 69 ETDRS letters (\geq 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 \geq 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).



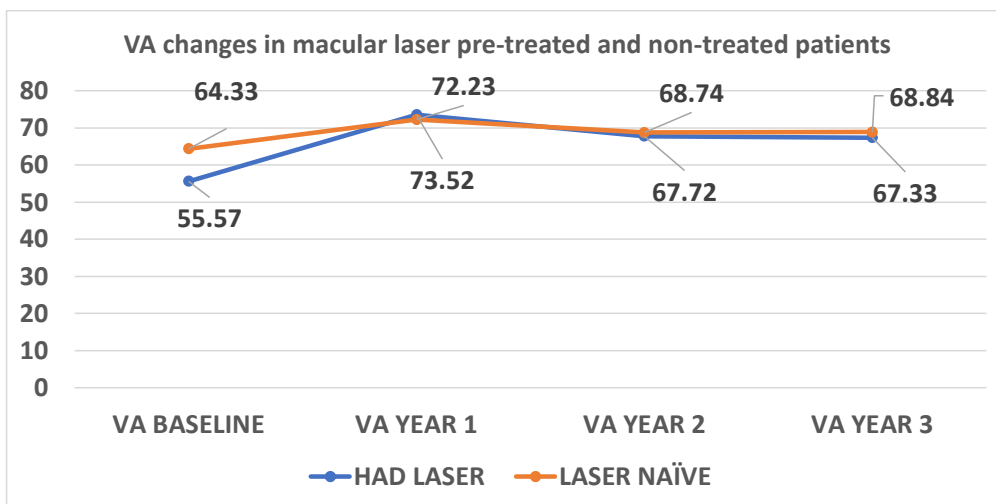
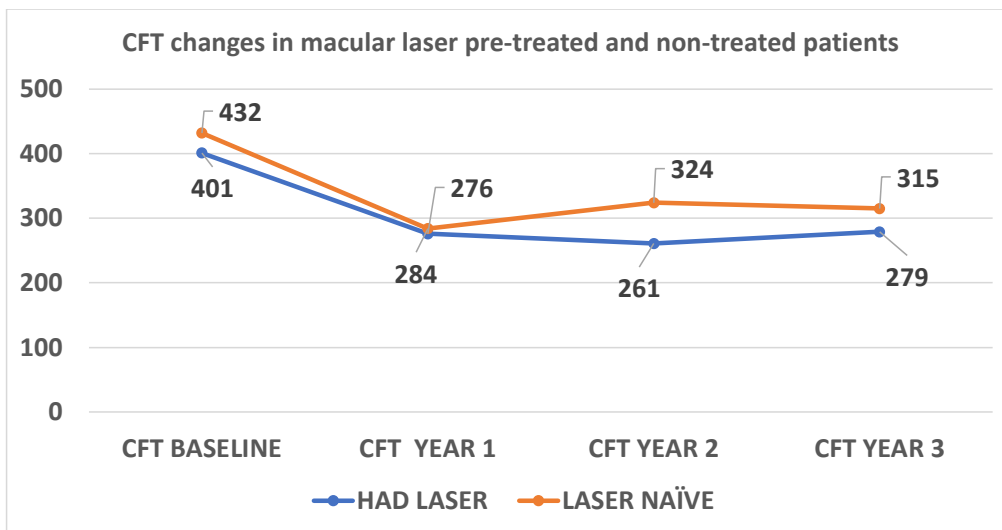
C) Subgroup analyses according to lens status and history on pre-treatment with macular laser

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 year 3
	Mean CFT baseline	Mean CFT year 1	Mean CFT year 2	
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.

10.3.5. References

1. Yau JW, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes care*. 2012; 35: 556-64.
2. Keenan T, Johnston R, Donachie P, Sparrow J, Stratton I and Scanlon P. United Kingdom National Ophthalmology Database Study: Diabetic Retinopathy; Report 1: prevalence of centre-involving diabetic macular oedema and other grades of maculopathy and retinopathy in hospital eye services. *Eye*. 2013; 27: 1397.
3. Diabetes Prevalence. Available at <https://www.diabetes.co.uk/diabetes-prevalence.html> (2019, accessed on 22nd of September 2019).
4. New CDC report: More than 100 million Americans have diabetes or prediabetes. Available at <https://www.cdc.gov/media/releases/2017/p0718-diabetes-report.html> (2017, accessed on 22nd of September 2019).
5. Diabetes: cases and costs predicted to rise. Available at <https://www.nhs.uk/news/diabetes/diabetes-cases-and-costs-predicted-to-rise/#> (2012, accessed on 22nd of September 2019).
6. The Costs of Diabetes. Available at <https://www.diabetes.org/resources/statistics/cost-diabetes>. (2018, accessed on 22nd of September 2019).
7. Whiting DR, Guariguata L, Weil C and Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes research and clinical practice*. 2011; 94: 311-21.
8. Wells JA, Glassman AR, Ayala AR, et al. Aflibercept, Bevacizumab, or Ranibizumab for Diabetic Macular Edema: Two-Year Results from a Comparative Effectiveness Randomized Clinical Trial. *Ophthalmology*. 2016; 123: 1351-9.

9. Bressler SB, Glassman AR, Almkhatar T, et al. Five-Year Outcomes of Ranibizumab With Prompt or Deferred Laser Versus Laser or Triamcinolone Plus Deferred Ranibizumab for Diabetic Macular Edema. *American journal of ophthalmology*. 2016; 164: 57-68.
10. Boyer DS, Nguyen QD, Brown DM, et al. Outcomes with as-needed ranibizumab after initial monthly therapy: long-term outcomes of the phase III RIDE and RISE trials. *Ophthalmology*. 2015; 122: 2504-13. e1.
11. Elman MJ, Aiello LP, Beck RW, et al. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology*. 2010; 117: 1064-77.e35.
12. Heier JS, Korobelnik J-F, Brown DM, et al. Intravitreal aflibercept for diabetic macular edema: 148-week results from the VISTA and VIVID studies. *Ophthalmology*. 2016; 123: 2376-85.
13. Rajendram R, Fraser-Bell S, Kaines A, et al. A 2-year prospective randomized controlled trial of intravitreal bevacizumab or laser therapy (BOLT) in the management of diabetic macular edema: 24-month data: report 3. *Archives of ophthalmology*. 2012; 130: 972-9.
14. Virgili G, Parravano M, Evans JR, Gordon I and Lucenteforte E. Anti-vascular endothelial growth factor for diabetic macular oedema: a network meta-analysis. *Cochrane Database of Systematic Reviews*. 2017.
15. Brown DM, Nguyen QD, Marcus DM, et al. Long-term outcomes of ranibizumab therapy for diabetic macular edema: the 36-month results from two phase III trials: RISE and RIDE. *Ophthalmology*. 2013; 120: 2013-22.

16. Kiss S, Liu Y, Brown J, et al. Clinical utilization of anti-vascular endothelial growth-factor agents and patient monitoring in retinal vein occlusion and diabetic macular edema. *Clinical ophthalmology (Auckland, NZ)*. 2014; 8: 1611.
17. Ziemssen F, Wachtlin J, Kuehlewein L, et al. Intravitreal Ranibizumab Therapy for Diabetic Macular Edema in Routine Practice: Two-Year Real-Life Data from a Non-interventional, Multicenter Study in Germany. *Diabetes Therapy*. 2018; 9: 2271-89.
18. Peto T and Chakravarthy U. New Findings From Diabetic Retinopathy Clinical Research Retina Network Protocol V Confirm a Role for Focal Laser Photocoagulation or Observation for Eyes With Center-Involved Diabetic Macular Edema and Good Visual Acuity: New Is Not Always Best. *JAMA ophthalmology*. 2019.

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 \geq 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

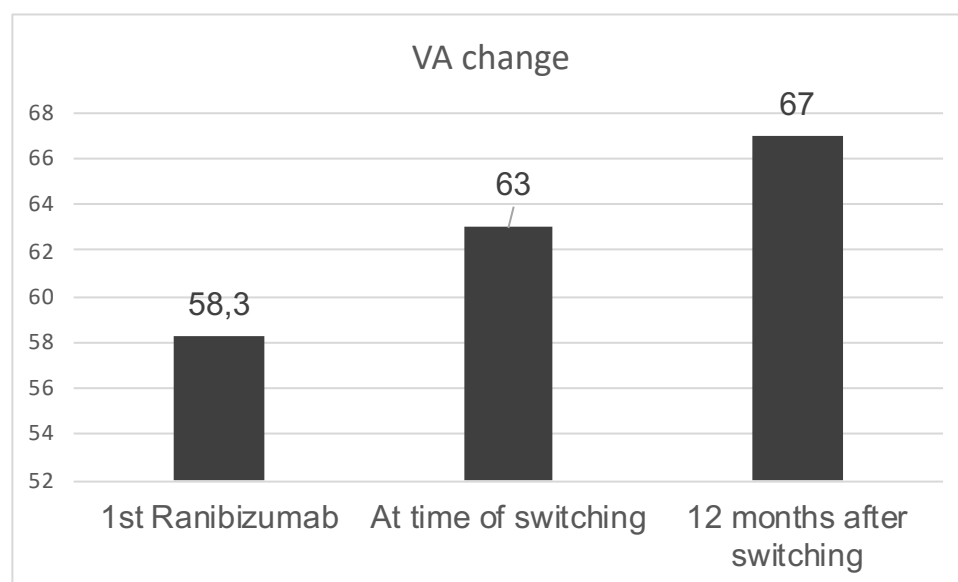


Figure 1. Change in visual acuity from 1st ranibizumab injection to end of 12-month follow up period post switching to aflibercept; VA = visual acuity

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 \geq 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 \pm 5.20) intravitreal ranibizumab injections (prior switching) and mean 6.58 (SD \pm 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

1. Yau JW, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes care*. 2012; 35: 556-64.
2. Wells JA, Glassman AR, Ayala AR, et al. Aflibercept, Bevacizumab, or Ranibizumab for Diabetic Macular Edema: Two-Year Results from a Comparative Effectiveness Randomized Clinical Trial. *Ophthalmology*. 2016; 123: 1351-9.
3. Bressler SB, Glassman AR, Almkhatar T, et al. Five-Year Outcomes of Ranibizumab With Prompt or Deferred Laser Versus Laser or Triamcinolone Plus Deferred Ranibizumab for Diabetic Macular Edema. *American journal of ophthalmology*. 2016; 164: 57-68.
4. Heier JS, Korobelnik J-F, Brown DM, et al. Intravitreal aflibercept for diabetic macular edema: 148-week results from the VISTA and VIVID studies. *Ophthalmology*. 2016; 123: 2376-85.
5. Boyer DS, Nguyen QD, Brown DM, Basu K and Ehrlich JS. Outcomes with As-Needed Ranibizumab after Initial Monthly Therapy: Long-Term Outcomes of the Phase III RIDE and RISE Trials. *Ophthalmology*. 2015; 122: 2504-13.e1.
6. Rajendram R, Fraser-Bell S, Kaines A, et al. A 2-year prospective randomized controlled trial of intravitreal bevacizumab or laser therapy (BOLT) in the management of diabetic macular edema: 24-month data: report 3. *Archives of ophthalmology*. 2012; 130: 972-9.
7. Virgili G, Parravano M, Evans JR, Gordon I and Lucenteforte E. Anti-vascular endothelial growth factor for diabetic macular oedema: a network meta-analysis. *Cochrane Database of Systematic Reviews*. 2017.

8. Schmidt-Erfurth U, Garcia-Arumi J, Bandello F, et al. Guidelines for the management of diabetic macular edema by the European Society of Retina Specialists (EURETINA). *Ophthalmologica*. 2017; 237: 185-222.
9. Stewart MW. Pharmacokinetics, pharmacodynamics and pre-clinical characteristics of ophthalmic drugs that bind VEGF. *Expert review of clinical pharmacology*. 2014; 7: 167-80.
10. Rahimy E, Shahlaee A, Khan MA, et al. Conversion to aflibercept after prior anti-VEGF therapy for persistent diabetic macular edema. *American journal of ophthalmology*. 2016; 164: 118-27. e2.
11. Shah CP and Heier JS. Aflibercept for diabetic macular edema in eyes previously treated with ranibizumab and/or bevacizumab may further improve macular thickness. *Ophthalmic Surgery, Lasers and Imaging Retina*. 2016; 47: 836-9.
12. Lim LS, Ng WY, Mathur R, et al. Conversion to aflibercept for diabetic macular edema unresponsive to ranibizumab or bevacizumab. *Clinical Ophthalmology (Auckland, NZ)*. 2015; 9: 1715.
13. Wood EH, Karth PA, Moshfeghi DM and Leng T. Short-term outcomes of aflibercept therapy for diabetic macular edema in patients with incomplete response to ranibizumab and/or bevacizumab. *Ophthalmic Surgery, Lasers and Imaging Retina*. 2015; 46: 950-4.
14. Mira F, Paulo M, Henriques F and Figueira J. Switch to aflibercept in diabetic macular edema patients unresponsive to previous anti-VEGF therapy. *Journal of ophthalmology*. 2017; 2017.

15. Bahrami B, Hong T, Zhu M, Schlub TE and Chang A. Switching therapy from bevacizumab to aflibercept for the management of persistent diabetic macular edema. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 2017; 255: 1133-40.

16. Bressler NM, Beaulieu WT, Glassman AR, et al. Persistent macular thickening following intravitreal aflibercept, bevacizumab, or ranibizumab for central-involved diabetic macular edema with vision impairment: a secondary analysis of a randomized clinical trial. *JAMA ophthalmology*. 2018; 136: 257-69.

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](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 2nd and 3rd 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 months	12 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 significant change in vision.

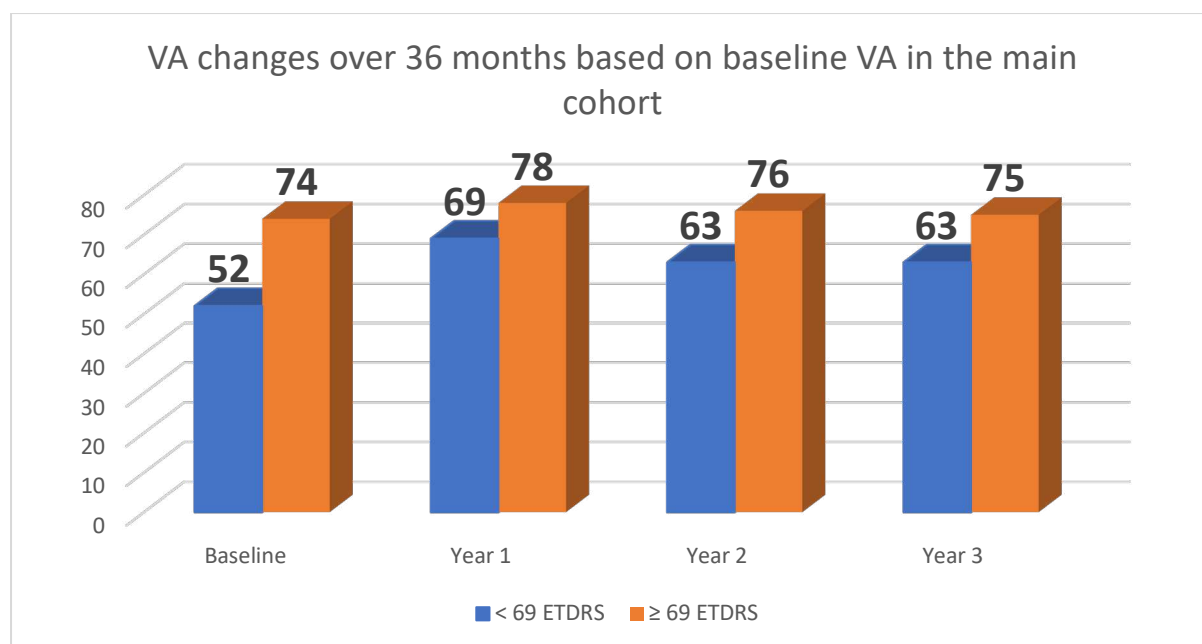


Figure 1. Visual acuity changes over 36 months based on baseline visual acuity (< 69 ETDRS letters at baseline and > 69 ETDRS letters at baseline).

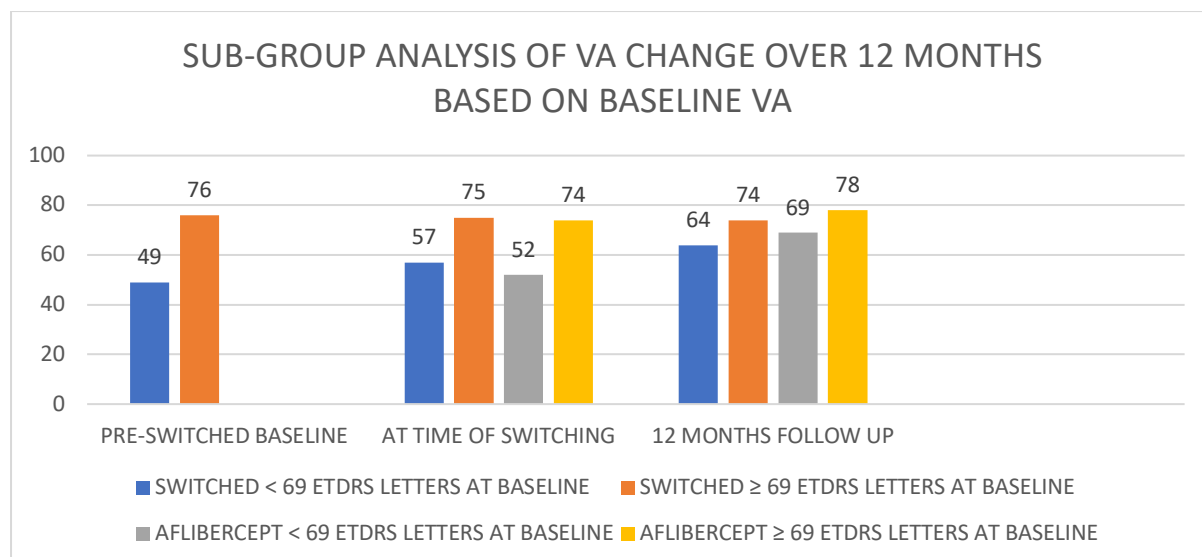


Figure 2. Visual acuity changes over 12 months based on baseline visual acuity (< 69 ETDRS letters at baseline and > 69 ETDRS letters at baseline). The results contain data from both cohorts, treatment-naïve and switched.

	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	-	-0.04	-
year 2			
CFT MEAN baseline	431 (129)	422 (138)	418 (158)
(SD) microns			
CFT MEAN 12 MONTHS	306 (122)	282 (100)	281 (110)
(SD) microns			
CFT change from baseline	-128	-140	-137
CFT MEAN 24 MONTHS	-	303 (93)	-
(SD) microns			
CFT CHANGE microns from baseline	-	-119	-
CFT CHANGE microns from year 1	-	21	-
CFT MEAN 36 MONTHS	-	303 (106)	-
(SD) microns			
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 CHANGE mm3 from baseline	MEAN	-	-1.24	-
MV CHANGE mm3 from year 1	MEAN	-	0.14	-
MV MONTHS (SD) mm3	MEAN 36	-	8.35 (1.62)	-
MV CHANGE mm3 from baseline	MEAN	-	-1.16	-
MV CHANGE mm3 from year 1	MEAN	-	0.22	-
MV CHANGE mm3 from year 2	MEAN	-	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. Therefore, 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)		
<i>ETDRS letters</i>	62.57 (14.37)	63.65 (17.79)
VA MEAN 12 MONTHS (SD)		
<i>ETDRS letters</i>	65.66 (15.66)	69.38 (15.96)
VA CHANGE <i>ETDRS letters</i>	3.09	5.73
CFT MEAN BASELINE (SD) <i>microns</i>	410 (167)	428 (147)
CFT MEAN 12 MONTHS (SD) <i>microns</i>	273 (123)	291 (91)
CFT CHANGE <i>microns</i>	-137	-137
MV MEAN BASELINE (SD) <i>mm3</i>	10.15 (2.60)	9.69 (2.20)
MV MEAN 12 MONTHS (SD) <i>mm3</i>	8.63 (2.08)	8.14 (1.31)
MV MEAN CHANGE <i>mm3</i>	-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 ETDRS 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-naïve 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.

15. REFERENCES

- 1 Diabetes Prevalence. Available at <https://www.diabetes.co.uk/diabetes-prevalence.html> (2019, accessed on 23rd of February 2021).
- 2 New CDC report: More than 100 million Americans have diabetes or prediabetes. Available at <https://www.cdc.gov/media/releases/2017/p0718-diabetes-report.html> (2017, accessed on 23rd of February 2021).
- 3 International Diabetes Federation on diabetes in Europe. Available at <https://idf.org/our-network/regions-members/europe/welcome.html> (2021, accessed on 23rd of February 2021).
- 4 Prevalence of diabetes mellitus in Croatia. Available at: <https://www.hzjz.hr/sluzba-epidemiologija-prevencija-nezaraznih-nezaraznih-bolest/dijabetes/> (2020, accessed on 23rd of February 2021).
- 5 Whiting DR, Guariguata L, Weil C, and Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes research and clinical practice*. 2011; 94: 311-21.
- 6 Hex N, Bartlett C, Wright D, Taylor M, Varley DJ. Estimating the current and future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs. *Diabetic medicine*. 2012 Jul;29(7):855-62.
- 7 The Costs of Diabetes. Available at <https://www.diabetes.org/resources/statistics/cost-diabetes>. (2018, accessed on 23rd of February 2021).
- 8 Šarić T, Poljičanin T, Metelko Ž. COST OF DIABETES COMPLICATIONS TREATMENT Effect of improving glycemic control, blood pressure and lipid status on the occurrence of complications and costs of disease treatment. *Liječnički vjesnik*. 2013 Jun 27;135(5-6):0-.

9 Yau JW, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes care*. 2012; 35: 556-64.

10 Keenan T, Johnston R, Donachie P, Sparrow J, Stratton I and Scanlon P. United Kingdom National Ophthalmology Database Study: Diabetic Retinopathy; Report 1: prevalence of centre-involving diabetic macular oedema and other grades of maculopathy and retinopathy in hospital eye services. *Eye*. 2013; 27: 1397.

11 EURETINA Report iz 2017: Available at:

https://www.euretina.org/downloads/EURETINA_Retinal_Diseases.pdf (2017, accessed on 23rd of February 2021).

12 Sabanayagam C, Banu R, Chee ML, Lee R, Wang YX, Tan G, Jonas JB, Lamoureux EL, Cheng CY, Klein BE, Mitchell P. Incidence and progression of diabetic retinopathy: a systematic review. *The lancet Diabetes & endocrinology*. 2019 Feb 1;7(2):140-9.

13 Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, Haffner S. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes care*. 2012 Mar 1;35(3):556-64.

14 Bahrami B, Zhu M, Hong T, Chang A. Diabetic macular oedema: pathophysiology, management challenges and treatment resistance. *Diabetologia*. 2016 Aug;59(8):1594-608.

15 Das A, McGuire PG, Rangasamy S. Diabetic macular edema: pathophysiology and novel therapeutic targets. *Ophthalmology*. 2015 Jul 1;122(7):1375-94.

16 Klaassen I, Van Noorden CJ, Schlingemann RO. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. *Progress in retinal and eye research*. 2013 May 1;34:19-48.

17 Dong N, Xu B, Chu L, Tang X. Study of 27 aqueous humor cytokines in type 2 diabetic patients with or without macular edema. *PloS one*. 2015 Apr 29;10(4):e0125329.

18 Funk M, Schmidinger G, Maar N, Bolz M, Benesch T, Zlabinger GJ, Schmidt-Erfurth UM. Angiogenic and inflammatory markers in the intraocular fluid of eyes with diabetic macular edema and influence of therapy with bevacizumab. *Retina*. 2010 Oct 1;30(9):1412-9.

19 Miller JW, Adamis AP, Shima DT, D'Amore PA, Moulton RS, O'Reilly MS, Folkman J, Dvorak HF, Brown LF, Berse B, Yeo TK. Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogenesis in a primate model. *The American journal of pathology*. 1994 Sep;145(3):574.

20 Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, and Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science*. 1983;219:983-5.

21 Pasqualetti G, Danesi R, Del Tacca M, Bocci G. Vascular endothelial growth factor pharmacogenetics: a new perspective for anti-angiogenic therapy.

22 Ng EW, Adamis AP. Targeting angiogenesis, the underlying disorder in neovascular age-related macular degeneration. *Canadian Journal of Ophthalmology*. 2005 Jun 1;40(3):352-68.

23 Zhang X, Zeng H, Bao S, Wang N, Gillies MC. Diabetic macular edema: new concepts in patho-physiology and treatment. *Cell & bioscience*. 2014 Dec;4(1):1-4.

24 Melder RJ, Koenig GC, Witwer BP, Safabakhsh N, Munn LL, Jain RK. During angiogenesis, vascular endothelial growth factor and basic fibroblast growth factor regulate natural killer cell adhesion to tumor endothelium. *Nature medicine*. 1996 Sep 1;2(9):992-7.

25 Jousen AM, Poulaki V, Qin W, Kirchhof B, Mitsiades N, Wiegand SJ, Rudge J, Yancopoulos GD, Adamis AP. Retinal vascular endothelial growth factor induces intercellular adhesion molecule-1 and endothelial nitric oxide synthase expression and initiates early diabetic retinal leukocyte adhesion in vivo. *The American journal of pathology*. 2002 Feb 1;160(2):501-9.

- 26 Ishida S, Usui T, Yamashiro K, Kaji Y, Ahmed E, Carrasquillo KG, Amano S, Hida T, Oguchi Y, Adamis AP. VEGF164 is proinflammatory in the diabetic retina. *Investigative ophthalmology & visual science*. 2003 May 1;44(5):2155-62.
- 27 Amadio M, Scapagnini G, Lupo G, Drago F, Govoni S, Pascale A. PKC β II/HuR/VEGF: a new molecular cascade in retinal pericytes for the regulation of VEGF gene expression. *Pharmacological research*. 2008 Jan 1;57(1):60-6.
- 28 Harhaj NS, Felinski EA, Wolpert EB, Sundstrom JM, Gardner TW, Antonetti DA. VEGF activation of protein kinase C stimulates occludin phosphorylation and contributes to endothelial permeability. *Investigative ophthalmology & visual science*. 2006 Nov 1;47(11):5106-15.
- 29 Soetikno V, Sari FR, Sukumaran V, Lakshmanan AP, Mito S, Harima M, Thandavarayan RA, Suzuki K, Nagata M, Takagi R, Watanabe K. Curcumin prevents diabetic cardiomyopathy in streptozotocin-induced diabetic rats: possible involvement of PKC–MAPK signaling pathway. *European Journal of Pharmaceutical Sciences*. 2012 Oct 9;47(3):604-14.
- 30 Okamoto T, Yamagishi SI, Inagaki Y, Amano S, Koga K, Abe R, Takeuchi M, Ohno S, Yoshimura A, Makita Z. Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin. *The FASEB Journal*. 2002 Dec;16(14):1928-30.
- 31 Akagi YP, Kador PF, Kuwabara T, Kinoshita JH. Aldose reductase localization in human retinal mural cells. *Investigative ophthalmology & visual science*. 1983 Nov 1;24(11):1516-9.
- 32 Tilton RG. Diabetic vascular dysfunction: links to glucose-induced reductive stress and VEGF. *Microscopy research and technique*. 2002 Jun 1;57(5):390-407.
- 33 Nakamura T, Ohta M, Sugiura M, Sugita M. Chloroplast ribonucleoproteins function as a stabilizing factor of ribosome-free mRNAs in the stroma. *Journal of Biological Chemistry*. 2001 Jan 5;276(1):147-52.

34 Xue M, Qian Q, Adaikalakoteswari A, Rabbani N, Babaei-Jadidi R, Thornalley PJ. Activation of NF-E2–related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. *Diabetes*. 2008 Oct 1;57(10):2809-17.

35 Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nature medicine*. 2003 Mar;9(3):294-9.

36 Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomarker insights*. 2016 Jan;11:BMIS38440.

37 Clinical information on role of the HbA1c. Available at: <https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/82080> (2017-2021, visited on 29th of August 2021).

38 HbA1c Test for Diabetes: Available at: <https://www.webmd.com/diabetes/guide/glycated-hemoglobin-test-hba1c> (2020, visited on 29th of August 2021).

39 New HbA1c units. Available at: <https://www.iddt.org/news/new-hba1c-units> (2021, visited on 29th of August 2021).

40 Mathur R, Bhaskaran K, Edwards E, Lee H, Chaturvedi N, Smeeth L, Douglas I. Population trends in the 10-year incidence and prevalence of diabetic retinopathy in the UK: a cohort study in the Clinical Practice Research Datalink 2004–2014. *BMJ open*. 2017 Feb 1;7(2):e014444.

41 Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *New England journal of medicine*. 1993 Sep 30;329(14):977-86.

- 42 Nathan DM, DCCT/Edic Research Group. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. *Diabetes care*. 2014 Jan 1;37(1):9-16.
- 43 King P, Peacock I, Donnelly R. The UK prospective diabetes study (UKPDS): clinical and therapeutic implications for type 2 diabetes. *British journal of clinical pharmacology*. 1999 Nov;48(5):643.
- 44 Stratton IM. UK Prospective Diabetes Study Group: Association of glycemia with macrovascular and microvascular complications in type 2 diabetes (UKPDS 35): prospective observational study. *BMJ*. 2000;31:405-12.
- 45 Ferris FL, Nathan DM. Preventing diabetic retinopathy progression. *Ophthalmology*. 2016 Sep 1;123(9):1840-2.
- 46 Chou TH, Wu PC, Kuo JZ, Lai CH, Kuo CN. Relationship of diabetic macular oedema with glycosylated haemoglobin. *Eye*. 2009 Jun;23(6):1360-3.
- 47 Singh RP, Wykoff CC, Brown DM, Larsen M, Terasaki H, Silva FQ, Saroj N, Gibson A, Vitti R, Kayshap S, Berliner AJ. Outcomes of diabetic macular edema patients by baseline hemoglobin A1c: analyses from VISTA and VIVID. *Ophthalmology Retina*. 2017 Sep 1;1(5):382-8.
- 48 Bansal AS, Khurana RN, Wieland MR, Wang PW, Van Everen SA, Tuomi L. Influence of glycosylated hemoglobin on the efficacy of ranibizumab for diabetic macular edema: a post hoc analysis of the RIDE/RISE trials. *Ophthalmology*. 2015 Aug 1;122(8):1573-9.
- 49 Shalchi Z, Okada M, Bruynseels A, Palethorpe D, Yusuf A, Hussain R, Herrspiegel C, Scazzariello A, Habib A, Amin R, Rajendram R. Effect of glycosylated hemoglobin on response to ranibizumab therapy in diabetic macular edema: real-world outcomes in 312 patients. *Canadian Journal of Ophthalmology*. 2018 Aug 1;53(4):415-9.

50 Yeung L, Sun CC, Ku WC, Chuang LH, Chen CH, Huang BY, Ting MK, Yang KJ. Associations between chronic glycosylated haemoglobin (HbA1c) level and macular volume in diabetes patients without macular oedema. *Acta ophthalmologica*. 2010 Nov;88(7):753-8.

51 Peng YJ, Tsai MJ. Impact of metabolic control on macular thickness in diabetic macular oedema. *Diabetes and Vascular Disease Research*. 2018 Mar;15(2):165-8.

52 Wise GN. Retinal neovascularization. *Transactions of the American Ophthalmological Society*. 1956;54:729.

53 Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, Nguyen HV. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *New England Journal of Medicine*. 1994 Dec 1;331(22):1480-7.

54 Gragoudas ES, Adamis AP, Cunningham Jr ET, Feinsod M, Guyer DR. Pegaptanib for neovascular age-related macular degeneration. *New England Journal of Medicine*. 2004 Dec 30;351(27):2805-16.

55 Sharma A, Parachuri N, Kumar N, Sharma R, Bandello F, Kuppermann BD, Loewenstein A. Brolucizumab—another anti-VEGF or beyond. *Eye*. 2020 Sep;34(9):1499-500.

56 EMA approval of brolucizumab. Available at:
<https://eyewire.news/articles/novartis-receives-european-approval-for-beovu-anti-veg-f-treatment-for-wet-amd/> (2021, visited on 4th of September 2021)

57 Brolicizumab in development for DMO. Available at:
<https://www.io.nihr.ac.uk/report/brolucizumab-for-visual-impairment-due-to-diabetic-macular-oedema/> (2020, visited on 4th of September 2021)

58 EMEA Scientific Communication on Bevaciumab. Available at:
https://www.ema.europa.eu/en/documents/scientific-discussion/avastin-epar-scientific-discussion_en.pdf (2005. Visited on 29th of August 2021.).

59 Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *New England journal of medicine*. 2004 Jun 3;350(23):2335-42.

60 Chen Y, Wiesmann C, Fuh G, Li B, Christinger HW, McKay P, de Vos AM, Lowman HB. Selection and analysis of an optimized anti-VEGF antibody: crystal structure of an affinity-matured Fab in complex with antigen. *Journal of molecular biology*. 1999 Nov 5;293(4):865-81.

61 Lien S, Lowman HB. Therapeutic anti-VEGF antibodies. *Therapeutic antibodies*. 2008:131-50.

62 Lucentis FDA Approval History. Available at: <https://www.drugs.com/history/lucentis.html> (2000-2021, visited on 29th of August 2021.).

63 Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, Boland P, Leidich R, Hylton D, Burova E, Ioffe E. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proceedings of the National Academy of Sciences*. 2002 Aug 20;99(17):11393-8.

64 Papadopoulos, N., Martin, J., Ruan, Q., Rafique, A., Rosconi, M.P., Shi, E., Pyles, E.A., Yancopoulos, G.D., Stahl, N. and Wiegand, S.J., 2012. Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. *Angiogenesis*, 15(2), pp.171-185.

65 Stewart MW, Rosenfeld PJ. Predicted biological activity of intravitreal VEGF Trap. *British Journal of Ophthalmology*. 2008 May 1;92(5):667-8.

66 Aflibercept FDA Approval History. Available at: <https://www.drugs.com/history/eylea.html> (2000-2021. Visited on 29th of August 2021.).

67 Fogli S, Del Re M, Rofi E, Posarelli C, Figus M, Danesi R. Clinical pharmacology of intravitreal anti-VEGF drugs. *Eye*. 2018 Jun;32(6):1010-20.

68 Lowe J, Araujo J, Yang J, Reich M, Oldendorp A, Shiu V, Quarmby V, Lowman H, Lien S, Gaudreault J, Maia M. Ranibizumab inhibits multiple forms of biologically active vascular endothelial growth factor in vitro and in vivo. *Experimental eye research*. 2007 Oct 1;85(4):425-30.

69 Babushkina EA, Belokopytova LV, Grachev AM, Meko DM, Vaganov EA. Variation of the hydrological regime of Bele-Shira closed basin in Southern Siberia and its reflection in the radial growth of *Larix sibirica*. *Regional Environmental Change*. 2017 Aug;17(6):1725-37.

70 Presta LG, Chen H, O'connor SJ, Chisholm V, Meng YG, Krummen L, Winkler M, Ferrara N. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer research*. 1997 Oct 15;57(20):4593-9.

71 Yang J, Wang X, Fuh G, Yu L, Wakshull E, Khosraviani M, Day ES, Demeule B, Liu J, Shire SJ, Ferrara N. Comparison of binding characteristics and in vitro activities of three inhibitors of vascular endothelial growth factor A. *Molecular pharmaceutics*. 2014 Oct 6;11(10):3421-30.

72 Yu L, Liang XH, Ferrara N. Comparing protein VEGF inhibitors: in vitro biological studies. *Biochemical and biophysical research communications*. 2011 May 6;408(2):276-81.

73 García-Quintanilla L, Luaces-Rodríguez A, Gil-Martínez M, Mondelo-García C, Maroñas O, Mangas-Sanjuan V, González-Barcia M, Zarra-Ferro I, Aguiar P, Otero-Espinar FJ, Fernández-Ferreiro A. Pharmacokinetics of intravitreal anti-VEGF drugs in age-related macular degeneration. *Pharmaceutics*. 2019 Aug;11(8):365.

74 Park SJ, Oh J, Kim YK, Park JH, Park JY, Hong HK, Park KH, Lee JE, Kim HM, Chung JY, Woo SJ. Intraocular pharmacokinetics of intravitreal vascular endothelial growth factor-Trap in a rabbit model. *Eye*. 2015 Apr;29(4):561-8.

75 Kushwaha SK, Saxena P, Rai A. The adsorption of proteins from aqueous solution on solid surfaces. *J Control Release*. 2014;13:208-21.

- 76 Ahn SJ, Ahn J, Park S, Kim H, Hwang DJ, Park JH, Park JY, Chung JY, Park KH, Woo SJ. Intraocular pharmacokinetics of ranibizumab in vitrectomized versus nonvitrectomized eyes. *Investigative ophthalmology & visual science*. 2014 Jan 1;55(1):567-73.
- 77 Kim H, Robinson SB, Csaky KG. FcRn receptor-mediated pharmacokinetics of therapeutic IgG in the eye. *Molecular vision*. 2009;15:2803.
- 78 Bakri SJ, Snyder MR, Reid JM, Pulido JS, Singh RJ. Pharmacokinetics of intravitreal bevacizumab (Avastin). *Ophthalmology*. 2007 May 1;114(5):855-9.
- 79 Boyer DS, Hopkins JJ, Sorof J, Ehrlich JS. Anti-vascular endothelial growth factor therapy for diabetic macular edema. *Therapeutic advances in endocrinology and metabolism*. 2013 Dec;4(6):151-69.
- 80 Diabetic Retinopathy Clinical Research Network. Randomized trial evaluating short-term effects of intravitreal ranibizumab or triamcinolone acetonide on macular edema following focal/grid laser for diabetic macular edema in eyes also receiving panretinal photocoagulation. *Retina (Philadelphia, Pa.)*. 2011 Jun;31(6):1009.
- 81 Bressler NM, Varma R, Suñer IJ, Dolan CM, Ward J, Ehrlich JS, Colman S, Turpcu A. Vision-related function after ranibizumab treatment for diabetic macular edema: results from RIDE and RISE. *Ophthalmology*. 2014 Dec 1;121(12):2461-72.
- 82 Schmidt-Erfurth U, Lang GE, Holz FG, Schlingemann RO, Lanzetta P, Massin P, Gerstner O, Bouazza AS, Shen H, Osborne A, Mitchell P. Three-year outcomes of individualized ranibizumab treatment in patients with diabetic macular edema: the RESTORE extension study. *Ophthalmology*. 2014 May 1;121(5):1045-53.
- 83 Lang GE, Berta A, Eldem BM, Simader C, Sharp D, Holz FG, Sutter F, Gerstner O, Mitchell P, RESTORE Extension Study Group. Two-year safety and efficacy of ranibizumab 0.5 mg in diabetic macular edema: interim analysis of the RESTORE extension study. *Ophthalmology*. 2013 Oct 1;120(10):2004-12.

84 Brown DM, Nguyen QD, Marcus DM, Boyer DS, Patel S, Feiner L, Schlottmann PG, Rundle AC, Zhang J, Rubio RG, Adamis AP. Long-term outcomes of ranibizumab therapy for diabetic macular edema: the 36-month results from two phase III trials: RISE and RIDE. *Ophthalmology*. 2013 Oct 1;120(10).

85 Nguyen QD, Brown DM, Marcus DM, Boyer DS, Patel S, Feiner L, Gibson A, Sy J, Rundle AC, Hopkins JJ, Rubio RG. Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. *Ophthalmology*. 2012 Apr 1;119(4):789-801.

86 Mitchell P, Bandello F, Schmidt-Erfurth U, Lang GE, Massin P, Schlingemann RO, Sutter F, Simader C, Burian G, Gerstner O, Weichselberger A. The RESTORE study: ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema. *Ophthalmology*. 2011 Apr 1;118(4):615-25.

87 Elman MJ, Bressler NM, Qin H, Beck RW, Ferris III FL, Friedman SM, Glassman AR, Scott IU, Stockdale CR, Sun JK, Diabetic Retinopathy Clinical Research Network. Expanded 2-year follow-up of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology*. 2011 Apr 1;118(4):609-14.

88 Nguyen QD, Shah SM, Khwaja AA, Channa R, Hatef E, Do DV, Boyer D, Heier JS, Abraham P, Thach AB, Lit ES. Two-year outcomes of the ranibizumab for edema of the macula in diabetes (READ-2) study. *Ophthalmology*. 2010 Nov 1;117(11):2146-51.

89 Elman MJ, Aiello LP, Beck RW, Bressler NM, Bressler SB, Edwards AR, Ferris III FL, Friedman SM, Glassman AR, Miller KM, Scott IU. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology*. 2010 Jun 1;117(6):1064-77.

90 Wells JA, Glassman AR, Ayala AR, Jampol LM, Aiello LP, Antoszyk AN, Arnold-Bush B, Baker CW, Bressler NM, Browning DJ, Elman MJ. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *The New England journal of medicine*. 2015 Mar;372(13):1193-203.

91 Do DV, Nguyen QD, Khwaja AA, Channa R, Sepah YJ, Sophie R, Hafiz G, Campochiaro PA, READ-2 Study Group. Ranibizumab for edema of the macula in diabetes study: 3-year outcomes and the need for prolonged frequent treatment. *JAMA ophthalmology*. 2013 Feb 1;131(2):139-45.

92 Glassman AR, Wells III JA, Josic K, Maguire MG, Antoszyk AN, Baker C, Beaulieu WT, Elman MJ, Jampol LM, Sun JK. Five-year outcomes after initial aflibercept, bevacizumab, or ranibizumab treatment for diabetic macular edema (Protocol T Extension Study). *Ophthalmology*. 2020 Sep 1;127(9):1201-10.

93 Pieramici DJ, Wang PW, Ding B, Gune S. Visual and anatomic outcomes in patients with diabetic macular edema with limited initial anatomic response to ranibizumab in RIDE and RISE. *Ophthalmology*. 2016 Jun 1;123(6):1345-50.

94 Wells JA, Glassman AR, Ayala AR, Jampol LM, Bressler NM, Bressler SB, Brucker AJ, Ferris FL, Hampton GR, Jhaveri C, Melia M. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema: two-year results from a comparative effectiveness randomized clinical trial. *Ophthalmology*. 2016 Jun 1;123(6):1351-9.

95 Boyer DS, Nguyen QD, Brown DM, Basu K, Ehrlich JS, Ride and Rise Research Group. Outcomes with as-needed ranibizumab after initial monthly therapy: long-term outcomes of the phase III RIDE and RISE trials. *Ophthalmology*. 2015 Dec 1;122(12):2504-13.

96 Pearce I, Banerjee S, Burton BJ, Chakravarthy U, Downey L, Gale RP, Gibson J, Pagliarini S, Patel J, Sivaprasad S, Andrews C. Ranibizumab 0.5 mg for diabetic macular edema with bimonthly monitoring after a phase of initial treatment: 18-month, multicenter, phase IIIB RELIGHT study. *Ophthalmology*. 2015 Sep 1;122(9):1811-9.

97 Ishibashi T, Li X, Koh A, Lai TY, Lee FL, Lee WK, Ma Z, Ohji M, Tan N, Cha SB, Shamsazar J. The REVEAL study: ranibizumab monotherapy or combined with laser versus laser monotherapy in Asian patients with diabetic macular edema. *Ophthalmology*. 2015 Jul 1;122(7):1402-15.

98 Elman MJ, Ayala A, Bressler NM, Browning D, Flaxel CJ, Glassman AR, Jampol LM, Stone TW, Diabetic Retinopathy Clinical Research Network. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: 5-year randomized trial results. *Ophthalmology*. 2015 Feb 1;122(2):375-81.

99 Elman MJ, Qin H, Aiello LP, Beck RW, Bressler NM, Ferris III FL, Glassman AR, Maturi RK, Melia M, Diabetic Retinopathy Clinical Research Network. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: three-year randomized trial results. *Ophthalmology*. 2012 Nov 1;119(11):2312-8.

100 Li X, Dai H, Li X, Han M, Li J, Suhner A, Lin R, Wolf S. Efficacy and safety of ranibizumab 0.5 mg in Chinese patients with visual impairment due to diabetic macular edema: results from the 12-month REFINE study. *Graefe's archive for clinical and experimental ophthalmology*. 2019 Mar;257(3):529-41.

101 Prünke C, Fajnkuchen F, Mahmood S, Ricci F, Hatz K, Studnička J, Bezlyak V, Parikh S, Stubbings WJ, Wenzel A, Figueira J. Ranibizumab 0.5 mg treat-and-extend regimen for diabetic macular oedema: the RETAIN study. *British Journal of Ophthalmology*. 2016 Jun 1;100(6):787-95.

102 Lang GE, Liakopoulos S, Vögeler J, Weiß C, Spital G, Gamulescu MA, Lohmann C, Wiedemann P. The RELATION study: efficacy and safety of ranibizumab combined with laser photocoagulation treatment versus laser monotherapy in NPDR and PDR patients with diabetic macular oedema. *Acta ophthalmologica*. 2018 May;96(3):e377-85.

103 Bressler SB, Glassman AR, Almkhatar T, Bressler NM, Ferris FL, Googe Jr JM, Gupta SK, Jampol LM, Melia M, Wells III JA, Diabetic Retinopathy Clinical Research Network. Five-year outcomes of ranibizumab with prompt or deferred laser versus laser or triamcinolone plus deferred ranibizumab for diabetic macular edema. *American journal of ophthalmology*. 2016 Apr 1;164:57-68.

104 Bressler NM, Beaulieu WT, Maguire MG, Glassman AR, Blinder KJ, Bressler SB, Gonzalez VH, Jampol LM, Melia M, Sun JK, Wells III JA. Early response to anti-vascular

endothelial growth factor and two-year outcomes among eyes with diabetic macular edema in protocol T. *American journal of ophthalmology*. 2018 Nov 1;195:93-100.

105 Korobelnik JF, Do DV, Schmidt-Erfurth U, Boyer DS, Holz FG, Heier JS, Midena E, Kaiser PK, Terasaki H, Marcus DM, Nguyen QD. Intravitreal aflibercept for diabetic macular edema. *Ophthalmology*. 2014 Nov 1;121(11):2247-54.

106 Terasaki H, Shiraki K, Ohji M, Metzigg C, Schmelter T, Zeitz O, Sowade O, Kobayashi M, Vitti R, Berliner A, Shiraga F. EFFICACY AND SAFETY OUTCOMES OF INTRAVITREAL AFLIBERCEPT FOCUSING ON PATIENTS WITH DIABETIC MACULAR EDEMA FROM JAPAN. *Retina (Philadelphia, Pa.)*. 2019 May;39(5):938.

107 Heier JS, Korobelnik JF, Brown DM, Schmidt-Erfurth U, Do DV, Midena E, Boyer DS, Terasaki H, Kaiser PK, Marcus DM, Nguyen QD. Intravitreal aflibercept for diabetic macular edema: 148-week results from the VISTA and VIVID studies. *Ophthalmology*. 2016 Nov 1;123(11):2376-85.

108 Brown DM, Schmidt-Erfurth U, Do DV, Holz FG, Boyer DS, Midena E, Heier JS, Terasaki H, Kaiser PK, Marcus DM, Nguyen QD. Intravitreal aflibercept for diabetic macular edema: 100-week results from the VISTA and VIVID studies. *Ophthalmology*. 2015 Oct 1;122(10):2044-52.

109 Ahmadi H, Ramezani A, Shoeibi N, Bijanzadeh B, Tabatabaei A, Azarmina M, Soheilian M, Keshavarzi G, Mohebbi MR. Intravitreal bevacizumab with or without triamcinolone for refractory diabetic macular edema; a placebo-controlled, randomized clinical trial. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 2008 Apr;246(4):483-9.

110 Diabetic Retinopathy Clinical Research Network. A phase II randomized clinical trial of intravitreal bevacizumab for diabetic macular edema. *Ophthalmology*. 2007 Oct 1;114(10):1860-7.

111 Lam DS, Lai TY, Lee VY, Chan CK, Liu DT, Mohamed S, Li CL. Efficacy of 1.25 MG versus 2.5 MG intravitreal bevacizumab for diabetic macular edema: six-month results of a randomized controlled trial. *Retina*. 2009 Mar 1;29(3):292-9.

112 Maturi RK, Pollack A, Uy HS, Varano M, Gomes A, Li XY, Cui H, Lou J, Hashad Y, Whitcup SM. Intraocular pressure in patients with diabetic macular edema treated with dexamethasone intravitreal implant in the 3-year MEAD study. *Retina*. 2016 Jun 1;36(6):1143-52.

113 Ip MS, Bressler SB, Antoszyk AN, Flaxel CJ, Kim JE, Friedman SM, Qin H, Diabetic Retinopathy Clinical Research Network. A randomized trial comparing intravitreal triamcinolone and focal/grid photocoagulation for diabetic macular edema: baseline features. *Retina (Philadelphia, Pa.)*. 2008 Jul;28(7):919.

114 Kim JE, Pollack JS, Miller DG, Mitra RA, Spaide RF, Isis Study Group. ISIS-DME: a prospective, randomized, dose-escalation intravitreal steroid injection study for refractory diabetic macular edema. *Retina*. 2008 May 1;28(5):735-40.

115 Cunha-Vaz J, Ashton P, Iezzi R, Campochiaro P, Dugel PU, Holz FG, Weber M, Danis RP, Kuppermann BD, Bailey C, Billman K. Sustained delivery fluocinolone acetonide vitreous implants: long-term benefit in patients with chronic diabetic macular edema. *Ophthalmology*. 2014 Oct 1;121(10):1892-903.

116 Callanan, David G., et al. "Dexamethasone intravitreal implant in combination with laser photocoagulation for the treatment of diffuse diabetic macular edema." *Ophthalmology* 120.9 (2013): 1843-1851.

117 Gillies MC, McAllister IL, Zhu M, Wong W, Louis D, Arnold JJ, Wong TY. Intravitreal triamcinolone prior to laser treatment of diabetic macular edema: 24-month results of a randomized controlled trial. *Ophthalmology*. 2011 May 1;118(5):866-72.

118 Lam DS, Chan CK, Mohamed S, Lai TY, Lee VY, Liu DT, Li KK, Li PS, Shanmugam MP. Intravitreal triamcinolone plus sequential grid laser versus triamcinolone or laser alone

for treating diabetic macular edema: six-month outcomes. *Ophthalmology*. 2007 Dec 1;114(12):2162-7.

119 Callanan DG, Loewenstein A, Patel SS, Massin P, Corcóstegui B, Li XY, Jiao J, Hashad Y, Whitcup SM. A multicenter, 12-month randomized study comparing dexamethasone intravitreal implant with ranibizumab in patients with diabetic macular edema. *Graefes Archive for Clinical and Experimental Ophthalmology*. 2017 Mar;255(3):463-73.

120 Danis RP, Sadda S, Li XY, Cui H, Hashad Y, Whitcup SM. Anatomical effects of dexamethasone intravitreal implant in diabetic macular oedema: a pooled analysis of 3-year phase III trials. *British Journal of Ophthalmology*. 2016 Jun 1;100(6):796-801.

121 Sarao V, Veritti D, Furino C, Giancipoli E, Alessio G, Boscia F, Lanzetta P. Dexamethasone implant with fixed or individualized regimen in the treatment of diabetic macular oedema: six-month outcomes of the UDBASA study. *Acta ophthalmologica*. 2017 Jun;95(4):e255-60.

122 Heng LZ, Sivaprasad S, Crosby-Nwaobi R, Saihan Z, Karampelas M, Bunce C, Peto T, Hykin PG. A prospective randomised controlled clinical trial comparing a combination of repeated intravitreal Ozurdex and macular laser therapy versus macular laser only in centre-involving diabetic macular oedema (OZLASE study). *British Journal of Ophthalmology*. 2016 Jun 1;100(6):802-7.

123 Stewart MW. Pharmacokinetics, pharmacodynamics and pre-clinical characteristics of ophthalmic drugs that bind VEGF. *Expert review of clinical pharmacology*. 2014; 7: 167-80.

124 Nguyen QD, Rodrigues EB, Farah ME, Mieler WF. *Retinal Pharmacotherapy E-Book*. Elsevier Health Sciences; 2010 Feb 26.

125 Nguyen QD, De Falco S, Behar-Cohen F, Lam WC, Li X, Reichhart N, Ricci F, Pluim J, Li WW. Placental growth factor and its potential role in diabetic retinopathy and other ocular neovascular diseases. *Acta ophthalmologica*. 2018 Feb;96(1):e1-9.

126 Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, Boland P, Leidich R, Hylton D, Burova E, Ioffe E. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proceedings of the National Academy of Sciences*. 2002 Aug 20;99(17):11393-8.

127 Yang J, Wang X, Fuh G, Yu L, Wakshull E, Khosraviani M, Day ES, Demeule B, Liu J, Shire SJ, Ferrara N. Comparison of binding characteristics and in vitro activities of three inhibitors of vascular endothelial growth factor A. *Molecular pharmaceutics*. 2014 Oct 6;11(10):3421-30.

128 Do DV, Schmidt-Erfurth U, Gonzalez VH, Gordon CM, Tolentino M, Berliner AJ, Vitti R, Rückert R, Sandbrink R, Stein D, Yang K. The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema. *Ophthalmology*. 2011 Sep 1;118(9):1819-26.

129 Holekamp NM, Campbell J, Almony A, Ingraham H, Marks S, Chandwani H, Cole AL, Kiss S. Vision outcomes following anti-vascular endothelial growth factor treatment of diabetic macular edema in clinical practice. *American journal of ophthalmology*. 2018 Jul 1;191:83-91.

130 Koç İ, Kadayıfçılar S, Eldem B. Real-world results of intravitreal ranibizumab, bevacizumab, or triamcinolone for diabetic macular edema. *Ophthalmologica*. 2018;239:85-93.

131 Farinha C, Martins A, Neves A, Soares R, Ruão M, Ornelas M, Neves P, Rodrigues FG, Coelho C, Silva R. Ranibizumab for the treatment of diabetic macular oedema in the real-world clinical setting in Portugal: a multicentre study. *Ophthalmologica*. 2019;241(1):1-8.

132 Ziemssen F, Wachtlin J, Kuehlewein L, Gamulescu MA, Bertelmann T, Feucht N, Voegeler J, Koch M, Liakopoulos S, Schmitz-Valckenberg S, Spital G. Intravitreal ranibizumab therapy for diabetic macular edema in routine practice: two-year real-life data from a non-interventional, multicenter study in Germany. *Diabetes Therapy*. 2018 Dec;9(6):2271-89.

133 Best AL, Fajnkuchen F, Nghiem-Buffer S, Grenet T, Quentel G, Delahaye-Mazza C, Cohen SY, Giocanti-Aurégan A. Treatment efficacy and compliance in patients with diabetic macular edema treated with ranibizumab in a real-life setting. *Journal of ophthalmology*. 2018 Apr 18;2018.

134 Patrao NV, Antao S, Egan C, Omar A, Hamilton R, Hykin PG, Sivaprasad S, Rajendram R, Moorfields Diabetic Macular Edema Study Group. Real-world outcomes of ranibizumab treatment for diabetic macular edema in a United Kingdom National Health Service setting. *American journal of ophthalmology*. 2016 Dec 1;172:51-7.

135 Van Aken E, Favreau M, Ramboer E, Denhaerynck K, MacDonald K, Abraham I, Brié H. Real-World Outcomes in Patients with Diabetic Macular Edema Treated Long Term with Ranibizumab (VISION Study). *Clinical Ophthalmology (Auckland, NZ)*. 2020;14:4173.

136 Menchini U, Bandello F, De Angelis V, Ricci F, Bonavia L, Viola F, Muscianisi E, Nicolò M. Ranibizumab for visual impairment due to diabetic macular edema: real-world evidence in the Italian population (PRIDE Study). *Journal of ophthalmology*. 2015 Jan 1;2015.

137 Massin P, Creuzot-Garcher C, Kodjikian L, Girmens JF, Delcourt C, Fajnkuchen F, Glacet-Bernard A, Guillausseau PJ, Ponthieux A, Blin P, Grelaud A. Real-world outcomes with Ranibizumab 0.5 mg in patients with visual impairment due to diabetic macular edema: 12-month results from the 36-month BOREAL-DME study. *Ophthalmic research*. 2019;62(2):101-10.

138 Massin P, Creuzot-Garcher C, Kodjikian L, Girmens JF, Delcourt C, Fajnkuchen F, Glacet-Bernard A, Guillausseau PJ, Guthux F, Blin P, Grelaud A. Real-world outcomes after 36 months treatment with ranibizumab 0.5 mg in patients with visual impairment due to diabetic macular edema (BOREAL-DME). *Ophthalmic Research*. 2020 Sep 15.

139 Ciulla TA, Bracha P, Pollack J, Williams DF. Real-world outcomes of anti-vascular endothelial growth factor therapy in diabetic macular edema in the United States. *Ophthalmology Retina*. 2018 Dec 1;2(12):1179-87.

140 Rahimy E, Shahlaee A, Khan MA, et al. Conversion to aflibercept after prior anti-VEGF therapy for persistent diabetic macular edema. *American journal of ophthalmology*. 2016; 164: 118-27. e2.

141 Shah CP and Heier JS. Aflibercept for diabetic macular edema in eyes previously treated with ranibizumab and/or bevacizumab may further improve macular thickness. *Ophthalmic Surgery, Lasers and Imaging Retina*. 2016; 47: 836-9.

142 Lim LS, Ng WY, Mathur R, et al. Conversion to aflibercept for diabetic macular edema unresponsive to ranibizumab or bevacizumab. *Clinical Ophthalmology (Auckland, NZ)*. 2015; 9: 1715.

143 Wood EH, Karth PA, Moshfeghi DM and Leng T. Short-term outcomes of aflibercept therapy for diabetic macular edema in patients with incomplete response to ranibizumab and/or bevacizumab. *Ophthalmic Surgery, Lasers and Imaging Retina*. 2015; 46: 950-4.

144 Mira F, Paulo M, Henriques F and Figueira J. Switch to aflibercept in diabetic macular edema patients unresponsive to previous anti-VEGF therapy. *Journal of ophthalmology*. 2017; 2017.

145 Bahrami B, Hong T, Zhu M, Schlub TE and Chang A. Switching therapy from bevacizumab to aflibercept for the management of persistent diabetic macular edema. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 2017; 255: 1133-40.

146 Peto T and Chakravarthy U. New Findings From Diabetic Retinopathy Clinical Research Retina Network Protocol V Confirm a Role for Focal Laser Photocoagulation or Observation for Eyes With Center-Involved Diabetic Macular Edema and Good Visual Acuity: New Is Not Always Best. *JAMA ophthalmology*. 2019.