

**Retinitis pigmentosa-associated
cystoid macular oedema**

MD (Res) thesis by Dr. Stacey A. Mamane

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Declaration

“I, Stacey Andrea Mamane confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.”

Abstract

Hereditary retinal diseases are now the leading cause of blindness certification in the working age population (age 16-64 years) in England and Wales, of which Retinitis Pigmentosa (RP) is the most common disorder. One complication of RP includes cystoid macular oedema (CMO), causing a reduction of central vision.

This thesis begins by reviewing retinal anatomy, retinal function and the visual cycle. It provides a description of the most common inherited retinal disorders (IRD) together with an update on structural, functional and molecular assessment of IRD. The possible underlying causes of RP-associated CMO (RP-CMO) are explored, including: 1) Breakdown of the blood-retinal barrier, 2) Failure (or dysfunction) of the pumping mechanism of the retinal pigment epithelium (RPE), 3) Muller cell oedema and dysfunction, 4) Anti-retinal antibodies, and 5) Vitreous traction. Current methods for the diagnosis and monitoring of RP-CMO are discussed. A literature review of all treatments attempted to date will be provided, including: oral and topical carbonic anhydrase inhibitors, oral, topical, intravitreal and periocular steroids, topical non-steroidal anti-inflammatory medications, photocoagulation, vitrectomy with internal limiting membrane peel, oral lutein, and intravitreal anti-vascular endothelial growth factor injections.

Extensive explanation is provided regarding the clinical trial undertaken entitled 'The AMOUR Study', which stands for 'Aflibercept for Macular Oedema in Underlying Retinitis Pigmentosa'. The purpose, methods, results and conclusion are provided.

Extensive explanation is provided regarding a second retrospective study undertaken entitled 'The CARMEL Study', which stands for 'Carbonic Anhydrase inhibitors for Retinitis Pigmentosa And Macular oEdema in various Layers'. The purpose, methods, results and conclusion are provided.

Everything is drawn together in the discussion and concluding remarks section, including a final chapter on future directions for the treatment of RP-CMO.

Impact statement

The work published herein has provided scientific impact by successfully contributing to the literature of cystoid macular oedema associated with retinitis pigmentosa (RP-CMO). This includes: 1 original research article in a peer reviewed journal, 1 review article in a peer reviewed journal, 1 case report in a peer reviewed journal and 1 article (in 2 parts) in a non-peer reviewed journal, 1 international poster presentation and 1 national oral presentation. A further manuscript has been submitted for publication and it is my hope to present the results of this research at the international meeting 'European Society of Retinal Specialists' (EURETINA), which is taking place in September 2019.

This thesis encompasses a continued focus on rare inherited retinal diseases, provides extensive review of the avenues of intervention used for RP-CMO to-date (1), poses a potential method to distinguish between patients with RP-CMO that are more, or less likely to respond to treatment with carbonic anhydrase inhibitors (CAIs) (2) and offers hope that, in selected cases of RP-CMO, intravitreal aflibercept can reduce macular thickness +/- improve vision (3, 4).

Inside of academia, this research offers a robust methodology that could be easily reproduced in order to carry out further studies using aflibercept for RP-CMO. With this in mind, a drug company in Japan has been approached to discuss the funding of a similar study using aflibercept for RP-CMO to allow comparisons to be drawn between patient populations. My hope for the future is that a larger, multi-site, randomised and controlled study will take place over a longer period of time to provide more statistically significant, longer-term data to improve our understanding of aflibercept in the treatment of RP-CMO.

The impact of this research outside of academia has been immediate, whereby, surplus vials of aflibercept have been approved for compassionate use in patients with RP-CMO at the hospital where this research was carried out. Indeed, the repurposing of this existing medication for a new indication will avoid the prolonged preclinical testing required of new compounds. Whilst aflibercept is currently licenced for use in neovascular age-related macular degeneration (AMD), macular oedema following retinal vein occlusion (RVO), diabetic macular oedema (DMO), and diabetic retinopathy (DR) in patients with DMO, it is my longer-term hope that this will extend to include RP-CMO.

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Acknowledgements

I would like to express my deep gratitude to Professor Michel Michaelides and Professor James Bainbridge, my research supervisors, for their professional guidance, enthusiastic encouragement and useful critiques of this research work.

I would also like to thank all of the patients involved with this research without whom none of this would have been possible.

I would also like to extend my thanks to the staff working on the Clinical Research Facility and Reading Centre at Moorfields Eye Hospital for their invaluable practical and technical support.

Finally, I wish to thank my husband, parents and children for their continued support and encouragement throughout my study.

List of abbreviations

µm – Microns

AAV – Adeno-associated virus

ABCR – Adenosine triphosphate binding cassette transporter

ACHM – Achromatopsia

AD – Autosomal dominant

AE – Adverse event

A2E – N-retinylidene-N-retinylethanolamine

AMD – Age-related macular degeneration

AMOUR – Aflibercept for Macular Oedema in Underlying Retinitis Pigmentosa

AO – Adaptive optics

AOSLO – Adaptive optics scanning laser ophthalmoscopy

AR – Autosomal recessive

Arr – Arrestin

ART – Automatic real time

ATF6 – Activating transcription factor 6

ATP - Adenosine triphosphate

Avastin – Bevacizumab

BCEA – Bivariate contour ellipse area

BCVA – Best corrected visual acuity

BMSC - Bone marrow derived stem cell

BP – Blood pressure

BRRB – Blood-retinal barrier

CA – Carbonic anhydrase

CAI – Carbonic anhydrase inhibitors

Ca²⁺ – Calcium

CARAMEL – Carbonic Anhydrase-inhibitors for Retinitis-pigmentosa And Macula oEdema in various Layers

Cd/m² – Candela per square metre

CE – European conformity

CI – Confidence interval

Cl⁻ – Chloride

CMO – Cystoid macular oedema

CMT – Central macular thickness

CNG - Cyclic nucleotide-gated

CNTF - Ciliary neurotrophic factor

CNV – Choroidal neovascular membrane

CO₂ – Carbon dioxide

COX – Cyclo-oxygenase

CRA – Central retinal artery

CRF – Case report form

CRRY – Complement receptor 1-like protein y

CS – Cystoid spaces

CSNB – Congenital stationary night blindness

CVA – Cerebrovascular accident

dB – Decibels

DEX implant – Intravitreal dexamethasone implant

DHA - docosahexaenoic

DM – Diabetes mellitus

DMO – Diabetic macular oedema

DNA – Deoxyribonucleic acid

DR – Diabetic retinopathy

DS – Dioptres sphere

ELM – External limiting membrane

EMA – European Medicines Agency

eMC - Electronic Medicines Compendium

EPR – Electronic patient record

EPT – Electrical phosphene threshold

ERG – Electroretinogram

ERM – Epiretinal membrane

ESC – Embryonic stem cell

ETDRS – Early Treatment Diabetic Retinopathy Study

Eylea – Aflibercept

FAF – Fundus autofluorescence

FDA – Food and drug administration

FFA – Fundus fluorescein angiography

fERG – Ficker electroretinogram

ffERG – Full field electroretinogram

FoxO – Forkhead box O

FTMH – Full-thickness macular hole

GC - Guanylate cyclase

GCAPs - Guanylate cyclase activating proteins

GCL – Ganglion cell layer

GCP – Good clinical practice

GDP – Guanosine di-phosphate

GEL – Genomics England

GMP - Guanosine monophosphate
cGMP - Cyclic guanosine monophosphate
5'GMP – 5 prime guanosine monophosphate
G_t – G protein, transducin
GRK1 – Rhodopsin kinase
GTP - Guanosine tri-phosphate
H⁺ - Hydrogen
hESC – Human embryonic stem cell
HFA – Humphrey field analyser
HM – Hand movement
HOV – Hill-of-vision
HR – Heart rate
hRPC – Human retinal progenitor cell
HTN – Hypertension
IDMC – Independent Data Monitoring Committee
IFT – Intraflagellar transport
IGF-1 – Insulin-like growth factor
IgG – Immunoglobulin G
IgM – Immunoglobulin M
ILM – Internal limiting membrane
INL – Inner nuclear layer
IOL – Intraocular lens
IOP – Intraocular pressure
IPL – Inner plexiform layer
iPSC – Induced pluripotent stem cells

IS/OS – Inner segment/outer segment

IQR – Interquartile range

IRB - Institutional review board

IRBP - interphotoreceptor retinoid binding protein

IRD – Inherited retinal disease

IS – Inner segments

ISe – Inner segment ellipsoid

IT – Information technology

IU – International units

IVTA – Intravitreal triamcinolone

K⁺ – Potassium

kb – Kilobase

kDa – Kilodalton

Kir – Inwardly-rectifying channels

KO – Knock out

LCA – Leber congenital amaurosis

LE – Left eye

LMH – Lamellar hole

LoA – Limits of agreement

LRAT - Lecithin retinol acyltransferase

Lucentis – Ranibizumab

m – Metres

mg – Milligram

Mg²⁺ – Magnesium

MI – Myocardial infarction

ml - millilitre

mm – Millimetres

mmHg – Millimetres of mercury

MP – Macular pigment

Na⁺ – Sodium

nAMD – Neovascular age-related macular degeneration

ng – Nanogram

NGF – Nerve growth factor

NGS – Next generation sequencing

NR2E3 - Nuclear Receptor Subfamily 2 Group E Member 30

NRL – Neural retina leucine zipper

NSAID – Non-steroidal anti-inflammatory

NV – Neovascularisation

OCT – Ocular coherence tomography

OCT-A – Optical coherence tomography angiography

ONL – Outer nuclear layer

OPL – Outer plexiform layer

OS- Outer segments

PERG – Pattern electroretinogram

PDE – Phosphodiesterase

PEDF – Pigment epithelium-derived factor

PEDF-NP – Pigment epithelium-derived factor-impregnated nanoparticle

PG – Prostaglandins

pg/ml – Picogram per millilitre

PI – Principal investigator

PIS – Patient information sheet

PL – Perception of light

PPV – Pars plana vitrectomy

PRPF - Pre-mRNA Processing Factor

PVD – Posterior vitreous detachment

QOL – Quality of life

R* - Photoexcited rhodopsin

rAAV – Recombinant adeno-associated virus

R&D – Research and Development

RBP – Retinal binding protein

RCT – Randomised controlled trial

RD – Retinal detachment

RDH – Retinol dehydrogenase

RE – Right eye

RHO – Rhodopsin

RIP – Receptor interacting protein

ROAD - Research On Anonymised Data

ROS – Reactive oxygen species

RP – Retinitis Pigmentosa

RP-CMO – Retinitis Pigmentosa-associated cystoid macular oedema

RPE – Retinal Pigment Epithelium

RPGR – Retinitis pigmentosa guanosine tri-phosphate ase regulator

RVO – Retinal vein occlusion

SAE – Serious adverse event

SAS – Dr. Stacey Andrea Strong

SD – Standard deviation

SDOCT – Spectral domain optical coherence tomography

SNRNP200 - small nuclear ribonucleoprotein U5 subunit 200

SOP – Standard operating procedure

SRF – Sub-retinal fluid

STATA – Statistics and data

STGD – Stargardt disease

TES – Transcorneal electrical stimulation

TIA – Transient ischaemic attack

TMG – Trial Management Group

TNF – Tumour necrosis factor

TSC – Trial Steering Group

TULP1 – Tubby like protein 1

UIC – Urinary Iodine concentration

USH2A – Usherin 2A

VA – visual acuity

VEGF – Vascular endothelial growth factor

VEP – Visual evoked potential

VF – Visual field

VFMA – Visual field modelling and analysis

VFT – Visual field testing

VH – Vitreous haemorrhage

VMA – Vitreo-macular adhesion

VMT – Vitreo macular traction

VPA – Valproic acid

VPU – Video processing unit

XL – X-linked

XLRS – X-linked retinoschisis

YAG – Yttrium-Aluminium-Garnet

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1.0 Introduction

1.1 Retinal anatomy and function

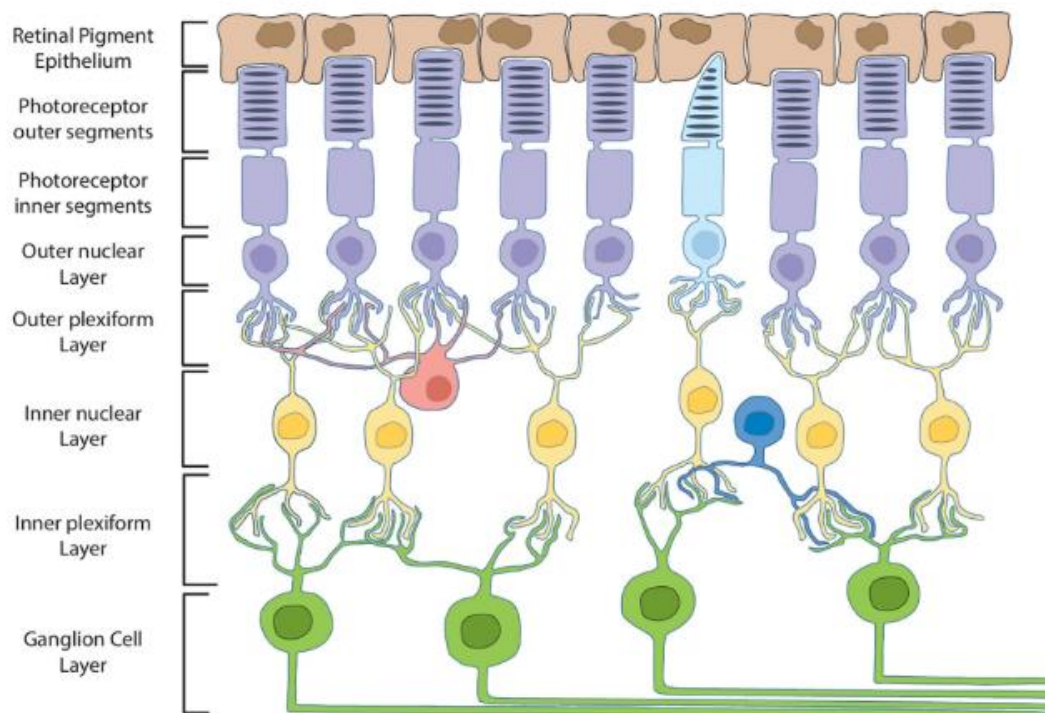
The retina is a layer of nerve tissue that lines the inside of the eye. It is made up of various layers, including (See Figure 1) (5):

- Retinal Pigment Epithelium (RPE)
- Photoreceptor outer segments (OS)
- Photoreceptor inner segments (IS)
- Outer nuclear layer (ONL)
- Outer plexiform layer (OPL)
- Inner nuclear layer (INL)
- Inner plexiform layer (IPL)
- Ganglion cell layer (GCL)

The INL contains the cell bodies of bipolar cells that synapse with and transfer information between photoreceptors and ganglion cells. Horizontal cells and amacrine cells act to integrate and regulate signal transduction throughout the retina (5). There are approximately 120 million rod photoreceptors and 6 million cone photoreceptors located within the outer retina of the human eye (6). Rod photoreceptor cells are located primarily in the periphery and peri-macular region of the retina and function to detect low-light and provide some element of night vision, also known as scotopic vision. Cone photoreceptors account for almost all photoreceptors in the central macula and are required for central, fine-resolution and colour vision under bright light conditions, also known as photopic vision (6). The RPE has several functions, including: maintaining the health of photoreceptors by recycling photopigments, metabolising and storing vitamin A, phagocytosing shed photoreceptor OS, as well as other functions (7). By sensing

light, photoreceptor cells are able to create nerve impulses that are sent to the brain, via the optic nerve, for processing.

Figure 1: Schematic illustration of retinal cell layers. Brown = RPE cells; Purple = Rod photoreceptors; Light blue = Cone photoreceptors; Yellow = Bipolar cells; Green = Ganglion Cells; Red = Horizontal cells; Dark blue = Amacrine cells. Taken from Sengillo et al. (2016) (5).

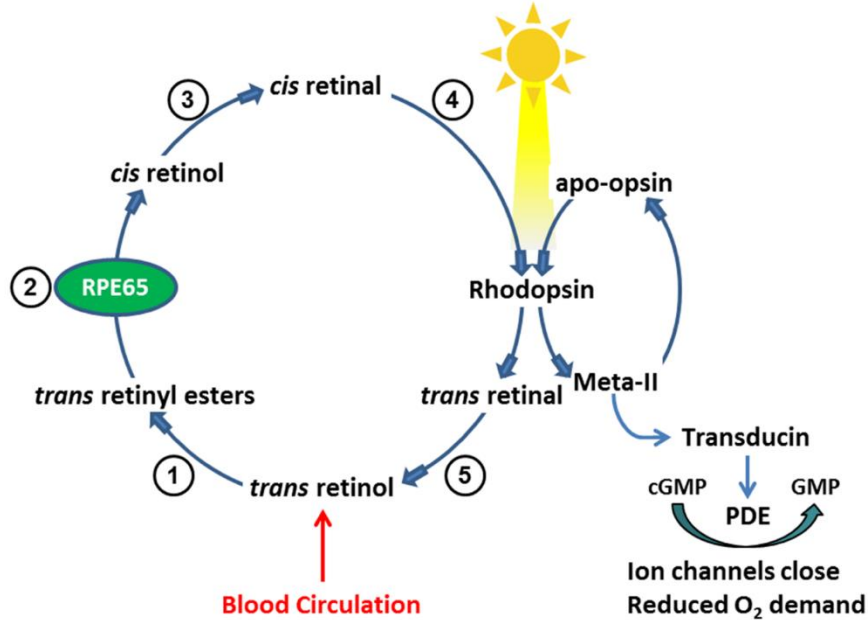


1.2 The visual cycle

The visual cycle (see figure 2) is a process designed to detect light and convert it into electrical signal (phototransduction) followed by de-activation of the phototransduction cascade and recycling/preparation of necessary molecules to enable phototransduction to re-occur (photoresponse recovery).

Figure 2: The Visual Cycle. Taken from Bavik et al. (2015) (8). 1 = All-*trans*-retinol retrieved from the blood circulation is converted into a retinyl ester within the RPE through the activity of lecithin retinyl acyltransferase (*LRAT*). 2 = RPE65 catalyses the retinyl esters to generate 11-*cis*-retinol. 3 = 11-*cis*-retinol is then oxidized by retinol dehydrogenase to form 11-*cis*-retinal. 4 = 11-*cis*-retinal is delivered to rod and cone outer segments where it combines with opsins to form rhodopsin. Light activation of rhodopsin initiates visual transduction and liberates all-*trans*-retinal as a photoproduct. 5 = Reduction of all-*trans*-retinal, via all-*trans*-retinal dehydrogenase, produces all-*trans*-retinol, which is transferred back to the RPE for recycling. The continued activity of RPE65 in the light state ensures sustained levels of rhodopsin, closure of ion channels through transducin activation, and reduced oxygen demand (8).

The Visual Cycle



1.2.1 Activation of the phototransduction cascade

Phototransduction is the process by which a photon of light detected by the retina creates a chain of events leading to the production of a neural signal. In order to appreciate the steps that take place following the arrival of a photon, it is first important to understand the baseline state of the photoreceptor in its absence. For simplicity, we will concentrate on the rod photoreceptors.

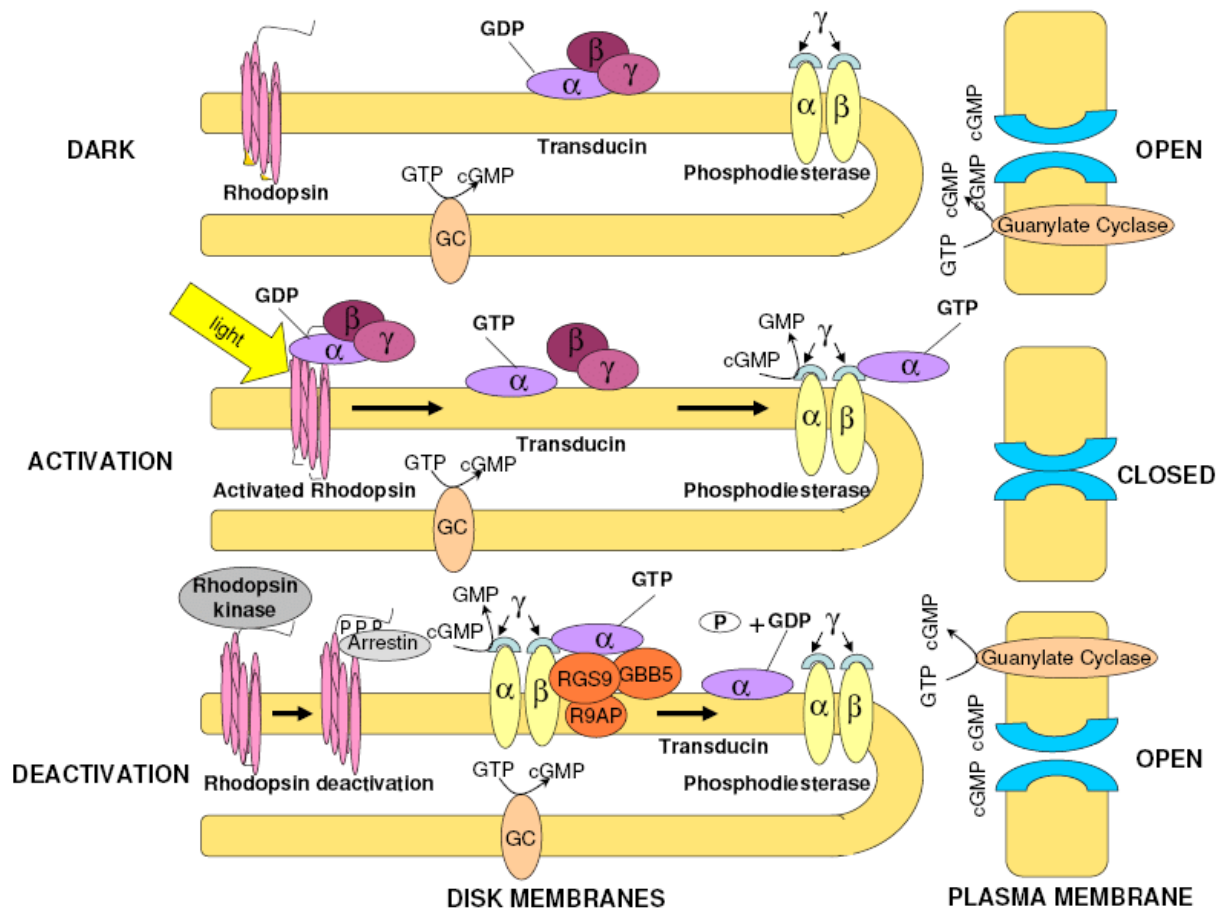
The outer segment of the rod photoreceptor is integral to the process of phototransduction, encompassing vast quantities of lipid bilayer membrane that have been arranged into flattened 'discs' (9). Within this membrane, a light sensitive molecule known as 'rhodopsin' can be found.

Rhodopsin is a G-protein-coupled receptor made up of 348 amino acids that has folded into a higher level structure to form 7 helical segments (9). Its concave shape extends across the cell membrane and

populates the disc membrane at a density of approximately 25,000 molecules per micrometre (9). This is equivalent to about 80% of the protein found in the disc membrane of rod OS (10). The chromophore termed '11-*cis*-retinal' is permanently and covalently attached to rhodopsin, which acts as an inverse agonist to prevent its apoprotein (in the case of rhodopsin, this is 'opsin') from being activated (11).

In the dark, a resting potential is generated in the photoreceptor cell due to open cyclic guanosine monophosphate (cGMP) -gated sodium (Na^+) channels (see figure 3), also known as cyclic nucleotide-gated (CNG) channels that are located in the outer membrane of the rod cell (9). The CNG channel is made up of 4 sub-units (3 sub-units of $\alpha 1$ and a single sub-unit of $\beta 1$). When cGMP is available it binds to these sub-units and opens the central pore in the channel thus allowing Na^+ and calcium (Ca^{2+}) to enter thus depolarising the cell membrane. The internal membrane voltage is negative.

Figure 3: Phototransduction activation in a rod photoreceptor. Taken from Burns et al. (2005) (12). The upper picture is demonstrating phototransduction activation. Photoexcited rhodopsin (R^*) activates transducin ($\text{G}_t\alpha$, $\text{G}_t\beta$ and $\text{G}_t\gamma$ sub-units) and PDE (α -, β - and γ - sub-units). cGMP synthesised by guanylate cyclase (GC) is hydrolysed by activated PDE. Phototransduction deactivation in a rod photoreceptor. R^* is quenched by phosphorylation by rhodopsin kinase followed by arrestin (Arr) binding. Transducin and PDE are deactivated by the RGS9-1-G β S-L.R9AP complex, which accelerates the rate of GTP hydrolysis on G α . cGMP synthesis by GC restores cGMP to its dark level (13).



Phototransduction activation (see figure 3) consists of the following steps:

- 1) **Rhodopsin activation:** When a photon of light strikes rhodopsin, it is absorbed by an electron in the 11-12 double bond position within 11-*cis*-retinal, which is promoted to a higher energy level. The pi bond is disrupted and the chain can rotate about a single axis of the sigma bond. The result is that the chain is temporarily free for rotation and isomerisation to its preferable 'straight' position before the double bond is re-established. All-*trans*-retinal triggers a cascade of conformational changes in the opsin, allowing rhodopsin to transition through its

intermediate forms: photorhodopsin, bathorhodopsin, lumirhodopsin and metarhodopsin I with ultimate production of meta-rhodopsin II*.

- 2) **Transducin activation:** Meta-rhodopsin II* is the activated form of rhodopsin that contains a structural pocket anatomically to enable the accessibility and coupling of transducin (G_t) – a trimeric G-protein attached to the cell membrane to maintain proximity with rhodopsin. Transducin is made up of α , β and γ sub-units found at a ratio of around 1:10 relative to rhodopsin (9). In its inactive form, a molecule of Guanosine di-phosphate (GDP) is found in association with the α sub-unit. When rhodopsin becomes active, a process called ‘nucleotide exchange’ occurs whereby a molecule of GDP is exchanged for Guanosine tri-phosphate (GTP) thus allowing separation and activation of the α sub-unit (9). One rhodopsin molecule activates as many as 200-400 transducin molecules per second (14).

- 3) **Phosphodiesterase activation:** The α sub-unit moves away to another membrane-bound protein called phosphodiesterase (PDE). PDE is a cyclic nucleotide consisting of 4 sub-units (1 α , 1 β and 2 γ sub-units) whose function is to hydrolyse cGMP. It is present at a ratio of approximately 1:100 relative to rhodopsin (9). The γ sub-units of PDE act to maintain its inactivation. The binding of transducin to the γ sub-units of PDE disrupts their function and subsequent activation of the α and β sub-units occurs to produce the active effector complex $G_t\alpha$ -PDE* (15).

- 4) **Hydrolysis of cyclic GMP:** $G_t\alpha$ -PDE* is able to convert the available supply of cGMP to its non-cyclic form 5 prime GMP (5'GMP) via hydrolysis. The end result is a reduction in the concentration of cGMP and increase in the concentration of 5'GMP (9).

5) **Cyclic nucleotide channel closure:** A decrease in the level of cGMP results in closure of CNG channels since cGMP is no longer bound to its sub-units and the central pore created cannot be maintained. When Na^+ and Ca^{2+} cations are no longer able to enter the cell, hyperpolarisation occurs due to voltage changes becoming more negative (16). This signals to its synaptic terminal to reduce the rate of glutamate release, thereby activating the 'on'-centre bipolar cell. This small change initiates the cellular events leading to vision. When rods turn off, 'on'-centre bipolar cells turn on. This activates retinal ganglion cells, which send axons to the optic nerve and then the brain (9).

1.2.2 De-activation of the phototransduction cascade

Phototransduction de-activation (see figure 3) consists of the following steps:

1) **Deactivation of R^* must occur:** After photoisomerisation, the addition of 3 phosphates to R^* 's C-terminal residues (phosphorylation) are essential for normal response recovery and must be mediated by rhodopsin kinase (GRK1) within 100ms of a flash (16). This allows for the protein Arr to bind with high affinity to phosphorylated rhodopsin in order to prevent further activation of G_t molecules and thus assist with the process of deactivation (16).

2) **cGMP hydrolysis by PDE must reduce:** Transducin remains active until GTP is hydrolysed – a process of catalysation involving a triumvirate complex of proteins including RGS9-1, Gbeta5-L, and R9AP (known as the RGS9 complex) (13).

3) cGMP-dependent CNG channels must re-open in order for electrical response to recover:

GC is a lyase enzyme located in the disc membrane that continuously generates cGMP in order to allow opening of CNG channels and depolarisation of the cell membrane following phototransduction. The activity of GC is controlled by GC-activating proteins (GCAPs) and its activity is dependent on the level of Ca^{2+} present. When phototransduction takes place, CNG channels close and Ca^{2+} influx is reduced. The resultant fall in Ca^{2+} activates GC-1 and GC-2 through GCAPs in order to produce more cGMP (16). In the dark, GC has low level activity with low cGMP to maintain the cell's resting potential.

1.2.3 Photoresponse recovery

Once a photon has been detected by rhodopsin, the molecule is unable to detect a subsequent photon until the pigment has been reset to its ground state (17). The by-product all-*trans*-retinal must be converted back into 11-*cis*-retinal in order to allow for photoresponse recovery.

The re-cycling process begins with release of all-*trans*-retinal from the activated opsin into the inner leaf of the disc bi-layer to form a complex with phosphatidylethanolamine. This complex is transported to the cytoplasmic disc surface by the retina specific adenosine triphosphate (ATP) binding cassette transporter (ABCR), and released into the cytoplasm (18). Here, the enzyme all-*trans*-retinol dehydrogenase acts to reduce all-*trans*-retinal to all-*trans*-retinol (vitamin A). The subsequent binding of all-*trans*-retinol to interphotoreceptor retinoid binding protein (IRBP), enables exportation from the photoreceptor outer segment to the adjacent layer of the RPE (17).

In the RPE, all-*trans*-retinol is used to generate all-*trans* retinyl-esters with Lecithin retinol acyltransferase (*LRAT*). These esters serve as the substrate for an isomerohydrolase reaction, likely

catalysed by RPE65, which generates the visual chromophore precursor 11-*cis* retinol (8). Oxidation of 11-*cis*-retinol, by 11 *cis*-specific retinal dehydrogenase, yields 11-*cis*-retinal, which is transferred from apical processes of the RPE to photoreceptor OS where it combines with the appropriate opsins to regenerate photosensitive visual pigment (See figure 2) (8).

All-*trans*-retinal cannot be synthesised by humans and further stores of vitamin A must therefore be ingested in order to keep up supplies. This can subsequently be retrieved from the blood and transferred to the RPE where conversion to all-*trans*-retinyl esters takes place via *LRAT* (8). When required, these stores can be mobilised and catalysed by the RPE-specific protein, RPE65 (8). Deficiency of vitamin A can lead to night blindness.

1.3 Inherited Retinal Disorders

1.3.1 Overview of Inherited Retinal Disease

Inherited Retinal Disease (IRD) is the leading cause of blindness certification in the working age population (age 16-64 years) in England and Wales (19) and the second most common cause of blindness certification in childhood (20).

The term IRD incorporates a large group of disorders that are clinically and genetically heterogeneous (20). Whilst we have the added advantage of genetic testing to confirm many IRDs, definitive diagnosis based on the history and clinical examination alone can often be challenging due to overlapping phenotypes and unclear inheritance patterns. For example, it is possible for disease-causing variants within the same gene to result in varying phenotypes, yet may occur within different genes and result in

more similar phenotypes (21). To complicate matters further, family members may carry identical genetic disease-causing variants yet vary in expressivity of phenotype (22). This, together with the large number of genes that have been identified in IRDs (>250) can make it challenging to diagnose a patient with a specific disorder (22).

When attempting to make a diagnosis of IRD, several factors are taken into consideration in order to steer the diagnosis in the right direction:

- **Identification of cell type affected:** This can be identified through detailed history taking, clinical examination, imaging and electrophysiological assessment. A rod dystrophy affects rod photoreceptors. A cone dystrophy affects cone photoreceptors. When both types of photoreceptor are involved the nomenclature reflects the order in which the photoreceptors are affected, for example, rod-cone dystrophies affect the rods prior to the cones (23).
- **Distribution of retinal involvement:** This will reflect the cell type involved and may be described as central, peri-central, sector or peripheral (24).
- **Natural history:** IRDs are described as 'predominantly stationary' if the condition is present at birth or from early infancy and tends to remain stable throughout life. A 'progressive' IRD tends to present in the first or second decades of life and worsens over time.
- **Inheritance:** IRDs may be inherited in an autosomal dominant (AD), autosomal recessive (AR), X-linked (XL) or mitochondrial manner (23). Digenic inheritance refers to the situation where disease-causing variants are required in two genes in order for the disease to be expressed (23).

Female carriers of XL variants may be unaffected or show less severe symptoms than an affected age-matched male (23).

- **Systemic involvement present or absent:** IRDs may have isolated ocular features, termed 'non-syndromic' or the addition of systemic features termed 'syndromic' (22).
- **Regional distribution:** IRDs may demonstrate regional distribution of retinal degeneration, for example, sectoral RP.

1.3.2 Mechanisms of photoreceptor cell death

Disease-causing variants triggering photoreceptor degeneration often affect the visual cycle or phototransduction cascade (25). Apoptosis is a form of programmed cell death considered to be the main pathway by which photoreceptors and/or RPE cells die (26). It is an active process provided with energy from mitochondria whereby a sequence of events triggered by the cell itself enables its removal without damaging adjacent healthy tissue (25). Enzyme activation is required in order to breakdown cellular deoxyribonucleic acid (DNA) and auto-digest intracellular components. A family of cysteine proteases known as caspases often drive these processes, leading to morphological changes such as: membrane blebbing, condensation of the nuclear chromatin and cytoplasm, fragmentation of the nucleus, and budding of the whole cell to produce membrane-bound bodies in which organelles are initially intact (25). These bodies are subsequently disposed of without inducing inflammation (25).

Necrosis is another pathway by which photoreceptor loss can occur. Whereas apoptosis uses signal transduction pathways to result in orderly cell death, necrosis results in a premature death following

exposure to pathogens such as toxins, trauma, ischemia and infection (26). This leads to an unregulated digestion of cellular components. *Necroptosis* also results in necrotic cell death, however, unlike necrosis it is carried out in a regulated way. Similar to apoptosis, necroptosis begins with the binding of tumour necrosis factor (TNF)- α and Fas ligand to their respective cell surface receptors. This kick-starts a signal transduction pathway that relies on the following critical mediators: receptor interacting protein (RIP)-1 kinase, RIP3 and mixed lineage kinase domain-like protein (27).

IRBP is an inter-photoreceptor matrix glycol-lipoprotein secreted by photoreceptors. It is known to play a pivotal role in photoreceptor survival, however, the mechanism by which this occurs is poorly understood. A variant in the human IRBP has been linked to RP. Animal models using IRBP $^{-/-}$ retinas have observed increased levels of RIP1, RIP3 and TNF- α receptor 1, an important membrane death receptor that mediates both programmed apoptosis and necrosis (28).

Oxidative stress is another major factor considered to trigger photoreceptor apoptosis in a variety of retinal diseases (29). Photoreceptor cells accumulate reactive oxygen species (ROS) and oxidative stress that is usually balanced by antioxidant defences. Photoreceptor degeneration is likely to happen if the balance is tipped in favour of ROS. The Forkhead box O (*FoxO*) proteins include transcription factors that promote oxidative stress resistance by binding to promoters of genes encoding manganese superoxide dismutase, catalase, and autophagy-related proteins (29). This pathway is emerging as an important family of proteins that modulate the expression of genes in the regulation of a variety of cellular processes including cell cycle, apoptosis, DNA repair, stress resistance, and metabolism (29). These scavenger proteins are therefore considered to play an essential role in oxidative detoxification in mammals (29).

Inflammation is also becoming a better understood pathway by which retinal cell death can occur.

Cubilla et al. observed increased photoreceptor apoptosis under basal, non-stress conditions using subcutaneous mifepristone - an antagonist of the glucocorticoid receptor - suggesting that glucocorticoids play a critical role in basal photoreceptor survival (26).

Selected IRDs will be discussed below to briefly illustrate the wide breadth of phenotypes and exemplify the application of clinical assessments in diagnosis and management:

1.3.3 Retinitis Pigmentosa

1.3.3.1 Overview

Retinitis Pigmentosa (RP), classified as a rod-cone dystrophy, is a genetically heterogeneous disorder considered to be a final common pathway arising from rod photoreceptor degeneration and RPE abnormalities (30). With an incidence of 1 : 3,500 – 1 : 4,000 in the USA and Europe, RP is the most common form of IRD (22).

Over 60 genes are known to cause RP and inheritance can be either AD, AR,XL or mitochondrial (10). AR inheritance accounts for 5 - 20% of RP and of these, 10-15% are due to variants in the *USH2A* gene (31, 32). AD inheritance accounts for 15-20% of RP and of these, 20-30% are due to variants in the Rhodopsin (*RHO*) gene (31, 32). XL inheritance is considered to be the most severe form of RP with onset in childhood and accounting for 5 - 15% of RP (31, 32). Of these, 70-90% are due to variants in the RP GTPase regulator (*RPGR*) gene. There is no known family history in 40-50% of patients, with the majority of these have AR RP (31). A rare form of RP known as 'digenic' occurs when simultaneous variants arise in both *PRPH2* (previously known as *RDS*) and *ROM1* genes (33).

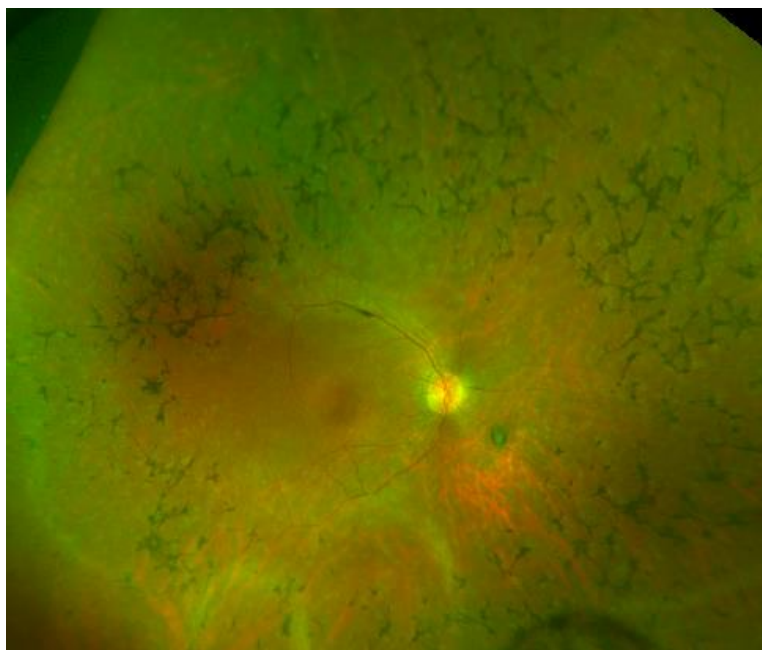
Additional terminology includes 'simplex' where an isolated case of RP occurs with an absence of family history and 'multiplex' where RP occurs in 2 or more family members (such as siblings) with no pre-existing family history (24).

1.3.3.2 Features

The classic ophthalmoscopic triad of RP (see figure 4) includes:

- 1) Attenuation of artery vessel calibre that occurs from vasoconstriction following vessel exposure to increased oxygen tension due to retinal thinning.
- 2) Pallor of the optic disc referred to as 'waxy pallor' or 'chamois yellow'.
- 3) Bone-spicule retinal pigmentation. This represents a diffuse abiotrophic process where disruption of the RPE allows pigment to migrate along the course of the vessels, which tend to become obliterated following hyaline degeneration (34).

Figure 4: Fundus photograph of a patient with retinitis pigmentosa..



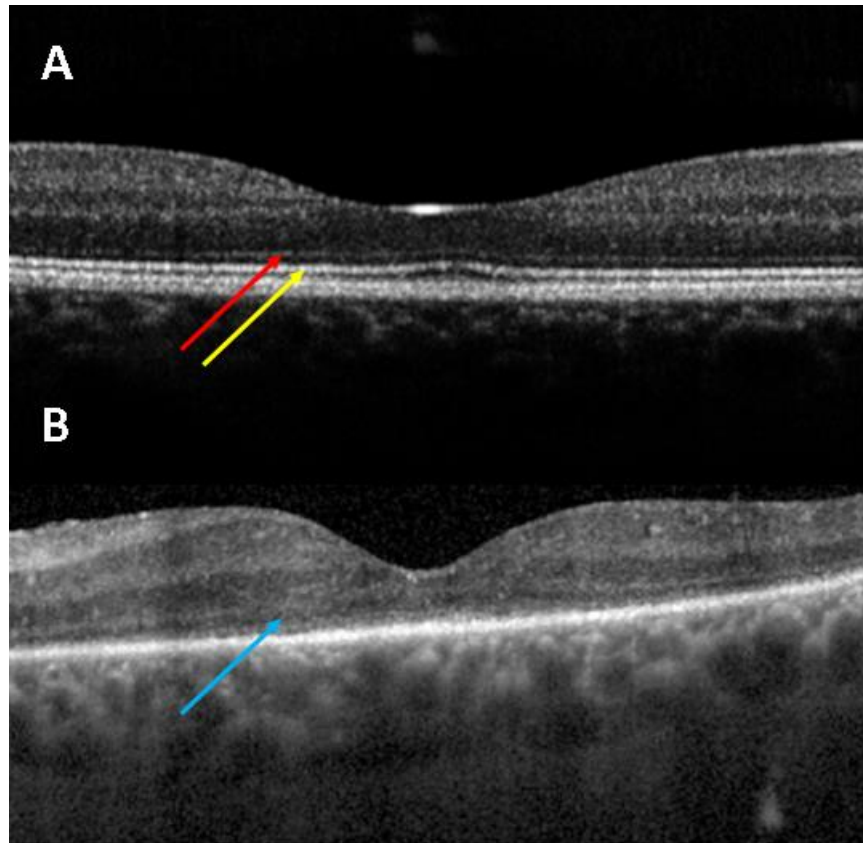
Another sign that can be easily seen on spectral-domain optical coherence tomography (SDOCT) (Spectralis, Heidelberg Engineering Ltd, Heidelberg, Germany) imaging is a change of retinal architecture whereby the degeneration of photoreceptors causes the ellipsoid zone, also known as the photoreceptor inner segment/outer segment (IS/OS) junction, to become indistinct (see figure 5) instead of being a clear, highly reflective line.

Symptoms such as nyctalopia and progressive concentric (centripetal) visual field (VF) loss vary widely between patients depending on the type and location of a variant. For example, a patient is more likely to retain better visual acuity (VA) and improved dark light adaptation if a variant affects amino acids in the parts of rhodopsin located in the intradiscal space rather than the cytoplasmic space (36). Genetic modifiers and/or environmental factors account for phenotypic variation seen within families that share a common disease-causing variant (37).

Other clinical signs associated with RP include: cataract (typically posterior sub-capsular), dust-like particles in the vitreous, cystoid macular oedema, white dots deep within the retina and hyaline bodies affecting the optic nerve (10).

Triolo et al. (2013) reported choroidal neovascularisation (NV) in 3 of 176 eyes (2%) with RP (38).

Figure 5: SDOCT images centered on the macula taken from a patient without RP (A) and a patient with RP (B). Red arrow: External limiting membrane (ELM); Yellow arrow: Ellipsoid zone (IS/OS junction); Blue arrow: Outer retinal layer thinning. Used with permission.



1.3.3.3 Associations

Examples of syndromic RP include:

- **Usher syndrome:** This is the most common syndromic form of RP, which is inherited in an AR manner (39). Usher Syndrome can be classified into 3 groups: Type 1 is the most common type (accounting for approximately 70% of patients with Usher Syndrome) comprising of profound congenital sensorineural hearing loss, absent vestibular function and RP (39). The RP typically occurs by the age of 10 years and progression is slow (39). Type 2 accounts for approximately 26% of patients with Usher Syndrome and comprises of moderate to severe congenital sensorineural hearing loss (predominantly for higher frequencies), normal vestibular function,

and RP with onset by the age of 20 years (39). Type 3 accounts for approximately 4% of all patients with Usher Syndrome and comprises of progressive sensorineural hearing loss and RP with onset in second decade (39). Vestibular function may, or may not be affected.

- **Bardet Biedl syndrome:** AR condition characterised by the association of RP, obesity, learning difficulties, polydactyly, hypogenitalism and renal abnormalities (40).
- **Kearns-Sayre syndrome:** Mitochondrial inheritance with RP, external ophthalmoplegia, ataxia and heart block (41).
- **Bassen-Kornzweig syndrome** (also known as **Abetalipoproteinemia**): This is a condition involving malformation of red blood cells with associated neuromuscular disturbances such as progressive ataxia. There is also associated fat malabsorption and subsequent reduction of fat-soluble vitamin absorption (A, E and K) leading to clotting abnormalities and RP (24).
- **Mucopolysaccharidoses types I-III:** This is a group of conditions characterised by RP in associated with facial and bony changes, learning difficulties and corneal clouding (42).

1.3.4 Leber Congenital Amaurosis

1.3.4.1 Overview

Leber Congenital Amaurosis (LCA) accounts for around 5% of all IRD (43). This heterogenous recessive disease is considered to be the most severe form of IRD with an incidence of 3 per 100 000 births (44) and prevalence of 1 : 30 000 (45) to 1 : 81 000 (44). Disease-causing variants have been identified in 25

genes, the most common of which include: *GUCY2D* (6-21%), *CEP290* (20%) and *CRB1* (9-13%), however, approximately 30% of LCA patients remain without a molecularly proven diagnosis (43).

1.3.4.2 Features

Patients present in the first year of life with profound vision loss (typically ranging from 20/200 to no perception of light), roving nystagmus and amaurotic pupils (30, 43). Fundus examination ranges from a normal appearance to signs including maculopathy, bone-spicule pigment migration (see figure 6) and white flecks/dots (43).

Figure 6: Fundus photograph of a patient with Leber Congenital Amaurosis (LCA) caused by a *GUCY2D* variant.



Additional signs and symptoms include: hypermetropia (or less often myopia), photophobia, nyctalopia, Franceschetti's oculodigital sign where a patient will repeatedly poke and rub their eyes, olfactory

dysfunction, keratoconus and cataract (43). These latter two signs may be associated with variants in the *AiPL1* and *CRB1* genes (43). Most cases are isolated, uncommonly LCA is part of a syndrome such as Senior-Loken or Joubert Syndrome.

1.3.4.3 Imaging and electrophysiology

OCT may demonstrate macular atrophy/thinning (43). Electroretinogram (ERG) is typically sub-normal or non-detectable and considered essential in the of diagnosis of LCA (43). It is also helpful in distinguishing LCA from conditions such as achromatopsia (ACHM) and congenital stationary night blindness (CSNB) that also present with poor vision, nystagmus and a normal-looking fundus (43).

1.3.4.4 Natural history

The cumulative data from several studies observing the natural history of LCA found that across 90 patients, 15% demonstrated deterioration of vision, 75% demonstrated stability of vision and 10% demonstrated appreciable improvement of vision (44, 46-48). Indeed, it seems that certain variants in specific LCA genes demonstrate distinctive VA among the different LCA sub-types (43).

1.3.5 Stargardt Disease

1.3.5.1 Overview

Stargardt disease (STGD) is the most common inherited macular dystrophy across all ages with a prevalence of 1 in 8 000 to 1: 10 000 (49-51). The most common form of disease is known as STGD1, which is associated with variants in the *ABCA4* gene (50). Dysfunctional *ABCA4* protein results in toxic accumulation of lipofuscin - a major component of which is the bis-retinoid N-retinylidene-N-retinylethanolamine (A2E) - within RPE cells and subsequent photoreceptor cell death (23). Over 1000

disease-causing sequence variants have been identified in the *ABCA4* gene alone (49, 52) and the carrier frequency is thought to be anywhere up to 1 : 20 (53). Whilst STGD1 demonstrates AR inheritance, there is also a rare dominantly inherited form of STGD known as STGD3 arising from variants in the *ELOV4* gene.

1.3.5.2 Features

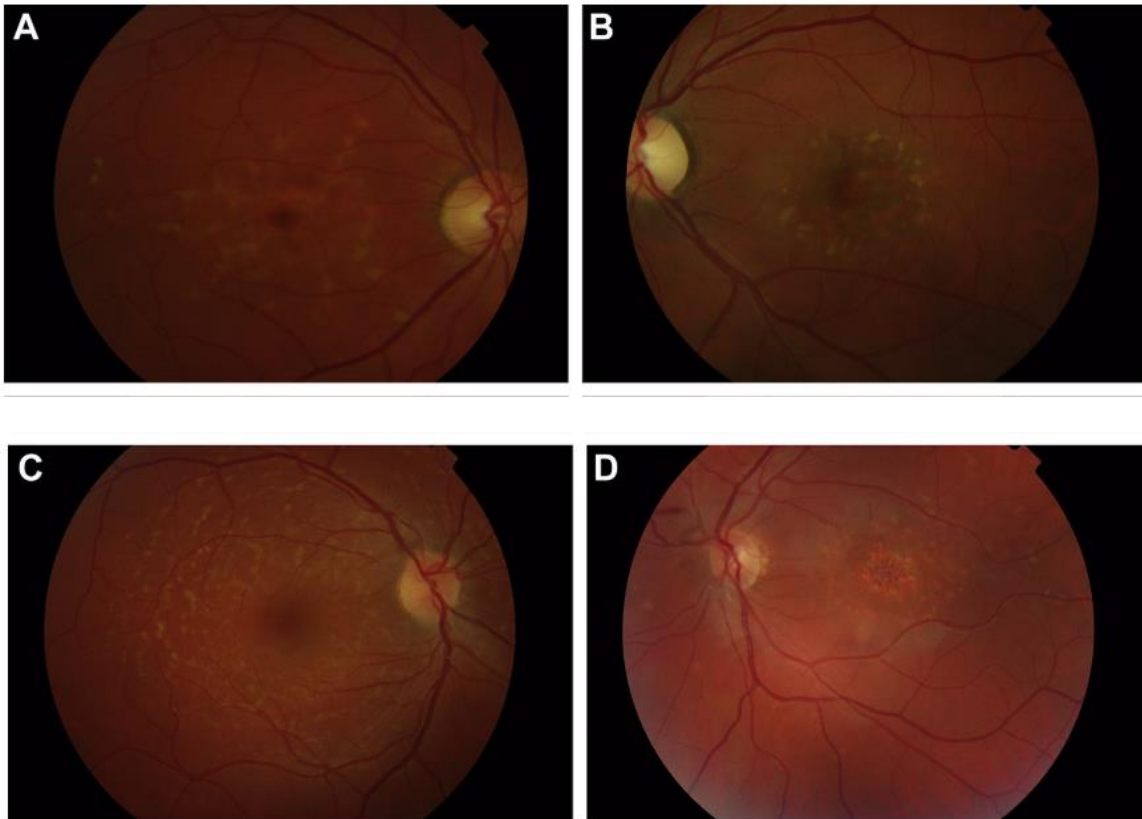
Due to the large number of disease-causing sequence variants, there is marked phenotypic heterogeneity amongst patients with STGD1 with variable age of onset and severity of disease. It is thought that the more severe sequence variants, such as nonsense variants cause earlier presentation of disease with increased severity; whereas adult onset/foveal-sparing disease is more frequently due to missense variants (50, 54-57).

Patients often present with bilateral central visual loss including dyschromatopsia and central scotomata (50).

On examination, features range anywhere from a normal fundus appearance and/or subtle loss of foveal reflex and/or RPE changes seen in early disease to the characteristic yellow-white flecks and/or macular atrophy associated with a bull's eye or beaten bronze appearance seen with advancing disease (23). See figure 7 for illustrative examples of eyes with different grades of visual impairment according to World Health Organization Criteria.

Figure 7: Illustrative examples of eyes with different grades of visual impairment according to World Health Organization Criteria. A = no visual impairment; best corrected visual acuity (BCVA) 20/16. B =

mild visual impairment; BCVA 20/32. C = moderate visual impairment; BCVA VA 20/120. D = Blindness; BCVA VA 20/400. Taken from Kong et al. (2016) (58).



1.3.5.3 Imaging and electrophysiology

Imaging, such as fundus autofluorescence (FAF) and SDOCT together with electrophysiological assessment, is of great benefit in assisting with the diagnosis of STGD in those who present without typical fundus features and in monitoring disease progression.

Fortunately, lipofuscin is a pigment that is able to demonstrate autofluorescence. As such, RPE with greater amounts of lipofuscin within its cells will appear as areas of increased signal on FAF (See figure 8 and 9). These areas of increased autofluorescence are at risk of photoreceptor cell loss (49). Prior to FAF, fundus fluorescein angiography (FFA) was helpful in demonstrating a dark (or 'silent') choroid -

occurring due to the blockage of choroidal fluorescence by lipofuscin within RPE, however, not all patients with STGD would demonstrate this feature (50, 55).

Figure 8: FAF image taken from a patient with Stargardt disease. Please note the characteristic autofluorescent flecks seen throughout the retina. Used with permission.

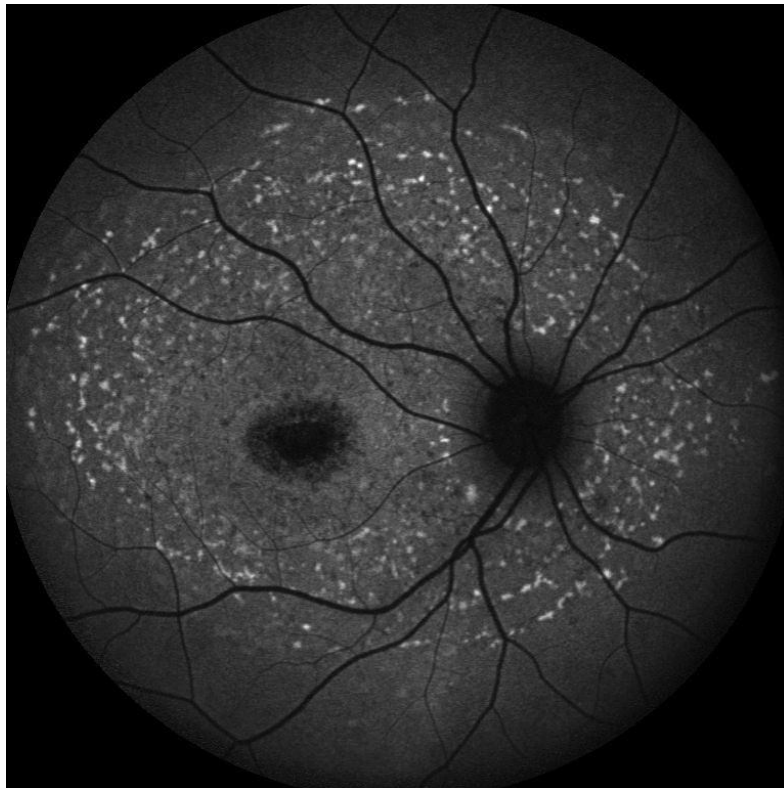
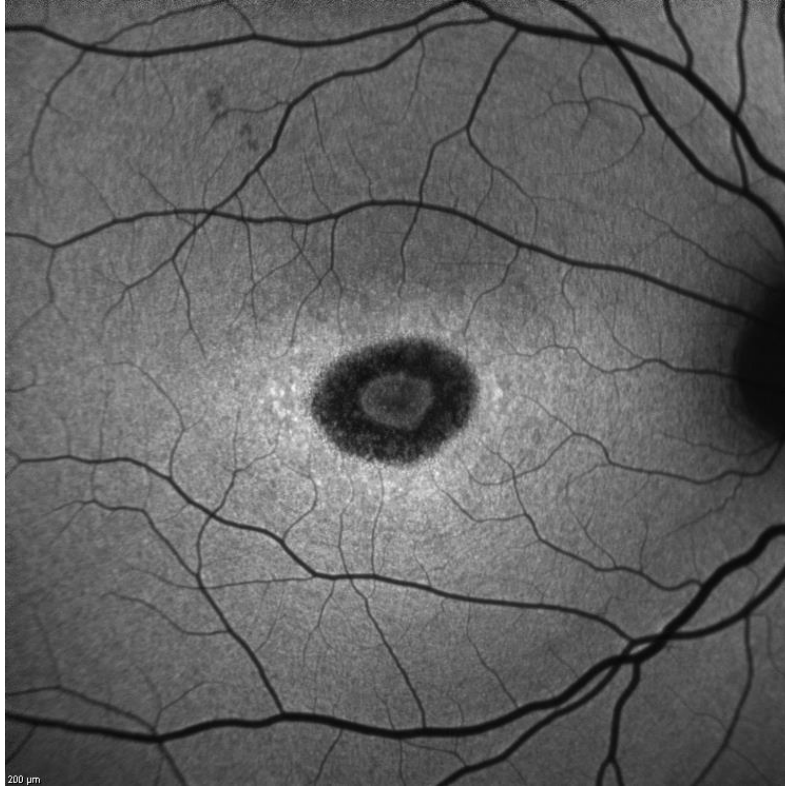


Figure 9: FAF image centred on the macula taken from a patient with Stargardt disease. Please note the characteristic areas of increased and decreased autofluorescence. Used with permission.



SDOCT is useful at highlighting areas where there has been loss of outer retinal architecture at the central macula (59).

Electrophysiology is useful for both diagnosis and prognosis. Lois et al. (2001) established the following classification system (60):

- Group 1: Normal full-field ERG (ffERG) in the presence of a diminished or undetectable pattern ERG (pERG)
- Group 2: Reduced cone function on ffERG in the presence of a diminished or undetectable pERG
- Group 3: Reduced rod and cone function on ffERG in the presence of a diminished or undetectable pERG

Fujinami et al. (2013) performed a longitudinal study of patients with Stargardt disease and found that prognosis seemed dependent on the presence (or absence) of initial rod involvement. All patients with initial rod ERG involvement demonstrated clinically significant electrophysiological deterioration (Group 3) (51). However, in direct contrast, clinically significant progression was observed in only 20% of patients with normal ffERG's at baseline (Group 1) (51).

1.3.5.4 Natural history

While STGD is considered a progressive disorder, patients may also experience a plateau of symptoms (60).

In the largest series to date (n = 68), Fujinami et al. (2013) retrospectively classified patients with STGD1 into 3 groups based on their baseline FAF and evaluated longitudinal FAF changes and patterns over a mean average of 9.1 years (51):

- Type 1: Localised low signal at the fovea with surrounding homogenous background demonstrated rate of atrophy enlargement as $0.06\text{mm}^2/\text{year}$.
- Type 2: Localised low signal at the macula with surrounding heterogenous background with numerous foci of abnormal signal demonstrated rate of atrophy enlargement as $0.67\text{mm}^2/\text{year}$.
- Type 3: Multiple low signal areas at the posterior pole with a heterogenous background demonstrated rate of atrophy enlargement as $4.37\text{mm}^2/\text{year}$.

1.3.6 Achromatopsia

1.3.6.1 Overview

Achromatopsia (ACHM), also known as rod monochromatism, is an AR disorder affecting S, M and L cone photoreceptors (61-66). The prevalence of ACHM is 1 : 30,000 – 1 : 50,000 (61). Around 80% of ACHM can be attributed to variants within the *CNGA3* and *CNGB3* genes, affecting phototransduction through dysfunction of the α and β sub-units of CNG channels, respectively (67). Less common causes of ACHM include variants within the *GNAT2*, *PDE6C* and *PDE6H* genes, affecting phototransduction through dysfunction of the α sub-unit of G_t and the α and γ sub-units of cGMP-phosphodiesterase, respectively (67). More recently variants within the activating transcription factor 6 (ATF6) gene have been identified and are believed to affect the maintenance of endoplasmic reticulum and cellular homeostasis (68).

1.3.6.2 Features

Two forms of ACHM exist – complete and incomplete:

Complete ACHM results from complete functional loss of cone photoreceptors. Patients typically present at birth or by early infancy with poor vision (20/200 or worse), pendular nystagmus, photophobia/photoaversion and reduced or absent colour vision (6, 69).

Patients with incomplete ACHM (far less common) tend to have similar, but milder clinical findings than those with complete ACHM (6). This occurs due to partial impairment of cone photoreceptors. Patients with incomplete ACHM may thus retain a degree of colour vision and demonstrate VA as good as 20/80 (6, 70).

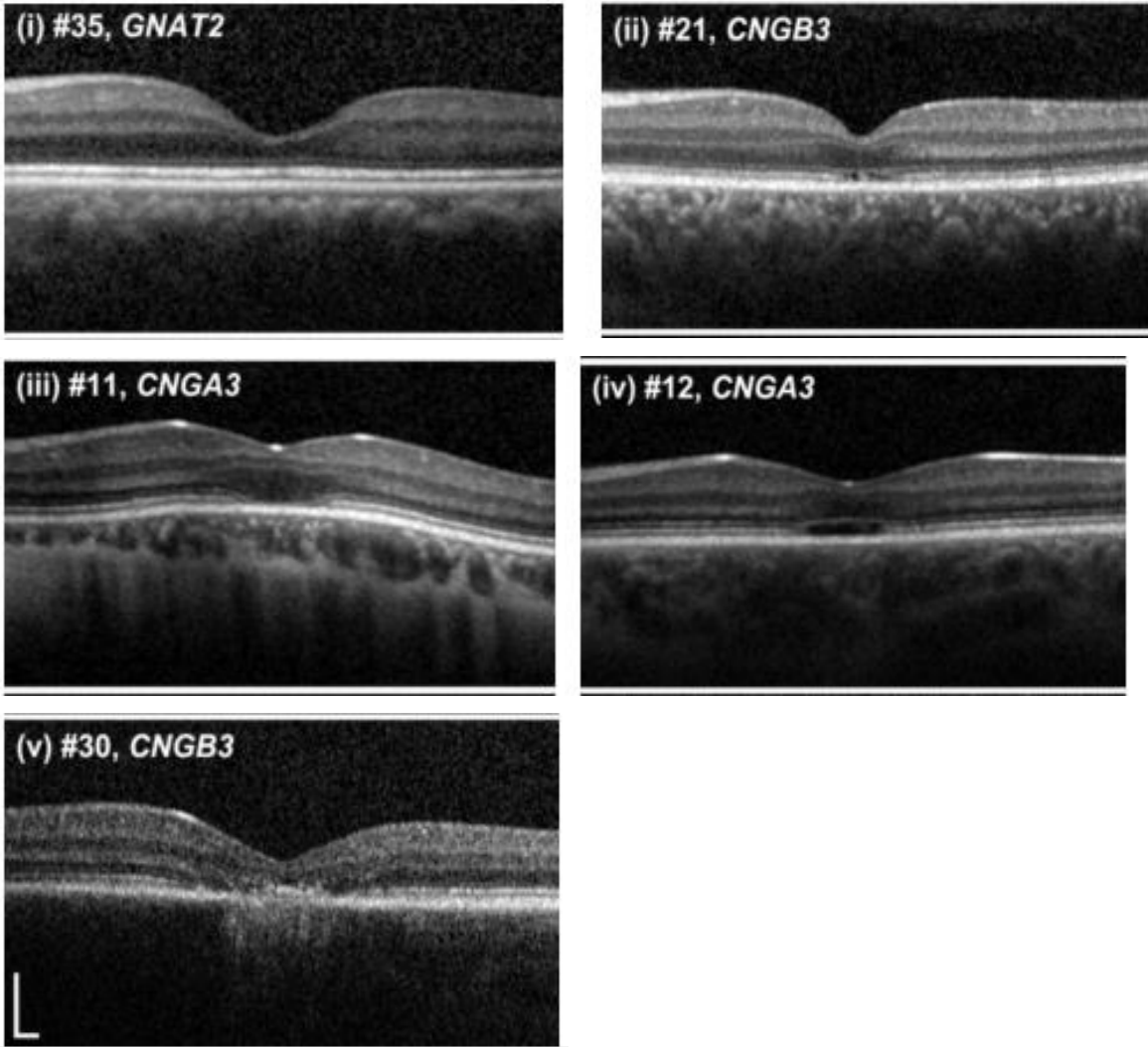
The fundus appearance in ACHM may reveal macular changes and vessel narrowing, however, most commonly it looks entirely normal (6).

1.3.6.3 Imaging and electrophysiology

Imaging remains an important adjunct in the classification of ACHM. Sundaram et al. (2014) describe a method of classifying outer retinal findings using SDOCT in patients with ACHM (See Figure 10) as follows (67):

- 1) Continuous inner segment ellipsoid (Ise)
- 2) Ise disruption
- 3) Absent Ise
- 4) Foveal hyporeflective zone
- 5) Outer retinal atrophy

Figure 10: SDOCT images of 5 patients with achromatopsia (ACHM) demonstrating various phenotypes. Taken from Sundaram et al. (2014) (67). (i) continuous ISe band, (ii) ISe disruption, (iii) ISe absence, (iv) hyporeflective zone present, and (v) outer retinal atrophy.



Electrophysiological testing demonstrates absent or markedly reduced cone responses, with normal rod responses, and is valuable in helping to distinguish from LCA (71).

1.3.6.4 Natural history

There is inconsistency between data published regarding the progressive nature of ACHM (6). Whilst ACHM is considered by many to be a stable condition, Thomas et al. (2012) observed progressive

changes in retinal morphology over time in children with ACHM (72) and other studies have demonstrated loss of cone photoreceptors over time (72, 73). Newer techniques of imaging such as adaptive optics (AO) are able to produce images that can be used to quantify cone photoreceptors. However, in order to better explore the natural history of ACHM, larger numbers of patients, assessed over longer periods of time will be needed.

1.3.7 X-linked Retinoschisis

1.3.7.1 Overview

X-linked retinoschisis (XLRS) is a condition resulting in abnormal splitting of the layers within the neurosensory retina. It has a prevalence between 1 : 5,000 – 1 : 20,000 people worldwide (74).

In 1997, Sauer et al. identified the gene responsible for XLRS known as RS1 (75). When functioning, RS1 is responsible for producing a protein called retinoschisin that binds to the surface of photoreceptors and bipolar cells in order to promote cell adhesion (69). Disease-causing variants within the RS1 gene therefore result in dysfunctional retinoschisin and loss of retinal layer integrity (69). To date, over 190 disease-causing variants of the RS1 gene have been identified (69).

1.3.7.2 Features

While penetrance is complete, clinical expression is variable in males. Foveal retinoschisis (see figure 11) is commonly seen in patients with XLRS and therefore reduction of central vision to various extents is typically present. If onset occurs at birth or in infancy, presenting features may include strabismus

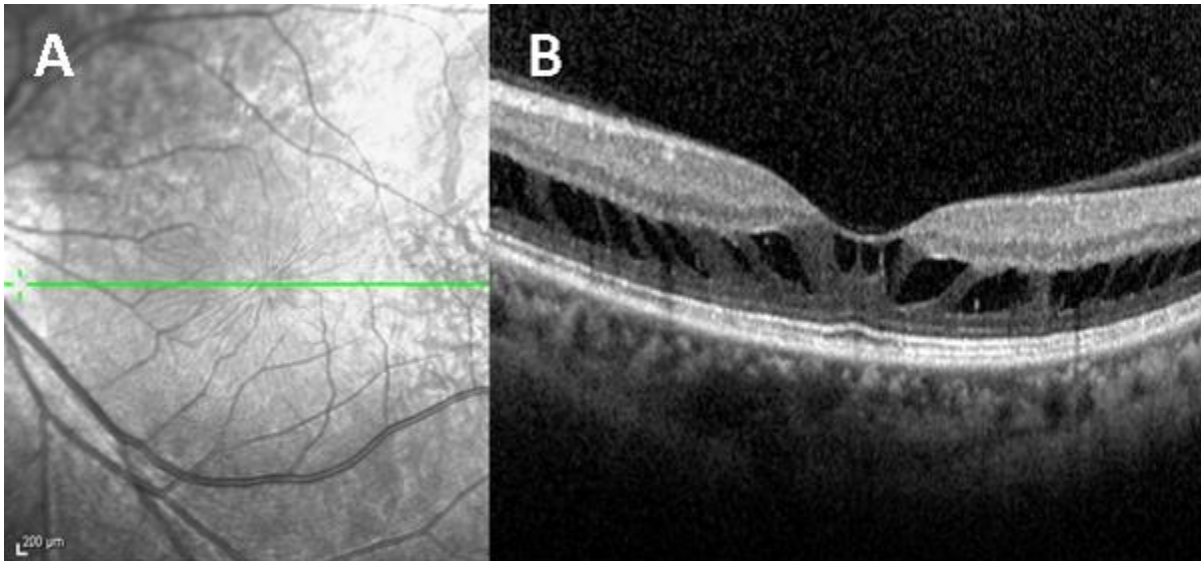
and/or nystagmus. In those that present later (around school age) bilateral central visual loss is typically the presenting feature (69). In adulthood, macular atrophy may occur (76). . Peripheral retinoschisis may also be seen in up to 50% of individuals and is typically located in the infero-temporal fundus (69). Hyperopia is also frequently seen (69). Complications such as retinal detachment (RD) or vitreous haemorrhage (VH) tend to occur within the first or second decade of life, and are associated with a poor prognosis (69). Female carriers have normal retinal structure and function (77).

1.3.7.3 Imaging and electrophysiology

SDOCT is an extremely useful tool that can demonstrate the presence of schisis even from a single line scan, for example, in an uncooperative child (69). The characteristic cartwheel pattern produced can also be demonstrated on FAF and is seen radiating out from the fovea, which occurs due to altered light transmission and is observed very commonly in XLRS (69, 74).

Electrophysiological testing typically identifies an 'electronegative' ERG: reduced b-wave (with preserved a-wave) that occurs due to inner retina dysfunction (76).

Figure 11: SDOCT image centered on the macula taken from a patient with retinoschisis. A: spokewheel-like maculopathy. B: retinoschisis as seen at the level of the green line observed in A. Used with permission.



1.3.7.4 Prognosis

RD and/or VH are complications of XLRS that tend to occur within the first or second decade of life. Most RDs are rhegmatogenous in origin and occur in up to 20% of patients whilst VH is seen in up to a third of patients (78, 79).

1.4 Update on Structural, Functional and Molecular assessment of IRD

1.4.1 Structural assessment of IRD

Over the years, imaging techniques such as SDOCT, FAF and AO scanning laser ophthalmoscopy (AOSLO) have been developed to provide adjuvant assessment of the retina in addition to history and clinical examination. Their role include:

- To provide early detection of disease as clinical assessment may initially appear normal.

- To provide serial data in order to monitor disease progression (50).

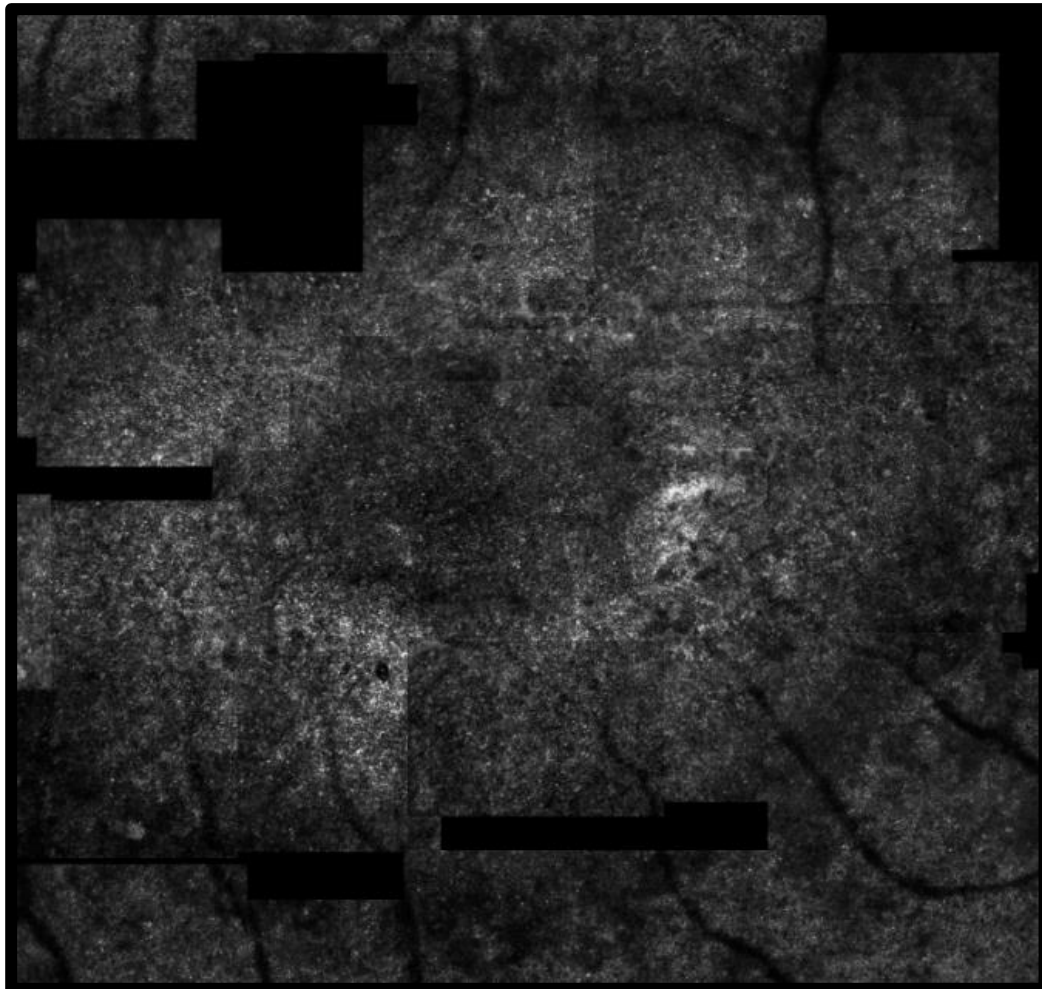
SDOCT is a rapidly evolving, non-invasive technique that uses simultaneous multiple wavelengths of reflected light to compile images of retinal architecture (23). This technique produces high resolution cross-sectional images that are able to detect signs such as outer retinal loss, retinoschisis and ELM thickening (59). ELM thickening that occurs prior to the development of atrophy in STGD has been detected in children as young as 5 years of age using SDOCT (23, 80). SDOCT has also been used to demonstrate that cataract surgery is safe and effective for patients with RP and that it does not seem to be associated with faster disease progression (81).

FAF is based on the property of 'autofluorescence' – a physiological phenomenon where certain molecules are able to emit light at a longer wavelength than that with which they were stimulated. Lipofuscin is a by-product of cell function that demonstrates autofluorescence and is thus able to be detected within RPE cells (59). Increased amounts of lipofuscin and other related metabolites within RPE cells therefore appear as areas of 'increased autofluorescence'. Whilst areas of reduced autofluorescence can be attributed to lower levels of RPE-containing lipofuscin, it may also represent areas of RPE and/or photoreceptor cell loss or atrophy. This test is particularly useful in STGD, where increased autofluorescence may highlight areas at risk of subsequent photoreceptor cell loss (23, 59).

AOSLO is a non-invasive technique that makes use of scanning laser ophthalmoscopy to produce high resolution photoreceptor and RPE mosaics in both normal and diseased eyes *in vivo* (23). To enhance the images obtained by this method, AO uses a wave-front corrector, usually a deformable mirror, to remove higher-order optical aberrations of the eye (23). Images are montaged by piecing together the best frame acquired from each video clip taken at various locations of the retina (see figure 12). The

locations are denoted by X and Y co-ordinates, for example central would be 0,0 and 1 degrees temporally and superiorly would be noted as 1S,1T. This process is usually undertaken manually, however computer based programmes have now been created to assist with this process.

Figure 12: Montage of the right macula of a patient with STGD created by myself using images acquired by AOSLO. Please note the black patches represent areas where either the image quality was too poor to include, or video clips did not overlap adequately to provide information for these segments.



1.4.2 Functional assessment of IRD

Accurate baseline assessment of visual function is crucial in patients with IRD in order to help diagnose and stage the disease, aid with providing an initial prognosis and for subsequent monitoring of disease progression. There are a variety of methods that can be employed to achieve this:

- Visual acuity:

VA is defined as “the relative ability of the visual organ to resolve detail that is usually expressed as the reciprocal of the minimum angular separation in minutes of two lines just resolvable as separate and that forms in the average human eye an angle of one minute” (82). VA is considered to have 3 components: 1) *Spatial acuity* is the ability to resolve two points in space. This is higher at the fovea than the periphery due to the difference in distribution of rods and cones. Increasing brightness also enables more cones to become responsible for VA and the ability to resolve a gap at the fovea increases (83). 2) *Temporal acuity* is the ability to distinguish visual events in time. Cones have a higher critical fusion frequency than rods at deciphering a flashing light from a continuous one (83). 3) *Spectral acuity* is the ability to distinguish differences in the wavelength of stimuli. Pilots exploit this natural phenomenon, for example, by using a background of red light in the cockpit; allowing high acuity tasks to be performed with stimulation of cones without bleaching the rods (83).

Numerous methods of VA testing are available. The Snellen and logMAR chart are two examples of methods used to test VA in adults. Preferential looking tests such as Keeler cards can be used to make an assessment of VA in pre-verbal children. Other examples for testing VA in older children include: the Kay picture test where pictures children are asked to name pictures presented to them that they are

familiar with and the Sheridan-Gardner test where the patient uses an identification card to match up the letter presented to them.

- Contrast sensitivity

Contrast sensitivity measures the ability to distinguish between finer and finer increments of light versus dark (84). It is related to the number of photoreceptor cells in a given area. It is possible for a patient with IRD to have a normal VA but reduced contrast sensitivity thus impacting on their quality of life (QOL). The Pelli-Robson chart is an example of a test that measures contrast sensitivity; it is carried out by asking the patient to read increasingly pale grey letters on a white background.

- Colour vision:

Colour is detected by short, middle and long (s, m and l)-wavelength sensitive cone photoreceptors in the macula. Dyschromatopsia is observed when IRD affects the macula. Pinkers et al. (1993), however, observed that the presence of CMO in RP affects mainly VA and not colour vision (85).

- Visual field testing:

VF testing (VFT) is a non-invasive technique designed to detect peripheral and/or central defects (23). It requires the patient to focus on a central target whilst lights of varying size and intensity are presented in different parts of the VF. 'Static perimetry' presents light stimuli as stationary targets and is typically performed by an automated machine such as the 'Octopus'. 'Kinetic perimetry' relies on an examiner to move a light stimulus from a non-seeing area to a seeing area. In either situation, a button is pressed by

the patient to inform the examiner (or computer) that they have seen the light. VFT is useful at baseline as well as at subsequent visits to help monitor progression of IRD.

- Microperimetry:

Microperimetry targets the central VF whilst simultaneously observing each point of retinal stimulation (86). Before the test begins, an infrared camera is used to define a reference frame. The stimulus position on the display can then be subsequently corrected if any eye movement is detected during the test. This produces information on how well a patient can fixate as well as allowing comparison of retinal sensitivity with retinal structure. Microperimetry is suggested to be a good measure for retinal function in IRD because retinal sensitivity in the macula correlates well with outer retinal thickness (86).

- 'Hill-of-vision':

'Hill-of-vision' (HOV) is a function of retinal sensitivity based on information acquired from VFT and microperimetry. On a topographical map, the highest peak represents an area of highest sensitivity, which is usually the fovea in eyes without disease. VF Modelling and Analysis (VFMA) is an innovative software currently being used to create detailed HOV maps that allow for a more thorough assessment of vision in IRD (23, 87).

1.4.3 Molecular assessment of IRD

Whilst construction of a family pedigree aids prediction of the inheritance pattern of a disease, confirmation of a mode of inheritance and identification of the specific underlying genetic variant(s) is

now possible with the help of molecular genetic testing. This is particularly useful when clinical diagnosis is uncertain, for example, in conditions with marked phenotypic variability.

Methods of molecular genetic testing include: single gene testing, which is useful for conditions with strong phenotype and genotype correlations, for example, XLR5 (RS1 gene) and STGD (ABCA4 gene) (88).

Other methods of testing such as APEX genotyping microarray chips can detect a fixed number of known variants from a fixed number of genes, however, as many of the IRD variants are novel this is not ideal for retinal dystrophies (10).

Next-generation sequencing (NGS) allows for large numbers of genes to be examined in a single sequencing assay in a relatively cheap and timely manner (10). This technique is employed for large gene panel testing, which is more appropriate for conditions such as RP where a large variety of genes can be responsible for the phenotypic picture (88). Panel testing also has the advantage over single gene testing in being able to detect modifiers, digenic variants, and multiallelic interactions that may complicate genetic diagnosis (88).

Despite these excellent techniques, the overall disease-causing variant detection rate of molecular diagnosis remains around 60% (22). In those patients where a molecular genetic diagnosis is confirmed, genetic counselling is tailored to provide information such as prognosis of their disease, considerations when starting a family as well as potential participation in studies and clinical trials (89). Factors such as the large numbers of genes responsible, variable expression, incomplete penetrance and oligogenic inheritance can make obtaining molecular diagnosis quite challenging (10). Genetic heterogeneity further complicates matters as some genes are implicated in other forms of retinal disease (10). Whole

exome sequencing, and to a greater extent whole genome sequencing, is now improving the rate of molecular diagnosis, and is now approaching 80%.

1.5 RP-associated cystoid macular oedema (RP-CMO)

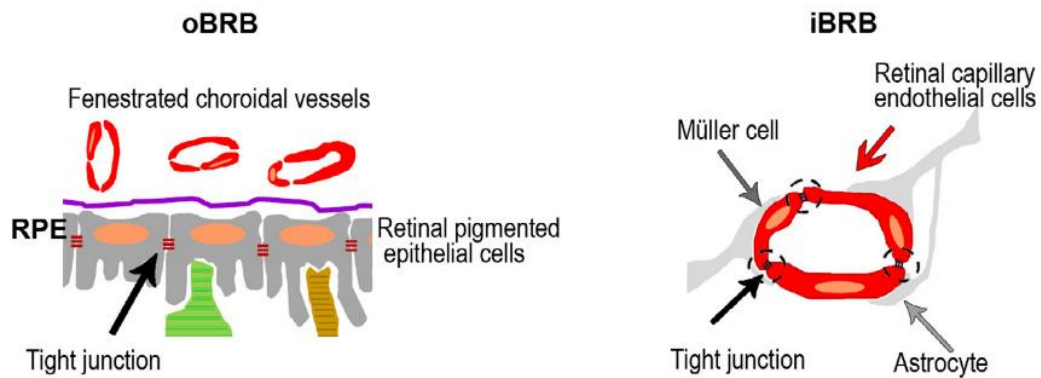
1.5.1 Pathogenesis of RP-CMO

RP-associated CMO (RP-CMO) may complicate RP and has been reported to occur in 10 - 50% of patients (90-93). Patients with RP-CMO may experience reduction of central vision either from the presence of fluid itself, or from underlying degeneration of retinal neurons due to their compression by the fluid (94). Whilst the literature does not confirm a single aetiology as cause for the pathogenesis of RP-CMO, several mechanisms have been proposed. It is plausible that RP-CMO may result from one, or a combination of these to a greater or lesser extent, depending on the genetic variant associated with RP.

1.5.1.1 Breakdown of the blood-retinal barrier

The blood-retinal barrier (BRB) exists to maintain homeostasis via the highly selective diffusion and active transport of molecules into and out of the retina thus preventing extravascular accumulation of fluid within the retina (95). This is achieved in two ways (see figure 13): (i) an outer barrier of apical tight junctions between RPE cells (96, 97), and (ii) an inner barrier of tight junctions between vascular endothelial cells (98). CMO can occur from BRB breakdown secondary to RPE and/or endothelial damage/dysfunction.

Figure 13: Schematic illustration of the blood-retina barrier (BRB). The outer barrier is composed of tight junctions between retinal pigment epithelial (RPE) cells. The inner barrier is composed of tight junctions between retinal capillary endothelial cells, however, glial cells and Müller cells surrounding blood capillaries also contribute to the formation of barrier properties. Taken from Cascio et al. (2015) (99).



Factors such as diabetes mellitus (DM), hypertension (HTN), aging and uveitis are known to cause weakening of the BRB (100). Indeed, the release of 'toxic products' released from the degenerating retina/RPE in RP may also cause weakening of the BRB and RP-CMO (101). These toxic products include: vascular endothelial growth factor (VEGF), adenosine (95), prostaglandins (PG) (100), histamine (102), Insulin-like growth factor-1 (IGF-1) (103), tumour necrosis factor alpha and interleukin 1 alpha and beta (104).

Vinores et al. (1995) performed a study using immunolocalisation of endogenous albumin to identify whether extravasation was greater from the inner or outer barrier in eyes with RP compared to normal eyes (105). In eyes with RP alone, albumin leakage was greatest from the inner barrier (105). In RP associated with other ocular complications (e.g. aphakia, glaucoma), leakage varied between the inner and outer barriers. No correlation was found between severity of photoreceptor degeneration and

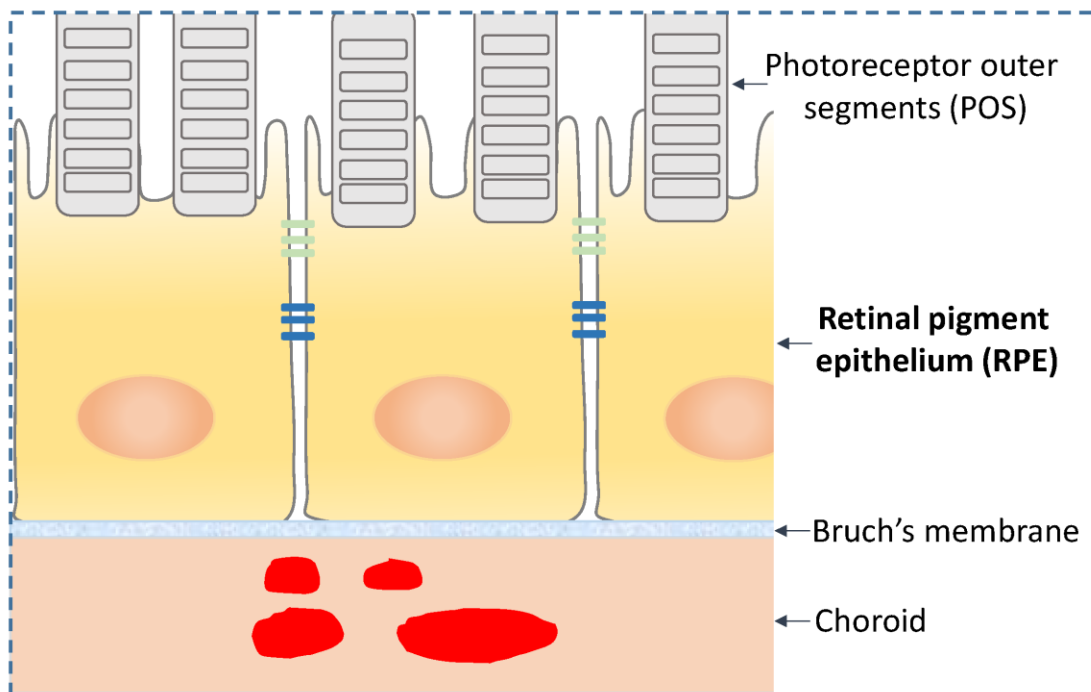
albumin leakage (105) suggesting that therapies for RP-CMO could be used regardless of underlying disease status.

1.5.1.2 Failure (or dysfunction) of the pumping mechanism in the RPE

The RPE constitutes a layer of cuboidal cells located directly underneath the photoreceptor cells, with extensions of microvilli from the apical surface that envelop their outer segments (see figure 14) (106).

This single layer of cells has a variety of essential functions in order to help maintain vision, including: the conversion and storage of retinoid, the phagocytosis of shed photoreceptor OS membrane, the absorption of scattered light, ion and fluid transport and RPE-photoreceptor apposition (106). This latter function is achieved by pumping fluid out from the sub-retinal space in order to maintain negative hydrostatic pressure (107).

Figure 14: Schematic illustration of the retinal pigment epithelium (RPE). Taken from Beranova-Giorgianni and Giorgiani (2018) (108).



RPE cells exhibit polarity, whereby cell components are asymmetrically distributed in apical or basolateral domains of the cell (106). Unlike other epithelia, the Na^+/K^+ -ATPase pump is expressed apically in the RPE cell. Under normal conditions, Cl^- enters the RPE cell via this pump and exits via Cl^- channels on the basolateral membrane that are modulated by intracellular Ca^{2+} (106). It is this active transport that drives water through aquaporin channels from the sub-retinal space into the choriocapillaris. Failure (or dysfunction) of this pumping mechanism may occur in RP, which could result in RP-CMO. Furthermore, the presence of CMO has been suggested to result in loss of polarised distribution of membrane-bound carbonic anhydrase (CA) IV in the RPE, thus further contributing to RP-CMO (98).

- 1.5.1.3 Muller cell oedema and dysfunction

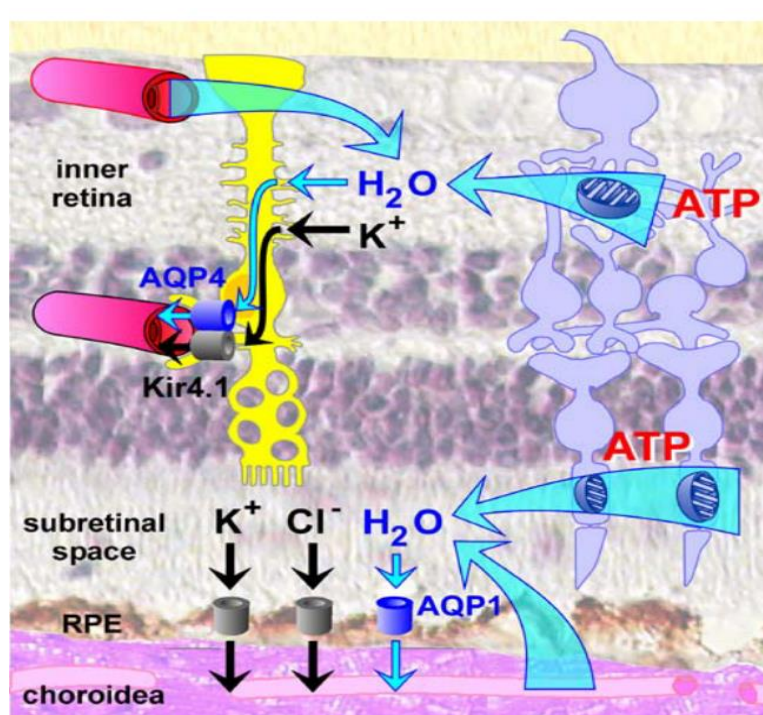
Regardless of the underlying pathogenesis, chronic CMO occurs only when the rate of fluid entry into the retina exceeds the rate of fluid absorption.

Fluid entry into the retina can either be directly from the blood, coupled to glucose up-take and/or as a by-product of aerobic metabolism; with the bidirectional movement of water osmotically coupled to the transport of osmolytes such as potassium (K^+) (See figure 15) (94). Whilst sub-retinal fluid absorption from the retina is via the RPE cell, the inner retina itself is dehydrated by the Muller cell. The Muller cell therefore not only plays an essential role in visual transduction but also in retinal homeostasis (fluid dynamics, ions, neurotransmitter molecules and pH) (94).

Under normal circumstances, K^+ released by activated retinal neurons are passively taken up by the Muller cells to prevent a build-up and potential excito-toxicity. This occurs via inwardly-rectifying channels (Kir), specifically Kir2.1, located in membranes with close proximity to the retinal neurons (94).

Release of K^+ occurs via Kir4.1 channels located in membranes with close contact to structures such as blood vessels (94). Kir channels consist of two transmembrane regions with cytosolic NH_2 and $COOH$ termini connected by a pore-forming loop. Molecules such as magnesium (Mg^{2+}) and polyamines are able to physically block the Kir4.1 channel pore from allowing outward movement of K^+ , while still able to accept inwards movement of K^+ (109). In addition, under pathological conditions such as inflammation and oxidative stress, Kir4.1 channels redistribute, becoming more evenly spread throughout the Muller cell and moving away from sites where ion and fluid release takes place. Kir2.1 channels, however, do not redistribute. Continued inward movement of K^+ therefore results in intracellular K^+ overload, increased osmotic pressure within the Muller cell, reduction in water efflux and ultimate Muller cell swelling (109). Indeed, it may be that the Muller Cell undergoes these changes and resultant swelling in retinal degenerative diseases such as RP.

Figure 15: Water fluxes through the retina. Taken from Reichenbach et al. (2007) (94). AQP1 = aquaporin1 channel. ATP = adenosine 5'-triphosphate. Kir4.1 = Inward rectifying potassium channel.



Makiyama et al. (2014) used OCT to investigate the prevalence and spatial distribution of cystoid spaces (CS) in patients with RP. Seventy-four of 275 patients (27%) demonstrated RP-CMO in at least one eye. INL CS were observed in 99% of eyes with CMO. The ONL/OPL was involved in 28% and GCL involved in 7% (110). These findings indirectly support the hypothesis of Muller cell swelling and dysfunction since CS were most frequently observed in the INL, which is where Muller cell bodies reside. It is also interesting to note that 79% of CS were located in areas of relatively well-preserved outer retina (110); in keeping with the observation that CMO is seen more commonly in less advanced RP compared to late stage RP.

Muller cells also release factors such as VEGF in response to hypoxic, inflammatory or glucose-deprived conditions thus contributing further to breakdown of the BRB and increased vascular permeability (94).

- 1.5.1.4 Anti-retinal antibodies

In 1970, Rahi demonstrated that photoreceptors of the retina are antigenic using haemagglutination and precipitation techniques (111). This raised the possibility that retinal degeneration could be wholly or partly due to immunologic injury (112).

Rahi (1973) subsequently managed to quantitatively estimate serum levels of immunoglobulin (Ig) G, A and M in 52 patients with RP compared to 40 controls (112). Significantly higher levels of IgM were found in a proportion of patients with RP as compared to controls, however, the exact role IgM plays in retinal degeneration could not be confirmed (112).

Spiro et al. (1978) investigated this further with genetic analysis to see whether a specific genetic class of RP had raised IgM levels. Unlike Rahi (1973), no difference in serum IgM levels was found between 75 patients with RP and 51 controls. Neither was there a difference between genetic classes of RP patients detected (113). Disagreement of results between these studies may have occurred due to variation of method/techniques used, environmental factors such as viral illness, or differences in RP patient population recruited (113).

Spalton et al. performed immunological studies on 17 RP patients with central and/or peripheral vascular leakage observed on FFA. IgG levels as well as complement C₃ and its breakdown product levels were within normal limits in all patients. Similar to Rahi (1973), 5 out of 17 patients had raised IgM unrelated to degree of vascular leakage. All patients demonstrated positive immunofluorescence to rat photoreceptors at 1:5 dilution of serum, however, this could be attributed to cross reactivity of smooth muscle antibodies with photoreceptor contractile organelles (101).

Anti-retinal antibodies have been prospectively studied by Heckenlively et al. (1996) in 30 RP patients with CMO compared to 30 RP patients without CMO. Anti-retinal antibodies were found in 27 of 30 RP-CMO patients compared to 4 of 30 RP patients without CMO (114). The most common retinal proteins showing antigenicity were CA II (30 kD) and enolase (46 kD) both occurring in 17 (63%) of 27 patients (114). In 1985, Chant et al. screened sera by indirect immunofluorescence on normal donor human eye sections to detect antibodies to human retinal antigens. They found 43 (37%) of 116 patients with RP to have binding of serum antibodies to normal donor retina compared to only 1 in 42 (2%) in the control group (115). Nevertheless, the role of anti-retinal antibodies in RP progression or RP-CMO remains unclear, with many unanswered questions including whether they are a secondary consequence of the

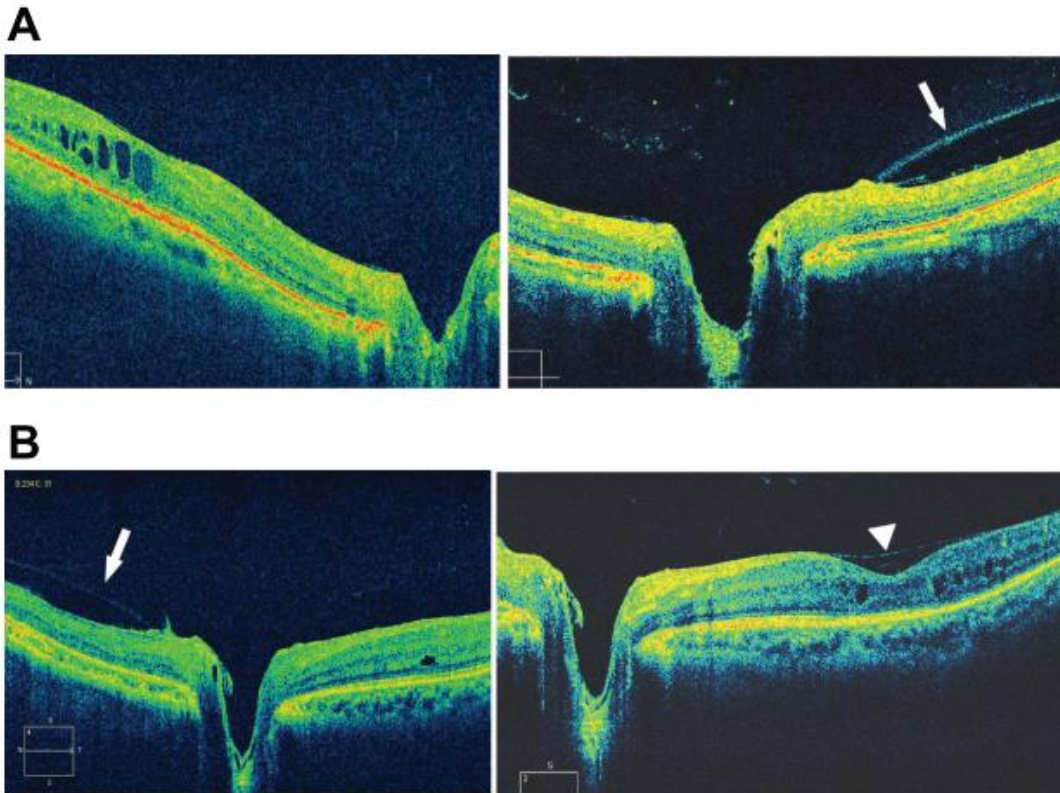
degenerative process, the wide range of auto-antibodies identified, and the high prevalence in normal controls (114, 115).

Whilst anti-retinal antibodies may play a role in RP-CMO, the exact mechanism through which this occurs is not fully understood.

- 1.5.1.5 Vitreous traction

It has been suggested that vitreous traction and epiretinal membrane (ERM) may contribute to RP-CMO by causing mechanical damage to Muller cells via an inflammatory reaction with subsequent capillary dilatation and leakage (116, 117). Schepens et al. and Takezawa et al. have reported cases of RP-CMO in the presence of vitreous traction (see figure 16) (117, 118). Interestingly, vitrectomy with posterior hyaloid removal has been seen to improve cases of diabetic macular oedema (DMO) whether or not there is presence of posterior vitreoretinal traction (119). Improvement of CMO in eyes with chronic uveitis has also been observed following vitrectomy (120).

Figure 16: Example of a patient with RP-CMO in the presence of vitreous traction. Taken from Takezawa et al. (2011) (118). Spectral domain optical coherence tomography images of the right A) and left B) eyes. In the right eye, cystoid macular oedema (CMO) at the fovea and posterior vitreoschisis (arrow) in the nasal quadrant is seen without a posterior vitreous detachment (PVD). In the left eye, minimal CMO at the fovea with a focal PVD (arrowhead) and posterior vitreoschisis (arrow) in the nasal quadrant are seen.



1.5.2 Diagnosis and monitoring of RP-CMO

Prior to the advent of OCT, monitoring of RP-CMO included slit-lamp biomicroscopy together with FFA.

OCT has since been shown to be more sensitive in detecting CMO compared to biomicroscopy in patients with diabetic retinopathy and RP-CMO (121, 122). OCT can detect CS in RP-CMO even when little, or no leakage is demonstrated on FFA (122, 123) and being non-invasive is ideal for monitoring RP-CMO. No studies have been performed using OCT-angiography (OCT-A) to investigate RP-CMO.

RP-CMO is not always associated with a reduction in VA (124). In 2009, Oishi et al. published a retrospective cross-sectional study carried out on 41 eyes of 25 patients with centre-involving RP-CMO. Cross-sectional scans obtained using Stratus OCT were reviewed to obtain information regarding foveal thickness, transverse/vertical lengths of foveal CS and photoreceptor layer thickness. These results were

compared with logMAR VA to see if there was a correlation with macular morphology. No correlation was found between VA and foveal thickness, photoreceptor layer thickness or vertical length of the CS. Whilst transverse length of CS demonstrated some statistical significance, the correlation coefficient was weak (125). The integrity of the IS/OS, however, has been shown to correlate well with VA and inform the likely prognosis (124-126).

Reduced VA in patients with RP-CMO may therefore be due to underlying retinal atrophy in addition to, or instead of from increased foveal thickness (127). Underlying retinal dysfunction/loss should therefore be considered when anatomical improvement is observed without complementary improvement of VA. Automated static perimetry may also be useful for monitoring RP-CMO given the documented correlation between changes in retinal thickness due to CMO and retinal sensitivity (128-130).

1.5.3 Inheritance patterns and specific associations with RP-CMO

A detailed clinical history together with construction of a pedigree chart can help provide insight into the mode of inheritance a patient with RP has. However, definitive conclusion can only be made with a molecular diagnosis.

In 1978, Spalton et al. investigated 25 patients with RP-CMO and concluded that oedema was no more common in any of the genetic groups when compared with any other: 6 patients with AD inheritance, 4 males with XL inheritance and 1 female heterozygote with the XL gene (131). Two other patients had a family history of RP but not enough information for formal classification. The remaining patients were classified as sporadic or AR (131).

Hajali et al. (2008) investigated 124 patients aged between 8 – 71 years-old with RP and found CMO in at least one eye to be most prevalent in the AD group (52%), followed by the AR group (39%), isolated group (39%) and Usher II group (35%), however, this was not statistically significant (91). RP-CMO also did not appear to be related to age-related, however, the numbers of patients within the youngest and oldest age groups were small (91).

Testa et al. (2014) carried out a retrospective cohort study involving 1161 eyes of 581 Italian patients with RP to assess for the presence of macular abnormalities. Data was collected using OCT images and medical records and found CMO to be the most frequent macular abnormality observed in 237/1161 (20.4%) eyes from 133/581 (23%) patients. This was significantly more frequent in females (27.5%) than in males (19.1%) and in the AD inherited pattern (34.1%) compared to the AR (13.9%) and XL (7.1%) groups (93). ERM was the next most frequent macular abnormality, observed in 181/1161 (15.6%) eyes from 115/581 (19.8%) patients (93).

In contrast, Liew et al. (2015) constructed pedigrees for RP-CMO patients in a retrospective cohort and found 55/81 (68%) patients with AR inheritance (4 of whom were molecularly proven), 25/81 (31%) patients with AD inheritance (16 of whom were molecularly proven) and 1/81 (1%) patients with XL inheritance (132).

A Korean family with AD-RP associated with the p.P347L variant in *RHO* has been reported, where all four children had bilateral CMO, suggesting this *RHO* variant may be associated with early-onset CMO (133). Despite severe bilateral CMO in all four children (aged between 11 - ...), VA remained good with preservation of the IS/OS line as confirmed by OCT. The mother (aged 44-years), however, had VA of light perception (PL) associated with marked foveal atrophy and a disruption of the IS/OS line confirmed

by OCT. The report suggests that early-onset CMO associated with the p.P347L variant in *RHO* may account for the severe visual prognosis (133).

In a recent cross-section prevalence study published by Liew et al. (2018), 338 eyes of 169 patients with RP were evaluated using SDOCT for CMO and/or ERM. CMO was observed in 50.9% of eyes, which was associated with younger age but not with gender (134). Patients with ERM and cataract/pseudophakia were less likely to have CMO (134). CMO was most prevalent in patients with AD inheritance (71.4% with CMO in at least one eye), followed by AR/sporadic inheritance (58.9%) and XL inheritance (12.5%) (134). ERM was found in 22.8% of eyes (134).

1.5.4 Avenues of intervention for RP-CMO

1.5.4.1 Pharmacological

- Carbonic Anhydrase Inhibitors: Oral and Topical

CA is an enzyme involved in hydrogen (H^+) ion transport located in various parts of the body (34). It increases renal elimination of Na^2+/HCO_3^- and K^+ , which results in a reduction of plasma bicarbonate and resulting metabolic acidosis (34). In the presence of CMO, membrane-bound CA IV is thought to lose its polarised distribution in the RPE. Inhibition of this enzyme results in acidification of the sub-retinal space, increased Cl^- transport, with subsequent movement of water across the RPE into the choroid (98). In addition, inhibition of CA increases tissue carbon dioxide (CO_2) concentrations and/or lowers tissue pH, resulting in vascular dilation and increased blood flow (135). Transvitreal placement of a polarographic oxygen electrode in anaesthetised pigs observed an increase in oxygen tension and

dilation of retinal arterioles and venules 30 minutes after intravenous injection of 500mg dorzolamide (136).

CAIs are often used for the treatment of RP-CMO despite no randomised controlled trials (RCTs) (comparing CAI with placebo) being published to provide evidence for their safety and efficacy.

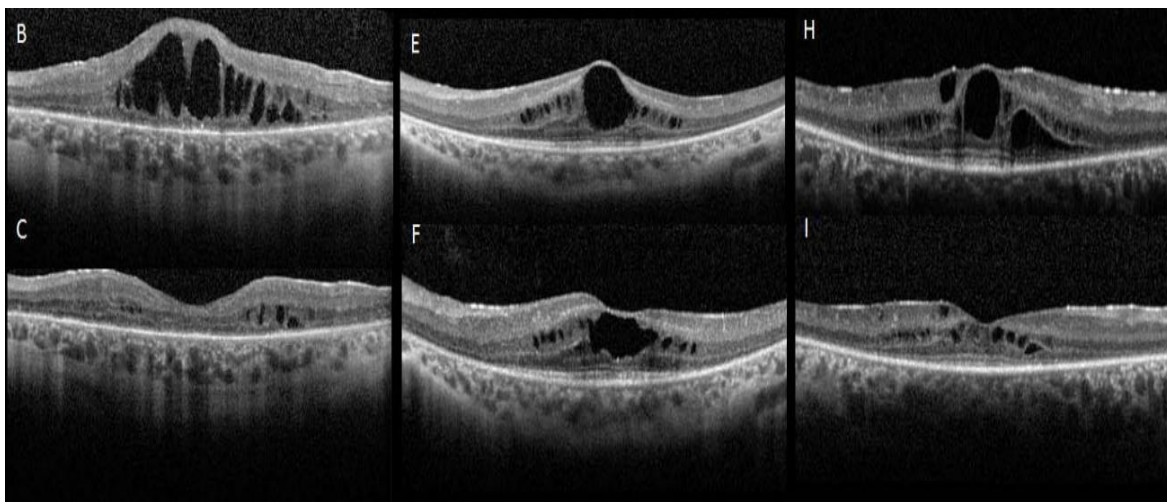
Notwithstanding this, several studies of varying quality have been published documenting response of RP-CMO following treatment with CAIs. The typical outcome measures used include central macular thickness (CMT) and VA.

A recent systematic review published by Bakthavatchalam et al. including 23 studies concluded that whilst both oral and topical CAIs are “effective first-line treatments” for RP-CMO, oral acetazolamide is superior to topical dorzolamide (137). The paper recommended that oral acetazolamide therefore be considered as first-line treatment for RP-CMO unless its adverse effects cannot be tolerated in which case topical dorzolamide can be used (137). Intravitreal steroids, oral corticosteroid, intravitreal anti-VEGF, grid laser photocoagulation, pars plana vitrectomy, or ketorolac were also deemed to be effective in improving RP-CMO for patients unresponsive to CAI treatment (137). Regardless of the type of therapy used, CMO recurrence was commonly seen long term (137).

The largest retrospective study to date was carried out by Liew et al. in 2015. One hundred and fifty seven eyes of 81 patients with RP-CMO were investigated with SDOCT before, and after treatment with CAIs. Eyes were considered as ‘responders’ if CMT decreased by at least 11% following treatment (132). Sixty four out of 81 (79%) patients were treated with 2% dorzolamide topically. Of these 125 eyes, 40.0% were deemed as ‘responders’ to treatment with a mean reduction CMT of 105 microns (μm). Mean VA improved from 6/15 at baseline to 6/12 after a median time on treatment of 3 months. Patients with AR

inheritance were found to be more likely to respond to topical dorzolamide treatment than those with AD inheritance. Seventeen out of 81 (21%) patients were treated oral acetazolamide. Of these 32 eyes, 28.1% were deemed as 'responders' to treatment with a mean reduction CMT of 115 μm . Mean VA improved from 6/15 at baseline to 6/12 over a median time on treatment of 4 months (132). Better response to treatment was observed in those patients with higher baseline CMT values. See figure 17 demonstrating examples of patients who have demonstrated response to topical or oral CAIs.

Figure 17: SDOCT of three patients with RP-CMO showing response to carbonic anhydrase inhibitors. B = Patient with *USH2A*-associated AR-RP; visual acuity 1/60 prior to treatment, C = The same patient after 9 months of treatment with topical dorzolamide three times a day showing marked improvement in cystoid macular oedema but without any improvement in visual acuity. E = Patient with Ushers syndrome type 1-associated AR-RP, F = The same patient after 4 months of treatment with topical dorzolamide three times a day showing improvement of CMO; visual acuity improved to 6/12. H = Patient with AR-RP; visual acuity was 6/36 prior to treatment, I = The same patient after 7 months of treatment with 250 mg slow release oral acetazolamide twice a day and improved visual acuity of 6/18. Taken from Liew et al. (2015) (132).



Grover et al. (2006) carried out a prospective non-randomised study of 15 patients with RP-CMO where each patient was treated with topical dorzolamide three times daily for at least 4 weeks. Thirteen out of 15 (87%) patients showed a reduction in CMT of at least 16% in at least one eye (138).

Similar findings were observed by Fishman et al. (2007) who also carried out a prospective non-randomised study of 8 patients with RP-CMO where each patient was treated with topical dorzolamide three times daily. All patients had a significant reduction of CMT after being on treatment for 1 -2 months (139). Three out of 6 (38%) also demonstrated statistically significant improvement of VA by at least 7 letters on the Snellen chart, in at least one eye (139).

Ikeda et al. (2013) carried out a prospective study in 10 patients with RP-CMO where each patient was treated with topical 1% dorzolamide three times daily for 18 months. Within 6 months, 9 out of 18 (50%) eyes demonstrated near complete resolution of CMO and another 5 out of 18 (28%) eyes demonstrated more than 20% reduction of CMT from baseline but this was not enough to classify them as near complete resolution (140). Improvement was sustained in 8 out of 9 (88.9%) eyes where near resolution of CMO was observed (140). Macular sensitivity improved by 18 months in all eyes in which initial CMO demonstrated near complete resolution (140).

Fishman et al. (1994) carried out a prospective, double-masked, crossover study using oral methazolamide 50mg twice daily versus placebo. A wash-out period of at least 28 days was included in between trialling each substance. Nine out of 17 (53%) patients using oral methazolamide demonstrated angiographic improvement of CMO, however, VA improved in at least one eye, by at least 2 lines in only 3 patients (141).

Fishman et al. (1989) also carried out a prospective, masked, crossover study using oral acetazolamide versus placebo for 2 week periods. VA improvement of at least one line, in at least one eye was observed in 10 out of 12 (83%) patients. However, 3 of these patients initiated on placebo demonstrated improvement only once switched to acetazolamide (142). Six out of 12 (50%) patients showed reduced leakage on FFA (142).

A recent meta-analysis published by Huang et al. (2017) included 11 studies where patients with RP-CMO had received topical or oral CAI therapy. Mean reduction in CMT was 46.02 μm , which was statistically significant. All but 3 studies demonstrated improvements in VA following CAI treatment (143).

Effect of location of CS on response to CAIs

Location of CS in RP-CMO may influence response to treatment with CAIs. Acetazolamide cannot readily enter the neurosensory retina making it potentially less effective at reducing INL CS (144). However, with good access to the RPE basolateral membrane (145), acetazolamide may be more effective at reducing ONL CS (146).

After gathering information from the above studies, I designed a retrospective cohort series entitled 'Carbonic Anhydrase inhibitors for Retinitis Pigmentosa And Macular oEdema in various Layers (the CARMEL study)' using SDOCT to: 1) detect which layer(s) CS's are present within in patients with RP-CMO, and 2) to review the effect that topical versus oral CAIs have on these CS's to see if there is a difference noted between treatments.

RP-CMO recurrence

Rebound CMO has been observed after stopping CAIs.

Grover et al. (2006) observed worsening of CMO with continued topical 2% dorzolamide three times daily treatment in 4 out of 13 (31%) patients demonstrating initial improvement (138).

Similarly, Fishman et al. (2007) describe worsening of CMO in both eyes of 2 patients who initially improved using topical 2% dorzolamide three times daily treatment (139).

Ikeda et al. (2013) observed RP-CMO recurrence in 4 out of 5 (80%) eyes by 18 months where an initial reduction of CMT by at least 20% from baseline had been observed within the first 6 months (140).

Thobani and Fishman (2011) carried out a retrospective chart review on 3 patients (1 patient with type 2 Usher syndrome, 1 patient with AD-RP and 1 patient with XLR5) using CAI therapy for CMO. All 3 patients experienced a recurrence of CMO whilst on CAI medication, which was thus discontinued. After a discontinuation period of between 1 – 6 months, all 3 patients demonstrated improvement of CMO following re-introduction of CAI treatment (147).

Side-effects:

Unfortunately, systemic absorption of CAIs can result in side-effects such as fatigue, loss of appetite, limb paraesthesia, kidney stones, aplastic anaemia and electrolyte disturbance including hypokalaemia with potential associated cardiac arrhythmia (148). Fortunately, topical CAIs are available with both liposolubility and hydrophilicity to allow the easy passing of the drug through the cornea, together with high affinity for the enzyme allowing 98% of CA inhibition (34). Side-effects are greatly reduced if using

topical treatment due to less systemic absorption. It can, however, lead to a bitter taste in the mouth due to inhibition of CA in the taste buds (139). Lacrimal sac compression during usage can lessen this effect.

Several studies have compared efficacy of topical versus oral CAI therapy as it would appear optimal to use topical therapy to lessen the risk of side-effects:

Grover et al. (1997) carried out a prospective, double-masked, cross-over study on 5 patients with RP-CMO who were randomised to receive either topical 2% dorzolamide or placebo eye-drops three times daily for 4 weeks. This was followed by a 4 week wash out period before receiving the alternative eye-drop for another 4 weeks. Following a second wash out period of 4 weeks, patients were treated with oral acetazolamide 500mg daily for 2 weeks. No objective improvement of VA was found in patients using topical 2% dorzolamide, however, 2 out of 5 (40%) patients had reduction of leakage seen on FFA. In comparison, an improvement of VA by 7 ETDRS letters or more was observed in 3 out of 5 (60%) patients when taking oral acetazolamide, with improvement of leakage documented by FFA in all patients (149).

In contrast to this, a more recent publication by Pacella et al. (2014) described 3 case reports of patients with RP-CMO using topical 2% dorzolamide versus oral acetazolamide. It is important to note that only patients 1 and 2 were treated with three times daily topical 2% dorzolamide following discontinuation of oral acetazolamide due to side-effects and insufficient quantitative data is provided overall in order to allow direct comparison between these treatments: Patient 1 had an initially good response to once daily oral acetazolamide 250mg with resolution of CMO and improvement of VA from 4/10 at baseline to 8/10 in the right eye (RE) and 3/10 at baseline to 7/10 in the left eye (LE). Due to renal colic, this was

stopped and replaced with three times daily topical 2% dorzolamide after relapse of CMO. Reduction (but not resolution) of CMO was observed after 7 days of use as documented by Spectralis HRA-OCT. Images and figures are provided for the RE only; baseline CMT 551 μ m, 3 weeks after starting treatment CMT 401 μ m, 6 months after starting treatment CMT 376 μ m (34). Patient 2 was also initiated on three times daily 'half a tablet' oral acetazolamide. After 7 days, resolution of CMO and improvement of vision from 5/10 at baseline to 8/10 was observed in the RE, with reduction of CMO and improvement of vision from 3/10 at baseline to 6/10 in the LE. Treatment was stopped due to paraesthesia and asthenia and replaced with topical three times daily 2% dorzolamide in each eye following recurrence of CMO. According to the paper, "considerable improvement" of the oedema (RE more than LE) was observed, however, images and figures were provided for the LE only; baseline CMT 463 μ m, 3 weeks after starting treatment CMT 330 μ m, 6 months after starting treatment CMT 322 μ m. The third patient had a history of renal calculi so was initiated on three times daily topical 2% dorzolamide. Significant reductions of CMT were observed: Baseline median 459 μ m, range 449 – 551 μ m; 3 weeks after starting treatment median 373 μ m, range 318 – 410 μ m; 6 months after starting treatment median 348 μ m, range 270 – 395 μ m (P = 0.002), however, no significant improvement of BCVA was observed in either eye (34).

- Steroids: Oral, Periocular and Intravitreal

Steroids inhibit production of arachidonate indirectly through the induction of lipocortin synthesis and subsequent inhibition of phospholipase resulting in reduced synthesis and release of pro-inflammatory cytokines, including: PG's, leukotrienes, VEGF and intercellular adhesion molecule 1 (150-154). An additional action of steroids that non-steroidal anti-inflammatories (NSAIDs) are not able to do, includes the ability to reduce the migration of macrophages and neutrophils, thereby reducing vascular permeability (155). This results in an improvement of BRB function and reduction of CMO, which has been documented in several intraocular neovascular, proliferative and oedematous diseases (92, 156-

171). Triamcinolone has also been shown to resolve CMO by preventing osmotic swelling of Muller cells. It achieves this by inducing the release of endogenous adenosine and subsequent A1 receptor activation, thus enabling the opening of ion channels (94).

- Oral deflazacort

Deflazacort is a third generation synthetic glucocorticoid with fewer side effects than prednisolone and longer immunomodulating and anti-inflammatory effects (160). Giusti et al. (2002) undertook a 1 year pilot study using oral deflazacort in 10 patients with RP-CMO. The treatment regime included the following: 30 milligrams (mg)/day for a week, 15 mg/day for two weeks, 6 mg/day for one month, 6 mg every other day for two months, 6 mg/day every three days for four months, 6 mg/day every three days for four more months. Significant improvements in near VA, retinal sensitivity, and angiographic findings were observed within 4 months of treatment with no ocular or systemic side-effects recorded (160). Despite some recurrence of CMO that persisted throughout the study, 47% of patients had reduced leakage on FFA compared to baseline, at the end of the study (160).

- Topical betamethasone

Kitahata et al. (2018) carried out a retrospective cohort study including 16 eyes of 10 patients with RP-CMO in whom treatment response with CAIs had been unsatisfactory. Topical 0.1% betamethasone was given daily following a 3-month course of topical brinzolamide in 14 patients and topical 2% dorzolamide in 2 patients. Mean CMT decreased over the first 7 months, but not thereafter compared to baseline. No statistically significant improvement of BCVA was demonstrated throughout the course of the study (172). Treatment was stopped in 3 patients in whom IOP was elevated.

- Intravitreal triamcinolone acetonide (IVTA)

Five patients aged 25 – 41 years (mean 33.2 years) with RP-CMO that had failed treatment with 250mg oral acetazolamide twice daily for 1 month underwent unilateral intravitreal injection of 4mg (0.1ml) of triamcinolone acetonide. Oral acetazolamide was stopped once the injection had been received. CMT improved from 418 μ m (range 376–626 μ m) at baseline, to 224 μ m (range 214–326 μ m) at 1 month post-injection, 275 μ m (range 215–584 μ m) at 3 months post-injection and 312 μ m (range 239–521 μ m) at 6 months post-injection. VA improved in 2 patients by 1 month post-injection but was not maintained. IOP measurement did not exceed 21 mmHg during the follow-up period. Re-treatment was performed where CMO recurrence occurred in 1 patient at 3 months post-injection and in another 2 patients at 6 months post-injection (92).

Scorolli et al. (2007) carried out a prospective, non-randomised trial comparing 20 eyes of 20 patients with RP-CMO treated with 0.1ml IVTA with 20 eyes of 20 RP-CMO patients who declined treatment (controls). All treated patients demonstrated anatomical improvement, which was greatest at 3-months post-injection. No statistical improvement in VA was observed. At day-1 post-IVTA, 10 eyes (50%) had a raised IOP (>21mmHg) including 2 patients (10%) measuring between 30-35mmHg. All IOPs returned to baseline within 6 months (168).

A case report published by Barge et al. (2013) describes a 32-year-old male with bilateral RP-CMO refractory to oral acetazolamide and topical ketoralac, who received bilateral IVTA. VA improved from 20/50 at baseline in the RE to 20/40 and 20/100 at baseline in the LE to 20/50 at 1 week post- injection with bilateral resolution of CMO (158). A rise in IOP was noted at 1 month post-injection in both eyes, which was controlled with topical timolol and brimonidine (158). At 2- and 5- months post-injection in the RE and LE respectively, VA reduced secondary to CMO recurrence. A second IVTA was performed in

the LE with resultant improvement of VA and CMO, however, CMO recurrence happened again. Bilateral sub-tenon injections of triamcinolone (40mg) were performed, again, resulting in improvement of VA and CMO. However, CMO recurrence occurred in the LE at 3 months (158).

A case report published by Saraiva et al. (2003) describes a 30-year-old male with bilateral RP-CMO, treated with bilateral 0.1 mL IVTA (0.4%) solution following treatment failure with oral acetazolamide. In the RE, baseline BCVA remained unchanged from 20/40 despite resolution of CMO. In the LE, baseline BCVA improved from 20/80 to 20/50 with resolution of CMO. It should be noted that the paper does not state the exact timeframe at which these results were obtained post-injection. It does confirm, however, that any visual improvement gained was lost by 6 months post-injection due to recurrence of CMO (166).

- Intravitreal dexamethasone

Intravitreal dexamethasone (DEX implant; Ozurdex, Allergan, Inc., Irvine, CA) has also been used in 4 eyes of 3 patients with RP-CMO refractory to oral CAI's and/or sub-tenon triamcinolone and/or topical NSAID. Mean CMT improved from 443µm at baseline, to 234µm at 1 month post-injection. Mean BCVA improved from 20/160 (range 20/50–20/200) at baseline, to 20/100 (range 20/40–20/125) at 1 month post-injection. At 3 months post-injection, mean CMT was 332µm and BCVA was 20/125 (range 20/100–20/200), requiring re-treatment in 2 patients due to CMO recurrence. No serious ocular or systemic adverse events (SAE) occurred (169).

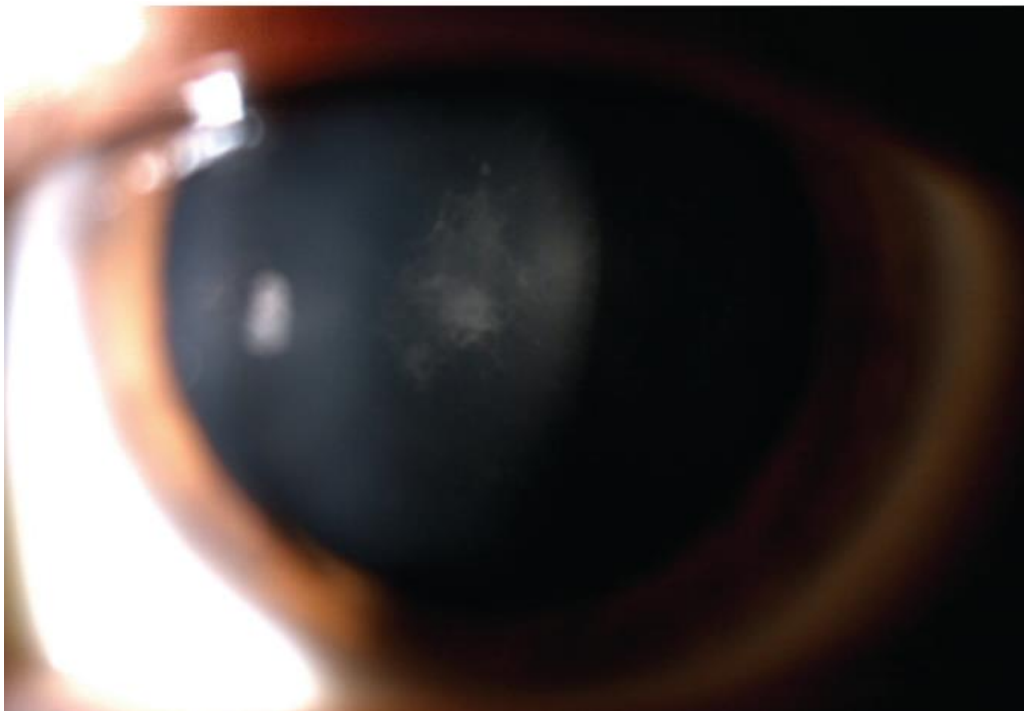
Another case report published in 2013 had similar outcomes; a 36-year-old male showing no VA improvement whilst using topical 2% dorzolamide three times daily received bilateral 0.7mg DEX implants. Complete resolution of CMO was documented in both eyes at 1 week post-injection and VA

improved from 2/10 at baseline, to 4/10 at 1 week post-injection in both eyes. Unfortunately, recurrence of CMO occurred at 2- and 3-months post-injection in the LE and RE respectively (164).

- Limitations to the use of steroids

Whilst the incidence of side-effects relating to the use of steroids in RP-CMO is unknown, common side-effects 'seen in more than 1 patient in 10' following DEX implant, include: IOP rise, conjunctival bleeding and cataract (see figure 18). DEX implant appears, however, to have a lower incidence of cataract and raised IOP compared to IVTA in treatment of retinal vein occlusion (RVO)-CMO (173, 174). There has been one reported case of central serous retinopathy following DEX implant in a 46-year-old suffering from DMO (175). The regular use of steroids for RP-CMO will therefore be significantly limited by its side-effect profile.

Figure 18: Mild sub-capsular cataract in the left eye. Taken from Barge et al. (2013) (158).



- Topical Non-Steroidal Anti-Inflammatory

NSAIDs act by inhibiting cyclo-oxygenase (COX) thus reducing the formation of PG's (154). Additional anti-inflammatory actions include suppression of polymorphonuclear cell locomotion and chemotaxis, reduced expression of inflammatory cytokines and the ability to act as free radical scavengers (176-178).

RP-CMO is not a common condition and the majority of research on the mechanism behind CMO generation has therefore been in association with cataract and intra-ocular lens (IOL) surgeries (100). Increased levels of PG's have been demonstrated in the aqueous following stimulation of the iris or other structures during surgery (100) and are highest when vitreous loss occurs (179). It is hypothesised that these PG's diffuse into the vitreous causing breakdown of the BRB and resultant CMO. Results from a meta-analysis undertaken in 1998 confirmed that fluorescein-angiographic CMO can be prevented by pre- and post-operative use of the topical NSAID, indomethacin (180).

With this in mind, NSAIDs have been trialled for use in RP-CMO (albeit minimally), which may arise from similar pathology of BRB breakdown. Non-selective COX inhibitors for ophthalmic use include indole, phenylacetic and phenylalkanoic acids, which are compounds easily converted into eye-drop formulation due to their highly water-soluble nature (177). Other chemical classes of NSAID, such as salicylates, fenamates and pyrazolone derivatives are considered too toxic to be used in the eye (177). Local side-effects from topical NSAID usage include stinging and conjunctival hyperaemia, however, systemic absorption is also possible (177). Studies in rabbits have found that up to 74% of the administered topical dose reaches the systemic circulation through absorption by nasolacrimal drainage (181). Effects such as exacerbation of bronchial asthma need to therefore be considered before initiating this treatment (182).

In 2015, a 12-month prospective and randomised study compared the topical effect of topical dorzolamide versus topical ketorolac on 28 eyes of 18 patients with RP or Usher syndrome-associated CMO. No significant change in CMT was observed in either group. VA improved in both groups at 6 months, however, this improvement was lost in the dorzolamide group by 12 months (183). Sample size was a limitation to the study.

- Combination of topical NSAID together with topical steroid or topical CAI

A case report by Park et al. (2013) describes an 85-year-old lady with unilateral RP-CMO in whom CAI usage was contraindicated due to a history of chronic renal impairment. As an alternative option, topical steroid (prednisolone acetate 1%) together with topical NSAID (ketorolac trometamol 0.5%) was prescribed four times daily in the LE only. Complete resolution of CMO was observed on SDOCT and BCVA improved from 20/200 at baseline to 20/60 at 3 months in the LE. Medication was stopped, however, 6 months following cessation, re-treatment was required. Again, this resulted in complete resolution of CMO and improvement of vision from 20/120 at the start of treatment to 20/80 after 3 months in the LE (184).

- Intravitreal Anti-Vascular Endothelial Growth Factor (anti-VEGF)

The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF) (185). In addition to promoting angiogenesis, VEGF-A reduces endothelial barrier function and increases permeability of choroidal vessels, both of which cause CMO (185). Indeed, anti-VEGF agents are routinely used to treat CMO and NV in a variety of retinal diseases such as age-related macular

degeneration (AMD), RVO and DMO. The following anti-VEGF agents will be discussed in detail regarding treatment for RP-CMO: pegaptanib sodium, bevacizumab, ranibizumab and aflibercept (see figure 19).




Whilst no studies have assessed vitreous levels of VEGF in patients with RP or RP-CMO, anatomical and/or functional improvement of RP-CMO has been observed following intravitreal anti-VEGF medication (3, 4, 186-189), which would support the hypothesis that VEGF contributes to RP-CMO formation.

Salom et al. (2008), however, identified markedly lower levels of VEGF-A in the aqueous humour of 16 eyes of 16 patients with RP (94.9 +/- 99.8 (mean +/- standard deviation (SD) picogram per millilitre (pg/mL)) compared to the same number of controls (336.5 +/- 116.8 pg/mL). Relative hyperoxia of the inner retina due to photoreceptor cell death may reduce VEGF production from retinal cells such as pericytes, endothelial cells, glial cells, Muller cells, and ganglion cells (190). The potential complications associated with the use of anti-VEGF therapy must therefore be considered.

- Pegaptanib sodium

Querques et al. (2009) published a case report regarding a 33-year-old male with RP-CMO refractory to treatment with oral acetazolamide in whom a single intravitreal injection of 0.3mg pegaptanib sodium (*Macugen, Eyetech Pharmaceuticals, Inc. and Pfizer Inc, New York, NY*) was trialled in the LE. Oral acetazolamide 500mg daily was continued throughout and stopped 1 month post-injection. BCVA improved from 2/200 at baseline to 20/40 in the LE and complete resolution of CMO was observed on OCT (no quantitative measurements of CMT are provided in the paper). Improvement of CMT and VA was maintained at 4 months post-injection despite withdrawal of oral acetazolamide (187).

Figure 19: Molecular properties of anti-VEGF agents. Taken from Klufas and D'Amico (2018) (191).

Agent	Bevacizumab (Avastin, Roche/Genentech) ¹	Ranibizumab (Lucentis, Roche/Genentech) ¹	Aflibercept (Eylea, Regeneron) ¹
Format	Full antibody (IgG1)	Monoclonal humanized antibody fragment	VEGF receptor 1/2-Fc fusion protein
Molecular structure			
Molecular Weight	149 kDa	48 kDa	115 kDa
Clinical dose for nAMD	1.25 mg (off-label use)	0.5 mg	2 mg
Equivalent molar dose ³	0.8	Reference	1.7 to 2

- Bevacizumab

Bevacizumab (Avastin, *Genentech/Roche*, South San Francisco, California) has been used off-label to treat RP-CMO with varying results.

In 2007, Melo et al. observed neither anatomical nor functional improvement in 2 eyes of 2 patients following treatment of RP-CMO with a single injection of intravitreal 1.25mg bevacizumab: Case 1 maintained VA of 20/200 both pre- and post-bevacizumab injection with no further improvement following IVTA. No significant difference was observed in CMT; pre-injection 524µm versus 529µm at 1 month post-injection. Case 2 had a baseline VA of 20/100, which worsened at 1 month post-injection to 20/200. No significant difference was observed in CMT; pre-injection 282µm versus 299µm at 1 month post-injection. Due to worsening cataract, the second patient subsequently underwent phacoemulsification plus IVTA and VA at 3 months post-injection improved to 20/25 (192).

More optimistic results were seen, however, in 2009 when Yuzbasioglu et al. treated 13 eyes of 7 patients with RP-CMO with an average of 3.3 (range, 1-8) injections of 1.25mg bevacizumab over 10.3

(range, 6-14) months. CMT reduced from 370.15 μ m (range, 245-603 μ m) at baseline to 142.53 μ m (range, 124-168 μ m). Pre- and post-treatment VA ranged from 5/400 - 20/100 and 20/200 - 20/63, respectively (189).

Whilst choroidal NV is rare in RP, it is of interest to note that intravitreal bevacizumab has also been observed to improve RP-choroidal NV (193, 194).

- Ranibizumab

In 2009, a cohort study using off-label 0.5mg intravitreal ranibizumab (LUCENTIS; Genetech, South San Francisco, California, USA) was performed by Artunay et al. Thirty eyes of 30 patients with RP-CMO refractory to treatment with oral acetazolamide for at least 6 months were enrolled. Fifteen eyes of 15 patients were treated with a single intravitreal injection of 0.5mg ranibizumab. Fifteen eyes of 15 patients that declined intravitreal ranibizumab were used as a control group. Thirteen out of 15 eyes (87%) in the treatment group demonstrated a significant reduction of CMO at 6 months post-injection. No statistically significant difference in VA was seen between the groups (186).

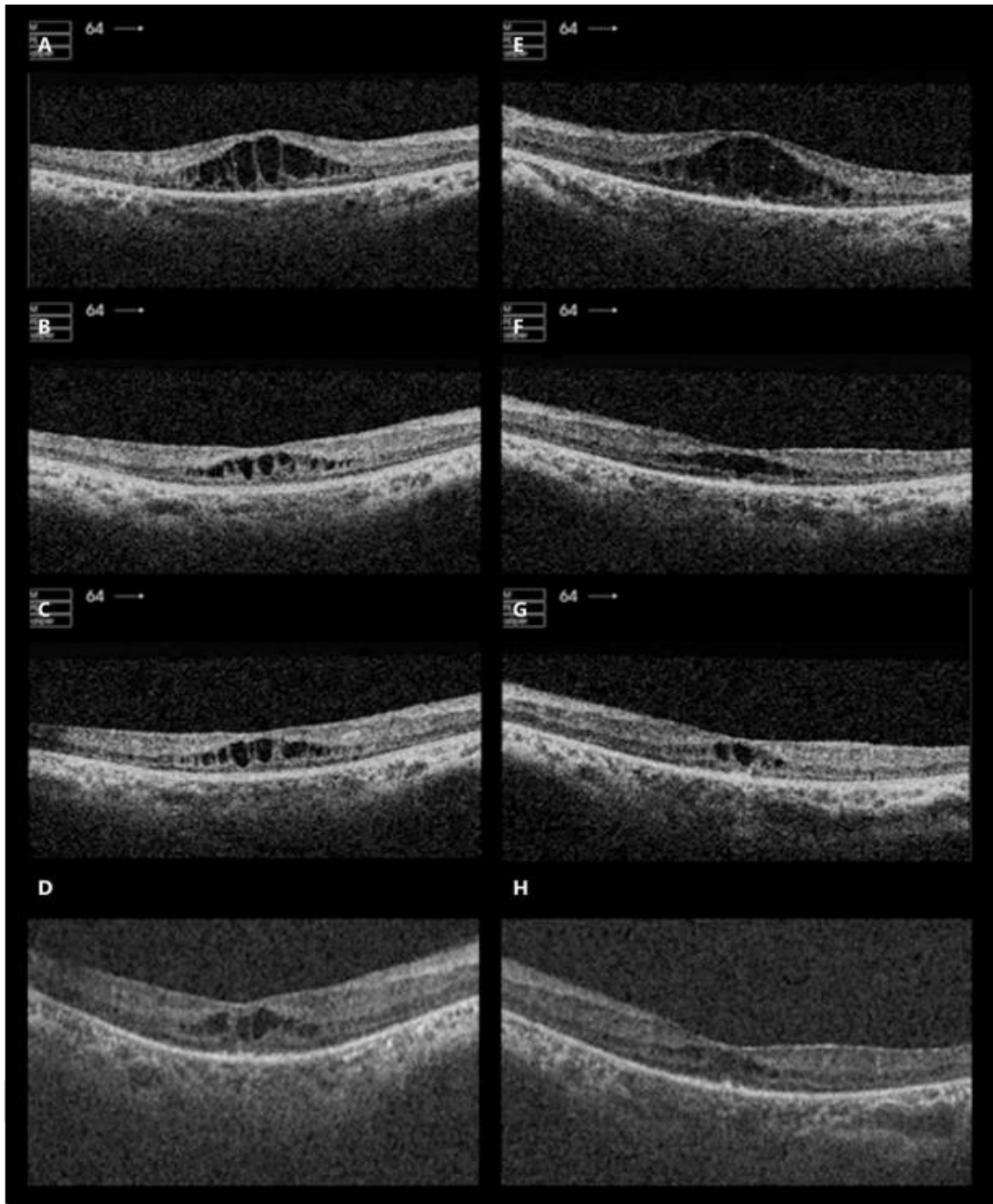
- Aflibercept

Aflibercept (EYLEA; Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA and Bayer Healthcare Pharmaceuticals, Berlin, Germany) may be superior to other anti-VEGF medications due to its intermediate size (115 kilodaltons (kDa)) and higher binding affinity (3). Its longer duration of action compared to other anti-VEGF medications is of interest as the frequency of repeat injections may be reduced (195). Whilst aflibercept is currently approved by the US Food and Drug Administration (FDA) for the management of neovascular AMD (nAMD) (2011), DMO (2014) and RVO-CMO (2014), it is not yet approved for usage in RP-CMO as the evidence surrounding its ability to treat RP-CMO is lacking (4).

A case report published in 2015 demonstrated improvement of CMT and VA following a single unilateral intravitreal injection of aflibercept in a 52-year-old Caucasian man with RP-CMO (4). At baseline, the vision in the RE was 3/10. One month post-injection, vision improved to 4/10 and the CMO resolved. Documented visual improvement was maintained at both the 2- and 6-month reviews. (4).

Our group subsequently published a case report regarding a 52-year-old male patient from Dubai, United Arab Emirates, who presented to the UK with a 3-year history of bilateral RP-CMO. Previous treatment had been with topical 2% dorzolamide, oral acetazolamide, and intravitreal ranibizumab, which had demonstrated only minimal reduction of CMO. Following re-confirmation of the diagnosis by clinical examination and OCT imaging, bilateral loading doses of intravitreal aflibercept were given (see figure 20). CMT reduced and the patient returned to Dubai. After 6 months, the patient was treated with intravitreal ranibizumab due to re-accumulation of fluid and unavailability of aflibercept in Dubai. Only minimal reduction of CMT was observed. Once available in Dubai, intravitreal aflibercept was administered bilaterally with further reduction of CMT observed. VA remained stable throughout (3).

Figure 20: OCT of both eyes before and after intravitreal injections of aflibercept given in the UK. OCT in the right eye before injection of aflibercept (A), 1 month after the first injection of aflibercept (B), 1 month after the second injection of aflibercept (C), and 8 weeks after the third injection of aflibercept (D). OCT in the left eye before injection of aflibercept (E), 1 month after the first injection of aflibercept (F), 1 month after the second injection of aflibercept (G), and 8 weeks after the third injection of aflibercept (H). Taken from Strong et al. (2016) (3).



Having demonstrated potential to treat RP-CMO following injection(s) with intravitreal aflibercept, my research group commenced a 12-month prospective study entitled 'Aflibercept for Macular oedema in underlying retinitis pigmentosa (AMOUR)' to determine the safety and efficacy of intravitreal aflibercept in RP-CMO using a 'treat and extend' regime (ClinicalTrials.gov identifier NCT02661711).

- Long-term safety of anti-VEGF

Miyata et al. (2018) published an article demonstrating no negative effects related to the progression of VF loss during continuous treatment with anti-VEGF agents (34 injections received in total; bevacizumab × 2, pegaptanib × 2, ranibizumab × 11, aflibercept × 19) for 8 years in a 56-year-old patient with choroidal neovascular membrane (CNV) associated with RP (196).

1.5.4.2 Nutritional

- Oral Lutein

Lutein and zeaxanthin are carotenoid pigments that contribute to the formation of macular pigment (MP). MP is thought to be protective against oxidative damage being most densely packed within the central 1° to 2° and located in the receptor axon layer (197).

Adackapara et al. (2008) published a 48-week study using lutein supplementation for 77 eyes of 39 patients with RP over 11 months. OCT scans were carried out every 6 weeks together with clinical examination. CMO was present in 19 out of 39 (49%) patients, which was bilateral in 17 out of 19 (89.5%) of these patients. Interestingly, baseline VA did not differ significantly between the groups of patients with and without CMO. Lutein was found to have no statistically significant effect on CMT in patients with RP either with, or without CMO (90).

- Oral Iodine

Iodine has been shown to promote tight junctions between RPE cells. Theoretically, it is possible that increasing iodine intake could improve RP-CMO by improving outer BRB integrity and fluid absorption (198). To date, however, there are no prospective trials using oral iodine for RP-CMO. This may be partly due to previous studies in mammals documenting RPE toxicity in sufficient concentrations (198). However, at a low intravenous concentrations, iodine has been observed to enhance RPE integrity in albino and pigmented rabbits (199).

Sandberg et al. (2014) performed a cross-sectional observational study of 212 non-smoking patients aged 18 to 69 years with RP to determine whether CMT in the presence of CMO is related to dietary iodine intake inferred from urinary iodine concentration (UIC). A total of 201 patients returned sufficient urine samples, however, 2 of these samples contained outliers for UIC and were not considered in the analysis. CMO was detected in at least one eye in 72 out of 199 (36.2%) of patients.

Higher UIC levels have been observed to be significantly associated with reduced CMT in non-smoking adults with RP-CMO (198). Although many patients were also taking vitamin A and/or docosahexaenoic (DHA) supplements throughout the study, further analysis found no confounding effect on the relationship of CMT and UIC. It would be interesting to perform a prospective randomised trial to review the effect of oral iodine on patients with RP and CMO versus those with RP without CMO.

- Laser

The retina receives oxygen and nutrients from the choriocapillaris via diffusion. In order for this to reach the inner retina, molecules must first pass through the outer retina where photoreceptors reside with a very high density of mitochondria and high oxygen consumption. Laser acts by causing thermal

destruction to both the RPE and adjacent photoreceptor cells with replacement by glia. This reduces oxygen consumption and allows oxygen to diffuse more easily from the choroid through the photoreceptor layer into the inner retina (200). Improved oxygenation of the inner retina should lead to constriction of retinal vessels (autoregulation) with decreased blood flow following laser treatment (200). According to Poiseuille's law, vessel resistance is inversely proportional to the radius to the fourth power. Vasoconstriction thus causes an increase in resistance and reduction of hydrostatic pressure downstream. As per Starling's law, this should reduce water flux from the vessel into the tissue allowing the oncotic pressure to drive water back into the vessels thus reducing CMO (200). In addition, the removal of hypoxic degenerating retina by laser reduces levels of VEGF and may improve leakage of fluid from vessels.

In 1987, grid laser photocoagulation was undertaken in one eye of 16 patients with bilateral RP-CMO. Six treated eyes gained one or more lines of vision, while none of the untreated eyes did. Seven untreated eyes lost one or more lines of vision, while none of the treated eyes did. Thirteen of 16 eyes showed decreased fluorescein leakage after treatment (201).

- Vitrectomy

In 2003, Garcia-Arumi et al. published a prospective non-randomised case series evaluating vitrectomy combined with inner limiting membrane (ILM) peel in 12 eyes of 8 patients with RP-CMO. All patients had been deemed refractory to treatment with acetazolamide. The presence or absence of pre-operative vitreo-macular traction (VMT), however, was not confirmed. All patients had surgery performed by the same surgeon. Mean CMT improved from 478 μ m (range, 380 - 570 μ m) pre-operatively to 342 μ m (range, 310–432 μ m) at 3 months post-vitrectomy and 260 μ m (range, 177 - 424 μ m) at 6 months post-vitrectomy. Mean VA improved from 20/115 (range, 20/60 – 20/400) pre-

operatively to 20/45 (range, 20/30 – 20/100) at 6 months post-vitreotomy (116). These results suggest that vitrectomy combined with ILM peel may reduce CMT with improvement of vision in patients with RP-CMO.

1.6 Approaches to therapy for IRDs

There are currently no cures for IRD. However, research is underway to better understand these diseases and to offer potential therapies that may slow or stop disease progression and/or reverse sight loss in people with IRD (20).

1.6.1 Supportive management of IRD

1.6.1.1 Visual rehabilitation

Various types of visual rehabilitation are available for patients depending on their type and extent of visual impairment. Chotikavanich et al. (2018) recently published a 5-year retrospective record review of hospital-based low-vision rehabilitation in Thailand. It comprised of 992 patient records, of which 760 were aged over 15 years (“adults”) and 232 were 115 years or younger (“children”) (202). Problems affecting the retina affected 534 out of 760 (70.3%) adults and 100 out of 232 (41.3%) children. RP was the most common ocular cause of vision loss in adults accounting for 215 out of 760 (28.3%). This study is interesting because it highlights QOL-related goals and what services patients are accessing for visual rehabilitation. Adults most commonly wished to be able to ‘read, write, and perform near tasks’ (503 out of 1,449 (34.7%) eyes), and to have ‘independent mobility’ (309 out of 1,449 (21.3%) eyes), whereas children wished to have ‘visual and developmental stimulation’ (134 out of 349 (38.4%) eyes) (202). The services most commonly accessed by adults included ‘visual aid devices’ 436 out of 842 (51.8%) services

provided and 'orientation and mobility training' 343 out of 842 (40.7%) services provided, whereas children mainly received 'visual and developmental stimulation' by multidisciplinary teams 125 out of 273 (33.8%) services provided (202). Studies such as this can help to guide future low-vision patient care and rehabilitation services.

Common visual aid devices such as glasses, contact lenses and magnifying glasses can improve the use of residual vision. Computers (including specialised computer software) allow patients to easily adjust their screen display and text size as well as offering additional features such as speech synthesis and portable Braille devices.

Photophobia may also be lessened through the use of dark or special filtered glasses or red-tinted contact lenses (6).

'Eccentric fixation' is a technique adopted by patients with central vision loss; fixation occurs at the edge of the lesion within a normal area of retina instead of at the fovea (203). Although resolution is reduced, magnifying aids can be used to compensate for this. Whilst many patients naturally adopt the use of eccentric fixation, training is available if desired for patients who continue to fixate centrally (203).

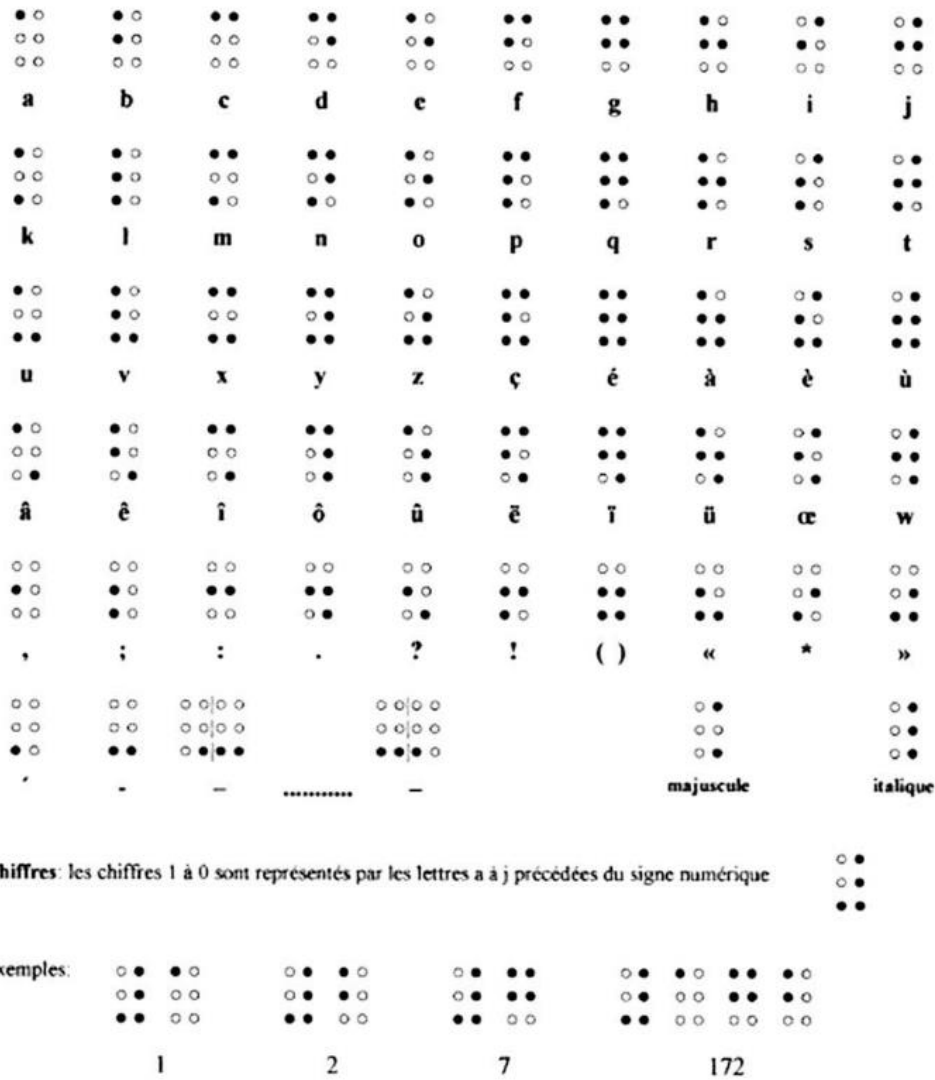
The history of the invention of Braille dates back to the early 1800's. Charles Barbier, a man serving in Napoleon Bonaparte's French army, wished to create a method of communication between soldiers that could not be easily intercepted by the enemy - as opposed to previous visual methods of communication using lamps that drew attention to their position (204). The system created was thus tactile-based, using a 12-dot cell, which had two dots across and six dots down. Unfortunately, it was deemed too complex for use in the battlefield, however, Barbier believed there was a place for his system amongst civilians. A

lecture Barbier gave at the Royal Institute for Blind Youths in Paris in 1821 was attended by Louis Braille (see figure 21), a boy born in 1809, who had lost his vision after accidentally stabbing himself in the eye with his father's awl (a gadget used to create holes in leather goods). Braille was inspired by Barbier's military code and spent time thereafter creating a more simplified version based on a 6-cell dot system, known as 'Braille', which to date is available in over 120 languages (see figure 22) (204).

Figure 21: Portrait of Louis Braille by Lucienne Filipi (Braille Museum, France). Taken from Jimenez et al. (2009) (205).



Figure 22: Braille alphabet as it currently exists. Taken from Jimenez et al. (2009) (205).



Guide dog services are provided by 'Guide Dogs', which is solely charity based (206). There are approximately 5000 guide dogs in the UK (206). Guide dogs have been demonstrated to reduce journey avoidance in patients with visual impairment; a study by Lloyd et al. (2008) noted 34 out of 50 (68%) participants avoided one or more journeys (i.e., environments, routes and destinations) before they received a guide dog (207). Common advantages for having a guide dog as reported by participants,

included: Facilitates independent mobility, Obstacle avoidance, Safer travel, Can dispense with cane (stigmatising through sight and sound, and prods abdomen) and Dignified travel (less stumbling) (207).

Common disadvantages for having a guide dog as reported by participants, included: Previous cane skills deteriorate, Less safe/efficient or disorienting if dog is not working well, Obstacles are not located or identified and Mobility is reduced as dog ages/slows down or becomes sick (207).

There are two types of cane used by patients with visual impairment: The 'symbol or identification' cane is made from a series of white aluminium tubes connected together with an elastic cord, which is mainly used to make others aware that the person carrying it has a mobility disability (208). The 'long' cane is a longer white cane with a plastic ball at the bottom end, used in a sweeping motion from side-to-side to identify obstacles along someone's path (208). Advantages for using a cane, include: being easily replaceable, affordable and allowing a person to receive tactile information about the environment around them. Disadvantages for using a cane, include: getting caught, for example, in cracks along the road and being negatively affected by adverse weather conditions such as snow.

1.6.2 Prevention of cell death (retinal neuroprotection)

As mentioned in section 1.3.2 Mechanisms of photoreceptor death, apoptosis plays a significant role in IRD and is considered the main pathway by which photoreceptors and/or RPE cells die (26). Various retinal disease models have therefore looked at novel ways of directly inhibiting apoptosis:

1.6.2.1 Growth factors

Neurotrophic factors have the ability to inhibit the apoptotic cascade and are therefore of interest as potential therapies for IRD (209).

Pharmacological treatment with nerve growth factor (NGF) has been trialled and shown to promote photoreceptor survival in animal models of RP (210).

A pilot study published by Falcini et al. (2016) administered daily murine NGF eye-drops for 10 days in 16 eyes of 8 patients with RP. Whilst some mild corneal irritation was noted, no SAEs were reported. No significant visual loss was observed as measured by flicker electroretinogram (fERG) and BCVA (209). Three patients reported subjective improvement of vision that was associated with temporary enlargement of the VF with improved fERG (209).

A multicenter, sham-controlled study published in 2016 by Birch et al. evaluated the long-term efficacy of ciliary neurotrophic factor (CNTF) delivered via an intraocular encapsulated cell implant for the treatment of RP (211). Thirty-six patients at 3 CNTF4 sites were randomised to receive a high- or low-dose implant in 1 eye and sham surgery in the fellow eye. Eyes retaining the implant showed significantly greater VF loss from baseline than either explanted eyes or sham eyes by 42 months (211). By 60 months and continuing through 96 months, VF loss was comparable among sham-treated eyes, eyes retaining the implant, and explanted eyes, as was VA and macular volume (211).

This reduction of photoreceptor function associated with the use of neurotrophic factors occurs due to the down-regulation of phototransduction cascade enzymes (209). It would therefore only be clinically useful if its benefits outweighed these risks.

1.6.2.2 Neuroprotective peptides

Somatostatin is an endogenous neuroprotective peptide produced in large quantities by the retina, most specifically the RPE (212). It acts as a neuromodulator through pathways such as: intracellular Ca^{2+} signalling, nitric oxide function, and glutamate release from photoreceptors (212). In addition to neuroprotection, somatostatin has potent anti-angiogenic properties and regulates various ion/water transport systems (212). Topical somatostatin has been observed to reduce ERG abnormalities and glial activation as well as reduction of photoreceptor apoptosis (212).

Akiyama et al. (2012) analysed the effect of pigment epithelium-derived factor-impregnated nanoparticles (PEDF-NPs) on photoreceptors in a retinal degeneration model using rats. At 8 weeks post-intravitreal injection, significantly increased numbers of cells in the ONL (due to inhibition of apoptosis) with preservation of a- and b-wave amplitudes in ERG studies were observed in eyes treated with PEDF-NP, compared to those treated with PEDF alone (213). This suggests that nanoparticles could be used as a long-term delivery system for growth factors to delay photoreceptor degeneration (213). Careful quantification of neuroprotective peptide is essential since the overexpression of IGF-1 in a mouse model resulted in loss of photoreceptors, bipolar, ganglion and amacrine cells (214).

1.6.2.3 Antioxidants

- Studies using RD models

Studies using RD models may provide insight for the treatment of IRD since RD also generates ROS that leads to photoreceptor apoptosis. This occurs when the physical separation of photoreceptors from underlying RPE leads to reduced oxygen and nutrient supply to photoreceptor OS (29). Resveratrol is a

potent antioxidant and small molecule activator of the *FoxO* pathway – affecting the cell cycle, apoptosis, DNA repair, stress resistance, metabolism and oxidative stress (215). Huang et al. (2013) observed that resveratrol given via intraperitoneal injection was able to reduce caspase activation and photoreceptor apoptosis in a RD model (29).

- Studies using glaucoma models

Studies using glaucoma models may provide insight for the treatment of IRD since glaucoma is another ocular condition associated with apoptosis (of retinal ganglion cells) leading to progressive loss of vision (215). Lulli et al. (2012) observed that topical administration of coenzyme Q10 in a mouse model was capable of penetrating through to the retina to provide a neuroprotective effect against apoptosis of retinal ganglion cells (215).

Berson et al. (2010) carried out a randomised, controlled, double-masked trial to evaluate the effect of lutein supplementation on the VF of patients with RP. The trial involved 225 non-smoking patients who received either 12mg of lutein or a control tablet daily over a 4-year period. All patients were given 15 000 International units (IU)/day of vitamin A palmitate. Total point score for the Humphrey Field Analyzer (HFA) was used as the primary outcome measure. Mean decline with the 60-4 programme was slower among those with the highest serum lutein levels. Lutein may therefore offer protection from oxidative damage and supplementation of 12 mg/day has been observed to slow loss of mid-peripheral VF in non-smoking adults with RP taking vitamin A (216).

- Oral lutein

In 2000, Danelie et al. published a study using lutein supplementation for a total of 26-weeks in 13 patients with RP as well as 3 patients with other retinal degenerations. Dosage was initiated at

40mg/day for 9 weeks and reduced to 20mg/day thereafter. Ten patients taking vitamin A and/or beta-carotene before the study commenced, continued this throughout. VA was tested by the patients themselves using a computer; Mean VA improved by 0.7 decibels (dB) but gains were most notable in those patients with blue eyes. Central VF area was tested by the patients themselves using a wall-chart; Mean VF area improved by 0.35dB but gains were most notable in those patients who received previous supplements. This short-term improvement plateaued at 6 to 14 weeks (217).

In 2001, Aleman et al. used heterochromatic flicker photometry to measure MP optical density profiles of 47 patients with clinically diagnosed RP, 11 patients with Usher syndrome and 29 controls. Following this, a subset of patients (23 with retinal degeneration versus 8 controls) were provided with 20mg/day lutein supplementation over a 6-month period to determine whether baseline serum and MP density could be modified (197). It should be noted that this study was neither masked nor placebo-controlled. Thirteen out of 58 (22.4%) of patients with retinal degeneration were taking vitamin A orally at 15,000 IU/day before the study commenced and were encouraged to continue taking this throughout. No difference in MP density levels were found between patients with retinal degeneration and controls. Lutein supplementation had no significant effect, positive or negative, towards foveal vision parameters (197).

- Systemic edaravone

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI186, Radicut) is a free radical scavenger with antiapoptotic and anti-inflammatory effects (218). It has been used via intravenous infusion to treat acute ischemic stroke and has been found to be effective in mouse models against retinal degeneration both in vivo and in vitro (219, 220).

1.6.2.4 Inhibition of necrosis

Inhibition of RIP1 has been observed to be successful in preventing cone and rod photoreceptor degeneration. RIP3 kinase inhibition has been observed to prevent cone necrosis. Inhibition of the RIP pathway could therefore be a therapeutic target to prevent retinal degeneration, at least in some disease models (28).

1.6.2.5 Anti-inflammatory mediators

Various anti-inflammatory mediators such as steroids have been studied as potential therapies for IRD.

Mo et al. (2013) reported that intravitreal 17 beta-estradiol significantly reduced neuronal apoptosis in a light-induced model (221).

Cubilla et al. (2013) found that sub-cutaneous injections of mifepristone, an antagonist of the glucocorticoid receptor, caused increased apoptotic signals in retinal extracts and apoptosis of the photoreceptors under basal, non-stress conditions (222). This suggests that glucocorticoids play a critical role in basal photoreceptor survival.

1.6.2.6 Other pharmacological therapies

Valproic acid (VPA) is a potent inhibitor of histone deacetylase and the inflammatory response pathway via apoptosis of microglial cells. It can also down-regulate complement proteins and increase levels of various neurotrophic factors (223). It is currently prescribed as an anti-convulsant, mood stabiliser and for the prevention of migraine but has shown neuroprotective potential for IRD. Clemson et al. (2011) carried out a retrospective chart review of 13 eyes of 7 patients with RP who had taken between 500 - 750 mg/day of VPA for 2 – 6 months. Statistically significant improvement of Goldman VF was observed

in 9 eyes. Two eyes had decreased VF and 2 eyes demonstrated no change. All 13 eyes demonstrated statistically significant improvement of BCVA (223).

Unoprostone isopropyl is a large-conductance Ca^{2+} -activated K^+ channel inhibitor currently used in topical form as a treatment for glaucoma. Due to its neuroprotective mechanism and ability to improve macular blood flow, unoprostone isopropyl shows potential as a therapeutic treatment for patients with RP (224). A phase III, multicenter trial carried out in Japan reviewed the efficacy and safety of unoprostone in the treatment of RP. The primary endpoint, however, found no significant difference in the value of mean retinal sensitivity at four central points through HFA (10-2) compared with placebo (clinicaltrials.gov identifier NCT01786395).

Brimonidine is a topical α_2 -agonist that may offer neuroprotection by slowing the progression of VF loss in patients with RP. Merin et al. (2008) carried out a placebo-controlled, double-masked, randomised study in 26 patients with retinal dystrophies. One eye received brimonidine tartrate 0.2% twice-daily whilst the other eye received artificial tears. Only 17 patients out of 26 (65%) completed follow-up. Whilst no difference was observed in VA, colour vision, or contrast sensitivity between the treated and placebo eyes, a trend toward a lesser degree of VF loss was found in the brimonidine-treated eyes (most strongly observed in a subgroup of patients with RP) (225). An exploratory, 12-month, ascending-dose study has also been carried out to evaluate the safety and efficacy on visual function following a single injection of brimonidine intravitreal implant in one eye of patients with RP (clinicaltrials.gov identifier NCT00661479). Details of statistical analysis are pending.

Fenretinide is a synthetic derivative of vitamin A that can be taken orally. It forms a complex with retinal binding protein (RBP), which is then excreted in the urine. With reduced levels of circulating RBP,

Vitamin A (retinol) is unable to bind to form the vitamin RBP-retinol complex resulting in decreased vitamin A concentrations in the eye and deceleration of A2E formation (226). Fenretinide has been demonstrated to reduce A2E production in the eye in knockout (KO) mice (*ABCA4*^{-/-}) thus offering a potential therapy for STGD (226). This is of particular interest for a subset of patients with AR-RP due to variants in the *ABCA4* gene (227).

QLT091001 is a synthetic retinoid replacement for 11-*cis*-retinal that has the potential to improve visual function by restoring this key component of the visual cycle. Wen and Birch (2015) carried out a phase I study to evaluate whether once-daily treatment with oral QLT091001 for 7 days can improve visual function in 14 patients with LCA and 18 patients with RP due to *RPE65* or *LRAT* variants (228). Nineteen of 28 eyes (68%) with LCA and 13 of 36 eyes (36%) with RP were classed as 'responders', whereby improvement of Goldmann VF retinal area of the primary isopter by $\geq 20\%$ was observed at two consecutive study visits starting within 2 months of treatment (228). Baseline thickness of the OS layer in responders was 13.5 μm in those with LCA and 11.7 μm in those with RP. Baseline thickness of the OS layer in non-responders was less than 4.6 μm in both groups. This suggests that patients with a greater baseline outer segment thickness are more likely to respond to treatment with QLT091001.

CU239 is non-retinoid compound able to specifically target and inhibit RPE65, a key enzyme in the visual cycle. Shin et al. (2018) observed a protective effect on the retina by CU239 in a light-induced retinal damage murine model (229).

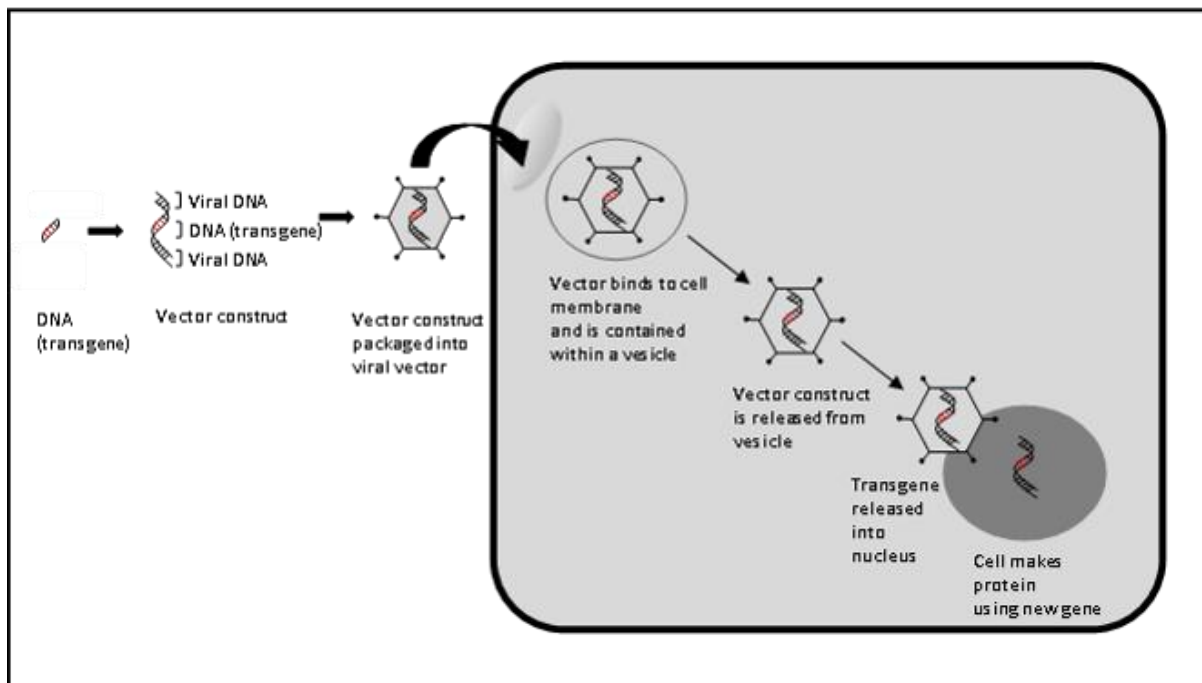
1.6.2.7 Gene therapy

Gene therapy describes a technique whereby a defective gene creating an abnormal or deficient product is replaced with a functioning gene. It is more applicable to the earlier stages of disease before significant retinal deterioration has occurred (20).

Common viral vectors used include adeno-associated virus (AAV) and lentivirus. AAV is a small, non-enveloped virus consisting of a protein shell surrounding and protecting a small, single-stranded DNA genome of approximately 4.8 kilobases (kb) (230). They are particularly useful as a viral vector (see figure 23) as they are: non-pathogenic, provide long-term transgene expression, produce minimal host immune response, and infect both dividing and quiescent cells (230).

Figure 23: Gene therapy using an adenovirus vector. Taken from Strong and Michaelides (2017) (231).

The defective gene creating an abnormal or deficient product is replaced with a functioning gene.



Animal models of IRD have enabled significant progress to be made in the field of gene therapy. They have provided insight into the specific gene defects causing retinal disease and the mechanisms involved, as well as aiding the design of molecules for translational research and gene-based therapy (230). Mice are the most popular models as transgenic technology is far advanced and animal housing is inexpensive (230).

The eye is considered an excellent organ for gene therapy. This is for several reasons: 1) Its immune privilege created by the BRB prevents a florid inflammatory reaction following introduction of a vector, 2) The organ is of small size, which minimises the amount of vector required, 3) The eye has easy access and compartmentalisation allowing treatment to be given using a variety of techniques, including: intravitreal, intracameral, subretinal or suprachoroidal (232). The contralateral eye can also serve as a control, which is useful when undertaking RCTs (5).

While sub-retinal injection following PPV and retinotomy causes a temporary focal separation of the neurosensory retina from RPE that spontaneously resolves, complications such as macular holes, sub-retinal haemorrhage, sub-retinal fibrosis, RD and rarely extension of the vector to other sites can occur (232). Intravitreal injection is more cost-effective and indeed safer, although endophthalmitis and RD remain a risk factor and the vector may struggle to enter the retina. This can be improved using techniques such as preliminary argon laser to initially disrupt the ILM (232). The suprachoroidal space can be reached via microneedle puncture of the sclera but has only been performed in rabbits (233).

Selected gene therapy trials for IRD

- *LCA*

Several trials for LCA have assessed viral vectors to induce transduction in the RPE65 gene:

LUXTURNA™, also known as voretigene neparvovec (AAV2-hRPE65v2) has been recently FDA and European Medicines Agency (EMA)-approved following a randomised, controlled, open-label, phase 3, clinical trial published by Spark Therapeutics Inc. in 2017 using bilateral, sub-retinal injections of LUXTURNA™ for patients with *RPE65*-related IRD, including LCA and RP (234). Significant improvement of light sensitivity, VFs and navigational ability under dim lighting conditions were observed in the intervention group versus the control group (234). No product-related serious adverse events or immune responses occurred (234).

Bainbridge et al. (2015) published a phase 1/2, open-label, dose-escalation study using recombinant AAV 2/2 (rAAV2/2) vector carrying the RPE65 complementary DNA for patients with LCA. Although no improvement was observed on ERG, an improvement of retinal sensitivity was found in 6 patients, peaking at 6 - 12 months, which lasted for up to 3 years (235).

A longer-term follow-up study for patients who have been administered AAV2/5-OPTIRPE65 in the phase I/II, open label, non-randomised, two-centre, dose escalation trial in adults and children with LCA associated with defects in RPE65 is currently underway (ClinicalTrials.gov Identifier: NCT02946879).

A phase III study evaluating sub-retinal injection of SPK-RPE65 in patients with LCA (an AAV2 gene therapy that delivers the RPE65 gene) observed statistically significant improvement in full-field light sensitivity threshold testing as well as ability to navigate a mobility course under various lighting

conditions in 21 treated patients compared with 10 controls (236). Unfortunately, no significant improvement of VA was observed.

- *STGD*

SAR422459, formerly known as StarGen™, is a gene-based therapy using the lentivirus vector to deliver a corrected ABCR gene via sub-retinal injection in patients with STGD. A pre-clinical 6-month study has demonstrated efficacy following a single administration. A phase I/IIa, dose-escalation study investigating the safety and efficacy of SAR422459 in STGD1 over a 48-week follow-up period is being undertaken (NCT01367444 results pending). There have been no safety concerns in the first three cohorts of subjects with relatively advanced disease, and no definite evidence of efficacy. Another cohort with less severe disease is also being recruited and may indeed show greater potential to benefit (231, 237).

ABCA4^{-/-} mice have been studied as a model for STGD1 in which rAAV-mediated delivery of a complement negative regulatory protein, known as complement receptor 1-like protein y (*CRRY*) into the subretinal space resulted in: reduced complement factors C3/C3b in the RPE, a two-fold reduction in bis-retinoid accumulation and 30% fewer lipofuscin granules/increased photoreceptor nuclei in the ONL compared to sham-injected ABCA4^{-/-} mice after 1 year (238).

- *Retinitis Pigmentosa*

A dose escalation, phase 1/2 clinical trial of retinal gene therapy for XLRP using an AAV-encoding *RPGR* is currently recruiting 24 patients at Manchester and Oxford Eye Hospital (ClinicalTrials.gov Identifier: NCT03116113).

Another open label, multi-centre, phase I/II dose escalation trial of a rAAV (AAV2/5-hRKp.*RPGR*) for gene therapy of adults and children with XL-RP owing to defects in *RPGR* is currently recruiting 36 patients at Moorfields Eye Hospital, London (ClinicalTrials.gov Identifier: NCT03252847).

- *XLRS*

rAAV-mediated delivery of the normal RS1 gene to the retina of young KO mice has demonstrated long-term retinoschisin expression and rescue of the retinal structure and function (69, 231). There are currently two phase I/II trials assessing the safety and tolerability of a two virus vectors delivered *intravitreally* specifically targeting RS1 gene to express retinoschisin (clinicaltrials.gov identifier NCT02416622 and NCT02317887) (232).

1.6.3 Tissue replacement:

1.6.3.1 Retinal stem cells and transplantation

Two scientists, John B. Gurdon and Shinya Yamanaka, were jointly awarded 'The Nobel Prize in Physiology or Medicine 2012' following their discovery that mature, specialised cells can be reprogrammed to become immature cells capable of developing into all tissues of the body.

In 1962, John B. Gurdon was able to replace the immature cell nucleus of an egg cell from a frog with the nucleus of a mature intestinal cell (239). A survey of over 150 frogs was performed obtained by the transplantation of donors ranging from late blastulae to swimming tadpoles (239). His results demonstrated at least 30% of blastula nuclei and at least 4% of hatched tadpole gut-cell nuclei contained a complete range of the genetic information required for formation and functioning of a

normal frog (239). Another observation noted was that transplant frogs derived from nuclei of differentiating cells were more often abnormal than those from embryonic cell nuclei (239).

Embryonic stem cells (ESCs), obtained from the inner cell mass of blastocysts, are pluripotent meaning they can potentially give rise to cells from all three germ layers including ectoderm, endoderm and mesoderm (240). In 1981, Evans and Kauffman were the first to discover how to culture ESCs from mouse blastocysts (241). In 1998, Thomson et al. published the first report regarding the successful isolation of human embryonic stem cells (hESCs) from human blastocysts (242). This generated much controversy due to this process involving the destruction of human embryos.

The ability to isolate hESCs (see figure 24) means that, theoretically, transplantation of specialised cells in order to replace damaged tissue in patients suffering from various degenerative diseases, is possible. However, the signalling mechanisms involved in lineage restriction of ESC to adopt various cellular phenotypes are still under investigation (243). Furthermore, for progression of hESC-based therapies towards clinical applications, appropriate culture conditions must be developed to generate genetically stable homogenous populations of cells, to avoid possible adverse effects following transplantation (243).

In 2006, Takahashi and Yamanaka's ground-breaking research demonstrated that adult somatic cells (using mouse fibroblasts) can be reprogrammed to generate induced pluripotent stem cells (iPSCs), using specific factors, back into an embryonic stem cell-like state (244). By using fibroblasts - the most commonly used primary somatic cell type for the generation of iPSCs - many of the ethical and technical hurdles associated with obtaining ESCs have been overcome.

Patient-specific iPSCs have since enabled scientists to investigate specific disease-causing variants and their associated pathophysiologic mechanisms, evaluate novel gene augmentation, gene silencing, small molecule therapies, and restore function through the transplantation of manufactured cells and tissues (231, 245).

One major advantage of iPSCs is the high availability of fibroblasts which can be easily isolated from skin biopsies. Being derived directly from adult tissue, other advantages of iPSCs include: reduced risk of host immune system rejection and the need for embryos can be bypassed. Due to the plasticity of stem cells and their unlimited capacity for self-renewal, however, adverse events (AE) such as tumour formation, immune rejection, and the risk of differentiating into unwanted cell types are possible (7, 231). Studies to date have therefore mainly focused on the safety and tolerability.

The first-in-human, phase I/IIa, open-label, prospective study of the safety and tolerability of sub-retinally transplanted human retinal progenitor cells (hRPC), created by ReNeuron, is being carried out in 15 patients with RP in the USA (clinicaltrials.gov identifier NCT02464436) (231). This followed on from rat model studies where treatment with hRPCs resulted in better VA compared with untreated eyes and greater preservation of the ONL on histological analysis (231, 246). ReNeuron claim that their hRPCs offer low immunogenicity and the potential for large-scale production using a patented and highly efficient cell expansion process (231, 246).

Studies such as Lund et al. (2006) have demonstrated extensive photoreceptor rescue in an animal model of retinal disease following sub-retinal transplantation of hESC-derived RPE (see figure 25) (247). In 2015, Schwartz et al. published the results of two phase I/II, open-label, multi-centre, prospective studies carried out to determine the safety and tolerability of sub-retinal transplantation of hESC-

derived RPE cells in patients with STGD and atrophic AMD (7, 231). hESC-derived cells were well tolerated for up to 37 months after transplantation in patients with either disease. No evidence of adverse proliferation, rejection, or serious ocular or systemic safety issues related to the transplanted tissue were found (7). The AEs, such as Staphylococcus Epidermidis-related endophthalmitis experienced in 1 patient, were specifically associated with vitreoretinal surgery and immunosuppression (7).

Figure 24: hESC-derived cone cells. Image provided by Dr. Anai Gonzalez Cordero at The UCL Institute of Ophthalmology, London, UK. Used with permission.

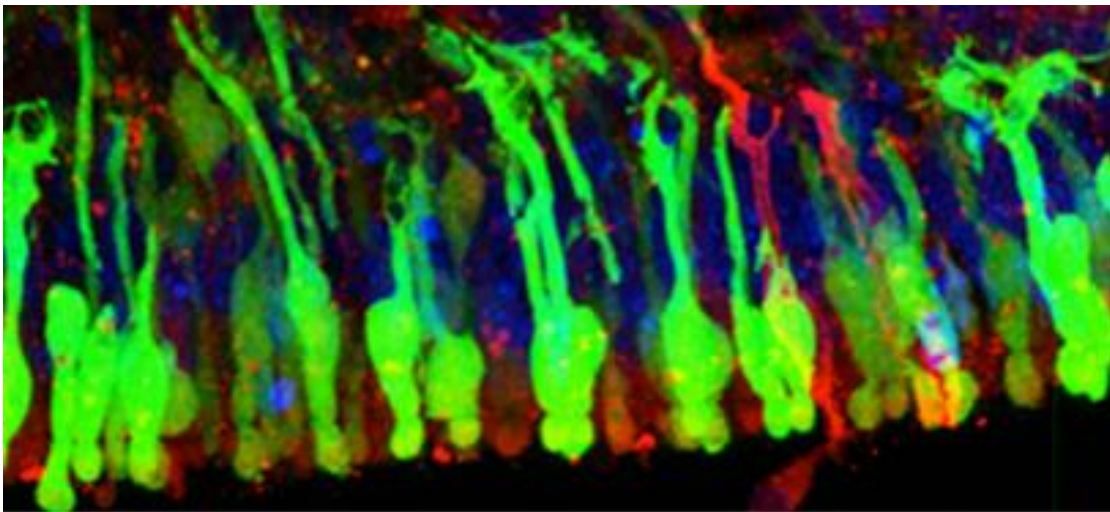
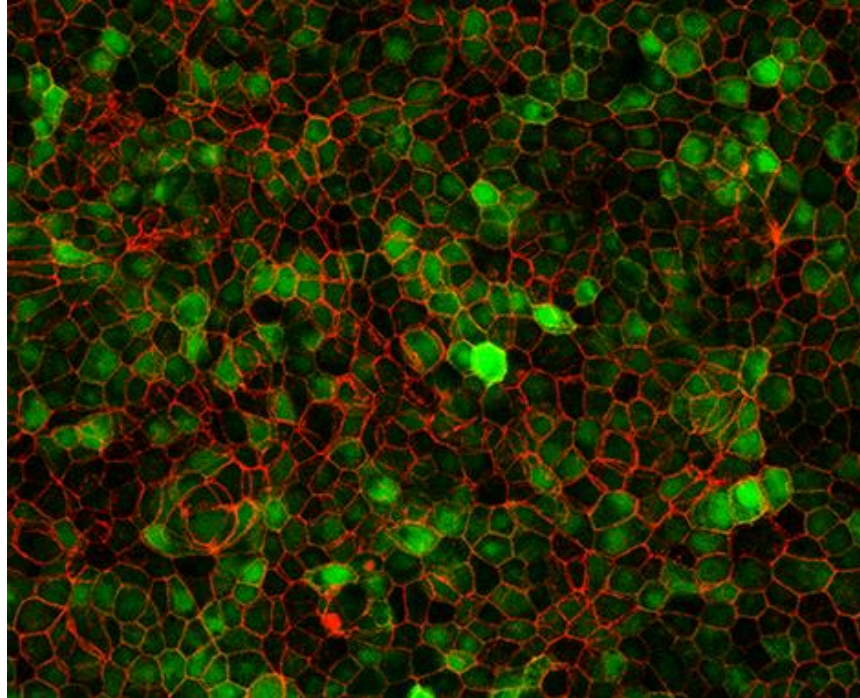


Figure 25: hESC-derived RPE cells. Image provided by Dr. Anai Gonzalez Cordero at The UCL Institute of Ophthalmology, London, UK. Used with permission.



1.6.4 Artificial Vision - Retinal Implants

Medical devices aim to improve quality of vision for patients with IRD. The restoration of useful vision can be achieved by bypassing diseased retina and sending signals directly to the brain, or by improving the clarity and magnification of patients' surroundings (231). Current devices for patients with RP that are European conformity (CE) marked and available for use in the UK, include (20):

The Alpha IMS is a 3.2 x 3.1mm² wireless sub-retinal microchip containing 1500 electrodes contained in a 50 x 50µm square arrangement. The device is able to capture light, stimulate the optic nerve and

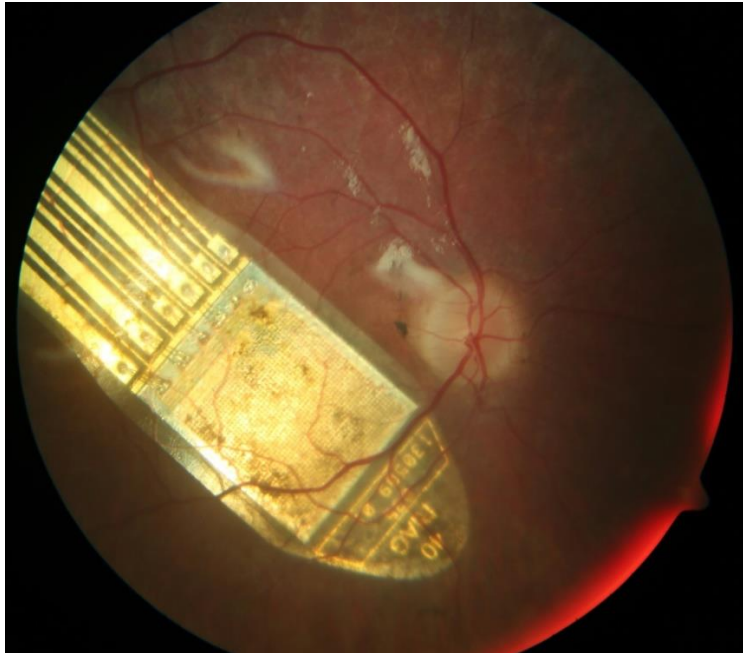
deliver signals to the brain. The device is powered by a wireless pocket battery and patients adjust the brightness using a dial fitted behind the ear. A clinical trial interim report suggests that sub-retinal implants can restore very-low vision or low vision in IRD patients with PL vision or worse (248). The cost of the Alpha IMS device together with the surgical implantation is estimated at \$130,000.

A Clinical trial interim report published by Stingl et al. (2015) referred to 29 blind patients with outer retinal degeneration in whom the Alpha IMS device had been implanted (248). The results of this international, multicenter clinical trial found that almost half of the patients could recognise object shapes and detail in daily life and almost three-quarters could localise high-contrast objects (248). The Alpha IMS is therefore able to restore low, but useful vision, in patients blind from hereditary degenerations of the photoreceptors with VAs up to 20/546 (249).

The durability and longevity of the Alpha IMS, however, was sub-optimal, mainly due to technical failures which occurred in some implants within the 12 month clinical trial observation period. A newer device, known as the Alpha AMS (see figure 26), was therefore created to address these technical glitches with improved materials and design (249). The Alpha AMS is a 3.2 x 4.0mm² wireless sub-retinal microchip containing 1600 electrodes in a 30µm round arrangement.

The results of 15 blind patients in whom the Alpha AMS was implanted in one eye, were published in 2017. This 12-month study, carried out at four sites, was not only found to be reliable, well tolerated and able to restore limited visual functions in blind patients with retinal degeneration, but demonstrated improved longevity compared to its predecessor, the Alpha IMS device (249).

Figure 26: The Alpha AMS device. Taken from RETINA IMPLANT. Used with permission. A colour fundus photograph of the RE demonstrating the sub-retinal visual implant.

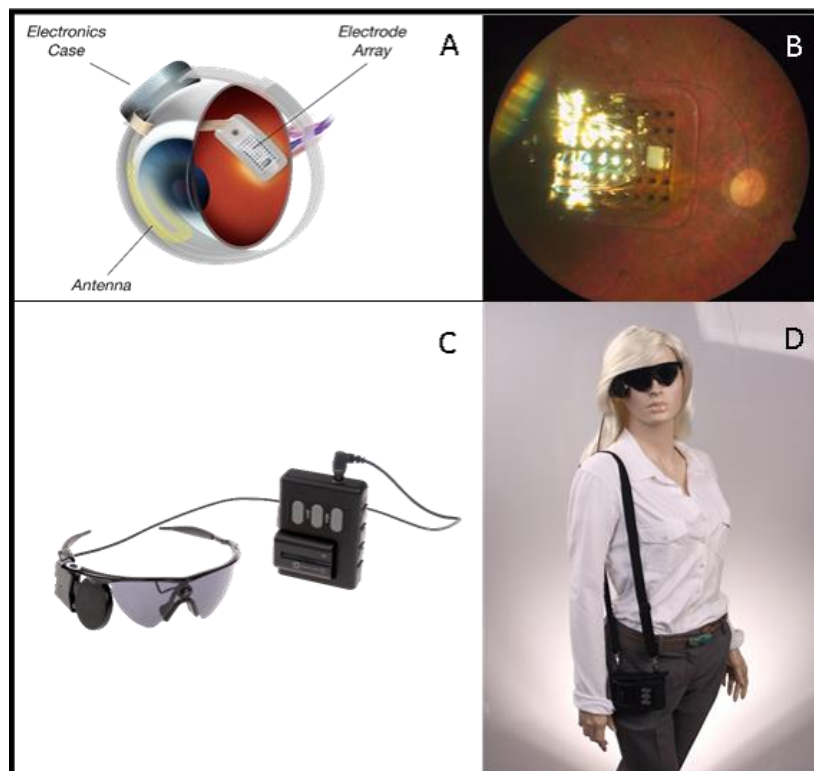


The Argus® II device (see figure 27), CE marked in 2011 and approved for use by the FDA in 2013, acts in place of degenerated outer retina i.e. photoreceptors by direct stimulation of the relatively preserved inner retina via epiretinal microelectrodes (250). The device aims to facilitate patients with: a) form discrimination/recognition; b) target localisation; c) motion detection; and d) navigation (250). A small camera mounted on a pair of glasses captures images, which are subsequently converted to a pixelated image by an external video processing unit (250). A wireless sub-retinal receiver receives these signals at the macula and is able to transmit directly to the visual cortex where they are interpreted as visual images. The cost of the device alone is \$100,000.

The International, multi-centre, phase II, Argus® II Retinal Prosthesis System clinical trial began in 2006 and has since implanted the device in 30 patients with profound visual loss from conditions such as RP,

choroideraemia and geographic atrophy secondary to AMD (251). Three devices have since been explanted, for example, following a SAE involving hypotony with 360 degree choroidal effusions and RD. Twenty-four of the remaining 27 patients remained implanted with functioning Argus® II Systems at 5 years post-implant (251). Patients performed significantly better on all visual function tests and functional vision tasks when the with the Argus® II System was 'on' as opposed to being 'off' (251).

Figure 27: The Argus® II Retinal Prosthesis System. Taken from Second Sight Medical Products. Used with permission. A = Placement of the electronic implant in and around the eye; B = Fundus photograph demonstrating the implant in-situ; C = A miniature video camera is mounted on a pair of glasses, which sends information to the video processing unit (VPU) via a cable. The VPU processes the information and sends instructions back to the glasses via a cable. Theses instructions are transmitted wirelessly to an antenna in the retinal implant; D = Model demonstrating how the device is worn.



2.0 The AMOUR study

2.1 Methods

2.1.1 Overview

The AMOUR study stands for 'Aflibercept for Macular Oedema in Underlying Retinitis Pigmentosa'. This is a prospective, non-randomised, exploratory, phase II study to assess the safety and efficacy of aflibercept in RP-CMO.

As discussed in section 1.5.4 Avenues of intervention, CAIs are the mainstay of treatment for RP-CMO, however, varying levels of response to treatment have been published. Anti-VEGF medication is currently licenced for use in neovascular age-related macular degeneration (AMD), macular oedema following retinal vein occlusion (RVO), diabetic macular oedema (DMO), and diabetic retinopathy (DR) in patients with DMO. Since VEGF has also been proposed as one of the toxic products released from degenerating retina that may contribute to weakening of the BRB and RP-CMO, the idea for a study using anti-VEGF for RP-CMO came about. Bayer LTD were approached and agreed to fund the study using aflibercept.

The protocol of the study adhered to the provisions of the Declaration of Helsinki. This was submitted by the PI Professor Michel Michaelides and Miss. Stacey Andrea Strong (SAS). SAS attended the local ethics committee meeting, which took place in Brighton and thereafter the study was approved. Informed consent was obtained from all patients. The study was undertaken at Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom. The study was registered with ClinicalTrials.gov and assigned the following unique identifier: NCT02661711.

2.1.2 Inclusion criteria

- CMO in association with RP
- At least 16 years of age
- Uni- or bilateral CMO (where patients have bilateral CMO, the worse eye only will be treated – defined as the eye with a greater CMT on OCT)
- No previous oral treatment for CMO for last 3 months
- No previous peribulbar or intravitreal treatment for CMO in the study eye for last 3 months
- No previous topical treatment for CMO in the study eye for last 1 month
- Central visual impairment that in the view of the principal investigator (PI) is due to CMO
- BCVA better than 3m/60

2.1.3 Exclusion criteria

- Insufficient patient cooperation or media clarity to allow adequate fundus imaging
- Evidence of visually significant VMT or ERM on OCT that in the PI's opinion is highly likely to significantly limit the efficacy of intravitreal therapy
- History of cataract surgery within prior 3 months or cataract surgery anticipated within 6 months of starting the study
- Any anti-VEGF treatment to study eye within 3 months
- History of YAG capsulotomy performed within 3 months
- Uncontrolled IOP ≥ 24 mmHg for ocular hypertension (on topical IOP lowering medications)
- Advanced glaucoma (in the opinion of a glaucoma specialist)
- Patients with active or suspected ocular or periocular infections
- Patients with active severe intraocular inflammation

- Patients with a new, untreated retinal tear or RD
- Patients with a stage 3 or 4 macular hole
- Thromboembolic event (myocardial infarction (MI)/cerebrovascular accident (CVA)/Unstable Angina) within 6 months
- Pregnancy or family planned within 15 months
- Females who are breast feeding
- Known allergy or hypersensitivity to anti-VEGF products

2.1.4 Name of committees involved in the study

Several committees were put in place as part of this study:

- The Trial Management Group (TMG) was responsible for overseeing recruitment and data management.
- The Trial Steering Committee (TSC) provided overall supervision of the study (including monitoring trial progress and conduct, checking of protocol compliance during the trial and deciding if the trial should be stopped early for safety or efficacy reasons).
- The Independent Data Monitoring Committee (IDMC) was responsible for ensuring that the risk-benefit ratio of the study was appropriate for all patients involved and that the scientific integrity of the trial was maintained throughout.

2.1.5 Patients and methods

2.1.5.1 Identification of patients

An electronic search was performed to identify all patients seen at Moorfields Eye Hospital NHS Foundation Trust, London, UK, between 1st December 2012 and 30th November 2015 with the phrases 'retinitis pigmentosa' and 'cystoid macular oedema' appearing in their electronic patient record (EPR). This time period was chosen as it was deemed a manageable period of time and number of patients' notes/images to review in the first instance. This initial search identified 295 patients; however, after review of each EPR, 165 patients were excluded from the study for the following reasons: no/minimal CMO (111), visually significant ERM (17), VA too poor (24), VA too good (4), macular hole (2), visually significant cataract (2), under 16 years of age (4) and pregnant (1).

A total of 130 patients were therefore found to be potentially suitable for the study. Patients were then contacted by the research fellow, Dr. Stacey Andrea Strong (SAS), carrying out the clinical trial either in person at the medical retina clinic, by telephone or letter. The aims, methods, anticipated benefits and potential hazards of the study were explained to each patient and a patient information sheet (PIS) was provided. Patients were given a minimum of 24 hours to consider whether they wished to attend a baseline evaluation/screening visit. The patients were informed that they were under no obligation to enter the trial and that they could withdraw at any time during the trial, without having to give a reason.

Out of 130 patients found to be suitable to enter the trial: 18 could not be contacted/did not reply, 1 was found to be deceased, 32 wished to be in the study and 79 declined to participate for reasons including: did not wish to have injections into their eye, happy with their current treatment and/or vision, or unable to commit to the study visits (due to distance from the hospital or concerns about the impact it would have on their job).

Fifteen patients were being treated with topical CAI (dorzolamide or brinzolamide) at point of contact, which was required to be stopped in the study eye for at least 1 month prior to arranging their screening appointment. Five patients were being treated with oral CAI (acetazolamide) at point of contact, which was required to be stopped at least 3 months prior to arranging their screening appointment. Ten patients were not using any treatment at point of contact and were able to attend a screening visit at their earliest convenience.

2.1.5.2 Screening and recruitment of patients

Within a week of being approached either at medical retina clinics or by telephone and having been provided with information about the trial, our research manager contacted the patient and invited them to attend a screening appointment. Screening appointments occurred within 28 days of being contacted, unless there was a reason that the patient could not attend, for example, they were abroad. If the patient was deemed ineligible, for example, because they were being treated with an oral carbonic anhydrase inhibitor (CAI), they were contacted again to re-check eligibility and a screening appointment was booked within 28 days.

At the screening appointment, patients had the opportunity to ask any further questions before informed consent was taken, their medical/drug history reviewed and vital signs including blood pressure (BP), heart rate (HR) and temperature measured. If they were deemed fit to enter the trial, the patient was 'recruited' and their 1st aflibercept intravitreal injection was given on the same day ('Visit 1'). All patients were recruited over a 6 month period.

2.1.5.3 Follow-up visits

At each follow-up visit, patients had their vital signs checked and a medication review performed. Tests of visual function carried out at every visit included: BCVA, colour vision, contrast sensitivity and SDOCT. In addition, microperimetry and FAF were also undertaken at the 6- and 12- month (exit) visits. Please refer to table 1 for an overview of the steps taken during study visits.

Table 1: An overview of the steps taken during study visits

Patient demographics confirmed (at all scheduled visits)
General medical and ocular history (taken at baseline only)
Medication review (at all scheduled visits)
Pregnancy status confirmed (taken at baseline only)
Eligibility check (taken at baseline only)
Informed consent (taken at baseline only)
Vital signs including: BP, HR and temperature (at all scheduled visits)
BCVA (at all scheduled visits)
Refracted BCVA (Baseline, 6 and 12 months)
Colour vision (at all scheduled visits)
Contrast Sensitivity (at all scheduled visits)
Microperimetry (Baseline, 6 and 12 months)
Dilation of the patient (at all scheduled visits)
Slit lamp examination to check for cataract (at all scheduled visits)
Pre-injection IOP check (at all scheduled visits)

SDOCT (at all scheduled visits)
FAF Imaging (at all scheduled visits)
Administration of Eylea (at all scheduled visits)
Post-injection IOP check (at all scheduled visits)

The ‘treat and extend’ regime is selected in clinical trials in order to simulate achieving optimal visual outcomes whilst simultaneously balancing the burden of long-term, frequent and high-cost treatment. Studies such as ‘PrONTO’, whereby anti-VEGF was used for the treatment of neovascular AMD, increased intervals between appointments by 2 weeks when no activity was present, for example, a new haemorrhage. There is no universally accepted treat and extend regimen, however, for AMD and a treat and extend regime for RP-CMO is not directly comparable with AMD. The AMOUR study therefore included the following protocol for its treatment regime as guided by the PI: IvA was administered every four weeks for the first three months (loading phase), followed by a treat and extend protocol up to 12 months. Extension from monthly to 6, 8, 10 and 12 week follow-up occurred when there was no reduction in macular oedema compared with the previous visit.

A consort flow diagram has been constructed to illustrate the flow of patients throughout the study (See Figure 28). Please refer to table 2 for a schedule of assessments.

Figure 28: Consort flow diagram

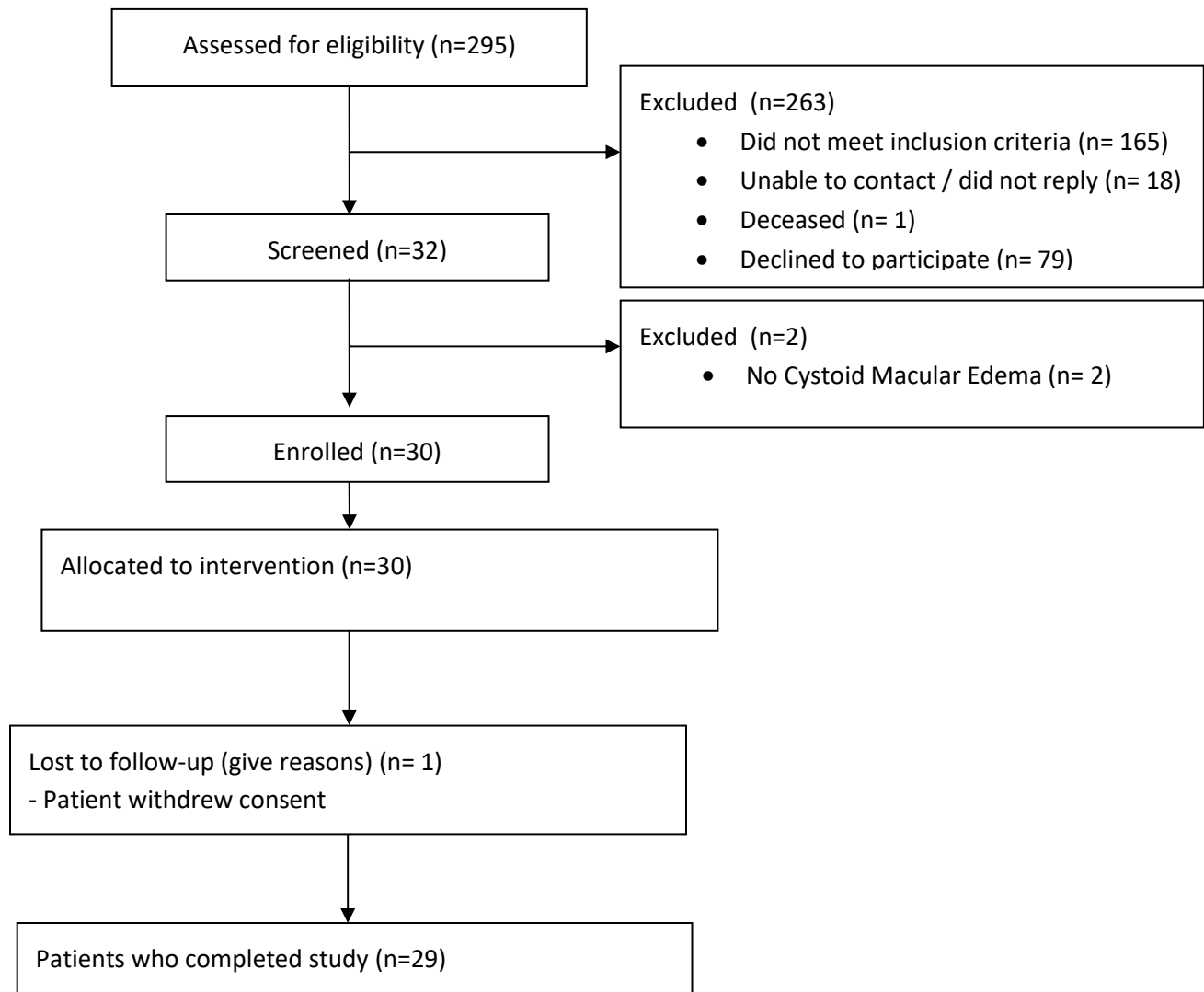


Table 2: Schedule of assessments

	Screening and Baseline	Treatment phase and follow-up*											
Visit number	1	2	3	4	5	6	7	8	9	10	11	12	13
Weeks	0 wks	4 wks post baseline	8 wks post baseline	4 - 12 wks post visit 3	4 - 12 wks post visit 4	4 - 12 wks post visit 5	4 - 12 wks post visit 6	4 - 12 wks post visit 7	4 - 12 wks post visit 8	4 - 12 wks post visit 9	4 - 12 wks post visit 10	4 - 12 wks post visit 11	4 - 12 wks post visit 12
Patient demographics confirmed	X	X	x	X	x	x	X	x	x	x	x	x	X
General medical and ocular history	X												
Medication review	X	x	x	x	x	x	X	x	x	x	x	x	X
Pregnancy status confirmed	X												
Eligibility check	X												
Informed Consent	X												
Vital signs: Blood pressure, heart rate and temperature	X	X	x	X	x	x	X	x	x	x	x	x	X
Best corrected ETDRS visual acuity	X	X	X	X	x	x	X	x	x	x	x	x	x
Refracted best corrected ETDRS visual acuity	X						X (at 6 months)						X (at 12 months)
Colour vision	X	X	X	X	x	x	X	x	x	x	x	x	x
Contrast sensitivity	X	X	X	X	x	x	X	x	x	x	x	x	x
Microperimetry	X						X (at 6 months)						X (at 12 months)
Dilation of the patient	X	X	X	X	x	x	X	x	x	x	x	x	x
Slit lamp examination	X	X	X	X	x	x	X	x	x	x	x	x	x
IOP check (pre-injection)	X	X	X	X	x	x	X	x	x	x	x	x	x
SDOCT in both eyes	X	X	X	X	x	x	X	x	x	x	x	x	x
Fundus Autofluorescence	X	X	X	X	x	x	X	x	x	x	x	x	x
Administration of Eylea	Possibly	X	X	X	x	x	X	x	x	x	x	x	x
IOP check (post-injection)	X	X	X	X	x	x	X	x	x	x	x	x	x

*Number of total visits will vary between patients as follow-up appointments will be any time between 4 – 12 weeks.

2.1.5.4 Informed consent

SAS was delegated the duty of taking informed consent by the PI, Professor Michel Michaelides. If patients were deemed suitable to participate in the trial at the end of their screening visit, written informed consent was obtained from each subject prior to participation in the trial. This took place following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. Consent did not denote enrolment into trial. Patients were informed that they could withdraw at any time during the trial, without having to give a reason. A copy of the signed consent form was given to each participant. The original signed form was retained at the study site and a copy placed in the medical notes.

It was discussed that if new safety information resulted in significant changes in the risk/benefit assessment during the study period, the consent form would be reviewed and updated if necessary and subjects would be re-consented as appropriate.

2.1.5.5 Randomisation

The study consisted of only 1-arm and all trial patients received the active drug, aflibercept via intravitreal injection. Whilst the study in 2009 carried out by Artunay et al. included 15 patients who declined to undertake intravitreal injections of ranibizumab as controls, the AMOUR study was an exploratory study using descriptive statistics only that therefore did not require controls.

2.1.5.6 Study size

Artunay et al. (2009) included 30 patients in a study using anti-VEGF for RP-CMO, however, only a single injection of ranibizumab was given to 15 patients (the other 15 patients had declined treatment and

were hence used as controls). No previous studies using anti-VEGF for RP-CMO have therefore been published using a treat and extend regime for which the sample size could be powered on. Our team of statisticians felt a sample size of 30 patients was therefore justified on the basis that 30 subjects will provide an estimate of the mean change in CMT from baseline to 12 months with reasonable precision as advocated by Browne (1995)(252) and Hertzog (2008) (253).

2.1.5.7 Masking

This was an open-label study and therefore no masking took place.

2.1.5.8 Discontinuation/withdrawal of participants and stopping rules

Circumstances in which subjects would have been withdrawn from the trial, included: mortality, CVA/transient ischaemic attack (TIA)/MI/unstable angina, hypersensitivity/allergy to anti-VEGF and if for any reason the patient no longer wished to be involved with the trial. This would have been confirmed in writing and documentation sent to both patient and GP informing them why they were withdrawn from the trial and thanking them for their participation up to that point.

Withdrawn subjects would only have been replaced if their withdrawal was deemed unrelated to the trial, for example, if a patient needed to relocate for work purposes/sick family member and could no longer physically attend their appointments. Replacement patients would have been recruited in an identical fashion to those who originally enrolled in the study. Recruitment of these patients would have been required to occur within the designated 8 month recruitment period. Patients who may have withdrawn for reasons deemed related to the trial e.g. intolerable side effects, would not have been

replaced. Patients who may have withdrawn from the study would have been asked to attend an exit follow-up review at 12 months, which would have involved the same tests to be undertaken as those still remaining in the study. In the event of a retinal tear, the dose of aflibercept would have been withheld and treatment not resumed until the break was adequately repaired.

The trial would have been stopped prematurely if SAEs were shown to be caused by aflibercept or if the TSC had any safety concerns.

2.1.5.9 Data management

The completed paper case report forms (CRFs) were checked for completion by the research nurse / research manager and data officer before data entry. All trial data were double entered by two independent data officers using the database created by the research and development (R&D) information technology (IT) team. The first and second data entries were compared for completion and consistency. Discrepancies were checked against the original CRF for entry errors, which were subsequently corrected. Sense checks, logic checks and range checks were also performed. Data queries were corrected and data were cleaned. The database was then locked and data transferred for data to be analysed by trial statisticians using statistics and data (STATA) statistical software. The data management process followed Moorfields Eye Hospital standard operating procedures (SOPs) for data management.

2.1.5.10 Statistical analysis

The primary analysis was an available case analysis but baseline characteristics of those who were lost to follow up were compared with those who were not.

If the findings from this study were favourable, these data would be used to plan a larger phase III multi-site study based on the same anti-VEGF regime. If safety and efficacy data were deemed favourable following this, our hope would be for the licencing of aflibercept to be extended to include the use of RP-CMO.

Descriptive statistics have been used to report the findings of this study due to its modest sample size and single arm design.

'Responders' would be considered as participants demonstrating a reduction of CMT by 11% or more between baseline and 12 months thus allowing comparison with previous studies that used the same definition (138, 254).

2.1.6 Name and description of all drugs used in the trial

2.1.6.1 Aflibercept (Eylea)

Aflibercept is currently licenced for use in nAMD and CMO secondary to RVO and DMO. It is a recombinant fusion protein consisting of portions of human VEGF receptor 1 and 2 extracellular domains fused to the Fc portion of human IgG1 (255). Aflibercept is produced in Chinese hamster ovary K1 cells by recombinant DNA technology. Aflibercept acts as a soluble decoy receptor that binds VEGF-A

and PlGF with higher affinity than their natural receptors, and thereby can inhibit the binding and activation of these VEGF receptors (195). VEGF-A and PlGF are members of the VEGF family of angiogenic factors that can act as potent mitogenic, chemotactic, and vascular permeability factors for endothelial cells. VEGF acts via two receptor tyrosine kinases; VEGFR-1 and VEGFR-2, present on the surface of endothelial cells. PlGF binds only to VEGFR-1, which is also present on the surface of leucocytes. Excessive activation of these receptors by VEGF-A can result in pathological neovascularisation and excessive vascular permeability. PlGF can synergise with VEGF-A in these processes, and is also known to promote leucocyte infiltration and vascular inflammation.

The following information has been taken from the electronic Medicines Compendium (eMC) (256): A total of 3,102 patients constituted the safety population of 8 phase III studies. Among those, 2,501 patients were treated with the recommended dose of 2 mg.

Serious ocular AEs related to the injection procedure have occurred in less than 1 in 1,900 intravitreal injections with aflibercept and included blindness, endophthalmitis, retinal detachment, cataract traumatic, vitreous haemorrhage, cataract, vitreous detachment, and intraocular pressure increase. The most frequently observed AEs – defined as occurring in at least 5% of patients treated with aflibercept – included: conjunctival haemorrhage (25%), VA reduction (11%), eye pain (10%), IOP increase (8%), vitreous detachment (7%), vitreous floaters (7%) and cataract (8%) (256).

Table 3 includes safety data of all adverse events reported from 8 phase III studies in the indications wet AMD, CRVO, BRVO and DMO with a reasonable possibility of causality to the injection procedure or medicinal product. In the wet AMD phase III studies, there was an increased incidence of conjunctival

haemorrhage in patients receiving anti-thrombotic agents. This increased incidence was comparable between patients treated with ranibizumab and aflibercept.

There is a theoretical risk of arterial thromboembolic events following intravitreal use of VEGF inhibitors.

A low incidence rate of arterial thromboembolic events was observed in the aflibercept clinical trials involving patients with AMD, DMO, RVO and myopic CNV. Across indications no notable difference between the groups treated with aflibercept and the respective comparator groups were observed.

As with all therapeutic proteins, there is a potential for immunogenicity with aflibercept.

Table 3: Adverse drug reactions associated with the use of intravitreal aflibercept. All treatment-emergent adverse drug reactions reported in patients in phase III studies (pooled data of the phase III studies for the indications wet AMD, CRVO, BRVO and DME). The adverse reactions are listed by system organ class and frequency using the following convention: Very common ($\geq 1/10$), common ($\geq 1/100$ to $< 1/10$), uncommon ($\geq 1/1,000$ to $< 1/100$), rare ($\geq 1/10,000$ to $< 1/1,000$). Within each frequency grouping, adverse drug reactions are presented in order of decreasing seriousness (256).

System Organ Class	Very common	Common	Uncommon	Rare
Immune system disorders			Hypersensitivity***	
Eye disorders	Visual acuity reduced, Conjunctival haemorrhage, Eye pain	Retinal pigment epithelial tear*, Detachment of the retinal pigment epithelium, Retinal degeneration, Vitreous haemorrhage, Cataract, Cataract cortical, Cataract nuclear,	Endophthalmitis**, Retinal detachment, Retinal tear, Iritis, Uveitis, Iridocyclitis, Lenticular opacities, Corneal epithelium defect,	Blindness, Cataract traumatic, Vitritis Hypopyon

		Cataract subcapsular, Corneal erosion, Corneal abrasion, Intraocular pressure increased, Vision blurred, Vitreous floaters, Vitreous detachment, Injection site pain, Foreign body sensation in eyes, Lacrimation increased, Eyelid oedema, Injection site haemorrhage, Punctate keratitis, Conjunctival hyperaemia, Ocular hyperaemia	Injection site irritation, Abnormal sensation in eye, Eyelid irritation, Anterior chamber flare, Corneal oedema	
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* Conditions known to be associated with wet AMD. Observed in the wet AMD studies only.

** Culture positive and culture negative endophthalmitis

*** including allergic reactions

2.1.6.2 Tropicamide 1% eye drops:

Tropicamide is an eye drop used to dilate the pupil of the eye allowing for easier retinal examination.

Tropicamide also effects the muscle that controls the lens of the eye, resulting in reduced accommodation. Due to the effects of tropicamide, patients may have photophobia and visual disturbance following installation, which may affect their ability to drive. The effects of tropicamide are only temporary lasting between 4-6 hours, however, it is possible for the effects to last longer.

Each carton of tropicamide contains 20 minim units, measuring approximately 0.5 millilitre (ml) of the active ingredient tropicamide 1%. Other ingredients include: sodium hydroxide, hydrochloric acid (for pH adjustment) and purified water. There is no preservative.

Tropicamide should not be used in patients with known allergy to tropicamide or any of its ingredients.

It should also not be used in patients with a history of acute angle closure glaucoma or patients with a

narrow anterior chamber. This can be confirmed on examination by an ophthalmologist prior to its usage. Tropicamide can cause temporary stinging upon administration into the eye. It is also possible for this medication to cause a patient to have a dry mouth.

2.1.6.3 Phenylephrine 2.5% eye drops:

Phenylephrine 2.5% is an eye drop used to dilate the pupil of the eye allowing for easier retinal examination. Due to the effects of phenylephrine, patients may have photophobia and visual disturbance following installation, which may affect their ability to drive. The effects of phenylephrine are only temporary lasting between 4-6 hours, however, it is possible for the effects to last longer.

Each carton of phenylephrine 2.5% contains 20 minim units, measuring approximately 0.5ml solution of phenylephrine hydrochloride 2.5%.

Other ingredients include: sodium metabisulphite, disodium edetate and purified water. There is no preservative.

Phenylephrine should not be used in patients with known allergy to phenylephrine or any of its ingredients. It should also not be used in patients with a history of heart disease, tachycardia, raised BP, aneurysms or thyrotoxicosis. Caution should be taken when used in patients with asthma or DM. It should also not be used in patients with a history of acute angle closure glaucoma or patients with a narrow anterior chamber at the front of the eye. This can be confirmed on examination by an ophthalmologist prior to its usage.

2.1.6.4 Proxymetacaine eye drops:

Proxymetacaine is an eye-drop used before the giving of an intravitreal injection, in order to produce an anaesthetic effect on the eye. Each pack contains 20 minim units containing the active ingredient proxymetacaine hydrochloride. Each unit contains approximately 0.5ml eye drops solution of proxymetacaine hydrochloride 0.5% (2.5 mg). The other ingredients include: hydrochloric acid, sodium hydroxide and purified water. This medicine does not contain a preservative as it is a sterile single use unit.

Allergic reactions can occasionally occur that affect the cornea and the iris. Patients may experience the following symptoms before the drug wears off: pupil dilation, reduced accommodation and conjunctival irritation. In rare circumstances, a defect or inflammation of the cornea and/or inflammation of the iris may occur.

2.1.6.5 Iodine eye drops and skin preparation

Iodine has a powerful bactericidal action and is used for disinfecting unbroken skin before operations. Iodine is active against fungi, viruses, protozoa, cysts and spores. The product is suitable for use by adults, children and the elderly. Excipients include: purified water and ethanol (96%). Its use is contraindicated in patients with hypersensitivity to iodine or iodides, newborn infants and in patients with thyroid disorders or those receiving lithium therapy.

Allergic reactions that can occur, include: urticaria, angioedema, cutaneous haemorrhage or purpuras, fever, arthralgia, lymphadenopathy and eosinophilia.

2.1.6.6 Chloramphenicol eye drops

Chloramphenicol eye-drops are used to reduce the risk of infection following intravitreal injection. It is contra-indicated for use in patients with a known hypersensitivity to chloramphenicol or to any other component of the preparation and if there is a family or personal history of blood dyscrasias including aplastic anaemia.

Adverse local effects include sensitivity reactions such as transient irritation, burning, stinging, itching and dermatitis. Sometimes the eye-drops can be tasted or affect taste as they drain from the eye into the back of the mouth. The prolonged use of eye-drops containing phenylmercuric preservative has been associated with skin irritation, primary atypical band keratopathy and mercurialentis (pigmentation of the anterior capsule of the lens).

Adverse systemic effects: Rarely cases of adverse haematological events (bone marrow depression, aplastic anaemia and death) have been reported following ocular use of chloramphenicol.

2.1.7 Preparation and labelling of the investigational medicinal products

Preparation and labelling of the investigational medicinal products were completed in accordance with the relevant GMP guidelines. Due to the primary packaging containing a small vial of medication on which particulars cannot be displayed, a second sheet displayed a label containing the following information:

(a) name of sponsor, or investigator

(b) route of administration, the name/identifier and strength/potency

(c) batch and/or code number to identify the contents and packaging operation

(d) a trial reference code allowing identification of the trial, site, investigator and sponsor if not given elsewhere

(e) the trial subject identification number/treatment number and where relevant, the visit number

2.1.8 Protocol for intravitreal injection of aflibercept

Intravitreal injections were carried out by SAS who was Good clinical practice (GCP) trained and suitably qualified and experienced to deliver intravitreal injections. Another locum consultant, Dr. Simona Esposti, was formally included on the delegation log to provide cover if SAS was absent due to annual, study or sick leave.

Eylea 2 mg (0.05mL) was administered by intravitreal injection every 4 weeks for the first 3 injections, followed by 2mg once every 4 - 10 weeks depending on whether there was evidence of OCT stability in the view of the PI (i.e. there was no further reduction in macular fluid compared to the previous visit).

2.1.9 Post-intravitreal injection management

Small volume injections (0.05ml) are unlikely to cause a significant rise in IOP (257). All patients were tested for hand movement (HM) vision immediately after injection:

- *If HM vision was not achieved*, indirect ophthalmoscopy was performed by SAS to check for perfusion status of the central retinal artery (CRA). Immediate anterior chamber paracentesis was performed if the CRA was considered to be non-perfused. Patients were then re-tested for HM vision. If this was achieved, IOP was checked using Goldmann tonometry after 30 minutes. If this was not achieved, a second anterior chamber paracentesis would be performed and the above process repeated.
- *If HM vision was achieved*, patients would be asked to wait for 30 minutes in the waiting area, after which time, IOP was re-checked using Goldmann tonometry.

If post-intravitreal IOP was 30mmHg or less, patients were discharged home with instructions regarding topical antibiotic usage and advice about when (and how) to seek help, for example, if the eye became red or painful or if the vision reduced. Patients were informed, however, that some blurring of vision is common immediately post-injection; often described as 'seeing spots floating in the eye', which usually resolve after a few days to a week (257). Patients were advised not drive until their visual function had recovered sufficiently.

Since this study was carried out, the Royal College of Ophthalmologists have published updated guidelines for intravitreal injection therapy. This states that “the use of peri-injection antibiotics is no longer recommended. There is no evidence that their use reduces the risk of post-operative

endophthalmitis, but there is evidence that their use can contribute to the emergence of drug-resistant pathogenic bacteria” (257).

2.1.10 Outcome measures

2.1.10.1 Primary outcome

There were two primary outcome measures: (i) To report the safety of aflibercept throughout the study (17 months in total); via the documentation of AEs deemed related to the trial drug; (ii) To report the efficacy of aflibercept via mean CMT on SDOCT at 12 months after baseline in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol.

2.1.10.2 Secondary outcomes

To report mean CMT at 6 months as measured with SDOCT in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol between baseline and 12 months.

To report mean change in CMT as measured with SDOCT in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol between baseline and 6 months, and baseline and 12 months.

To report mean BCVA ETDRS letter score at 6 and 12 months in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol between baseline and 12 months.

To report mean change in BCVA ETDRS letter score in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol between baseline and 6 months, and baseline and 12 months.

To report mean macular volume at 6 and 12 months as measured with SDOCT in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol between baseline and 12 months.

To report mean change in macular volume as measured with SDOCT in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol between baseline and 6 months, and baseline and 12 months.

To report all AEs and SAEs at any time point during the 12 month study of using intravitreal Eylea in eyes of patients with RP-CMO.

To report mean retinal sensitivity at 6 and 12 months using microperimetry in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol between baseline and 12 months.

To report mean change in retinal sensitivity using microperimetry in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol between baseline and 6 months, and baseline and 12 months

To report mean number of intravitreal injections administered in eyes of patients with RP-CMO treated with three loading doses of Eylea at monthly intervals followed by a treat and extend protocol between baseline and 12 months.

2.1.11 Methodology of assessments of visual function

2.1.11.1 VA

At the baseline, 6- and 12- month visits, all patients within the study were subjectively refracted by an optician in order to obtain any spectacle correction required to achieve their BCVA.

At all study visits, the lens correction from the most recent subjective refraction was placed in the trial frame to correct for any refractive error. VA was tested using the ETDRS chart, which incorporates specific design criteria to make it more accurate than the Snellen VA. Two ETDRS charts were used in this study (chart 1 for the RE and chart 2 for the LE) that were retro-illuminated using a lightbox containing 2 Cool Daylight 20 watt fluorescent tubes. The room lights were turned off whilst the test was being performed. The RE was always tested first and the non-tested eye was occluded throughout the test. Each patient sat at a starting distance of 4 metres (m), however, if a patient was unable to read 20 letters or more at this distance, the test would be repeated at 1m. In this case, only the first 6 rows

would be attempted. If the patient was wearing a trial frame, +0.75 dioptres sphere (DS) was added to the prescription to correct for the closer test distance. The VA score was the number of letters read correctly at 4m, plus the number of letters read correctly at 1m. If the patient did not require testing at 1m, i.e. they read 20 or more letters at 4m, then the score was the number of letters read correctly at 4m, plus 30.

If the patient was unable to read any letters on the ETDRS chart at 1m, then their ability to count fingers, detect HM or PL was measured.

2.1.11.2 CMT

The Heidelberg Spectralis OCT was used to obtain images of macular volume in order to measure CMT. The scan was performed by a technician who had been approved on the delegation log. It was performed on patients following pharmacological dilatation, but before intravitreal injection. Patients were asked to look directly into the camera lens, where they saw a bright blue dot representing the internal fixation target. Patients were encouraged to blink regularly throughout the examination, however, to maintain focus on the internal fixation target at all times. The macular volume scan protocol took images as follows: 20°x20°, 49 Sections, High Speed, 29 Frames automatic real time (ART). The technician would inspect each scan after acquisition to ensure high quality images had been taken.

2.1.11.3 Colour vision

Ishihara colour vision testing is a rapid and simple test involving the use of pseudo-isochromatic plates in order to measure colour vision.

Before seeing trial patients, staff were required to be signed off on the delegation log by the PI. The protocol stipulated that patients should be tested, before being pharmacologically dilated, in an adequately lit room. This was achieved by using a light box, meaning that testing was performed in a very controlled fashion. The Ishihara book contained 17 plates. Patients were adequately corrected for reading vision using plus lenses if required. Each eye was tested individually in turn (monocular testing starting with the RE, then the LE). Plates were held at 75 cm from the patient and tilted so that the plane of paper was at right-angles to the line of vision. Each patient was asked to state the numeral seen on each plate between plates 1 - 17. The answer was only counted as correct if given without more than three seconds delay. The number of numerals correctly identified was recorded out of 17 plates.

2.1.11.4 Contrast sensitivity

Contrast sensitivity was performed by an optician using the Pelli-Robson chart (Clement Clarke Inc., Columbus, OH). The patient was seated at a distance of 1m. Each eye was tested in turn (the RE was tested before the LE) with the non-tested eye being patched. As a standard, +0.75DS was added to each patient's refraction when performing the test. The luminance of the chart was between 80 - 120 candela per square metre (cd/m^2).

The patient was asked to name each letter on the chart, starting with the high-contrast letters on the upper left-hand corner and reading horizontally across the entire line. As low-contrast letters can take some time to appear, the patient was given instructions to keep looking and not give up too soon. The optometrist circled each letter read correctly and crossed out any letter read incorrectly, with letters not attempted left unmarked. The test was completed when the patient failed to correctly identify two or more letters in a triplet.

2.1.11.5 Retinal sensitivity

Fundus-driven perimetry, commonly known as microperimetry, is a technique for measuring VF sensitivity, whilst simultaneously viewing the fundus (258). It is deemed superior at evaluating the function of the macular area in patients with unstable or extra-foveal fixation (258).

In this study, standard mesopic microperimetry was used to test retinal sensitivity using the MP-1 microperimeter (Nidek Instruments, Inc, Padua, Italy). At baseline, microperimetry was carried out *twice* on each eye, whereas at 6- and 12- months, microperimetry was carried out only once on each eye.

To carry out the test, spherical error was initially accounted for in all patients using the following calculation: sphere + $\frac{1}{2}$ cylindrical error. Patients were dark-adapted for 10 minutes before performing the test and throughout the test, the non-tested eye was patched. Regardless of where the fixation cross was situated, the grid was centred over the fovea. The microperimetry protocol included: Cross 2 degrees, Goldmann III Stimulus 200ms, 4-2 strategy and 30 seconds of tracked fixation. At the end of the test, a photograph of each eye was taken using retinography. A local defect map, including -9:1 setting in order to provide mean sensitivity and mean defect together with bivariate contour ellipse area

(BCEA) value (numeric and fixation), was printed off at the end of the test and placed in the patient's study folder.

2.1.11.6 FAF

Autofluorescence describes the capacity for certain molecules, known as fluorophores, to emit light when they have been excited by suitable wavelengths (259). The endogenous fluorophores include the cornea, crystalline lens, RPE, uveal melanocytes and scleral collagen (259).

FAF images were acquired during this study at the baseline, 6- and 12- month visits using the Heidelberg Spectralis OCT machine. For each eye, an infrared reflectance image and blue autofluorescence image of standard field 2 (centred on the fovea) was acquired. Both 30 and 55 degree fields were acquired using high resolution at 50 frames (ART).

2.2 Results

2.2.1 Overview and baseline characteristics

Results are summarised in tables 4 to 14.

Thirty eyes of 30 patients were enrolled into the study. The first patient was recruited in March 2016 and the final patient had their 52-week visit in August 2017. Two patients were screened who did not satisfy the criteria for enrolment (study ID 29 and 31); the reason being that they no longer had presence of CMO. The mean age of the patients was 43.3 years (SD 11.5 years, range 20 – 61 years) consisting of 17 male (56.7%) and 13 female (43.3%) subjects. The ethnicity of the patients in the study were as follows: 26 White, 1 Asian, 1 Black, 1 Mixed and 1 Other. The study eye involved the LE in 16 (53.3%) cases and the RE in 14 (47.7%) cases. The median duration of CMO in the study eye was 252 weeks and the interquartile range (IQR) was 156 - 296 weeks. All patients enrolled in the study received the active drug, aflibercept. All patients enrolled in the study received the active drug, aflibercept. The median number of injections given across all patients in the study was 7 (IQR 6 - 9); with the minimum number of injections given being 4, and the maximum number of injections given being 11.

Likely disease-causing sequence variants were identified in 16 of 30 (53.3%) study participants (see figure 29, table 4), which included: (i) AD inheritance: neural retina leucine zipper (*NRL*) gene (1 patient), *RHO* gene (2 patients), pre-mRNA processing factor (*PRPF*) 31 gene, also known as RP-11 (3 patients), *PRPF8* gene (1 patient), small nuclear ribonucleoprotein U5 subunit 200 (*SNRNP200*) gene (1 patient); (ii) AR inheritance: usherin 2A (*USH2A*) gene (3 patients including the following variants: c.1841-2A>G homozygous; c.11700C>A, p.Tyr3900Ter; c.4618G>A, p.Asp1540Asn; c.2299del, p.Glu767Serfs*21 hom),

tubby like protein 1 (*TULP1*) gene (1 patient), *RP-1* gene (1 patient), retinol dehydrogenase (*RDH*)-12 (*RDH12*) gene (1 patient), intraflagellar transport (*IFT*)-140 (*IFT140*) gene (1 patient); and (iii) X-linked inheritance: *RPGR* gene (1 patient). The other 14 patients were recruited into the Genomics England (GEL), Specialist Pathology: Evaluating Exomes in Diagnostic (SPEED) or Manchester 176 panel studies, however, identification of disease-causing variants remain unsolved to date.

Figure 29: The known or putative localisation of proteins translated by genes associated with RP within the rod photoreceptor or RPE cell. Taken from Dias et al. (2018) (260).

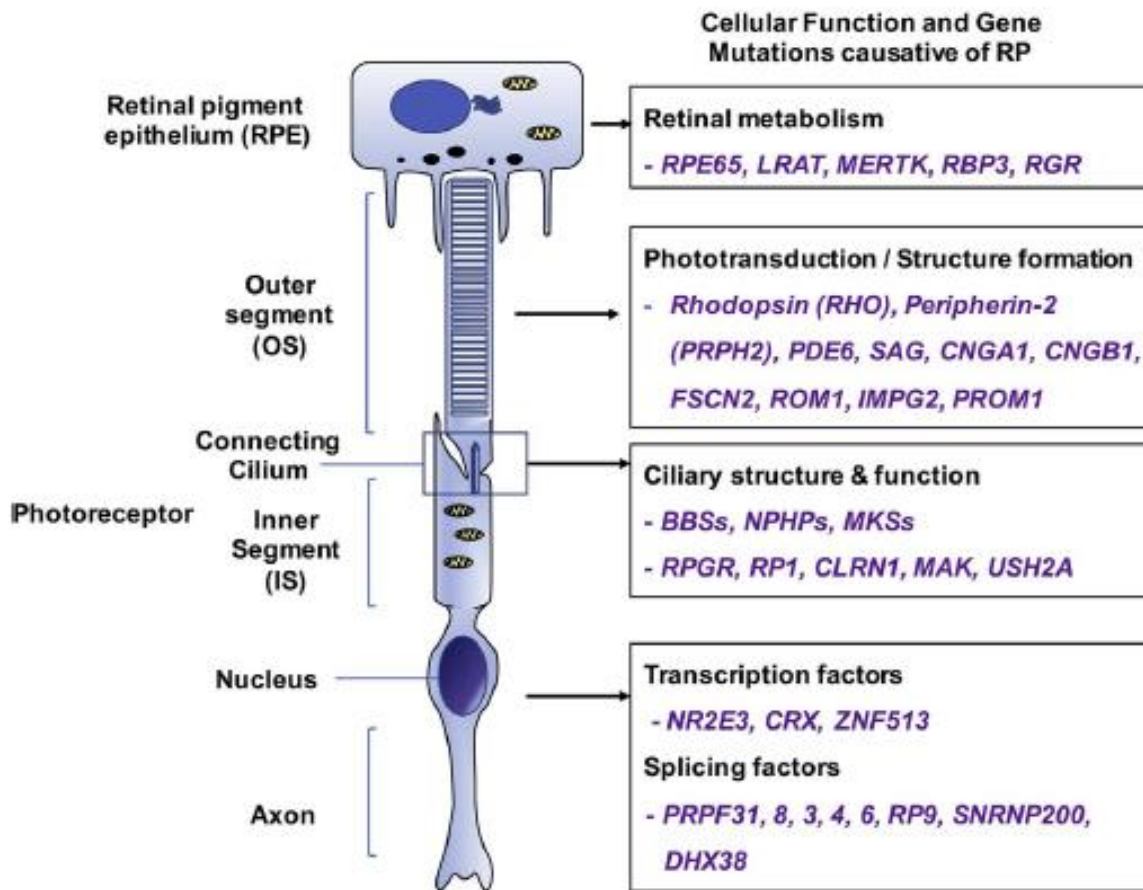


Table 4: Genetic data of all patients in the study

Study ID	Symbol	Protein	Inheritance pattern
1	PFPR31	pre-mRNA processing factor 31	AD
2	PFPR8	pre-mRNA processing factor 8	AD
3	USH2A Location: c.1841-2A>G homozygous	usherin 2A	AR
4	Unsolved	Unsolved	Unsolved
5	RP11	retinitis pigmentosa-11	AD
6	RP1	retinitis pigmentosa-1	AD
7	TULP1	tubby like protein 1	AR
8	Unsolved	Unsolved	Unsolved
9	NRL	neural retina leucine zipper	AD
10	IFT140	intraflagellar transport-140	AR
11	Unsolved	Unsolved	Unsolved
12	RHO/RP4	Rhodopsin	AD
13	Unsolved	Unsolved	Unsolved
14	Unsolved	Unsolved	Unsolved
15	RHO	Rhodopsin	AD
16	Unsolved	Unsolved	Unsolved
17	Unsolved	Unsolved	Unsolved
18	SNRNP200	small nuclear ribonucleoprotein U5 subunit 200	AR
19	Unsolved	Unsolved	Unsolved
20	USH2A Location: c.2299del, p.Glu767Serf s*21 hom	usherin 2A	AR
21	Unsolved	Unsolved	Unsolved

22	Unsolved	Unsolved	Unsolved
23	Unsolved	Unsolved	Unsolved
24	PRPF31	pre-mRNA processing factor 31	AD
25	Unsolved	Unsolved	Unsolved
26	Unsolved	Unsolved	Unsolved
27	Unsolved	Unsolved	Unsolved
28	USH2A Location: c.11700C>A, p.Tyr3900Ter ; c.4618G>A, p.Asp1540As n	usherin 2A	AR
29	n/a	n/a	n/a
30	RPGR	retinitis pigmentosa GTPase regulator	XL
31	n/a	n/a	n/a
32	RDH12	retinol dehydrogenase-12	AD

The baseline characteristics for all participants in the study are summarised in tables 5 and 6. The following results are representative of the study eye for the cohort overall: mean baseline ETDRS BCVA was 64 letters (SD 11.5 letters) with a mean CMT of 458.7µm (SD 84.6µm); median baseline macular volume was 8.0mm³ (IQR 7.5 – 8.8); median baseline colour vision was 15 plates (IQR 6 – 16); mean baseline contrast sensitivity was 1.58 log CS (SD 0.35); mean baseline IOP was 12.5mmHg (SD 2.9); mean baseline retinal sensitivity was 6.3 dB (SD 3.6). Twenty-four (80%) patients were phakic in their study eye compared with 6 (20%) patients who were pseudophakic. No patients were aphakic.

Table 5: Non-Ocular Baseline Characteristics (whole cohort)

	Aflibercept
Number of Patients (Eyes)	30 (30)
Male / Female, n (%)	17 (57)/ 13(43)
Age (years), Mean (SD)	43.3 (11.5)
Ethnicity, n (%):	
White	26 (87)
Asian	1 (3)
Black	1 (3)
Mixed	1 (3)
Other	1 (3)

Table 6: Ocular Baseline Characteristics (whole cohort)

	Aflibercept (n=30)
Study Eye, Left/Right, n(%)	16 (53)/ 14 (47)
Duration of CME (weeks), Median (IQR)	252 (156-296)
Lens status, n (%):	
Aphakic	0 (0)
Pseudophakic	6 (20)
Phakic	24 (80)
ETDRS BCVA (letters), Mean (SD)	64 (11.5)
Ishihara colour vision (out of 17 plates), Median (IQR)	15 (6-16)
Contrast sensitivity (cd/m ²), Mean (SD)	1.58 (0.35)
IOP (mmHg), Mean (SD)	12.5 (2.9)
Central macular thickness on SDOCT (µm), Mean (SD)	458.7 (84.6)

Macular Volume on SDOCT (mm ³), Median (IQR)	8.0 (7.5-8.8)
Mean Retinal sensitivity on microperimetry (dB), Mean (SD)	6.3 (3.6)

CME = cystoid macular oedema; μm = microns; ETDRS = early treatment diabetic retinopathy study; BCVA = best corrected visual acuity; SD = standard deviation; IQR = Interquartile range; cd/m^2 = candela per square meter; IOP = intraocular pressure; mmHg = millimetre of mercury; SDOCT = Spectral domain optical coherence tomography; mm^3 = millimetres cubed; dB = decibels

Nine of 29 (31.0%) patients were graded as having either questionable or definite presence of ERM within 3mm of the fovea. No patients were found to have vitreo-macular traction (VMT) on their baseline OCT scan. One of 29 (3.4%) patients was found to have vitreo-macular adhesion on their baseline OCT scan. Nine of 29 (31.0%) patients were graded as having either questionable or definite disruption of the ellipsoid zone within 1mm of the fovea on their baseline OCT scan.

One participant did not complete 12 months of follow-up due to a relapse of mental illness and withdrew from the study. The GP was informed and asked to contact the patients' social worker. This patient was last reviewed at visit 2 with these data being carried forward and an intention-to-treat analysis undertaken. The baseline characteristics for this participant who withdrew from the study were not different to patients who continued in the study. Twenty-nine out of 30 (96.7%) patients therefore completed 12 months of follow-up for the study.

A post-hoc exploratory analysis of responders-only was also undertaken. Baseline characteristics for responders are summarised in Tables 7 and 8. Sub-group analysis of responders demonstrated similar baseline characteristics to the group taken as a whole, with mean baseline ETDRS BCVA of 63.6 letters (SD 11.3 letters), mean CMT of 489.8 μm (SD 105.9 μm) and median duration of CME was 264 weeks (IQR

228-416). The median number of injections for this group was 7 (IQR 6 - 10) where the minimum number of injections given was 5 and the maximum number of injections given was 11.

Table 7: Non-Ocular Baseline Characteristics (Responders only)

	Aflibercept
Number of Patients (Eyes)	11 (11)
Male / Female, n (%)	8 (73)/3(27)
Age (years), Mean (SD)	42.7 (15.6)
Ethnicity, n (%):	
White	11 (100)
Asian	0 (0)
Black	0 (0)
Mixed	0 (0)
Other	0 (0)

Table 8: Ocular Baseline Characteristics (Responders only)

	Aflibercept (n=11)
Study Eye, Left/Right, n(%)	6 (55)/5 (45)
Duration of CME (weeks), Median (IQR)	264 (228, 416)
Lens status, n (%):	
Aphakic	0 (0)
Pseudophakic	1 (9)
Phakic	10 (91)
ETDRS BCVA (letters), Mean (SD)	63.6 (11.3)
Ishihara colour vision (out of 17 plates), Median (IQR)	10 (3-14)
Contrast sensitivity (cd/m ²), Mean (SD)	1.42 (0.38)
IOP (mmHg), Mean (SD)	12.4 (3.4)

Central macular thickness on SDOCT (μm), Mean (SD)	489.8 (105.9)
Macular Volume on SDOCT (mm^3), Median (IQR)	8.9 (8.3-9.9)
Mean Retinal sensitivity on microperimetry (dB), Mean (SD)	5.8 (3.7)

CME = cystoid macular oedema; μm = microns; ETDRS = early treatment diabetic retinopathy study; BCVA = best corrected visual acuity; SD = standard deviation; IQR = Interquartile range; cd/m^2 = candela per square meter; IOP = intraocular pressure; mmHg = millimetre of mercury; SDOCT = Spectral domain optical coherence tomography; mm^3 = millimetres cubed; dB = decibels

2.2.2 Outcome measures

2.2.2.1 Efficacy: analysis of all study participants

The primary and secondary efficacy outcomes for all patients (responders and non-responders) within the study are summarised in Tables 9 and 10. Mean CMT at 12 months was $413.4\mu\text{m}$ (SD $98.2\mu\text{m}$, 95% CI $376.0 - 450.7\mu\text{m}$) corresponding to a reduction in CMT of $47.6\mu\text{m}$ (SD $86.6\mu\text{m}$, 95% CI -80.5 to $-14.6\mu\text{m}$) or 9.61 % (17.56 %) between baseline and 12 months. Mean macular volume at 12 months was 8.0mm^3 (SD 0.7, 95% CI $7.7 - 8.2$) corresponding to a change in macular volume of -0.3mm^3 (SD 0.7, 95% CI $-0.6 - -0.1$) between baseline and 12 months. Mean CMT at 6 months was similar at $414.8\mu\text{m}$ (SD $96.4\mu\text{m}$, 95% CI $378.1 - 451.4\mu\text{m}$) corresponding to a reduction in CMT of $46.2\mu\text{m}$ (SD $108.7\mu\text{m}$, 95% CI -87.6 to $-4.9\mu\text{m}$) or 8.13 % (23.3 %) between baseline and 6 months. Mean macular volume at 6 months was 7.9mm^3 (SD 0.6, 95% CI $7.7 - 8.2$) corresponding to a change in macular volume of -0.3mm^3 (SD 0.8, 95% CI $-0.7 - 0.0$) between baseline and 6 months. Figure 30 is a graph demonstrating mean change in CMT from baseline to 6 months, and baseline to 12 months in the group overall.

Table 9: Primary outcome measures

	Aflibercept (n=29)	95% CI
Central Macular thickness on SDOCT (μm), Mean (SD) at Baseline	458.7 (84.6)	
Central Macular thickness on SDOCT (μm), Mean (SD) at 12 months	413.4 (98.2)	376.0 - 450.7

SDOCT = Spectral domain optical coherence tomography; μm = microns; SD = standard deviation

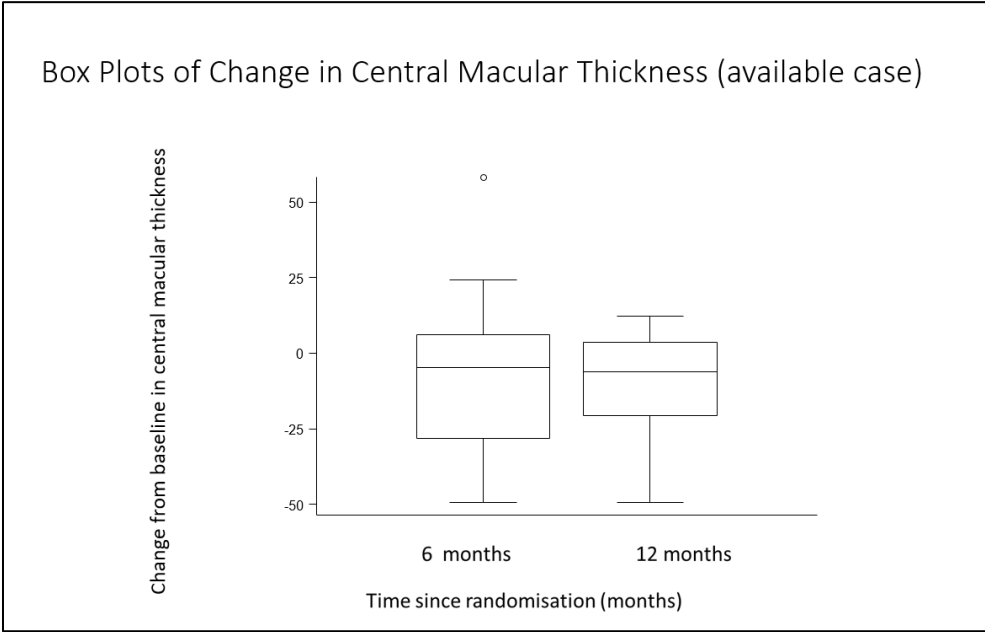
Table 10: Secondary outcome measures

	Aflibercept (n =29)	95% CI
Central Macular thickness on SDOCT (μm), Mean (SD) at 6 months	414.8 (96.4)	378.1 – 451.4
Change in Central Macular thickness on SDOCT (μm) from		
- Baseline to 12 months, Mean (SD)	-47.6 (86.6)	-80.5 - -14.6
- Baseline to 6 months, Mean (SD)	-46.2 (108.7)	-87.6 - -4.9
ETDRS BCVA (letters), Mean (SD) at 6 months	66.9 (10.6)	62.8 – 70.9
ETDRS BCVA (letters), Mean (SD) at 12 months	68.0 (11.1)	63.8 – 72.3
Change in ETDRS BCVA (letters) from		
- Baseline to 12 months, Mean (SD)	4.3 (6.9)	1.7 – 6.9
- Baseline to 6 months, Mean (SD)	3.1 (6.6)	0.6 – 5.6
Macular Volume on SDOCT (mm^3), Mean (SD) at 6 months	7.9 (0.6)	7.7 – 8.2
Macular Volume on SDOCT (mm^3), Mean (SD) at 12 months	8.0 (0.7)	7.7 – 8.2
Change in Macular Volume on SDOCT (mm^3) from		
- Baseline to 12 months, Mean (SD)	-0.3 (0.7)	-0.6 – -0.1
- Baseline to 6 months, Mean (SD)	-0.3 (0.8)	-0.7 – 0.0

Retinal Sensitivity (dB), Mean (SD) at 6 months	4.92 (3.49)	3.56 – 6.27
<i>Missing, n(%)</i>	1 (3)	
Retinal Sensitivity (dB), Mean (SD) at 12 months	4.93 (3.48)	3.55 – 6.31
<i>Missing, n(%)</i>	2 (6)	
Change in Retinal Sensitivity (dB) from		
- Baseline to 12 months, Mean (SD)	-1.09 (2.10)	-1.90 - -0.27
- Baseline to 6 months, Mean (SD)	-1.23 (2.24)	-2.10 - -0.37
Total number of injections received over the study period (12 months), Median (IQR)	7 (6-9)	-

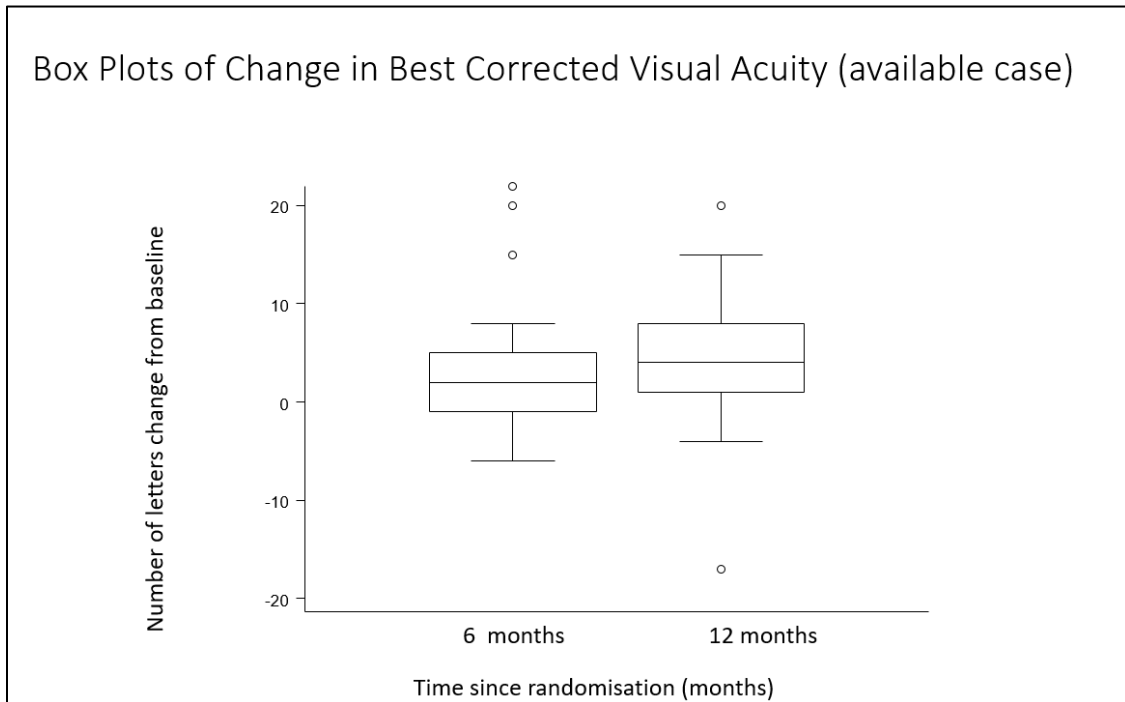
CME = cystoid macular oedema; μm = microns; ETDRS = early treatment diabetic retinopathy study; BCVA = best corrected visual acuity; SD = standard deviation; IQR = Interquartile range; cd/m^2 = candela per square meter; IOP = intraocular pressure; mmHg = millimetre of mercury; SDOCT = Spectral domain optical coherence tomography; mm^3 = millimetres cubed; dB = decibel

Figure 30 : A graph demonstrating mean change in CMT from baseline to 6 months, and baseline to 12 months in the group overall.



Mean ETDRS BCVA was 66.9 letters (SD 10.6, 95% CI 62.8 – 70.9) at 6 months and 68.0 letters (SD 11.1, 95% CI 63.8 – 72.3) at 12 months. This equated to a gain of 3.1 letters (SD 6.6, 95% CI 0.6 – 5.6) and 4.3 letters (SD 6.9, 95% CI 1.7 – 6.9) respectively at 6 and 12 months. No patients lost ≥ 30 letters. Please see figure 31 for box plots of change in BCVA demonstrating mean change in BCVA from baseline to 6 months, and baseline to 12 months in the group overall.

Figure 31 : Box plots of change in BCVA demonstrating mean change in BCVA from baseline to 6 months, and baseline to 12 months in the group overall.



Mean retinal sensitivity at 6 months was 4.92 dB (SD 3.49, 95% CI 3.56 – 6.27) corresponding to a change in retinal sensitivity of -1.23dB (SD 2.24, 95% CI -2.1 - -0.37). Data were missing for 1 (3%) patient. Mean retinal sensitivity at 12 months was 4.93 dB (SD 3.48, 95% CI 3.55 – 6.31) corresponding

to a change in retinal sensitivity of -1.09dB (SD 2.10, 95% CI -1.9 - -0.27). Data were missing for 2 (6%) patients.

3.1.2.2 Efficacy: sub-group analysis of responders only

The primary and secondary efficacy outcomes using descriptive statistics for sub-group analysis of responders within the study are provided in table 11.

Table 11: Descriptive statistics for responders

	Aflibercept (n=11)
Central Macular thickness on SDOCT (μm), Mean (SD) at 12 months	350.3 (93.3)
Central Macular thickness on SDOCT (μm), Mean (SD) at 6 months	360.7 (85.2)
Change in Central Macular thickness on SDOCT (μm) from	
- Baseline to 12 months, Mean (SD)	-139.5 (65.8)
- Baseline to 6 months, Mean (SD)	-129.1 (125.1)
ETDRS BCVA (letters), Mean (SD) at 6 months	67.5 (10.1)
ETDRS BCVA (letters), Mean (SD) at 12 months	68.4 (11.8)
Change in ETDRS BCVA (letters) from	
- Baseline to 12 months, Mean (SD)	4.7 (9.5)
- Baseline to 6 months, Mean (SD)	3.8 (6.8)
Macular Volume on SDOCT (mm^3), Mean (SD) at 6 months	8.5 (0.6)
Macular Volume on SDOCT (mm^3), Mean (SD) at 12 months	8.5 (0.8)
Change in Macular Volume on SDOCT (mm^3) from	
- Baseline to 12 months, Mean (SD)	-0.6 (0.6)
- Baseline to 6 months, Mean (SD)	-0.6 (0.6)
Retinal Sensitivity (dB), Mean (SD) at 6 months	4.93 (4.06)

Retinal Sensitivity (dB), Mean (SD) at 12 months	4.48 (3.83)
Change in Retinal Sensitivity (dB) from	
- Baseline to 12 months, Mean (SD)	-0.97 (1.92)
- Baseline to 6 months, Mean (SD)	-0.92 (2.03)
Total number of injections received over the study period (12 months), Median (IQR)	7 (6-10)

CME = cystoid macular edema; μm = microns; ETDRS = early treatment diabetic retinopathy study; BCVA = best corrected visual acuity; SD = standard deviation; IQR = Interquartile range; cd/m^2 = candela per square meter; IOP = intraocular pressure; mmHg = millimetre of mercury; SDOCT = Spectral domain optical coherence tomography; mm^3 = millimetres cubed; dB = decibels

Eleven out of 29 (36.7%) patients were considered responders having demonstrated a reduction in CMT of 11% or more at 12 months compared to baseline. These same patients were also classed as responders at 6 months when applying the same criteria. Please see figure 32 for 2 examples of responders in the study. Genetic variants were identified and confirmed in 5 of 11 (45.5%) of responders, which included: *RPGR* (1 patient), *PRPF31* (1 patient), *USH2A* (c.11700C>A, p.Tyr3900Ter; c.4618G>A, p.Asp1540Asn) (1 patient), *RHO* (1 patient) and *RDH12* (1 patient). The other 6 patients were recruited into the GEL or SPEED studies, however, identification of variants remain unsolved.

Following sub-analysis of these 11 patients, mean CMT at 12 months was $350.3\mu\text{m}$ (SD $93.3\mu\text{m}$) corresponding to a change in CMT of $-139.5\mu\text{m}$ (SD $65.8\mu\text{m}$) or 28.1% (12.9 %) between baseline and 12 months. Mean macular volume at 12 months was 8.5mm^3 (SD 0.8) corresponding to a change in macular volume of -0.6mm^3 (SD 0.6) between baseline and 12 months. Mean CMT at 6 months was similar at $360.7\mu\text{m}$ (SD $85.2\mu\text{m}$) corresponding to a change in CMT of $-129.1\mu\text{m}$ (SD $125.1\mu\text{m}$) or 22.9% (29.7 %)

(See Figure 32) between baseline and 6 months. Mean macular volume at 6 months was 8.5mm^3 (SD 0.6) corresponding to a change in macular volume of -0.6mm^3 (SD 0.6) between baseline and 6 months. Figure 33 demonstrates SDOCT images of 2 responders taken at baseline and at 1 month post-baseline (after having received only a single intravitreal injection of aflibercept).

Figure 32 : A graph demonstrating mean change in CMT from baseline to 6 months, and baseline to 12 months in responders-only.

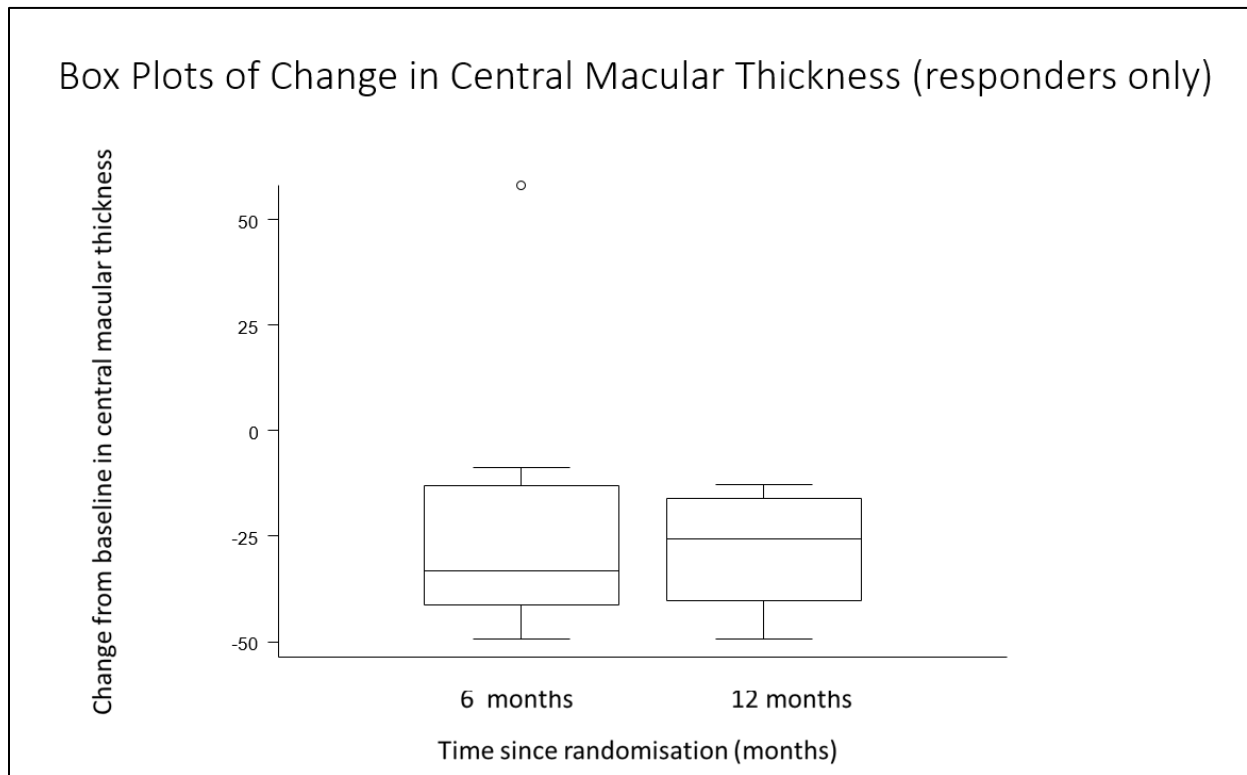
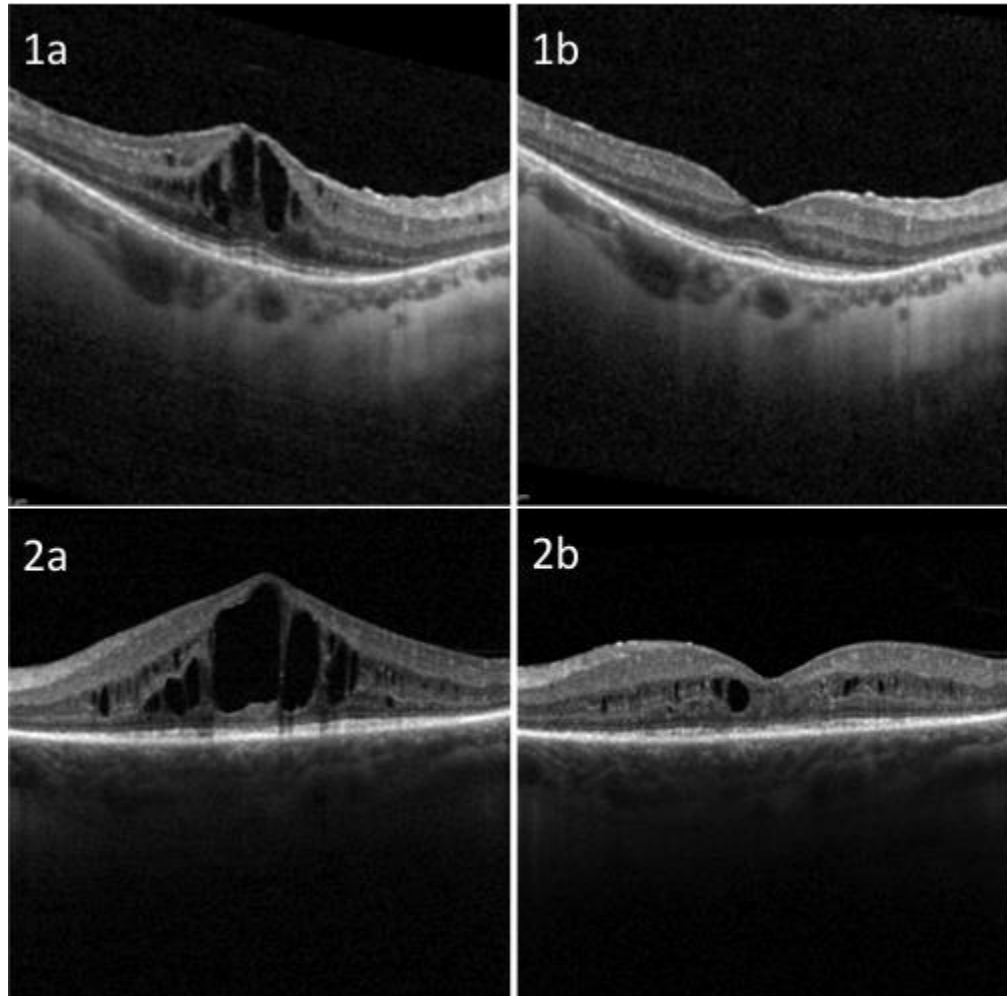


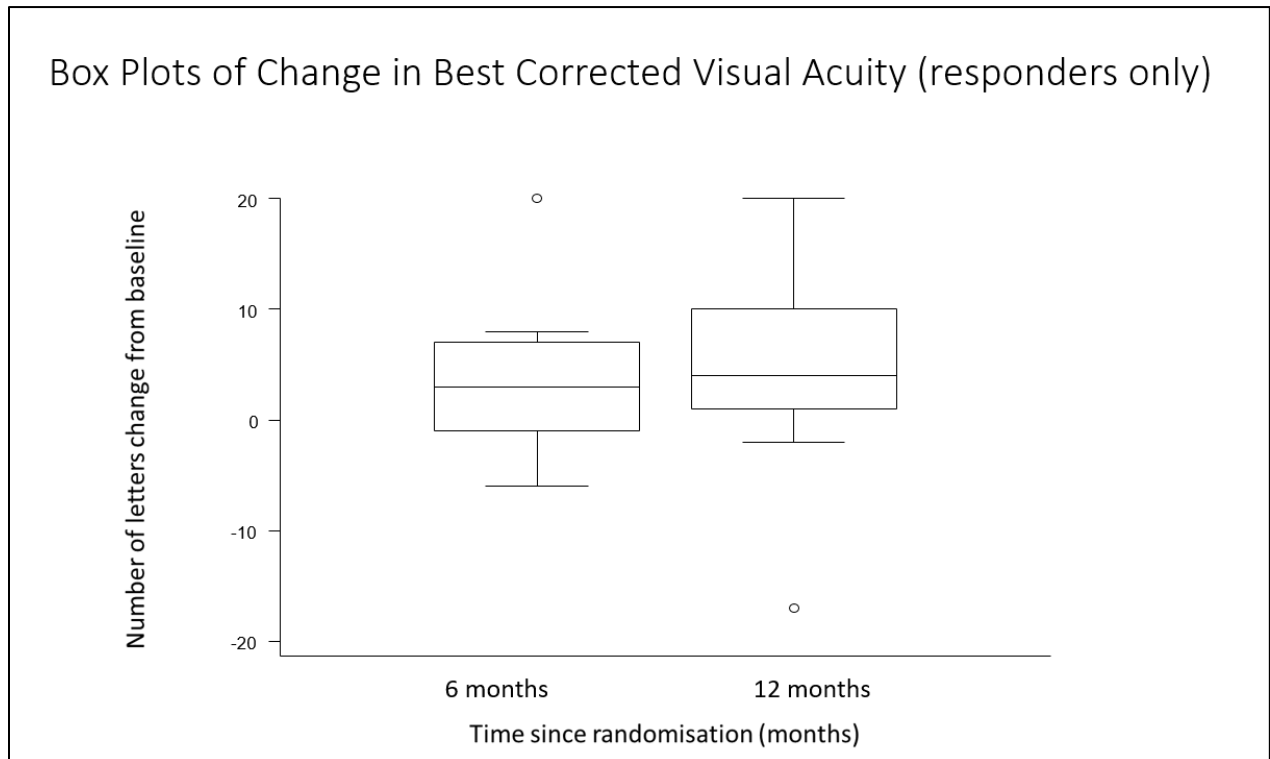
Figure 33: Two representative examples of responders: 1a and 2a show SDOCT baseline images of two study participants (study IDs: 04 and 14); 1b and 2b are SDOCT images taken at 1 month post 1st aflibercept injection in the same two participants, respectively



Mean ETDRS BCVA at 6 months was 67.5 letters (SD 10.1) corresponding to a gain of 3.8 letters (SD 6.8). Mean ETDRS BCVA at 12 months was 68.4 letters (SD 11.8) corresponding to a gain of 4.7 letters (SD 9.5) (see figure 34). It should be noted that 3 of 11 (27.3%) responders were graded as having disruption of the ellipsoid zone within 1mm of the fovea on their baseline OCT scan. No improvement of vision was

found in all 3 of these patients. Four of 11 (36.4%) responders were graded as having questionable presence of ERM within 3mm of the fovea.

Figure 34: Box plots of change in BCVA demonstrating mean change in BCVA from baseline to 6 months, and baseline to 12 months in responders-only.



Mean retinal sensitivity at 6 months was 4.93dB (SD 4.06) corresponding to a change in retinal sensitivity of -0.92dB (SD 2.03) between baseline and 6 months. Mean retinal sensitivity at 12 months was 4.48dB (SD 3.83) corresponding to a change in retinal sensitivity of -0.97dB (SD 1.92) between baseline and 12 months.

The median number of injections given in responders was 7 (IQR 6 - 10).

3.1.2.3 Additional data of non-responders

Eighteen out of 29 (62.1%) patients were classified as non-responders. Genetic variants were identified and confirmed in 10 of 18 (55.6%) non-responders, which included: *NRL* (1 patient), *RHO* (1 patient), *PRPF31* (1 patient), *PRPF8* (1 patient), *SNRNP200* (1 patient), *USH2A* (2 patients), *TULP1* (1 patient), *RP1* (1 patient) and *IFT140* (1 patient). Six of 18 (33.3%) non-responders were graded as having disruption of the ellipsoid zone within 1mm of the fovea on their baseline OCT scan.

2.2.3 Safety

Ocular and non-ocular AEs and SAEs are summarised in tables 12 - 14.

Table 12. Ocular and Non-Ocular Adverse Events (AEs) and Serious Adverse Events (SAEs) – 0-6 months after baseline

ID	Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious
1	Floater in RE	11/08/2016	05/09/2016	Mild	Probably	None	Resolved	Yes	No
2	Sub-conjunctival haemorrhage	04/04/2016	05/04/2016	Mild	Not Related	None	Resolved	Yes	No
2	Blurring of vision	25/08/2016	06/09/2016	Mild	Possibly	None	Resolved	No	No
3	Contacted by pt to say similar blurring to 1st injection	07/06/2016	15/06/2016	Mild	Definitely	None	Resolved	Yes	No
3	Corneal epithelial defect post injection	11/04/2016	12/04/2016	Mild	Definitely	None	Resolved	Yes	No
3	Back pain after bending down	10/10/2016	14/10/2016	Mild	Not Related	None	Resolved	No	No

ID	Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious
4	Dry cornea	12/08/2016	14/08/2016	Mild	Definitely	None	Resolved	Yes	No
4	LUL lesion (chalazion)	19/10/2016	On-going	Mild	Not Related	None	AE ongoing	No	No
4	Grittiness / Dry eye	09/07/2016	16/07/2016	Mild	Probably	None	Resolved	Yes	No
5	Headache post - IVT	26/07/2016	26/07/2016	Mild	Probably	None	Resolved	Yes	No
5	Feeling lethargic	25/09/2016	30/11/2016	Mild	Unlikely	None	AE ongoing	No	No
5	Raised IOP post - IVT	03/05/2016	03/05/2016	Mild	Definitely	None	Resolved	Yes	No
5	Sub-conjunctival haemorrhage (5 days post IVT)	05/06/2016	15/06/2016	Mild	Definitely	None	Resolved	Yes	No
5	Tinnitus	10/07/2016	On-going	Mild	Not Related	None	AE ongoing	No	No
8	Dry ocular surface + pain	10/11/2016	16/11/2016	Moderate	Definitely	Discontinued permanently	Resolved	Yes	No
9	Headache	24/06/2016	26/06/2016	Mild	Not Related	None	Resolved	Yes	No
10	Sub-conjunctival haemorrhage	16/05/2016	19/05/2016	Mild	Definitely	None	Resolved	Yes	No
10	Sub-conjunctival haemorrhage	22/08/2016	26/08/2016	Mild	Definitely	None	Resolved	Yes	No
13	Bad back	15/08/2016	19/08/2016	Mild	Unlikely	None	Resolved	No	No
14	Viral cold with headache	18/10/2016	27/10/2016	Mild	Not Related	None	Resolved	No	No
14	Corneal abrasion	24/06/2016	25/06/2016	Mild	Definitely	None	Resolved	Yes	No
15	Grittiness/Blurring using laptop	01/10/2016	18/10/2016	Mild	Unlikely	None	Resolved	No	No
16	Conversion of prostate biopsy from benign to low-grade neoplasia	20/09/2016	On-going	Mild	Unlikely	None	AE ongoing	No	No
16	Vision is not as sharp	04/07/2016	06/12/2016	Mild	Possibly	None	AE ongoing	No	No

ID	Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious
17	Bad back	14/11/2016	01/01/2017	Mild	Not Related	None	Resolved	Yes	No
17	Yag capsulotomy (on non-study eye LE)	28/10/2016	28/10/2016	Mild	Not Related	None	Resolved	Yes	No
17	Feeling Low	01/12/2016	01/05/2017	Mild	Not Related	None	Resolved	Yes	No
18	Viral Cold	22/09/2016	30/09/2016	Mild	Not Related	None	Resolved	No	No
19	Grittiness both eyes	12/09/2016	13/09/2016	Mild	Unlikely	None	Resolved	Yes	No
20	Labyrinthitis	02/08/2016	01/04/2017	Mild	Unlikely	None	Resolved	No	No
21	Sub-conjunctival haemorrhage	01/07/2016	03/07/2016	Mild	Definitely	None	Resolved	Yes	No
21	Soreness of eye	05/10/2016	07/10/2016	Mild	Definitely	None	Resolved	Yes	No
22	UTI	24/10/2016	28/10/2016	Mild	Not Related	None	Resolved	No	No
22	Dry corneal surface	02/11/2016	03/11/2016	Mild	Definitely	None	Resolved	Yes	No
22	Viral Illness	28/12/2016	22/01/2017	Mild	Not Related	None	Resolved	Yes	No
22	Sub-conjunctival haemorrhage	04/07/2016	09/07/2016	Mild	Definitely	None	Resolved	Yes	No
22	Dry corneal surface	04/07/2016	05/07/2016	Mild	Definitely	None	Resolved	Yes	No
22	Dry Eye	05/10/2016	30/10/2016	Mild	Definitely	None	Resolved	Yes	No
23	Bitten by mosquito	10/08/2016	17/08/2016	Mild	Not Related	None	Resolved	No	No
24	Flare up of mental health issues	15/09/2016	On-going	Moderate	Unlikely	Discontinued permanently	AE ongoing	Yes	No
25	Heartburn	31/08/2016	25/09/2016	Mild	Unlikely	None	Resolved	No	No
25	Sub-conjunctival haemorrhage	06/12/2016	24/12/2016	Mild	Definitely	None	Resolved	Yes	No
26	Viral cold (nasal congestion)	19/11/2016	24/12/2016	Mild	Not Related	Delayed Dose	Resolved	Yes	No
27	Exacerbation of mental health illness	04/11/2016	17/11/2016	Mild	Unlikely	None	Resolved	No	No
27	Viral Illness	02/11/2016	09/11/2016	Mild	Not Related	None	Resolved	Yes	No

ID	Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious
27	Punched in non-study eye (LE) with bruise under eye	27/08/2016	31/08/2016	Mild	Not Related	None	Resolved	No	No
28	Sub-conjunctival haemorrhage	05/10/2016	13/10/2016	Mild	Definitely	None	Resolved	Yes	No

Table 13. Ocular and Non-Ocular Adverse Events (AEs) and Serious Adverse Events (SAEs) – 6- 12 months after baseline

ID	Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious
4	Perforated Ear Drum	26/02/2017	03/03/2017	Mild	Not Related	None	Resolved	No	No
4	Ear Infection	26/02/2017	03/03/2017	Mild	Not Related	None	Resolved	No	No
4	Ear Infection	03/04/2017	09/04/2017	Mild	Not Related	None	Resolved	No	No
4	Viral cold	03/12/2016	05/12/2016	Mild	Not Related	None	Resolved	No	No
4	Vision not as clear	15/11/2016	06/12/2016	Mild	Definitely	None	Resolved	Yes	No
11	Sub-conjunctival haemorrhage	14/12/2016	17/12/2016	Mild	Definitely	None	Resolved	Yes	No
15	Low Mood	20/05/2017	On-going	Mild	Not Related	None	AE ongoing	No	No
15	Viral cold	13/12/2016	18/12/2016	Mild	Not Related	None	Resolved	Yes	No
16	Floater Right Eye	20/04/2017	27/04/2017	Mild	Definitely	None	Resolved	Yes	No
16	Grittiness after injection	06/02/2017	12/02/2017	Mild	Definitely	None	Resolved	Yes	No
16	Dry ocular surface	19/04/2017	20/04/2017	Mild	Definitely	None	Resolved	Yes	No

17	Viral Gastric Bug	09/04/2017	11/04/2017	Mild	Not Related	None	Resolved	No	No
17	Reduced central vision due to progression of underlying disease	12/05/2017	On-going	Moderate	Unlikely	Discontinued permanently	AE ongoing	Yes	No
21	Posterior vitreous detachment	17/03/2017	On-going	Mild	Possibly	None	AE ongoing	Yes	No
21	Anxiety	03/03/2017	On-going	Mild	Not Related	None	AE ongoing	No	No
21	Corneal abrasion + dry cornea	13/01/2017	16/01/2017	Mild	Definitely	None	AE ongoing	Yes	No
27	Punched in the face just below RE (no sequelae)	20/03/2017	20/03/2017	Mild	Not Related	None	Resolved	No	No
27	Viral illness	21/01/2017	31/01/2017	Mild	Not Related	None	Resolved	Yes	No
28	Posterior vitreous detachment	16/02/2017	On-going	Mild	Possibly	None	AE ongoing	Yes	No
28	Vitreous floater	05/06/2017	On-going	Mild	Definitely	None	AE ongoing	Yes	No
30	Feeling low	17/02/2017	28/02/2017	Mild	Not Related	None	Resolved	Yes	No
30	Right eye posterior sub-capsular cataract (non-study eye)	17/02/2017	On-going	Mild	Not Related	None	AE ongoing	Yes	No

Table 14. Ocular and Non-Ocular Adverse Events (AEs) and Serious Adverse Events (SAEs) – More than 12 months after baseline

ID	Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious
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11	Feeling tired from fasting	05/06/2017	06/06/2017	Mild	Not Related	None	AE ongoing	No	No
15	Vitreous Floaters	16/06/2017	On-going	Mild	Possibly	None	AE ongoing	Yes	No
17	Reduced central vision	25/10/2017	On-going	Mild	Possibly	Discontinued permanently	Resolved	No	Yes
27	Exacerbation of mental health illness	17/11/2017	23/11/2017	Mild	Unlikely	None	Resolved	No	No
30	Viral illness	05/12/2017	12/12/2017	Mild	Not Related	Delayed Dose	Resolved	Yes	No

3.1.3.1 Ocular adverse events

Ocular AEs included: floaters, sub-conjunctival haemorrhage, blurring/reduced/not as sharp vision, epithelial dystrophy/abrasion immediately following injection, grittiness, dry eye, chalazion, raised IOP immediately following injection, posterior vitreous detachment (PVD), yttrium aluminium garnet (YAG) capsulotomy on the non-study eye, cataract in the non-study eye, history of being punched in the face not involving the eye and history of being punched in the non-study eye. There were no cases of endophthalmitis or RD reported during this study.

3.1.3.2 Ocular serious adverse events

One participant reported sub-acute reduction of vision at week 32 despite being a 'responder' with testing demonstrating a reduction in vision of 14 ETDRS letters. This was reported as a SAE and injections were immediately discontinued. Further assessments were undertaken including SDOCT, FAF, microperimetry and OCT-A in order to help determine the cause for this reduction in vision. There was no demonstrable change in outer retinal lamination compared to baseline, with also no change in

microperimetry or FAF compared to baseline, and no abnormality detected on OCT-A. The non-study eye had a baseline vision of 30 ETDRS letters due to advanced photoreceptor loss and it was therefore concluded that the reduction in vision was most likely to be secondary to progression of underlying RP rather than as a consequence of receiving aflibercept injections. This patient was happy to remain within the study and attended their 6- and 12- month follow-up appointments.

3.1.3.3 Non-ocular / systemic adverse events

All non-ocular/systemic AEs were reported during the study whether or not they were considered to be secondary to aflibercept. Non-ocular/systemic AEs included: back pain, headache, lethargy, tinnitus, viral cold, conversion of prostate biopsy from benign to low-grade neoplasia, feeling low/low mood, labyrinthitis, urinary tract infection, flare up of mental health, heartburn, perforated ear drum, ear infection, viral gastric illness, anxiety and mosquito bite.

Whilst the participant who developed labyrinthitis during the study was reassured that it was unlikely to be secondary to aflibercept, they decided that they would prefer to discontinue receiving injections. This patient remained in the study and attended their 6- and 12- month follow-up appointments.

3.0 The CAMEL study

3.1 Methods

3.1.1 Overview

The CAMEL study stands for 'Carbonic Anhydrase-inhibitors for Retinitis-pigmentosa And Macula oEdema in various Layers'. This was a retrospective cohort study to determine if there was an association between the spatial distribution of CS in RP-CMO and response to CAIs.

This study was institutional review board (IRB) approved.

3.1.2 Inclusion criteria

Patients were included in the study if they met the following inclusion criteria:

- 1) Confirmed diagnosis of RP-CMO
- 2) Uni- or bilateral (if bilateral, each eye was evaluated individually)
- 3) Commenced on treatment with either a topical and/or oral CAI within the period 1st January 2013 – 31st December 2014. Please note that this start date was selected because a publication by Liew et al. (2015) included patient data from the same institution between January and December 2012 and we did not wish for data to overlap.
- 4) Pre-treatment OCT scan acquired within 3 months of initiating treatment AND post-treatment OCT scan acquired between 3 – 9 months after initiation of treatment
- 5) Any age
- 6) Heyex machine images only

3.1.3 Exclusion criteria

Patients were excluded if any of the following applied:

- 1) A diagnosis of CMO not considered to be related to RP
- 2) Treatment for RP-CMO received within 3 months of initiation of CAI e.g. IVTA/anti-VEGF
- 3) Pre-treatment OCT scan not acquired within 3 months of initiating treatment
- 4) Post-treatment OCT scan acquired greater than 9 months from initiation of treatment
- 5) Images taken on a Topcon machine

3.1.4 ROAD application form

A form entitled 'Research On Anonymised Data' (ROAD) form was completed and submitted to the R&D department at Moorfields Eye Hospital before this retrospective study was carried out. Only once permissions were received was the study undertaken.

3.1.5 Patients and methods

3.1.5.1 Identification of patients

This retrospective cohort study carried out at Moorfields Eye Hospital NHS Foundation Trust, London, UK between 1st January 2013 – 31st December 2014 made use of a computer-based search to identify all patients with 'retinitis pigmentosa' and 'cystoid macular oedema' appearing in their EPR. This time period was chosen as it was deemed a manageable period of time and number of scans to analyse. This initial search identified 103 patients, however, after review of each patient record, 78 patients were excluded from the study due to having 'no' CMO.

After accounting for the inclusion and exclusion criteria, the total number of patients included in the study totalled 25. Of these, 18 had bilateral RP-CMO and 7 had unilateral RP-CMO; 43 eyes were therefore graded in total.

2.2.5.2 Images and consent

SDOCT was undertaken in all recruited subjects as part of a patient's standard care at their medical retina clinic appointment. All patients across the cohort underwent scan acquisition according to the standard of care procedure used for out-patient clinics at Moorfields Eye Hospital. All images were anonymous therefore additional consent from individual patients was not required.

2.2.5.3 Grading system

Two independent graders experienced in SDOCT interpretation were selected to grade pre-and post-treatment scans (SAS, NH). Both graders were blinded to the treatment that each patient received and whether they were classed as a 'responder' or not.

Each grader began by performing re-centration of the images if deemed necessary in order to optimise results for the study. The following variables were graded for their presence within 3600µm of the foveal centre: sub-retinal fluid (SRF), INL fluid, ONL fluid, GCL fluid, ERM, vitreo-macular adhesion (VMA), VMT, lamellar macular hole (LMH) and full-thickness macular hole (FTMH). Each of these variables was graded as either: present (>90% certainty), questionably present (50-90% certainty), absent (<50% certainty) or ungradable. The presence of ELM within 1200µm of the foveal centre was

also graded and a comment made as to whether it was felt to be intact throughout or disrupted. A further 'yes' or 'no' response was required for the grading of whether there was felt to be intact ELM and/or fluid present (in any lamination) directly under the foveal centre. Pre- and post- treatment CMT and macular volume were also documented.

If both graders agreed on a variable, the grading was complete. If the second grader (NH) disagreed with the first grader (SAS), adjudication was performed by a consultant retinal specialist (MM).

CMT values were considered to be in agreement if graded within 50µm of each other. Macular volume values were considered to be in agreement if graded within 1.5 millimetres (mm)³ of each other. In total, graders 1 and 2 correlated on 1146 out of 1290 (88.8%) points thus requiring adjudication of 144 out of 1290 (11.2%) points. The mean of the grader scores for CMT was used for analysis.

2.2.5.4 Statistical analysis

Descriptive statistics are used to describe the results of the CARMEL study. For categorical variables, Kappa statistic was computed with respective 95% confidence interval (CI) for assessing inter-rater agreement as Kappa is thought to be a more robust measure than simple percent agreement (Kappa takes into account the possibility of agreement occurring by chance). For continuous variables Bland-Altman agreement methods were used to quantify limits of agreement (LoA). Analysis was performed in STATA version 13 (STATA Corp., Texas, USA) and 95% CIs for Kappa were computed using the kapci package using 1000 bootstrap replicates.

No natural history studies have taken place to assess change of CMT over time in patients with RP-CMO who are not receiving treatment. Artunay et al. (2009) demonstrated no statistical change of CMT

throughout the duration of their 6-month study in their control group (n = 15), however, these patients had still previously received acetazolamide for at least 6-months. Whilst diurnal variation in CMT measurements has been evaluated for conditions such as DMO, there is nothing in the literature with regards to RP-CMO. Patients were therefore considered to be a 'responder' if they demonstrated a reduction of CMT of at least 11% or more following treatment, thus allowing comparison with previous studies that used the same definition (138, 254). Please note that for the purposes of this study, all values over 10.5% were rounded up to 11%.

3.1.6 Outcome measures

3.1.6.1 Primary outcome measure

To report mean CMT as measured with SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

3.1.6.2 Secondary outcome measures

To report the presence of SRF within 3600µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

To report the presence of INL fluid within 3600µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

To report the presence of ONL fluid within 3600µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

To report the presence of GCL fluid within 3600µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

To report the presence of ERM within 3600µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

To report the presence of VMA within 3600µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

To report the presence of VMT within 3600µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

To report the presence of LMH or FTMH within 3600µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

To report the presence of ELM within 1200µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

To report the integrity of ELM within 1200µm of the foveal centre using SDOCT in eyes of patients with RP-CMO acquired between 3 – 9 months after initiation of oral or topical CAI treatment.

3.2 Results

Forty three eyes (22 right; 21 left); were included in the study consisting of 18 patients with bilateral RP-CMO and 7 patients with unilateral RP-CMO. Seventeen of these patients were male and 8 were female. Median age was 48 and ranged between 17 and 79 years. Four out of 43 (9.3%) eyes were treated with oral acetazolamide 250mg twice a day versus 39 out of 43 (90.7%) of eyes treated with topical 2% dorzolamide or brinzolamide three times a day. All 43 eyes in the study were graded as having INL fluid present on their pre-treatment OCT scan. Thirty three out of 43 eyes (76.7%) in the study demonstrated co-existing ONL fluid present on their pre-treatment OCT scan. Eleven out of 43 eyes (25.6%) in the study demonstrated co-existing GCL fluid present on their pre-treatment OCT scan. No patients demonstrated presence of SRF.

Estimates of agreement for all variables assessed by the two graders are presented in figures 38 and 39. No single variable was found to have poor agreement. No evidence of bias was found in terms of inter-rater agreement for pre-treatment CMT (see figure 35), mean difference -0.74. 95% CI (-3.12, 1.63). Inter-rater LoA were -16.18 to 14.69 for pre-treatment CMT and -18.36 to 21.76 for post-treatment CMT, which was considered by the PI to be acceptable. Inter-rater agreement was -0.91 to 1.14 for pre-macular volume and -1.22 to 1.24 for post-macular volume, which was considered by the PI to be acceptable with no evidence of bias. Figure 36 demonstrates pre-CMT and post-CMT measurements made by the two graders using a box plot.

Figure 35: Bland-Altman graph illustrative of pre-CMT inter-rater agreement. CMT = central macular thickness

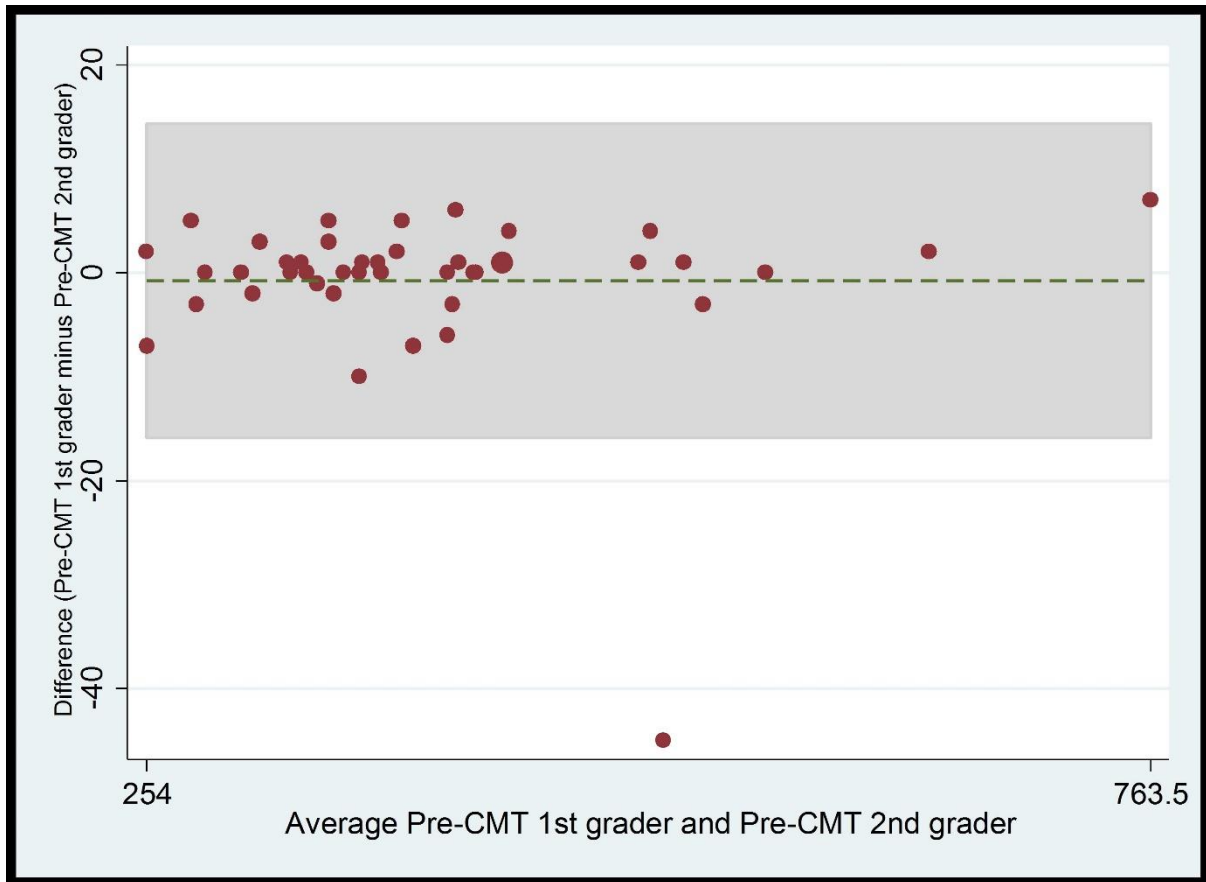
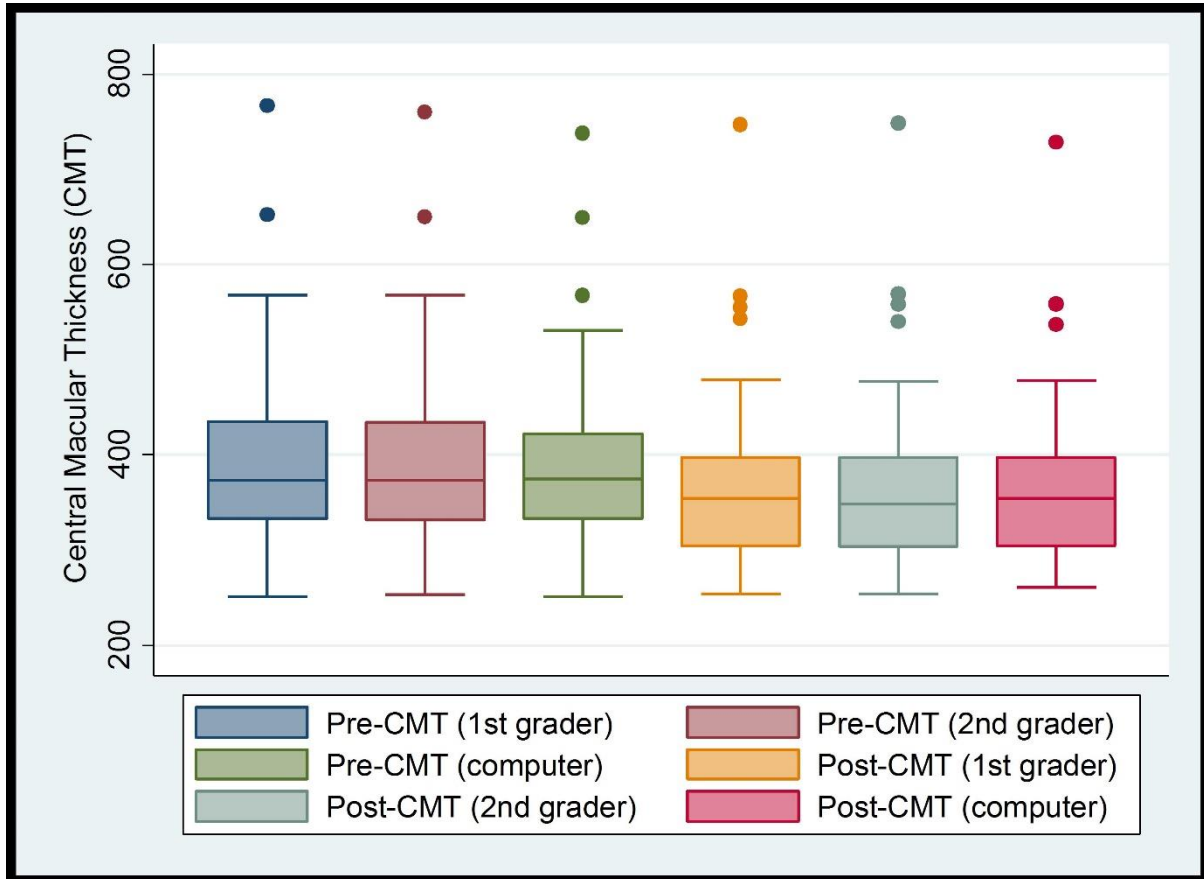


Figure 36: Box plot illustrative of pre-CMT and post-CMT measurements made by the two graders. CMT = central macular thickness.



Out of 43 eyes that were graded, 13 (30.2%) were classed as ‘responders’ having achieved a CMT reduction following treatment of at least 11%. All 13 responders demonstrated ONL fluid on their pre-treatment OCT and the presence of fluid (in any layer) directly under the fovea. ERM was ‘definitely present’ in 8 out of 13 (61.5%) responders and ‘questionably present’ in 2 out of 13 (15.4%) responders. No responder demonstrated VMA, VMT or a FTMH on their pre-treatment OCT scan. ELM was considered to be present (intact or disrupted) within 1200µm of the fovea and present directly under the fovea in all but 1 responder (92.3%). BCVA improved by at least 10 ETDRS letters in 2 out of 13

(15.4%) responders. No responders demonstrated a loss of 10 or more ETDRS letters. The remaining 11 out of 13 (84.6%) responders demonstrated no change in their BCVA following treatment. Interestingly, 4 out of 13 (30.8%) responders demonstrated total clearance of ONL fluid on their post-treatment OCT scan whilst INL cysts remained

Out of 30 non-responders, 20 (66.7%) eyes had ONL fluid on their pre-treatment OCT compared to 10 (33.3%) eyes without ONL fluid on their pre-treatment OCT. Sixteen out of 30 (53.3%) non-responder eyes had fluid (in any layer) directly under the fovea. Interestingly, there were three non-responders that demonstrated total clearance of ONL fluid on their post-treatment OCT scan whilst INL cysts remained (see Figure 3). VMA was definitely present in 7 out of 30 (23.3%) non-responders and questionably present in 2 out of 30 (6.7%) non-responders. ELM was considered to be present (intact or disrupted) within 1200µm of the fovea in 26 out of 30 (86.7%) non-responder eyes, however, was only present directly under the fovea in 20 out of 30 (67.0%) eyes. BCVA improved by at least 10 ETDRS letters in 2 out of 30 (6.7%) non-responders. No non-responders demonstrated a loss of 10 or more ETDRS letters. The remaining 28 out of 30 (93.3%) non-responders demonstrated no change in their BCVA following treatment.

Of note, all 4 patients (2 responders and 2 non-responders) who gained at least 10 ETDRS letters of BCVA, demonstrated ONL fluid on their pre-treatment OCT scans.

4.0 Discussion

IRD is the leading cause of blindness certification in the working age population (age 16-64 years) in England and Wales, occurring from abnormalities of retinal cell structure including photoreceptors as well as defects in phototransduction and the visual cycle (10). RP is the most common IRD to date with RP-CMO being a known complication of RP. However, the exact underlying pathogenesis of RP-CMO remains uncertain and thus challenging to treat.

A variety of treatments, including: CAI's, steroids (topical, oral, intravitreal, sub-tenon), NSAIDs, lutein, laser and vitrectomy have been attempted with varying success to address various hypotheses such as breakdown of the BRB, failure of the RPE pump, Muller cell oedema and dysfunction, anti-retinal antibodies and vitreous traction. The majority of studies published provide levels of evidence between 3 and 4; no large RCTs have been undertaken, thus the effect of known and unknown confounders cannot be excluded (1), many studies did not have a control group (level 4 evidence) inherently limiting the validity of findings (as these may be a result of natural history rather than the intervention) (1) and many studies were retrospective, which may thus be affected by recall bias. We therefore remain in a position where there are currently no studies able to provide high-level evidence for the treatment of RP-CMO.

The release of toxic products (including VEGF) from degenerating retina/RPE in patients with RP contributes to weakening of the BRB and RP-CMO formation (101). Anti-VEGF is thought to act by reversing proliferation and cell migration stimulated by VEGF and the delocalization of tight junction proteins induced by VEGF 165 (261). It therefore has the potential to treat RP-CMO, however, the evidence for its usage in this condition is limited.

In view of the above, two studies were carried out and reported in this thesis in order to achieve a better understanding of the following:

- 1) To explore whether an association exists between the spatial distribution of CS in RP-CMO and response to treatment with CAIs (the CAMEL study)

- 2) To explore the efficacy and safety of aflibercept as a treatment for RP-CMO (the AMOUR study)

The CAMEL Study

In keeping with Makiyama et al. (2014), the CAMEL study observed an overall higher frequency of INL compared to ONL fluid in patients with RP-CMO (110). This suggests that inner BRB dysfunction may have a greater role than the outer BRB in the development of RP-CMO (2).

It was interesting to note that 100% of responders demonstrated ONL fluid on their pre-treatment OCT, however, not every patient with pre-treatment ONL fluid responded. The presence of ERM graded as either 'questionably present' or 'definitely present' was similar in both groups (10 out of 13 (77.0%) responder eyes versus 24 out of 30 (80.0%) non-responder eyes) and therefore does not appear to have significantly affected response to treatment in our cohort. In contrast, the presence of VMA graded as either 'questionably present' or 'definitely present' was greater in the non-responder group (0 out of 13 (0.0%) responder eyes versus 9 out of 30 (30.0%) non-responder eyes) and may therefore play a role in limiting response to treatment due to its tractional component.

Overall, there were seven patients (including 'responders' and 'non-responders') that demonstrated total clearance of ONL fluid on their post-treatment OCT scan despite persistence of INL cysts. Our working hypothesis to explain this response includes the closer anatomical proximity of ONL fluid to the RPE where the action of CAIs take place. A greater percentage of eyes in the responder group demonstrated at least 10 ETDRS letter improvement of BCVA compared to the non-responder group (15.4% of responders versus 6.7% of non-responders). It is not uncommon for anatomical improvement to occur without significant functional improvement (125). Factors such as underlying photoreceptor loss and/or chronicity of RP-CMO may have an effect on visual outcome (1, 125). Whilst it was noted that patients with visual gain demonstrated ONL fluid on their pre-treatment OCT scans, a larger study with appropriate statistical analysis is required to be able to qualify whether this observation is significant.

Although there was only a limited vision-improving effect observed in this study, we still believe in treating RP-CMO to achieve anatomical improvement to prevent irreversible structural damage as well as to potentially decelerate underlying photoreceptor loss (1).

The CARMEL study was limited due to a small cohort size. Further studies with larger patient numbers are required in order to support our findings of OCT phenotypes that may help predict treatment response. This would help better inform patient counselling and management in this highly genetically heterogenous retinal dystrophy.

CAIs are also associated with side-effects such as: tingling/numbness of the limbs, fatigue, renal stones, aplastic anaemia, hypokalaemia and cardiac arrhythmia (148). To this end, it would be valuable to identify factors that might help predict response of RP-CMO to CAI treatment in order to tailor patient

care appropriately. Whilst the CAMEL study identified the presence of ONL fluid on pre-treatment OCT scan to possibly be a positive prognostic factor in the treatment of RP-CMO, we are limited by design and numbers to be able to provide accurate statistical analysis and it must therefore be interpreted as observation only.

Unfortunately, permission to obtain information regarding genetic data was not requested when setting up the CAMEL study and therefore could not be included. This would have been an interesting area to explore.

The AMOUR Study

The AMOUR study is the first prospective study to obtain safety and efficacy data on the use of serial intravitreal injections with aflibercept for the treatment of RP-CMO, employing a monthly loading phase of 3 injections followed by a treat-and-extend protocol for a total of 12 months of follow-up. No statistically or clinically significant improvement of vision was demonstrated in this cohort as a whole, or in sub-group analysis of responders. Responders gained 3.8 (SD 6.8) and 4.7 (SD 9.5) ETDRS letters respectively at 6 and 12 months.

In keeping with Moustafa and Moschos (2015), the AMOUR study observed a reduction of CMT +/- improvement of VA following intravitreal aflibercept within its cohort (4). When the cohort was analysed as a whole, the mean (SD) percentage change in CMT relative to baseline was -8.1% (23.3%) and -9.6% (17.6%) at 6- and 12- months respectively. In total, 11 out of 29 (37.9%) patients were classified as responders at both 6- and 12- months having demonstrated a reduction of at least 11% CMT on SDOCT compared to baseline. These patients experienced a mean percentage change in CMT relative to baseline of -22.9% (29.7 %) and -28.1% (12.9 %) at 6- and 12- months respectively. Responders also

demonstrated a greater change of macular volume over the study (-0.6mm^3 at 6 and 12 months) compared to non-responders (-0.3mm^3 at 6 and 12 months). An intriguing observation, unlike other disorders where anti-VEGF agents have been employed, is that all responders ($n=11$) achieved a notable reduction in CMO after their first injection ('early-responder'). There were no 'late-responders'. This is clinically very valuable as for the majority of patients it may be possible to decide at a very early stage whether injections should be pursued.

There were no significant safety concerns and serial injections were well tolerated.

Responders in this study were identified across all categories of inheritance pattern (AD, AR and XL). There was no association between response to anti-VEGF treatment and mode of inheritance. Whilst just over half of the patients in this study had a confirmed molecular diagnosis, no genotype was associated with response to treatment; including 1 *USH2A* patient responded, whilst 2 other *USH2A* patients did not, and 1 *PRPF31* patient responded - whilst 2 others did not. This study included only 1 patient with XL inheritance who was deemed a responder and we therefore cannot draw any comparison with other patients with XL-RP. More advanced disease, defined as disruption of the ellipsoid zone within 1mm of the fovea (seen in 27.3% of responders and 33.3% of non-responders) did not appear to affect likelihood of response to anti-VEGF.

The majority of patients (26 out of 30) in the study reported their ethnicity as 'White'. Whilst we cannot confirm the reason for this, it should be noted that no patient declined to be in the study based on religious or ethnic grounds. Since almost all patients in the study came from the medical retina clinics at Moorfields Eye Hospital, it would be interesting to perform an electronic search to analyse the ethnic

backgrounds of the patients in attendance at these clinics to see if this mirrors the patient ratios in our study. Unfortunately, the ethnicity of the cohort of patients included in the paper published by Liew et al. (2015) and Liew et al. (2018) was not reported. This would have been interesting to compare with as both studies were carried out at the same institution at a similar time (132, 262).

Mean retinal sensitivity was reduced by -0.92dB (SD 2.03) and -0.97dB (SD 1.92) at 6- and 12- months respectively compared to baseline despite an overall improvement of CMT and BCVA.

Whilst this slight reduction may be clinically significant, it should be noted that test-retest variability was not determined at baseline and it is therefore possible that the results remain within limits of normal variation.

Strengths of this study included excellent patient attendance throughout its duration, with a 96.7% participant retention rate at 12-months. The study drug was well tolerated and no cases of endophthalmitis occurred. The study design including an initial loading phase followed by a treat-and-extend regime, which allowed for the observation of both early and (potentially) late responders. Likely disease-causing sequence variants were also established in 16 of 30 (53.3%) study participants.

One of the limitations to our study was being unable to include treatment-naive patients with shorter duration of CME. Many patients were deemed eligible for the study yet declined intravitreal injections without first trialling topical and/or oral treatment. All patients in the study had therefore used topical CAI medication previously; 15 of whom were using topical CAI treatment up until 1 month prior to their screening appointment. Five of these patients were deemed responders. Five patients in the study were using oral CAI treatment up until 3 months prior to their screening appointment; 1 patient withdrew from the study, 2 patients were deemed responders, and 2 patients did not respond. No obvious trend

was demonstrated to suggest whether recent use of topical or oral CAIs influences response to anti-VEGF therapy.

Long-standing CMO duration was observed in many patients within this cohort, with the median duration being 252 weeks (IQR, 156 - 296 weeks). An interesting observation identified from this study was that duration of CMO did not appear to affect anatomical response to anti-VEGF; median CMO duration in responders was 264 weeks (IQR 228, 416), compared to the group overall (252 weeks (IQR 156, 296). Indeed, the patient with the longest standing CMO duration of the cohort (20 years) had complete resolution of CMO after a single intravitreal injection of aflibercept.

As long as patients achieved a BCVA better than 20/400 at baseline, they were considered eligible for the study. Our study therefore included patients with fairly advanced underlying disease as demonstrated by photoreceptor loss and outer retinal thinning - features that have been shown to hinder VA improvement despite reduction of CMT (127). It was therefore unsurprising to find that all 3 of 11 (27.3%) responders graded as having disruption of the ellipsoid zone within 1mm of the fovea on their baseline OCT scan demonstrated no improvement of vision. Greater improvement of VA may be demonstrated in patients with a relatively more intact photoreceptor layer at baseline.

It would be valuable to repeat this study in a larger cohort of patients, ideally naive to other treatment modalities, with shorter history of CMO duration and relatively intact photoreceptor layers at baseline. Only including patients with a molecularly confirmed genetic diagnosis would help to determine if genotype has a role in predicting response to anti-VEGF treatment. Additional suggestions to consider when designing a future study would include: to perform fundus fluorescein angiogram (FFA) at baseline to see whether active leakage is present and whether FFA is predictive of which patients will/will not

respond to aflibercept, to take baseline samples of vitreous in order to assess levels of VEGF in this cohort of patients, to include a control group (possibly using placebo), to randomise patients if there is an option of a control/placebo group, to blind patients and/or clinicians if there is an option of a control/placebo group, and to include OCT-A as an additional imaging modality.

This phase II exploratory study demonstrates that intravitreal aflibercept can be effective at reducing CMT in select patients with RP-CMO. The factors predicting who is likely to respond, however, remain to be clarified. Our data supports more studies to further investigate the role of VEGF blockade in RP-CMO.

5.0 Concluding remarks

To conclude, the successful management of RP-CMO should aim to both improve both quality and quantity of vision in the short-term as well as slowing the rate of vision loss long-term. It appears there are 2 approaches to tackle RP-CMO: the first involves identifying a therapeutic agent that is able to pinpoint and address the exact underlying mechanism(s); the second involves treating the underlying condition of retinal degeneration thus preventing any related complications from arising.

Unfortunately, with varying ideas regarding the mechanism of RP-CMO formation and a lack of high quality evidence for RP-CMO treatments, we are not yet in a position to treat RP-CMO with absolute clarity. We are, however, in an exciting era of opportunity to potentially address one, or both of these options in the future given enough time, funding and research.

An extensive literature review has been provided in this thesis regarding avenues of intervention for RP-CMO, which can be summarised as follows:

- CAIs are often used for the treatment of RP-CMO despite no RCTs (comparing CAI with placebo) being published providing evidence for their safety and efficacy. Before treating a patient with CAI, consideration should be given to the possible side-effects associated with its usage. These can be minimised by using CAIs topically, as opposed to orally, which may be as effective (34). Cases of rebound CMO have been documented with prolonged use (140).
- Topical use of steroid and/or NSAID can be effective at reducing CMO +/- improving vision in patients where CAIs are contraindicated. The effects of systemic absorption of these

medications need to be considered, however, and a thorough medical history should be taken before their usage. IOP can also rise therefore close monitoring of IOP during the course of steroid administration would be necessary (172). IVTA may be useful for selected cases of RP-CMO (166).

- Whilst no studies have assessed vitreous levels of VEGF in patients with RP or RP-CMO, anatomical and/or functional improvement of RP-CMO has been observed following intravitreal anti-VEGF medication (3, 4, 186-189). The high binding affinity and long duration of action of aflibercept may reduce the frequency of repeat injections (195).

The CAMEL study has highlighted the dilemma regarding the use of CAI treatment for RP-CMO; similar to Liew et al. (2015) where response to treatment with CAI was demonstrated in only 50 out of 125 (40.0%) eyes treated with topical dorzolamide and 9 out of 32 (28.1%) eyes treated with oral acetazolamide, the CAMEL study demonstrated response in only 13 out of 43 (30.2%) eyes. It has also drawn our attention to the trends shown towards: better improvement where pre-treatment OCT scans include CS in the ONL, greater chance of BCVA improvement in patients who respond and that the presence of ERM does not appear to significantly affect response to treatment.

The AMOUR study found improvement of CMT +/- VA using descriptive statistics only in certain patients with RP-CMO, although we have been unable to ascertain factors that might predict treatment success. Where CMO and CMT was seen to improve, subsequent improvement of vision was not necessarily demonstrated. This is not an uncommon finding and previous studies have also demonstrated lack of significant improvement on multifocal ERG (4). This might be explained by other factors that influence VA such as: atrophy of the retina and/or RPE and scarring.

Whilst concrete conclusions cannot be drawn, the evidence available to us as provided in this thesis through a combination of literature review and consideration of the results from both the CARMEL and AMOUR studies, suggests:

- 1) Topical CAI may be used as a first-line approach with review of response after 4 - 6 months.
- 2) a) After 4 - 6 months, if there has been minimal (but not significant) reduction of CMT or improvement of BCVA, consider switching to an alternative topical CAI or oral CAI.
b) After 4 - 6 months, if there has been no reduction of CMT or improvement of BCVA or if the spatial distribution of cysts are located only in the INL, consideration should be given to trialling alternative therapeutic options, such as: topical NSAID agents or considering intravitreal anti-VEGF (although these latter agents are currently unlicensed for RP-CMO).

A better understanding of the mechanisms underlying RP-CMO will facilitate better targeted and likely more efficacious and durable therapies (1).

6.0 Future directions

6.1 Diagnosis of IRD

Preliminary diagnosis of IRD based on the history and clinical examination can often be tricky due to overlapping phenotypes and unclear inheritance patterns. Genetic testing is therefore extremely useful for diagnosis as well as to further our understanding of IRD genetic associations and direct appropriate treatments. Molecular diagnosis has also helped propel the fields of stem cell research and genome editing technology (10).

Until recently, patients were unable to receive a molecular diagnosis due to the high level of cost and various inefficiencies involved in molecular genetic testing (263). Since around 2010, the development of NGS has offered the possibility of genome-mapping in order to help diagnose diseases such as retinal dystrophies (10). However, around half of all people tested remain without a molecular diagnosis. There are also many challenges that come with advances in genomic medicine, including (264):

- Developing skills and expertise in genomics within the wider health professional workforce
- Issues relating to patient communication, privacy and consent (particularly for genomic testing in children)
- Handling uncertain, unexpected or incidental findings from genomic tests in clinical practice

- Implications of significant results for other family members
- Bioinformatics provision and secure genomic data storage and access within the health service
- Impact of genomics on current healthcare services, resources and patient pathways (including equity of access to genomic tests)
- Developing intelligent decision support systems that allow the use of genomic and clinical information to aid in the prescribing of drugs at the right dose
- Clarifying risks and benefits associated with using genomic tests for opportunistic screening

Our hope for the future is to improve sequencing performance and interpretation while providing quicker turnaround times and reduction in cost (263).

6.2 Management of IRD

Future management of IRD will focus on identifying therapeutic options to reverse, or slow down, the rate of underlying retinal degeneration. This will vary according to each patient's rate and extent of disease:

6.2.1 Management of earlier stages of disease

Neuroprotection, antioxidants and other pharmacological therapies are useful for earlier stages of disease, being actively investigated and showing huge potential in preventing cell death. Photoreceptors in particular will most likely require the inhibition of both apoptosis and necroptosis (26).

Gene therapy is also best placed for earlier stages of disease where the opportunity still remains to alleviate the underlying defect, regenerate damaged retinal cells and prevent further retinal deterioration (20). There are many gene therapy studies currently being undertaken in humans to address IRD. We will therefore focus on those studies on the horizon, specifically for RP:

- At present, there are no gene therapy trials being carried out in ADRP. This is due to the greater complexity of gene silencing approaches needed in ADRP compared to gene supplementation strategies in AR/XL-RP (230).
- We eagerly await the results of three AAV human trials in progress for RP caused by variants in the *RPGR* gene [NCT03116113, NCT03316560, and NCT03252847] (230).
- AR-RP caused by variants in the *PDE6 β* gene account for approximately 1–2% of all RP cases (230). Gene therapy using rAAV-mediated *PDE6 β* has been demonstrated in both mouse and dog models to restore dim-light vision, providing great promise for human treatment, with anticipated clinical trials (230).

- Variants in cyclic nucleotide-gated channel $\beta 1$ (CNGB1) cause approximately 4% of AR-RP (230). Again, gene therapy using an AAV vector has been demonstrated in both mouse and dog models to improve vision at very low light levels, with a clinical trial in development (230).

6.2.2 Management of more advanced stages of disease

More advanced stages of disease would benefit from artificial vision and stem cell therapy. The indefinite self-renewal ability and plasticity of stem cells allows for *in vitro* generation of an unlimited number of distinct cell types, and has opened new avenues for regenerative medicine (243). There are many stem cell studies currently being undertaken in humans to address IRD including:

- A first-in-human, phase I/IIa, open-label, prospective study investigating the safety and tolerability of sub-retinally transplanted hRPC in patients with RP is currently recruiting in the USA (ClinicalTrials.gov Identifier: NCT02464436). The primary outcome measure is safety and secondary outcome measure is efficacy via VA, VF sensitivity, retinal photography, FAF and SDOCT.

Retinal implants or prosthetics rely on a patient having intact neural pathways whereby the transmission of information to the visual centres of the brain can be enhanced (10). Compared to gene therapy, systems such as the Argus® II are less costly, with demonstrated favourable outcomes, albeit though artificial vision (265). Future improvements of retinal prostheses such as this will include better programming and increased electrode count within the device, however, there are technological, material and biological limitations to this (266).

Further investigation of these potential therapies is required in order to address the various stages of IRD. Information regarding efficacy, applicability, acceptability, cost and long-term effects of these treatments, however, will be required before implementation into routine clinical practice (20).

6.3 Management of RP-CMO

Given the potentially reversible nature of RP-CMO, there is a real need to better understand disease mechanisms and undertake prospective clinical trials of therapeutic agents to provide the evidence base to improve treatment of RP-CMO (1). Setting up clinical trials for RP-CMO, however, is a challenge due to its low prevalence, highly variable course of disease progression, significant genetic and allelic heterogeneity, and very slow progression to visual loss (1).

Whilst anti-VEGF has been demonstrated to reduce RP-CMO in select cases of RP (for reasons that are yet unknown), the potential complications associated with the use of anti-VEGF therapy must be considered. Whilst Salom et al. (2008) observed lower aqueous levels of VEGF in eyes of patients with RP versus controls, it would be interesting to note levels of VEGF in the vitreous and whether there are significant differences between patients with RP versus controls, as well as patients with RP versus those with RP-CMO. This being an invasive procedure, however, would likely prove challenging to gain ethical approval for and is why we did not consider undertaking in the AMOUR study.

Suggestions to consider when designing a future study, include: to perform FFA at baseline to see whether there is active leakage present and whether FFA is predictive of which patients will/will not respond to aflibercept, to take baseline samples of vitreous in order to assess levels of VEGF in this

cohort of patients, to increase the number of patients in the study to potentially offer results with statistical significance, to include patients who already have a molecular diagnosis (or where this is not possible, obtain DNA samples from patients at baseline to attempt to obtain a molecular diagnosis), to include a control group (possibly using placebo), to randomise patients if there is an option of a control/placebo group, to blind patients +/- clinicians if there is an option of a control/placebo group and to include OCT-A as an additional outcome measure.

The free radical scavenger Edaravone, shown to be effective against retinal degeneration both in vivo and in vitro, may also have a role in inhibiting vascular leakage thus reducing Muller cell oedema (267). Studies to date include only mice / rat models. Hopefully, with continued research, we will see the results of this drug in human subjects.

A better understanding of the underlying mechanisms and response to treatments of RP-CMO is required to facilitate better targeted and more efficacious therapies (1).

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8.0 Publications

Article submitted, but not yet published

Title:

A prospective exploratory study to assess the safety and efficacy of aflibercept in cystoid macular edema associated with Retinitis Pigmentosa

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Abstract:

Purpose: A study to report the safety and efficacy at 12-months of intravitreal aflibercept (Eylea) (ivA) for cystoid macular edema (CME) associated with retinitis pigmentosa (RP).

Design: Prospective, exploratory, phase II, non-randomized, single-center, open-label, 12-month, 1-arm clinical trial.

Participants: A total of 30 eyes of 30 patients with center-involving RP-CME.

Methods: Participants received intravitreal aflibercept (ivA) every four weeks for the first three months (loading phase), followed by a treat and extend protocol up to 12-months. Extension from monthly to 6, 8, 10 and 12-week follow-up occurred when there was no further reduction in macula edema compared with the previous visit.

Main outcome measures:

(i) To report the safety of aflibercept in RP-CME throughout the study (17 months in total); via the documentation of adverse events (AEs) deemed related to the trial drug; (ii) To report the efficacy of aflibercept in RP-CME via mean central macular thickness (CMT) on Spectral domain OCT (SDOCT) at 12-months after baseline.

Results: Twenty-nine out of 30 (96.7%) patients completed 12-months of follow-up. A total of 4 to 11 injections per patient were given over the 12-month study. No statistically significant reduction of CMT or VA improvement was demonstrated in the group overall. Eleven out of 29 (37.9%) participants were considered as 'responders' demonstrating at least an 11% reduction of CMT at 12-months on SDOCT compared with baseline. A reduction of CMT by mean (SD) 28.1% (12.9 %) was observed in responders at 12 months, however, no statistically significant corresponding improvement in BCVA was seen. Baseline characteristics, including duration of CME were similar between the responder and non-responder groups. No clinically significant adverse events were deemed secondary to ivA.

Conclusions: This first prospective exploratory study demonstrates both the safety and acceptability of serial ivA in patients with RP-CME, effective at reducing CMT in 37.9% of patients. All patients demonstrating anatomical response did so after their first injection. Longer duration of CME did not negatively affect response to anti-VEGF. Further study in a larger cohort of patients with shorter CME duration would be valuable to better establish the utility of VEGF blockade in RP-CME.

Inherited retinal disease is the second commonest cause of visual loss in childhood and the commonest cause of visual loss in the working age population¹. Retinitis pigmentosa-associated cystoid macular edema (RP-CME) is a known complication of retinitis pigmentosa (RP), reported to occur in between 10 - 50% of patients with RP at some stage in their lifetime²⁻⁵. One of the most commonly reported ocular symptoms of RP is concentric peripheral visual field loss that is relentless and progressive for which there is currently no cure. Complications of RP such as cataracts and RP-CME interfere with central vision and are thereby particularly debilitating, making effective treatments for RP-CME highly valuable.

Several mechanisms have been proposed to explain why CME develops in RP, however, no single aetiology has been definitively established, and it is plausible that RP-CME may result from a combination of these⁶: (i) breakdown of the blood-retinal barrier^{7,8}, (ii) failure (or dysfunction) of the retinal pigment epithelium (RPE) pump mechanism⁹, (iii) Müller cell edema and dysfunction¹⁰, (iv) anti-retinal antibodies¹¹, and (v) vitreous traction^{12,13}. With this in mind, many treatment approaches for RP-CME have been employed including: laser therapy, topical carbonic anhydrase inhibitors (CAIs), oral CAIs, peri-ocular and intravitreal steroids, and intravitreal anti-vascular endothelial growth factor (anti-VEGF) agents⁶. However, the vast majority of the published literature is retrospective and thereby inherently limited, often involving small numbers of participants and short duration of follow-up. The

presence of CME in RP has been associated with younger age but not with gender¹⁴. RP-CME is most prevalent in patients with autosomal dominant (AD) inheritance (71.4% with CME in at least one eye), followed by autosomal recessive (AR)/sporadic inheritance (58.9%) and XL inheritance (12.5%)¹⁴. Patients with epiretinal membrane (ERM) and cataract/pseudophakia are less likely to develop CME¹⁴.

Whilst the current mainstay of treatment for RP-CME is topical/oral CAIs, there is no level 1 evidence supporting their use and studies have demonstrated highly variable efficacy. Liew et al. recently carried out a 12-month retrospective review of 81 patients with RP-CME seen at Moorfields Eye Hospital (UK) on treatment with either topical dorzolamide (64 patients, 125 eyes) or oral acetazolamide (17 patients, 32 eyes)¹⁴. Forty percent of eyes (53.1% of patients) following treatment with topical dorzolamide and 28.1% of eyes (41.2% of patients) following treatment with oral acetazolamide demonstrated response (defined as a reduction of central macular thickness (CMT) on OCT of at least 11% between visits)¹⁵. A cross-sectional study performed on this same cohort of patients (n = 81) identified older age, earlier age of onset of symptoms, and thicker CMT to be associated with lower VA¹⁶. Gender and inheritance pattern were not found to be associated with VA¹⁶.

Several publications have observed a variable effect of anti-VEGFs in RP-CME, including: pegaptanib sodium (Macugen, (OSI) Eyetech Pharmaceuticals and Pfizer Inc.)¹⁷, bevacizumab (Avastin, Genentech/Roche, South San Francisco, California, USA)^{18,19}, ranibizumab (LUCENTIS; Genentech, South San Francisco, Calif., USA)^{20,21} and aflibercept (EYLEA; Regeneron Pharmaceuticals, Inc., Tarrytown, New York, N.Y., USA, and Bayer Healthcare Pharmaceuticals, Berlin, Germany)^{21,22}. The largest study to date by Artunay et al. enrolled 30 eyes of 30 patients with RP-CME refractory to treatment with oral acetazolamide for at least 6 months. Fifteen eyes of 15 patients were treated with a single intravitreal injection of ranibizumab (ivR). Fifteen eyes of 15 patients that declined ivR were used as a control group.

Thirteen out of 15 eyes (87%) in the treatment group demonstrated significant reduction of CME at 6 months post-injection although the definition of 'significant reduction' is not stated in the paper. No statistically significant difference in VA was demonstrated in this cohort as a whole, or in sub-group analysis of responders. Moustafa and Moschos published a case report demonstrating improvement of CMT and VA following a single unilateral intravitreal injection of aflibercept (ivA) in a 52-year-old with RP-CME. At baseline, the vision in the RE was 3/10. One month post-injection, vision improved to 4/10 and the CME resolved. Documented visual improvement was maintained at both the 2- and 6-month reviews²². Our group subsequently published a case report regarding a 38-year-old patient, who presented with a 3-year history of bilateral RP-CME. Previous treatment had been with topical 2% dorzolamide, oral acetazolamide, and ivR, which had demonstrated only minimal reduction of CME. He had a good structural response to bilateral doses of ivA. He subsequently received serial ivR with further reduction of CMT observed. VA remained stable throughout²¹.

Given the aforementioned lack of high quality evidence for the use of any of the therapeutic options for RP-CME, we have undertaken a phase II exploratory prospective study to assess the safety and efficacy of ivA in a well characterized cohort of patients with RP-CME in order to help provide evidence towards this unmet medical need.

Patients and Methods

The protocol of the study adhered to the provisions of the Declaration of Helsinki and was approved by the local ethics committee. Informed consent was obtained from all patients. The study was undertaken at Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom. The study was assigned the number 2015-003723-65 by EudraCT and was registered with ClinicalTrials.gov and assigned the

following unique identifier: NCT02661711. A consort flow diagram illustrates the flow of patients through the study (see supplementary figure 1).

Patient eligibility

The following criteria were used to guide patient enrollment:

(A) *Inclusion criteria*: (1) patients of either gender aged ≥ 16 years; (2) CME in association with RP; (3) Unilateral or Bilateral CME (the worse eye only treated – defined as the eye with a greater central macular thickness (CMT) on OCT); (4) No previous oral treatment for CME for last 3 months; (5) No previous peribulbar or intravitreal treatment for CME in the study eye for last 3 months; (6) No previous topical treatment for CME in the study eye for last 1 month; (7) Central visual impairment that in the view of the Principal Investigator (PI) was due to CME; (8) BCVA better than 20/400.

(B) *Exclusion criteria* (ocular criteria were applied to the *study eye only*): (1) Insufficient patient cooperation or media clarity to allow adequate fundus imaging; (2) Evidence of visually significant vitreo-retinal traction or epiretinal membrane (ERM) on OCT that in the PI's opinion was likely to significantly limit the efficacy of intravitreal therapy; (3) History of cataract surgery within prior 3 months or cataract surgery anticipated within 6 months of starting the study; (4) Any anti-VEGF treatment to study eye within 3 months; (5) History of YAG capsulotomy performed within 3 months; (6) Uncontrolled IOP ≥ 24 mmHg for ocular hypertension (on topical IOP lowering medications); (7) Advanced glaucoma (in the opinion of a glaucoma specialist); (8) Patients with active or suspected ocular or periocular infections; (9) Patients with active severe intraocular inflammation; (10) Patients with a new, untreated retinal tear or detachment; (11) Patients with a stage 3 or 4 macular hole; (12)

Thromboembolic event (MI/CVA/Unstable Angina) within 6 months; (13) Pregnancy or family planned within 15 months; (14) Breast feeding; (15) Known allergy or hypersensitivity to anti-VEGF products.

Identification of suitable patients for the trial

An electronic search was performed to identify all patients seen at Moorfields Eye Hospital NHS Foundation Trust, London, UK, between 1st December 2012 and 30th November 2015 with the phrases 'retinitis pigmentosa' and 'cystoid macular edema' appearing in their electronic patient records. This initial search identified 295 patients; however, after review of each electronic patient record and latest SDOCT imaging, 165 patients were excluded from the study for the following reasons: no/minimal CME (111), visually significant ERM (17), VA too poor (24), VA too good (4), macular hole (2), visually significant cataract (2), under 16 years of age (4) and pregnant (1).

A total of 130 patients were therefore found to be potentially suitable participants. Patients were then contacted by the dedicated trial fellow (SAS) either in person at their routine medical retina clinic, by telephone or letter. The aims, methods, anticipated benefits and potential hazards of the study were explained to each patient and a patient information sheet provided. Patients were given a minimum of 24 hours to consider whether they wished to attend a baseline evaluation/screening visit. Out of these patients: 18 could not be contacted/did not reply, 1 was found to be deceased, 32 wished to be considered for the study, and 79 declined to participate for reasons including: did not wish to have injections into their eye (n = 42), happy with their current treatment and/or vision (n = 22), or unable to commit to the study visits (due to distance from the hospital or concerns about the impact it would have on their job) (n = 15).

Out of 32 patients who wished to be considered for the study, 15 patients were being treated with a topical CAI (dorzolamide or brinzolamide) and 5 patients were being treated with an oral CAI (acetazolamide) at time of contact. Patients were requested to stop using CAIs for at least 1 month in the study eye if being used topically, or at least 3 months if orally, before their screening appointment was made. Ten of these 32 patients were not using any treatment at time of contact and were able to attend a screening visit at their earliest convenience, but 2 patients no longer had CME at screening so had been excluded from the trial.

If patients were deemed suitable to participate in the trial at the end of their screening visit, written informed consent was obtained.

Recruitment period

All 30 patients were recruited over a 6 month period.

Baseline evaluation/screening visit

The baseline evaluation/screening visit took place within 28 days of the patient being contacted unless they were on CAIs. Patients on oral/topical CAI treatment were allowed sufficient time off treatment in order to fulfil the inclusion criteria for the trial, before being contacted again for re-confirmation that they still wished to attend a baseline evaluation/screening visit. At the screening appointment, each patient had the opportunity to ask any further questions before informed consent was taken.

Baseline tests of visual function included the following:

Subjective refraction and best-corrected visual acuity (BCVA)

All patients were subjectively refracted at baseline to obtain their spectacle correction. BCVA was tested monocularly at 4 metres (m) using 2 ETDRS charts (one chart for each eye) that were retro-illuminated using a light box containing 2 Cool Daylight 20 watt fluorescent tubes. If a patient was unable to read 20 letters or more at this distance, the test would be repeated at 1m. In this case, only the first 6 rows would be attempted. If the patient was wearing a trial frame, +0.75 dioptres sphere (DS) was added to the prescription to correct for the closer test distance. The VA score was the number of letters read correctly at 4m, plus the number of letters read correctly at 1m. If the patient did not require testing at 1m, i.e. they read 20 or more letters at 4m, then the score was the number of letters read correctly at 4m, plus 30.

Spectral Domain Optical Coherence Tomography (SDOCT)

The Heidelberg Spectralis SDOCT (Heidelberg Engineering, Heidelberg, Germany) was used to obtain macular volume scans in order to measure CMT. The macular volume scan protocol had the following settings: 20°x20°, 49 Sections, High Speed, 29 frames automatic real time (ART).

Ishihara colour vision testing

The Ishihara version used contained 17 plates held at 75 cm from the patient. A light box was used to achieve standardised lighting and patients were adequately corrected for reading vision using plus lenses if required performing the test monocularly.

Contrast sensitivity

Contrast sensitivity was performed monocularly using the Pelli-Robson chart (Clement Clarke Inc., Columbus, OH). The patient was seated at a distance of 1m. As a standard, +0.75DS was added to each patient's refraction when performing the test. The luminance of the chart was between 80 - 120 candela

per square metre (cd/m^2). The patient was asked to name each letter on the chart, starting with the high-contrast letters on the upper left-hand corner and reading horizontally across the entire line. The test was completed when the patient failed to correctly identify two or more letters in a triplet.

Retinal sensitivity

Mesopic microperimetry using the MP-1 microperimeter (Nidek Instruments, Inc, Padua, Italy) was carried out *twice* on each eye at baseline. Spherical error was accounted for in all patients who were then dark-adapted for 10 minutes before performing the test. The microperimetry protocol included: Cross 2 degrees, Goldmann III Stimulus 200ms, 4-2 strategy and 30 seconds of tracked fixation. The results were generated using a local defect map including -9:1 setting in order to provide mean sensitivity and mean defect together with bivariate contour ellipse area (BCEA) value (numeric and fixation).

Fundus autofluorescence (FAF)

FAF images were acquired using the Heidelberg Spectralis. For each eye, a near-infrared reflectance image and short-wavelength autofluorescence image of standard field 2 (centred on the fovea) were acquired. Both 30 and 55 degree field of view were acquired using high resolution at 50 frames (ART).

If a patient was deemed eligible to enter the trial, intra-ocular pressure (IOP) was measured using Goldmann tonometry and their 1st ivA given on the same day ('Visit 1'). The IOP was re-checked 30 minutes after ivA, and if the pressure was increased (≥ 30 mmHg) appropriate treatment was commenced.

Randomisation

The study consisted of only 1-arm and all trial patients received the active drug, aflibercept via intravitreal injection.

Follow-up visits

At each follow-up visit, patients had their vital signs checked and a medication review performed. Tests of visual function carried out at every visit included: BCVA, colour vision, contrast sensitivity and SDOCT. In addition, microperimetry and FAF were also undertaken at the 6- and 12- month (exit) visits. IvA was administered every four weeks for the first three months (loading phase), followed by a treat and extend protocol up to 12 months. Extension from monthly to 6, 8, 10 and 12 week follow-up occurred when there was no reduction in macular edema compared with the previous visit. Please refer to supplementary table 1 for a schedule of assessments.

Intravitreal Procedure

Aflibercept (Eylea; Regeneron, Tarrytown, New York, USA and Bayer Healthcare, Leverkusen, Germany) was supplied by Bayer LTD in vials containing 100 microlitres of 40mg/ml solution for injection, equivalent to 4mg aflibercept and stored by Moorfields Pharmaceuticals (London, UK). Each vial enabled a usable amount to deliver a single dose of 50 microlitres containing 2 mg aflibercept. In a designated intravitreal treatment room, under sterile conditions, using topical anesthesia and povidone-iodine 5% into the conjunctival sac and onto the lid margins, and following application of a drape and insertion of a lid speculum, injections were undertaken with a 30-gauge needle through the infratemporal quadrant, with a drop of preservative-free (PF) chloramphenicol placed in the fornix at the end of the procedure. Patency of the central retinal artery was determined by visual acuity (VA) of hand movements or better. After the injection, topical chloramphenicol was self-instilled 4 times per day for 5 days by the patients.

Primary outcome measures

There were two primary outcome measures : (i) To report the safety of aflibercept in RP-CME throughout the study (17 months in total); via the documentation of adverse events (AEs) deemed related to the trial drug; (ii) To report the efficacy of aflibercept in RP-CME via mean central macular thickness (CMT) on Spectral domain OCT (SDOCT) at 12 months after baseline.

Secondary outcome measures

The secondary outcome measures relating to efficacy were: (i) The mean CMT on SDOCT at 6 months after baseline; (ii) The mean change in CMT on SDOCT from baseline to 6 months after baseline and baseline to 12 months after baseline; (iii) The mean Best Corrected Visual Acuity (BCVA) using the ETDRS visual acuity chart at a starting distance of 4m at 6 and 12 months after baseline; (iv) The mean change in ETDRS BCVA from baseline to 6 months and baseline to 12 months; (v) The mean macular volume on SDOCT at 6 and 12 months; (vi) The mean change in macular volume on SDOCT from baseline to 6 months and baseline to 12 months; (vii) Report all AEs and serious adverse events (SAEs) throughout the study (17 months in total); (viii) The mean retinal sensitivity using microperimetry at 6 and 12 months; (ix) The mean change in retinal sensitivity using microperimetry from baseline to 6 months and baseline to 12 months; (x) The mean number of intravitreal injections administered throughout the study.

Sample size

No previous studies have been published for which the sample size could be powered. A sample size of 30 patients was therefore justified on the basis that 30 subjects will provide an estimate of the mean change in CMT from baseline to 12 months with reasonable precision as advocated by Browne (1995)²³ and Hertzog (2008)²⁴.

Masking

This was an open-label study and therefore no masking took place.

Data management

The completed paper case report forms (CRFs) were checked for completion by the research nurse / research manager and data officer before data entry. All trial data were double entered by two independent data officers using the database created by the R&D IT team. The first and second data entries were compared for completion and consistency. Discrepancies were checked against the original CRF for entry errors, which were subsequently corrected. Sense checks, logic checks and range checks were also performed. Data queries were corrected and data were cleaned. The database was then locked and data transferred for data to be analysed by trial statisticians using STATA statistical software. The data management process followed Moorfields Eye Hospital standard operating procedures (SOPs) for data management.

Statistical analysis

The primary analysis was an available case analysis but baseline characteristics of those who were lost to follow up were compared with those who were not. If the findings from this study were favourable, these data would be used to plan a definitive future randomised controlled trial.

Descriptive statistics have been used to report the findings of this study due to its modest sample size and single arm design.

‘Responders’ would be considered as participants demonstrating a reduction of CMT by 11% or more between baseline and 12 months, thus allowing comparison with previous studies that have used the same definition^{15,25-29}.

All statistical analyses were conducted using Stata Statistical Software version 15.0.

Results

Results are summarized in tables 1 to 3 and supplementary tables 2 to 9.

Table 1: Primary outcome measures

	Aflibercept (n=29)	95% CI
Central Macular thickness on SDOCT (μm), Mean (SD) at Baseline	458.7 (84.6)	
Central Macular thickness on SDOCT (μm), Mean (SD) at 12 months	413.4 (98.2)	376.0 to 450.7

SDOCT = Spectral domain optical coherence tomography; μm = microns; SD = standard deviation

Table 2: Secondary outcome measures

	Aflibercept (n =29)	95% CI
Central Macular thickness on SDOCT (μm), Mean (SD) at 6 months	414.8 (96.4)	378.1 – 451.4
Change in Central Macular thickness on SDOCT (μm) from		
- Baseline to 12 months, Mean (SD)	-47.6 (86.6)	-80.5 to -14.6
- Baseline to 6 months, Mean (SD)	-46.2 (108.7)	-87.6 to -4.9
ETDRS BCVA (letters), Mean (SD) at 6 months	66.9 (10.6)	62.8 to 70.9
ETDRS BCVA (letters), Mean (SD) at 12 months	68.0 (11.1)	63.8 to 72.3
Change in ETDRS BCVA (letters) from		
- Baseline to 12 months, Mean (SD)	4.3 (6.9)	1.7 to 6.9
- Baseline to 6 months, Mean (SD)	3.1 (6.6)	0.6 to 5.6
Macular Volume on SDOCT (mm^3), Mean (SD) at 6 months	7.9 (0.6)	7.7 to 8.2
Macular Volume on SDOCT (mm^3), Mean (SD) at 12 months	8.0 (0.7)	7.7 to 8.2

Change in Macular Volume on SDOCT (mm ³) from		
- Baseline to 12 months, Mean (SD)	-0.3 (0.7)	-0.6 to -0.1
- Baseline to 6 months, Mean (SD)	-0.3 (0.8)	-0.7 to 0.0
Retinal Sensitivity (dB), Mean (SD) at 6 months	4.92 (3.49)	3.56 to 6.27
<i>Missing, n(%)</i>	1 (3)	
Retinal Sensitivity (dB), Mean (SD) at 12 months	4.93 (3.48)	3.55 to 6.31
<i>Missing, n(%)</i>	2 (6)	
Change in Retinal Sensitivity (dB) from		
- Baseline to 12 months, Mean (SD)	-1.09 (2.10)	-1.90 to -0.27
- Baseline to 6 months, Mean (SD)	-1.23 (2.24)	-2.10 to -0.37
Total number of injections received over the study period (12 months), Median (IQR)	7 (6 to 9)	-

CME = cystoid macular edema; μm = microns; ETDRS = early treatment diabetic retinopathy study; BCVA = best corrected visual acuity; SD = standard deviation; IQR = Interquartile range; cd/m^2 = candela per square meter; IOP = intraocular pressure; mmHg = millimetre of mercury; SDOCT = Spectral domain optical coherence tomography; mm^3 = millimetres cubed; dB = decibels

Table 3: Descriptive statistics for responders

	Aflibercept (n=11)
Central Macular thickness on SDOCT (μm), Mean (SD) at 12 months	350.3 (93.3)
Central Macular thickness on SDOCT (μm), Mean (SD) at 6 months	360.7 (85.2)
Change in Central Macular thickness on SDOCT (μm) from	
- Baseline to 12 months, Mean (SD)	-139.5 (65.8)
- Baseline to 6 months, Mean (SD)	-129.1 (125.1)
ETDRS BCVA (letters), Mean (SD) at 6 months	67.5 (10.1)
ETDRS BCVA (letters), Mean (SD) at 12 months	68.4 (11.8)
Change in ETDRS BCVA (letters) from	
- Baseline to 12 months, Mean (SD)	4.7 (9.5)

- Baseline to 6 months, Mean (SD)	3.8 (6.8)
Macular Volume on SDOCT (mm ³), Mean (SD) at 6 months	8.5 (0.6)
Macular Volume on SDOCT (mm ³), Mean (SD) at 12 months	8.5 (0.8)
Change in Macular Volume on SDOCT (mm ³) from	
- Baseline to 12 months, Mean (SD)	-0.6 (0.6)
- Baseline to 6 months, Mean (SD)	-0.6 (0.6)
Retinal Sensitivity (dB), Mean (SD) at 6 months	4.93 (4.06)
Retinal Sensitivity (dB), Mean (SD) at 12 months	4.48 (3.83)
Change in Retinal Sensitivity (dB) from	
- Baseline to 12 months, Mean (SD)	-0.97 (1.92)
- Baseline to 6 months, Mean (SD)	-0.92 (2.03)
Total number of injections received over the study period (12 months), Median (IQR)	7 (6 to 10)

CME = cystoid macular edema; μm = microns; ETDRS = early treatment diabetic retinopathy study; BCVA = best corrected visual acuity; SD = standard deviation; IQR = Interquartile range; cd/m^2 = candela per square meter; IOP = intraocular pressure; mmHg = millimetre of mercury; SDOCT = Spectral domain optical coherence tomography; mm^3 = millimetres cubed; dB = decibels

Baseline characteristics and injection frequency

The baseline characteristics for all participants are summarized in supplementary tables 2 to 4. Thirty eyes of 30 patients were enrolled, with the first patient recruited in March 2016 and the final patient had their 52-week visit in August 2017. Two patients were screened who did not satisfy the criteria for enrolment (study ID 29 and 31); the reason being that they no longer had CME. The mean age of the patients was 43.3 years (SD 11.5 years, range 20 to 61 years), consisting of 17 male (56.7%) and 13

female (43.3%) patients. The study eye was the left eye in 16 (53.3%) patients and the right eye in 14 (47.7%). The median duration of CME in the study eye was 252 weeks and the interquartile range (IQR) was 156 to 296 weeks.

All patients enrolled in the study received the active drug, aflibercept. The median number of injections given across all patients in the study was 7 (IQR 6 to 9); with the minimum number of injections given being 4, and the maximum number of injections given being 11.

Likely disease-causing sequence variants were identified in 16 of 30 (53.3%) study participants (see supplementary table 2), which included: (i) AD inheritance: neural retina leucine zipper (NRL) gene (1 patient), rhodopsin (RHO) gene (2 patients), pre-mRNA processing factor 31 (PRPF31) gene (3 patients), pre-mRNA processing factor-8 (PRPF8) gene (1 patient), small nuclear ribonucleoprotein U5 subunit 200 (SNRNP200) gene (1 patient); (ii) AR inheritance: usherin 2A (USH2A) gene (3 patients), tubby like protein 1 (TULP1) gene (1 patient), retinitis pigmentosa-1 (RP1) gene (1 patient), retinol dehydrogenase-12 (RDH12) gene (1 patient), intraflagellar transport-140 (IFT140) gene (1 patient); and (iii) X-linked inheritance: retinitis pigmentosa GTPase regulator (RPGR) gene (1 patient).

The other 14 patients have undergone genetic screening (including whole genome sequencing) and remain unsolved to date.

Mean baseline ETDRS BCVA was 64 letters (SD 11.5 letters) with a mean CMT of 458.7 microns (SD 84.6 microns) in the study eye for the cohort overall. Twenty-four (80%) patients were phakic, compared with 6 (20%) patients who were pseudophakic in their study eye.

Nine of 29 (31.0%) patients were graded as having either questionable or definite presence of ERM within 3mm of the fovea. No patients were found to have vitreo-macular traction (VMT) on their baseline OCT scan. One of 29 (3.4%) patients was found to have vitreo-macular adhesion on their baseline OCT scan. Nine of 29 (31.0%) patients were graded as having either questionable or definite disruption of the ellipsoid zone within 1mm of the fovea on their baseline OCT scan.

One participant did not complete 12 months of follow-up due to illness and withdrew from the study. Since a single patient only withdrew, analysis was conducted using available case data. The baseline characteristics for this participant who withdrew from the study were not different to patients who continued in the study. Twenty-nine out of 30 (96.7%) patients therefore completed 12 months of follow-up for the study.

A post-hoc exploratory analysis of responders-only was also undertaken. Baseline characteristics for responders are summarized in supplementary tables 5 and 6. Sub-group analysis of responders demonstrated similar baseline characteristics to the group taken as a whole, with mean baseline ETDRS BCVA of 63.6 letters (SD 11.3 letters), mean CMT of 489.8 microns (SD 105.9 microns), and median duration of CME was 264 weeks (IQR 228 to 416) . The median number of injections for this group was 7 (IQR 6 to 10); where the minimum number of injections given was 5, and the maximum number of injections was 11.

Outcome measures

Efficacy: analysis of all study participants

The primary and secondary efficacy outcomes for all patients (responders and non-responders) within the study are summarised in tables 1 and 2. Mean CMT at 12 months was 413.4µm (SD 98.2µm, 95% CI

376.0 to 450.7 μ m), corresponding to a reduction in CMT of 47.6 μ m (SD 86.6 μ m, 95% CI -80.5 to -14.6 μ m) or 9.61 % (17.56 %) between baseline and 12 months. Mean macular volume at 12 months was 8.0mm³ (SD 0.7, 95% CI 7.7 to 8.2), corresponding to a change in macular volume of -0.3mm³ (SD 0.7, 95% CI -0.6 to -0.1) between baseline and 12 months. Mean CMT at 6 months was similar at 414.8 μ m (SD 96.4 μ m, 95% CI 378.1 to 451.4 μ m), corresponding to a reduction in CMT of 46.2 μ m (SD 108.7 μ m, 95% CI -87.6 to -4.9 μ m) or 8.13 % (23.3 %) (see supplementary figure 2) between baseline and 6 months. Mean macular volume at 6 months was 7.9mm³ (SD 0.6, 95% CI 7.7 to 8.2), corresponding to a change in macular volume of -0.3mm³ (SD 0.8, 95% CI -0.7 to 0.0) between baseline and 6 months.

Mean ETDRS BCVA was 66.9 letters (SD 10.6, 95% CI 62.8 to 70.9) at 6 months and 68.0 letters (SD 11.1, 95% CI 63.8 to 72.3) at 12 months. This equated to a gain of 3.1 letters (SD 6.6, 95% CI 0.6 to 5.6) and 4.3 letters (SD 6.9, 95% CI 1.7 to 6.9) respectively at 6 and 12 months (see supplementary figure 3). No patients lost \geq 30 letters.

Mean retinal sensitivity at 6 months was 4.92 dB (SD 3.49, 95% CI 3.56 to 6.27), corresponding to a change in retinal sensitivity of -1.23dB (SD 2.24, 95% CI -2.1 to -0.37). Data were missing for 1 (3%) patient. Mean retinal sensitivity at 12 months was 4.93 dB (SD 3.48, 95% CI 3.55 to 6.31), corresponding to a change in retinal sensitivity of -1.09dB (SD 2.10, 95% CI -1.9 to -0.27). Data were missing for 2 (6%) patients.

Efficacy: sub-group analysis of responders only

The primary and secondary efficacy outcomes using descriptive statistics for sub-group analysis of responders within the study are provided in table 3.

Eleven out of 29 (37.9%) patients were classified as responders having demonstrated a reduction in CMT of 11% or more at 12 months compared to baseline. These same patients were also classed as responders at 6 months when applying the same criteria. Genetic mutations were identified and confirmed in 5 of 11 (45.5%) responders, which included: RPGR (1 patient), PRPF31 (1 patient), USH2A (c.11700C>A, p.Tyr3900Ter; c.4618G>A, p.Asp1540Asn) (1 patient), RHO (1 patient) and RDH12 (1 patient). The other 6 patients are genetically unsolved to date.

Following sub-analysis of these 11 patients, mean CMT at 12 months was 350.3 μ m (SD 93.3 μ m), corresponding to a change in CMT of -139.5 μ m (SD 65.8 μ m) or 28.1% (12.9 %) between baseline and 12 months. Mean macular volume at 12 months was 8.5mm³ (SD 0.8), corresponding to a change in macular volume of -0.6mm³ (SD 0.6) between baseline and 12 months. Mean CMT at 6 months was similar at 360.7 μ m (SD 85.2 μ m), corresponding to a change in CMT of -129.1 μ m (SD 125.1 μ m) or 22.9% (29.7 %) (See figure 1) between baseline and 6 months. Mean macular volume at 6 months was 8.5mm³ (SD 0.6) corresponding to a change in macular volume of -0.6mm³ (SD 0.6) between baseline and 6 months. Figure 2 demonstrates SDOCT images of 2 responders taken at baseline and at 1 month post-baseline (after having received only a single ivA).

Figure 1: A graph demonstrating mean change in CMT from baseline to 6 months after baseline, and baseline to 12 months after baseline in responders-only (n=11).

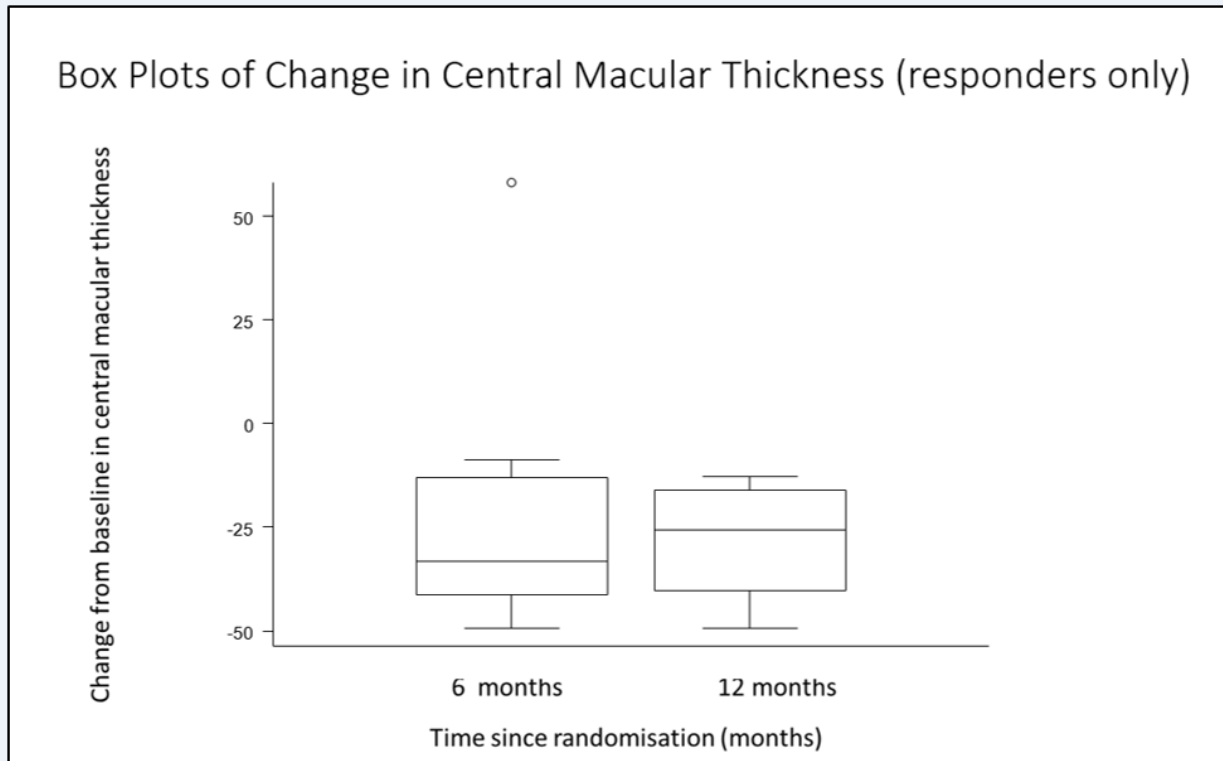
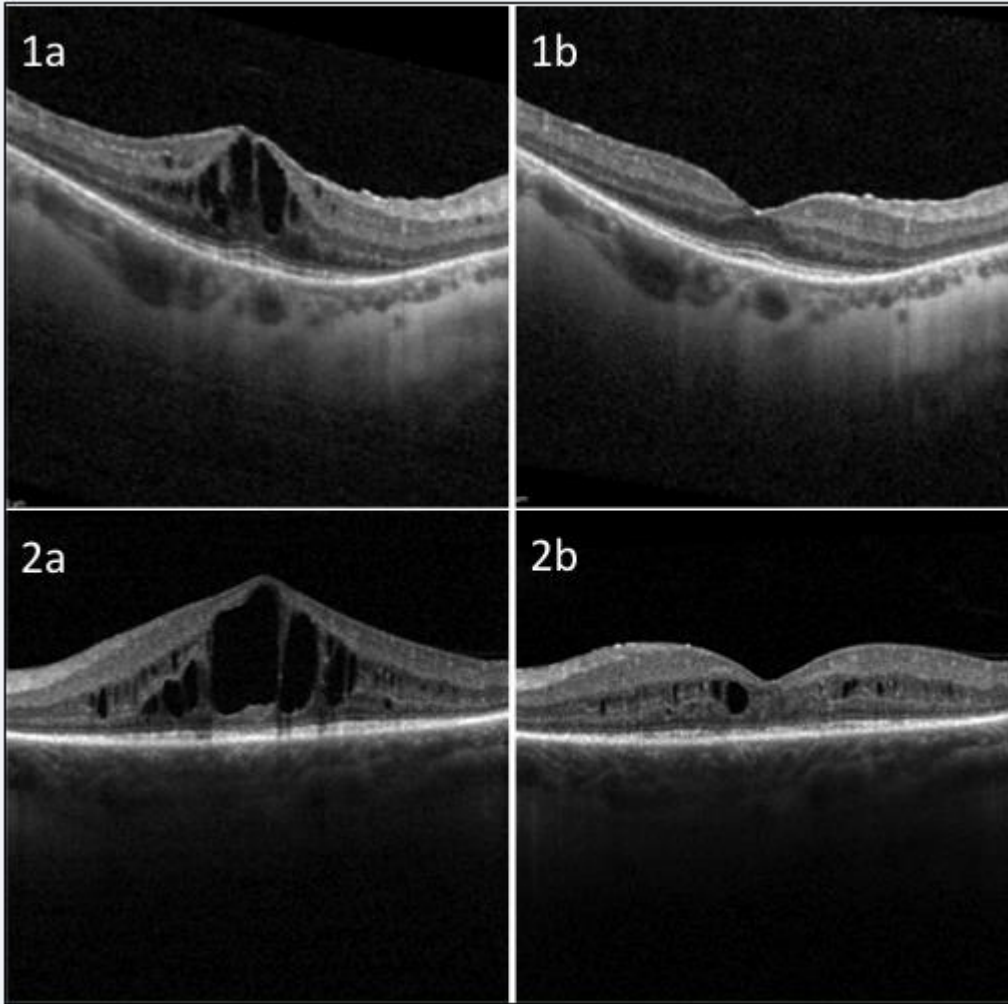
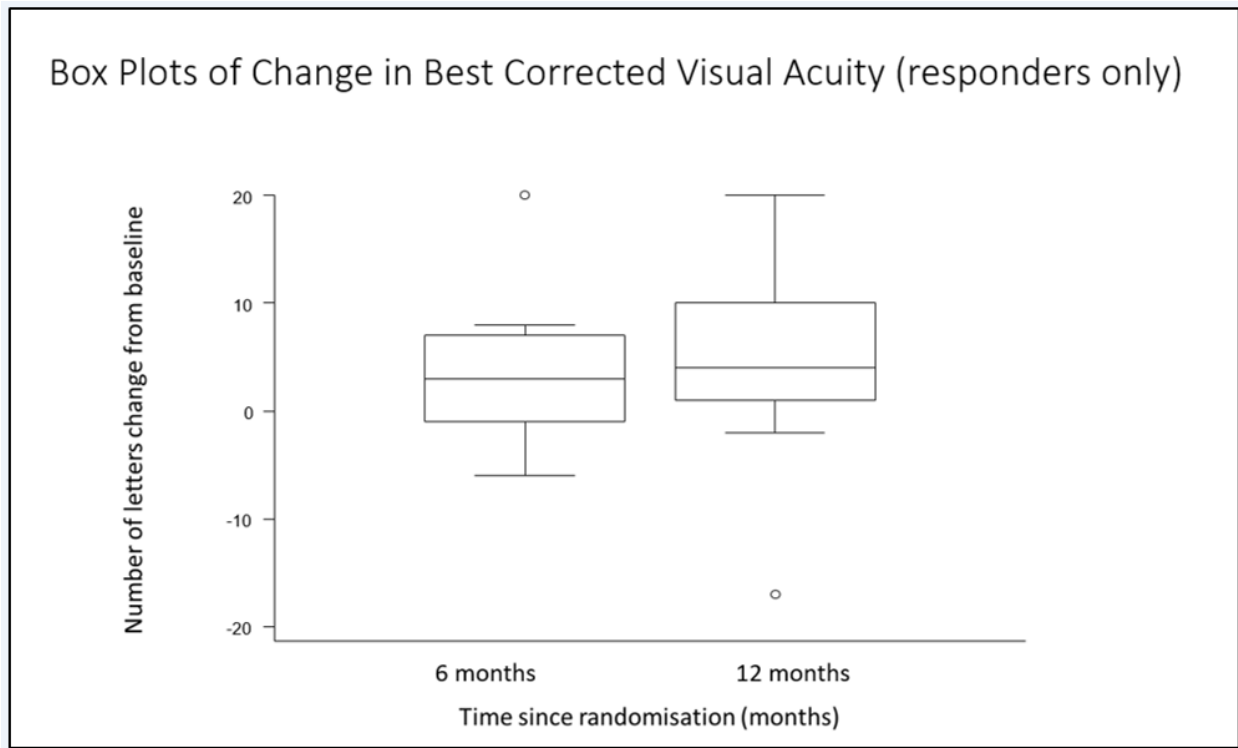


Figure 2: Two representative examples of responders: 1a and 2a show SDOCT baseline images of two study participants (study IDs: 04 and 14); 1b and 2b are SDOCT images taken at 1 month post 1st aflibercept injection in the same two participants, respectively



Mean ETDRS BCVA at 6 months was 67.5 letters (SD 10.1) corresponding to a gain of 3.8 letters (SD 6.8). Mean ETDRS BCVA at 12 months was 68.4 letters (SD 11.8) corresponding to a gain of 4.7 letters (SD 9.5) (Figure 3). It should be noted that 3 of 11 (27.3%) responders were graded as having disruption of the ellipsoid zone within 1mm of the fovea on their baseline OCT scan. No improvement of vision was found in all 3 of these patients. Four of 11 (36.4%) responders were graded as having questionable presence of ERM within 3mm of the fovea.

Figure 3: A graph demonstrating mean change in CMT from baseline to 6 months after baseline, and baseline to 12 months after baseline in responders-only (n=11).



Mean retinal sensitivity at 6 months was 4.93dB (SD 4.06), corresponding to a change in retinal sensitivity of -0.92dB (SD 2.03) between baseline and 6 months. Mean retinal sensitivity at 12 months was 4.48dB (SD 3.83), corresponding to a change in retinal sensitivity of -0.97dB (SD 1.92) between baseline and 12 months.

The median number of injections given in responders was 7 (IQR 6 to 10).

Additional data of non-responders

Eighteen out of 29 (62.1%) patients were classified as non-responders. Genetic mutations were identified and confirmed in 10 of 18 (55.6%) non-responders, which included: NRL (1 patient), RHO (1 patient), PRPF31 (1 patient), PRPF8 (1 patient), SNRNP200 (1 patient), USH2A (2 patients), TULP1 (1

patient), RP1 (1 patient) and IFT140 (1 patient). Six of 18 (33.3%) non-responders were graded as having disruption of the ellipsoid zone within 1mm of the fovea on their baseline OCT scan.

Safety

Ocular and non-ocular AEs and SAEs are summarized in supplementary tables 7 to 9.

Ocular AEs:

Ocular AEs were the expected standard range of AEs seen with intravitreal injections (see supplementary tables 7 to 9). There were no cases of endophthalmitis or retinal detachment.

Ocular SAE:

One participant reported sub-acute reduction of vision at week 32 despite being a 'responder', with testing demonstrating a reduction in vision of 14 ETDRS letters. Injections were immediately discontinued. Further assessments were undertaken including SDOCT, FAF, microperimetry and OCT-angiography (OCT-A). There was no demonstrable change in outer retinal lamination compared to baseline, with also no change in microperimetry or FAF compared to baseline, and no abnormality detected on OCT-A. The non-study eye had a baseline vision of 30 ETDRS letters due to advanced photoreceptor loss and it was therefore concluded that the reduction in vision was most likely secondary to progression of underlying RP rather than as a consequence of ivA. The patient remained in the study and attended the 6 and 12 month follow-up appointments.

Non-ocular/systemic AEs:

All non-ocular/systemic AEs were reported during the study whether or not they were considered to be secondary to aflibercept. Non-ocular/systemic AEs included: back pain, headache, lethargy, tinnitus,

viral cold, conversion of prostate biopsy from benign to low-grade neoplasia, feeling low/low mood, labyrinthitis, urine tract infection, relapse of mental illness, heartburn, perforated ear drum, ear infection, viral gastric illness, anxiety and mosquito bite.

Whilst the participant who developed labyrinthitis during the study was reassured that it was unlikely to be secondary to aflibercept, they decided that they would prefer to discontinue receiving injections. This patient remained in the study and attended the 6 and 12 month follow-up appointments.

Discussion

This is the first prospective study to obtain safety and efficacy data on the use of serial intravitreal injections with aflibercept for the treatment of RP-CME, employing a monthly loading phase of 3 injections followed by a treat-and-extend protocol for a total of 12 months of follow-up. There were no significant safety concerns and serial injections were well tolerated. Eleven out of 29 (37.9%) patients were classified as responders at both 6 and 12 months, having demonstrated a reduction of at least 11% CMT on SDOCT compared to baseline. These patients experienced a mean (SD) percentage change in CMT relative to baseline of -22.9% (29.7 %) and -28.1% (12.9 %) at 6 and 12 months respectively. Responders gained 3.8 (SD 6.8) and 4.7 (SD 9.5) ETDRS letters respectively at 6 and 12 months. Responders demonstrated a greater change of macular volume over the study (-0.6mm³ at 6 and 12 months) compared to non-responders (-0.3mm³ at 6 and 12 months). When the cohort was analysed as a whole, the mean (SD) percentage change in CMT relative to baseline was -8.1% (23.3%) and -9.6% (17.6%) at 6 and 12 months respectively. An intriguing observation, unlike other disorders where anti-VEGF agents have been employed, is that all responders (n=11) achieved a notable reduction in CME after their first injection ('early-responder'). There were no 'late-responders'. This is clinically very

valuable as for the majority of patients it may be possible to decide at a very early stage whether injections should be pursued.

Responders in this study were identified across all categories of inheritance pattern (AD, AR and XL). There was no association between response to anti-VEGF treatment and mode of inheritance. Whilst just over half of the patients in this study had a confirmed molecular diagnosis, no genotype was associated with response to treatment; including 1 USH2A patient responded, whilst 2 other USH2A patients did not, and 1 PRPF31 patient responded - whilst 2 others did not. This study included only 1 patient with XL inheritance who was deemed a responder and we therefore cannot draw any comparison with other patients with XL-RP. More advanced disease, defined as disruption of the ellipsoid zone within 1mm of the fovea (seen in 27.3% of responders and 33.3% of non-responders) did not appear to affect likelihood of response to anti-VEGF.

The release of toxic products (including VEGF) from degenerating retina/RPE in patients with RP contributes to weakening of the BRB and RP-CME formation⁷. Anti-VEGF is thought to act by reversing proliferation and cell migration stimulated by VEGF and the delocalization of tight junction proteins induced by VEGF¹⁶⁵³⁰. Intriguingly, Salom et al. observed lower aqueous levels of VEGF in eyes of patients with RP versus controls³¹. It would therefore be interesting to measure levels of VEGF in the vitreous and review whether there are significant differences between patients with RP versus controls, as well as patients with RP versus those with RP-CME. This being an invasive procedure, however, would likely prove challenging to gain ethical approval and is why we did not consider undertaking in this study.

Strengths of our study included excellent patient attendance throughout its duration, with a 96.7% participant retention rate at 12-months. The study drug was well tolerated and no cases of endophthalmitis occurred. The study design including an initial loading phase followed by a treat-and-extend regime, which allowed for the observation of both early and (potentially) late responders. We also had established likely disease-causing sequence variants in 16 of 30 (53.3%) study participants.

One of the limitations to our study was being unable to include treatment-naive patients with shorter duration of CME. Many patients were deemed eligible for the study yet declined intravitreal injections without first trialling topical and/or oral treatment. All patients in the study had therefore used topical CAI medication previously; 15 of whom were using topical CAI treatment up until 1 month prior to their screening appointment. Five of these patients were deemed responders. Five patients in the study were using oral CAI treatment up until 3 months prior to their screening appointment; 1 patient withdrew from the study, 2 patients were deemed responders, and 2 patients did not respond. No obvious trend was demonstrated to suggest whether recent use of topical or oral CAIs influences response to anti-VEGF therapy.

Long-standing CME duration was observed in many patients within our cohort, with the median duration being 252 weeks (IQR, 156 to 296 weeks). An interesting observation identified from this study was that duration of CME did not appear to affect anatomical response to anti-VEGF; median CME duration in responders was 264 weeks (IQR 228, 416), compared to the group overall (252 weeks (IQR 156, 296)). Indeed, the patient with the longest standing CME duration of the cohort (20 years) had complete resolution of CME after a single ivA.

As long as patients achieved a BCVA better than 20/400 at baseline, they were considered eligible for the study. Our study therefore included patients with fairly advanced underlying disease as demonstrated by photoreceptor loss and outer retinal thinning - features that have been shown to hinder VA improvement despite reduction of CMT³². It was therefore unsurprising to find that all 3 of 11 (27.3%) responders graded as having disruption of the ellipsoid zone within 1mm of the fovea on their baseline OCT scan demonstrated no improvement of vision. Greater improvement of VA may be demonstrated in patients with a relatively more intact photoreceptor layer at baseline.

It would be valuable to repeat this study in a larger cohort of patients, ideally naive to other treatment modalities, with shorter history of CME duration and relatively intact photoreceptor layers at baseline. Only including patients with a molecularly confirmed genetic diagnosis would help to determine if genotype has a role in predicting response to anti-VEGF treatment. Additional suggestions to consider when designing a future study would include: to perform fundus fluorescein angiogram (FFA) at baseline to see whether active leakage is present and whether FFA is predictive of which patients will/will not respond to aflibercept, to take baseline samples of vitreous in order to assess levels of VEGF in this cohort of patients, to include a control group (possibly using placebo), to randomise patients if there is an option of a control/placebo group, to blind patients and/or clinicians if there is an option of a control/placebo group, and to include OCT-A as an additional imaging modality.

This phase II exploratory study demonstrates that ivA can be effective at reducing CMT in patients with RP-CME, suggesting that aflibercept should be considered part of the armamentarium when selecting treatments for RP-CME. The factors predicting who is likely to respond, however, remain to be clarified. Our data supports more studies to further investigate the role of VEGF blockade in RP-CME.

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Supplementary table 1: Schedule of assessments

	Screening and Baseline	Treatment phase and follow-up*											
Visit number	1	2	3	4	5	6	7	8	9	10	11	12	13
Weeks	0 weeks	4 weeks post baseline	8 weeks post baseline	4 to 12 weeks post visit 3	4 to 12 weeks post visit 4	4 to 12 weeks post visit 5	4 to 12 weeks post visit 6	4 to 12 weeks post visit 7	4 to 12 weeks post visit 8	4 to 12 weeks post visit 9	4 to 12 weeks post visit 10	4 to 12 weeks post visit 11	4 to 12 weeks post visit 12
Patient demographics confirmed	X	X	x	X	x	x	x	x	x	x	x	x	X
General medical and ocular history	X												
Medication review	X	x	x	x	x	x	x	x	x	x	x	x	X
Pregnancy status confirmed	X												
Eligibility check	X												
Informed Consent	X												
Vital signs: Blood pressure, heart rate and temperature	X	X	x	X	x	x	x	x	x	x	x	x	X
Best corrected ETDRS visual acuity	X	X	X	X	x	x	x	x	x	x	x	x	x
Refracted best corrected ETDRS visual acuity	X					X (at 6 months)							X (at 12 months)
Colour vision	X	X	X	X	x	x	x	x	x	x	x	x	x
Contrast sensitivity	X	X	X	X	x	x	x	x	x	x	x	x	x
Microperimetry	X					X (at 6 months)							X (at 12 months)
Dilation of the patient	X	X	X	X	x	x	x	x	x	x	x	x	x
Slit lamp examination	X	X	X	X	x	x	x	x	x	x	x	x	x
IOP check (pre-injection)	X	X	X	X	x	x	x	x	x	x	x	x	x
SDOCT in both eyes	X	X	X	X	x	x	x	x	x	x	x	x	x
Fundus Autofluorescence	X	X	X	X	x	x	x	x	x	x	x	x	x
Administration of Eylea	Possibly	X	X	X	x	x	x	x	x	x	x	x	x
IOP check (post-injection)	X	X	X	X	x	x	x	x	x	x	x	x	x

*Number of total visits will vary between patients as follow-up appointments will be any time between 4 to 12 weeks.

Supplementary table 2: Genetic data of all patients in the study

Study ID	Symbol	Protein	Inheritance pattern
1	PFPR31	pre-mRNA processing factor 31	AD
2	PFPR8	pre-mRNA processing factor 8	AD
3	USH2A Location: c.1841-2A>G homozygous	usherin 2A	AR
4	Unsolved	Unsolved	Unsolved
5	RP11	retinitis pigmentosa-11	AD
6	RP1	retinitis pigmentosa-1	AD
7	TULP1	tubby like protein 1	AR
8	Unsolved	Unsolved	Unsolved
9	NRL	neural retina leucine zipper	AD
10	IFT140	intraflagellar transport-140	AR
11	Unsolved	Unsolved	Unsolved
12	RHO/RP4	rhodopsin	AD
13	Unsolved	Unsolved	Unsolved
14	Unsolved	Unsolved	Unsolved
15	RHO	rhodopsin	AD
16	Unsolved	Unsolved	Unsolved
17	Unsolved	Unsolved	Unsolved
18	SNRNP200	small nuclear ribonucleoprotein U5 subunit 200	AR
19	Unsolved	Unsolved	Unsolved
20	USH2A Location: c.2299del, p.Glu767Serf s*21 hom	usherin 2A	AR
21	Unsolved	Unsolved	Unsolved

Supplementary table 3: Non-Ocular Baseline Characteristics

	Aflibercept
Number of Patients (Eyes)	30 (30)
Male / Female, n (%)	17 (57)/ 13(43)
Age (years), Mean (SD)	43.3 (11.5)
Ethnicity, n (%):	
White	26 (87)
Asian	1 (3)
Black	1 (3)
Mixed	1 (3)
Other	1 (3)

Supplementary table 4: Ocular Baseline Characteristics

	Aflibercept (n=30)
Study Eye, Left/Right, n(%)	16 (53)/ 14 (47)
Duration of CME (weeks), Median (IQR)	252 (156 to 296)
Lens status, n (%):	
Aphakic	0 (0)
Pseudophakic	6 (20)
Phakic	24 (80)
ETDRS BCVA (letters), Mean (SD)	64 (11.5)
Ishihara colour vision (out of 17 plates), Median (IQR)	15 (6-16)
Contrast sensitivity (cd/m ²), Mean (SD)	1.58 (0.35)
IOP (mmHg), Mean (SD)	12.5 (2.9)
Central macular thickness on SDOCT (µm), Mean (SD)	458.7 (84.6)
Macular Volume on SDOCT (mm ³), Median (IQR)	8.0 (7.5 to 8.8)
Mean Retinal sensitivity on microperimetry (dB), Mean (SD)	6.3 (3.6)

CME = cystoid macular edema; µm = microns; ETDRS = early treatment diabetic retinopathy study; BCVA = best corrected visual acuity; SD = standard deviation; IQR = Interquartile range; cd/m² = candela per square meter; IOP = intraocular pressure; mmHg = millimetre of mercury; SDOCT = Spectral domain optical coherence tomography; mm³ = millimetres cubed; dB = decibels

Supplementary table 5: Non-Ocular Baseline Characteristics (Responders only)

	Aflibercept
Number of Patients (Eyes)	11 (11)
Male / Female, n (%)	8 (73)/3(27)
Age (years), Mean (SD)	42.7 (15.6)
Ethnicity, n (%):	
White	11 (100)
Asian	0 (0)
Black	0 (0)
Mixed	0 (0)
Other	0 (0)

Supplementary table 6: Ocular Baseline Characteristics (Responders only)

	Aflibercept (n=11)
Study Eye, Left/Right, n(%)	6 (55)/5 (45)
Duration of CME (weeks), Median (IQR)	264 (228, 416)
Lens status, n (%):	
Aphakic	0 (0)
Pseudophakic	1 (9)
Phakic	10 (91)
ETDRS BCVA (letters), Mean (SD)	63.6 (11.3)
Ishihara colour vision (out of 17 plates), Median (IQR)	10 (3 to 14)
Contrast sensitivity (cd/m ²), Mean (SD)	1.42 (0.38)
IOP (mmHg), Mean (SD)	12.4 (3.4)
Central macular thickness on SDOCT (µm), Mean (SD)	489.8 (105.9)
Macular Volume on SDOCT (mm ³), Median (IQR)	8.9 (8.3 to 9.9)
Mean Retinal sensitivity on microperimetry (dB), Mean (SD)	5.8 (3.7)

CME = cystoid macular edema; µm = microns; ETDRS = early treatment diabetic retinopathy study; BCVA = best corrected visual acuity; SD = standard deviation; IQR = Interquartile range; cd/m² = candela per square meter; IOP = intraocular pressure; mmHg = millimetre of mercury; SDOCT = Spectral domain optical coherence tomography; mm³ = millimetres cubed; dB = decibels

Supplementary table 7: Ocular and Non-Ocular adverse events (AEs) and serious adverse events (SAEs) – 0-6 months after baseline

Study ID	Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious
1	Floater in RE	11/08/2016	05/09/2016	Mild	Probably	None	Resolved	Yes	No
2	Sub-conjunctival haemorrhage	04/04/2016	05/04/2016	Mild	Not Related	None	Resolved	Yes	No
2	Blurring of vision	25/08/2016	06/09/2016	Mild	Possibly	None	Resolved	No	No
3	Contacted by pt to say similar blurring to 1st injection	07/06/2016	15/06/2016	Mild	Definitely	None	Resolved	Yes	No
3	Corneal epithelium dystrophy post injection	11/04/2016	12/04/2016	Mild	Definitely	None	Resolved	Yes	No
3	Back pain after bending down	10/10/2016	14/10/2016	Mild	Not Related	None	Resolved	No	No
4	Dry cornea	12/08/2016	14/08/2016	Mild	Definitely	None	Resolved	Yes	No
4	LUL lesion (chalazion)	19/10/2016		Mild	Not Related	None	AE ongoing	No	No
4	Grittiness / Dry eye	09/07/2016	16/07/2016	Mild	Probably	None	Resolved	Yes	No
5	Headache post - IVT	26/07/2016	26/07/2016	Mild	Probably	None	Resolved	Yes	No
5	Feeling lethargic	25/09/2016	30/11/2016	Mild	Unlikely	None	AE ongoing	No	No
5	Raised IOP post - IVT	03/05/2016	03/05/2016	Mild	Definitely	None	Resolved	Yes	No
5	Sub-conjunctival haemorrhage (5 days post IVT)	05/06/2016	15/06/2016	Mild	Definitely	None	Resolved	Yes	No
5	Tinnitus	10/07/2016		Mild	Not Related	None	AE ongoing	No	No
8	Dry ocular surface + pain	10/11/2016	16/11/2016	Moderate	Definitely	Discontinued permanently	Resolved	Yes	No
9	Headache	24/06/2016	26/06/2016	Mild	Not Related	None	Resolved	Yes	No
10	Sub-conjunctival haemorrhage	16/05/2016	19/05/2016	Mild	Definitely	None	Resolved	Yes	No
10	Sub-conjunctival haemorrhage	22/08/2016	26/08/2016	Mild	Definitely	None	Resolved	Yes	No
13	Bad back	15/08/2016	19/08/2016	Mild	Unlikely	None	Resolved	No	No
14	Viral cold with headache	18/10/2016	27/10/2016	Mild	Not Related	None	Resolved	No	No
14	Corneal abrasion	24/06/2016	25/06/2016	Mild	Definitely	None	Resolved	Yes	No
15	Grittiness/Blurring using laptop	01/10/2016	18/10/2016	Mild	Unlikely	None	Resolved	No	No
16	Conversion of prostate biopsy from benign to low-grade neoplasia	20/09/2016		Mild	Unlikely	None	AE ongoing	No	No
16	Vision is not as sharp	04/07/2016	06/12/2016	Mild	Possibly	None	AE ongoing	No	No

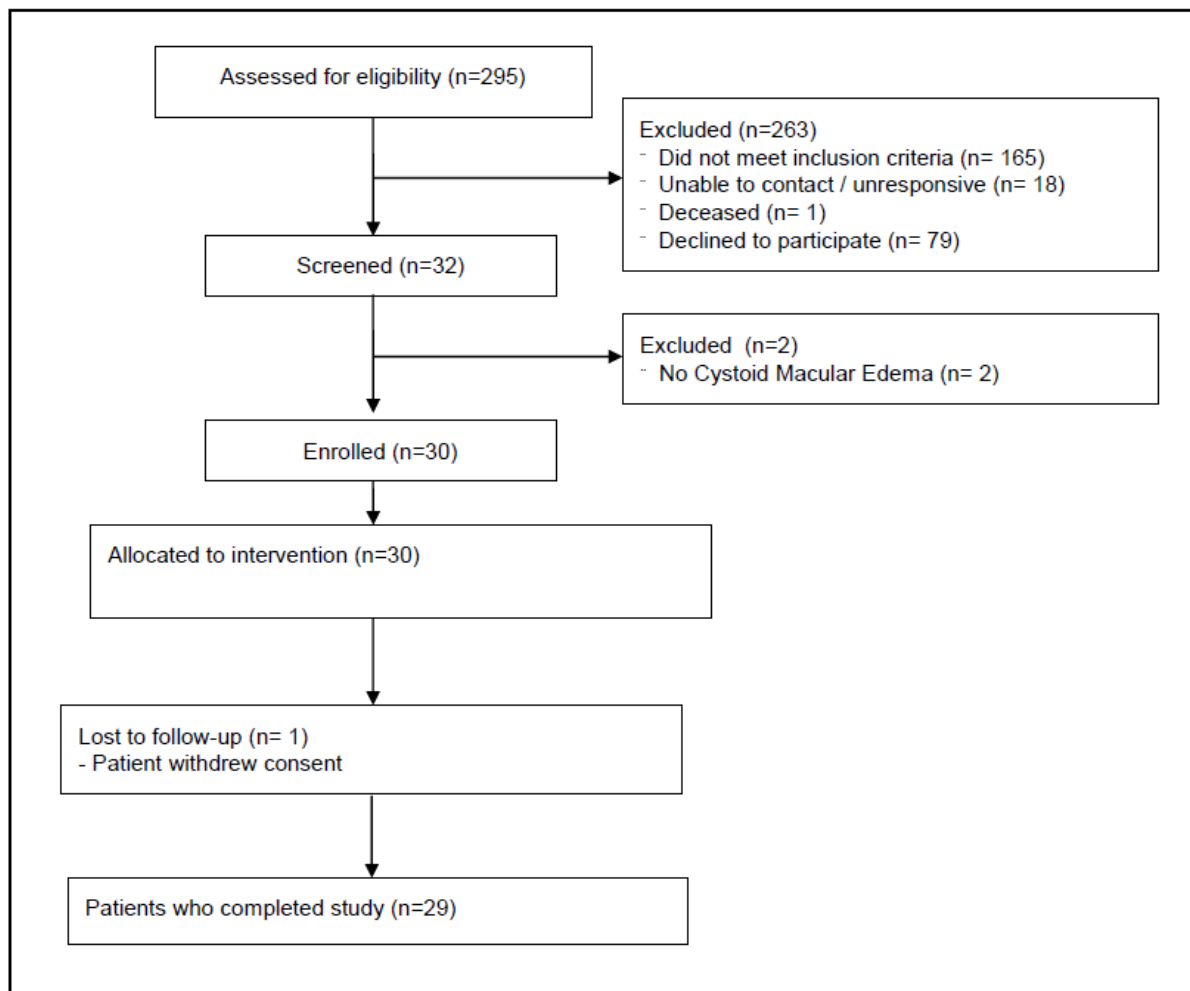
Supplementary table 8: Ocular and Non-Ocular Adverse Events (AEs) and Serious Adverse Events (SAEs) – 6- 12 months after baseline

Study ID	Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious
4	Perforated Ear Drum	26/02/2017	03/03/2017	Mild	Not Related	None	Resolved	No	No
4	Ear Infection	26/02/2017	03/03/2017	Mild	Not Related	None	Resolved	No	No
4	Ear Infection	03/04/2017	09/04/2017	Mild	Not Related	None	Resolved	No	No
4	Viral cold	03/12/2016	05/12/2016	Mild	Not Related	None	Resolved	No	No
4	Vision not as clear	15/11/2016	06/12/2016	Mild	Definitely	None	Resolved	Yes	No
11	Subconjunctival haemorrhage	14/12/2016	17/12/2016	Mild	Definitely	None	Resolved	Yes	No
15	Low Mood	20/05/2017		Mild	Not Related	None	AE ongoing	No	No
15	Viral cold	13/12/2016	18/12/2016	Mild	Not Related	None	Resolved	Yes	No
16	Floater Right Eye	20/04/2017	27/04/2017	Mild	Definitely	None	Resolved	Yes	No
16	Grittiness after injection	06/02/2017	12/02/2017	Mild	Definitely	None	Resolved	Yes	No
16	Dry ocular surface	19/04/2017	20/04/2017	Mild	Definitely	None	Resolved	Yes	No
17	Viral Gastric Bug	09/04/2017	11/04/2017	Mild	Not Related	None	Resolved	No	No
17	Reduced central vision due to progression of underlying disease	12/05/2017		Moderate	Unlikely	Discontinued permanently	AE ongoing	Yes	No
21	Posterior vitreous detachment	17/03/2017		Mild	Possibly	None	AE ongoing	Yes	No
21	Anxiety	03/03/2017		Mild	Not Related	None	AE ongoing	No	No
21	Corneal abrasion + dry cornea	13/01/2017		Mild	Definitely	None	AE ongoing	Yes	No
27	Punched in the face just below RE (no sequelae)	20/03/2017	20/03/2017	Mild	Not Related	None	Resolved	No	No
27	Viral illness	21/01/2017	31/01/2017	Mild	Not Related	None	Resolved	Yes	No
28	Posterior vitreous detachment	16/02/2017		Mild	Possibly	None	AE ongoing	Yes	No
28	Vitreous floater	05/06/2017		Mild	Definitely	None	AE ongoing	Yes	No
30	Feeling low	17/02/2017	28/02/2017	Mild	Not Related	None	Resolved	Yes	No
30	Right eye posterior sub-capsular cataract (non study eye)	17/02/2017		Mild	Not Related	None	AE ongoing	Yes	No

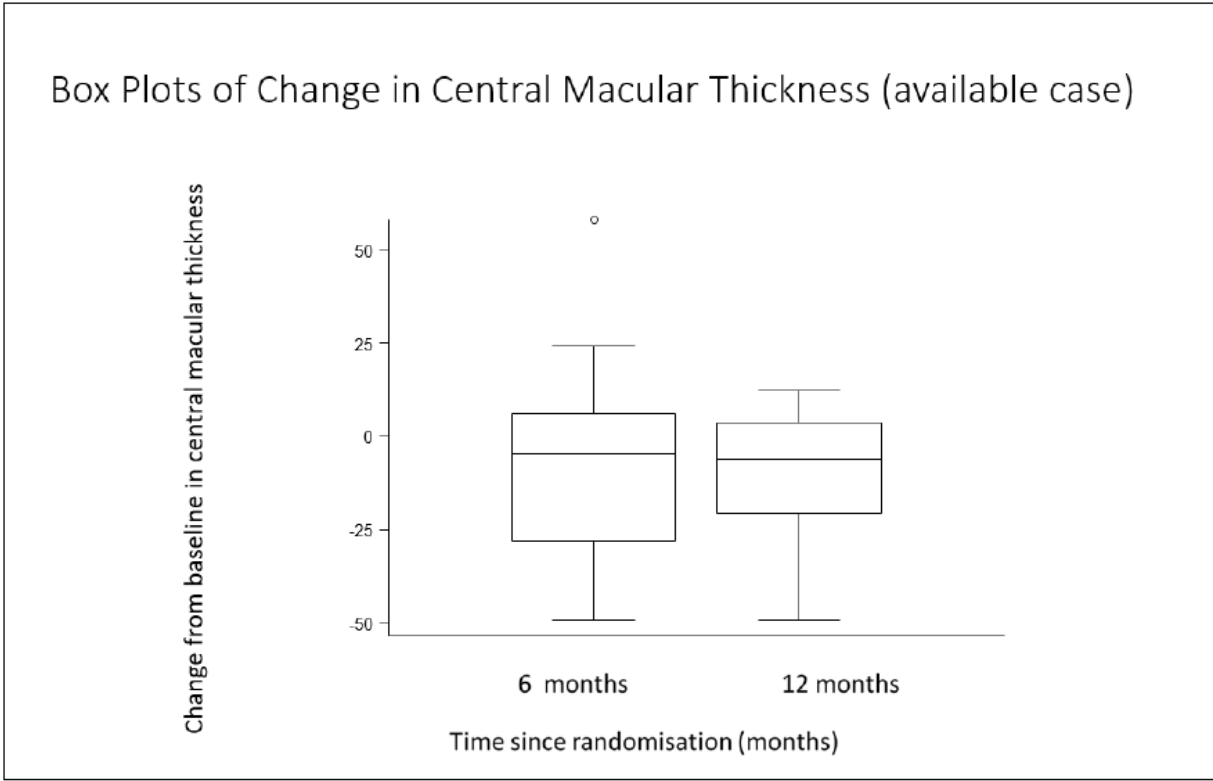
Supplementary Table 9: Ocular and Non-Ocular Adverse Events (AEs) and Serious Adverse Events (SAEs) – More than 12 months after baseline

Study ID	Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious
11	Feeling tired from fasting	05/06/2017		Mild	Not Related	None	AE ongoing	No	No
15	Vitreous Floaters	16/06/2017		Mild	Possibly	None	AE ongoing	Yes	No
17	Reduced central vision	25/10/2017	12/05/2017	Mild	Possibly	Discontinued permanently	Resolved	No	Yes
27	Exacerbation of mental health illness	17/11/2017	23/11/2017	Mild	Unlikely	None	Resolved	No	No
30	Viral illness	05/12/2017	12/12/2017	Mild	Not Related	Delayed Dose	Resolved	Yes	No

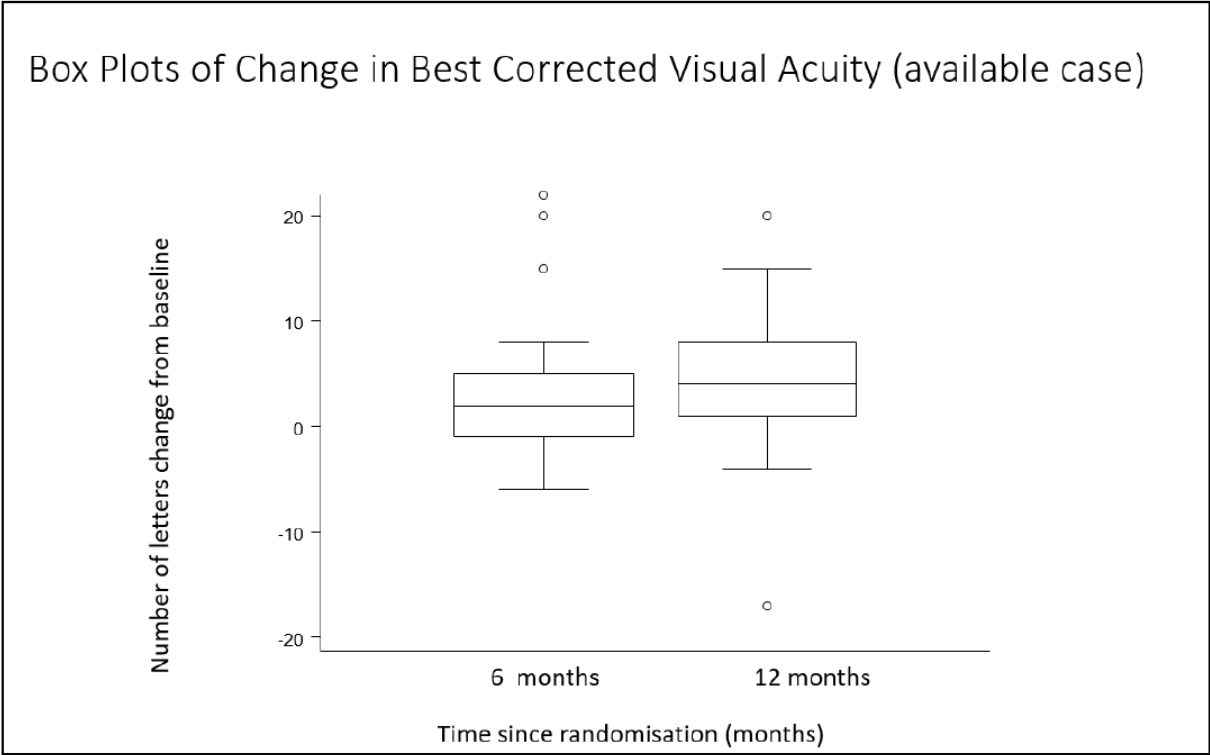
Supplementary figure 1: Consort flow diagram



Supplementary figure 2 : A graph demonstrating mean change in CMT from baseline to 6 months after baseline, and baseline to 12 months after baseline in the group overall (n=29).



Supplementary figure 3 : Box plots of change in BCVA demonstrating mean change in BCVA from baseline to 6 months after baseline, and baseline to 12 months after baseline in the group overall (n=29).



Citation:

Strong SA, Hirji N, Quartilho A, Kalitzeos A, Michaelides M. Retrospective cohort study exploring whether an association exists between spatial distribution of cystoid spaces in cystoid macular oedema secondary to retinitis pigmentosa and response to treatment with carbonic anhydrase inhibitors. Br J Ophthalmol. 2018.

Full title:

A retrospective cohort study exploring whether an association exists between spatial distribution of cystoid spaces in cystoid macular oedema secondary to Retinitis Pigmentosa and response to treatment with carbonic anhydrase inhibitors.

Abstract

Background: Carbonic anhydrase inhibitors (CAIs) are frequently used as an initial step to treat Retinitis Pigmentosa-associated cystoid macular oedema (RP-CMO). Interestingly, it has been postulated that CAIs might reduce outer nuclear layer (ONL) fluid more effectively than inner nuclear layer (INL) fluid due to better access to retinal pigment epithelium basolateral membrane than neurosensory retina. This retrospective cohort study explores if an association between spatial distribution of cystoid spaces in RP-CMO and CAI response exists.

Methods: Two independent graders reviewed pre- and post-treatment OCT images of 25 patients (43 eyes) initiated on topical and/or oral CAI's between January 2013 and December 2014. Documentation

included the presence/absence of: fluid (and layer(s) involved), external limiting membrane, epiretinal membrane (ERM), vitreomacular adhesion/traction, lamellar/full-thickness macular hole and central macular thickness (CMT)/volume.

Results: INL fluid was found in all study eyes. All 13 'responders' (at least 11% reduction of CMT after treatment) demonstrated pre-treatment ONL fluid. In 7 patients (4 responders and 3 non-responders) complete clearance of ONL fluid was achieved despite persistence of INL fluid. ERM presence was similar in responders and non-responders.

Conclusion: In this study, INL fluid was found to be the most common spatial distribution of RP-CMO. However, patients who were classed as a 'responder' to CAI treatment, all demonstrated co-existing ONL fluid on their pre-treatment OCT scan. This may be explained by CAIs having better access to retinal pigment epithelium basolateral membrane than neurosensory retina. Our study also suggests a minimal impact on response to CAIs by epiretinal membrane.

Introduction

Inherited retinal disease (IRD) is the leading cause of blindness certification in the working age population (age 16-64 years) in England and Wales ¹. Retinitis Pigmentosa (RP) is the most common group of IRD, causing progressive centripetal reduction of vision and associated with variants in over 80 genes to date, accounting for approximately 50-60% of cases, with an autosomal recessive, autosomal dominant or X-linked mode of inheritance ². Secondary complications associated with RP include cataracts and RP-associated cystoid macular oedema (RP-CMO), which further contribute to reduction of visual acuity ³. RP-CMO has been reported to occur in 10 - 50% of patients ⁴⁻⁷.

Proposed mechanisms for RP-CMO include: 1) breakdown of the blood-retinal barrier (BRB), 2) failure (or dysfunction) of the pumping mechanism in the retinal pigment epithelium (RPE), 3) Müller cell oedema and dysfunction, 4) anti-retinal antibodies and 5) vitreous traction ⁸.

Makiyama et al ⁹ used spectral domain optical coherence tomography (SD-OCT) to investigate the prevalence and spatial distribution of cystoid spaces (CS) in RP. Seventy-four of 275 patients (27%) demonstrated RP-CMO in at least one eye. Inner nuclear layer (INL) CS were observed in 99% of eyes with CMO. It is of note that Müller cell bodies reside in the INL, providing support for the aforementioned Müller cell oedema /dysfunction hypothesis. The outer nuclear layer (ONL)/outer plexiform layer was involved in 28% and ganglion cell layer involved in 7%. Interestingly, 79% of CS were located in areas of relatively well-preserved outer retina in keeping with the observation that CMO is seen more commonly in less advanced RP compared to late stage RP ⁹.

Several case-reports and small-scale studies have been published regarding the safety and efficacy of carbonic anhydrase inhibitors (CAIs) in the treatment of RP-CMO, however, to-date there is currently no level 1 evidence to support this ⁸. In the largest retrospective study to date of CAIs involving 81 patients (157 eyes) with RP-CMO, objective improvement on OCT was observed in 53% of patients (40% of eyes) using topical dorzolamide versus 41% of patients (28% of eyes) using oral acetazolamide ¹⁰. CAIs are associated with a variety of potential side effects, including: fatigue, loss of appetite, limb paraesthesia, kidney stones, aplastic anaemia, hypokalaemia and cardiac arrhythmia ¹¹. Their mechanism of action is through inhibition of the enzyme carbonic anhydrase IV (CA IV), resulting in acidification of the sub-retinal space, increased chloride ion transport, and subsequent movement of water across the RPE into the choroid ¹².

Acetazolamide cannot readily enter the neurosensory retina, yet has good access to the RPE basolateral membrane¹³. It has been previously suggested that CMO with RPE pathology may respond better to CAIs than CMO with retinal capillary pathology¹³⁻¹⁵

We have therefore undertaken a retrospective cohort study designed to determine if there is an association between the spatial distribution of CS in RP-CMO and response to CAIs.

Materials and methods

This retrospective cohort study identified all patients seen at Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom between January 2013 and December 2014 with 'Retinitis Pigmentosa' and 'Cystoid Macular Oedema' appearing in their electronic patient record. This time period was chosen as it was deemed a manageable period of time and number of scans to analyse. This initial search identified 103 patients, however, after review of each patient record, 78 patients were excluded from the study due to having 'no' cystoid macular oedema. This study was IRB approved.

Patients were subsequently included in the study if they met the following inclusion criteria (irrespective of age): (i) Confirmed diagnosis of RP-CMO; (ii) Unilateral or bilateral (if bilateral, each eye was evaluated individually) RP-CMO; (iii) Commenced on treatment with either a topical and/or oral CAI between January 2013 and December 2014; (iv) Pre-treatment SD-OCT scan acquired within 2 weeks of initiating treatment AND post-treatment SD-OCT scan acquired between 3 – 9 months after initiation of treatment. This time period was chosen because whilst patients in our clinics are typically reviewed at 3 – 4 months after treatment initiation, their appointments may be postponed due to various factors.

Patients were excluded if any of the following applied: (i) A diagnosis of CMO not considered to be related to RP; (ii) Other treatment for RP-CMO received within 3 months of initiation of CAI e.g.

intravitreal injection of triamcinolone/anti-VEGF agent; (iii) Pre-treatment SD-OCT scan not acquired within 2 weeks of initiating treatment and/or post-treatment OCT scan acquired greater than 9 months from initiation of treatment.

After accounting for the inclusion and exclusion criteria, the total number of patients included in the study totalled 25. Of these, 18 had bilateral RP-CMO and 7 had unilateral RP-CMO; 43 eyes graded in total.

SD-OCT (Spectralis, Heidelberg Engineering Ltd, Heidelberg, Germany) was undertaken in all recruited subjects. All patients across the cohort underwent scan acquisition according to the standard of care procedure used for out-patient clinics at Moorfields Eye Hospital, London.

Image Grading

Two independent graders experienced in SD-OCT interpretation graded pre-and post-treatment scans (SS, NH). Both graders were blinded to the treatment that each patient received and whether they were classed as a 'responder' or not.

Each grader began by performing re-centration of the images if deemed necessary. The following variables were graded for their presence within 3600 microns of the foveal centre: sub-retinal fluid (SRF), inner nuclear layer (INL) fluid, outer nuclear layer (ONL) fluid, ganglion cell layer fluid, epi-retinal membrane (ERM), vitreo-macular adhesion (VMA), vitreo-macular traction (VMT), lamellar macular hole and full-thickness macular hole (FTMH). Each of these variables was graded as either: present (>90% certainty), questionably present (50-90% certainty), absent (<50% certainty) or ungradable. The presence of external limiting membrane (ELM) within 1200 microns of the foveal centre was also graded

and a comment made whether it was felt to be intact throughout or disrupted. A further 'yes' or 'no' response was required for the grading of whether there was felt to be intact ELM and/or fluid present (in any lamination) directly under the foveal centre. Pre- and post- treatment CMT and macular volume were also documented.

If both graders agreed on a variable, the grading was complete. If the second grader (NH) disagreed with the first grader (SS), adjudication was performed by a consultant retinal specialist (MM). CMT values were considered to be in agreement if graded within 50 microns of each other. Macular volume values were considered to be in agreement if graded within 1.5mm³ of each other. The mean of the grader scores for CMT was used for analysis. Patients were considered to have responded to treatment if they achieved a CMT reduction of 11.0% or greater in keeping with previous studies^{16,17}. Although these studies were published over 8 years ago, no studies have since been published providing an alternative percentage reduction of CMT. We therefore chose this figure to allow comparison with other studies.

Pre- and post-treatment visual acuity was also documented.

Statistical Analysis

Descriptive statistics have been used to describe the results of the CARMEL study. For categorical variables, Kappa statistic was computed with respective 95% confidence interval (CI) for assessing inter-rater agreement as Kappa is thought to be a more robust measure than simple percent agreement (Kappa takes into account the possibility of agreement occurring by chance). For continuous variables Bland-Altman agreement methods were used to quantify limits of agreement (LoA). Analysis was performed in STATA version 13 (STATA Corp., Texas, USA) and 95% CIs for Kappa were computed using the kapci package using 1000 bootstrap replicates.

Results

Forty three eyes (22 right; 21 left); were included in the study consisting of 18 patients with bilateral RP-CMO and 7 patients with unilateral RP-CMO. Seventeen of these patients were male and 8 were female. Median age was 48 and ranged between 17 and 79 years. Four out of 43 (9.3%) eyes were treated with oral acetazolamide 250mg twice a day versus 39 out of 43 (90.7%) of eyes treated with topical dorzolamide or brinzolamide three times a day. All 43 eyes in the study were graded as having INL fluid present on their pre-treatment OCT scan. Thirty three out of 43 eyes (76.7%) in the study demonstrated co-existing ONL fluid present on their pre-treatment OCT scan. Eleven out of 43 eyes (25.6%) in the study demonstrated co-existing RGC layer fluid present on their pre-treatment OCT scan. No patients demonstrated the presence of sub-retinal fluid.

Estimates of agreement for all variables assessed by the two graders are presented in Table 1, Figure 1 and Figure 2. No single variable was found to have poor agreement. No evidence of bias was found in terms of inter-rater agreement for pre-treatment CMT, mean difference -0.74. 95% CI (-3.12, 1.63). Inter-rater LoA were -16.18 to 14.69 for pre-treatment CMT and -18.36 to 21.76 for post-treatment CMT, which was considered by the chief investigator to be acceptable. Inter-rater agreement was -0.91 to 1.14 for pre-macular volume and -1.22 to 1.24 for post-macular volume, which was considered by the chief investigator to be acceptable with no evidence of bias.

Figure 1 Bland-Altman graph for pre-CMT inter-rater agreement. CMT, central macular thickness.

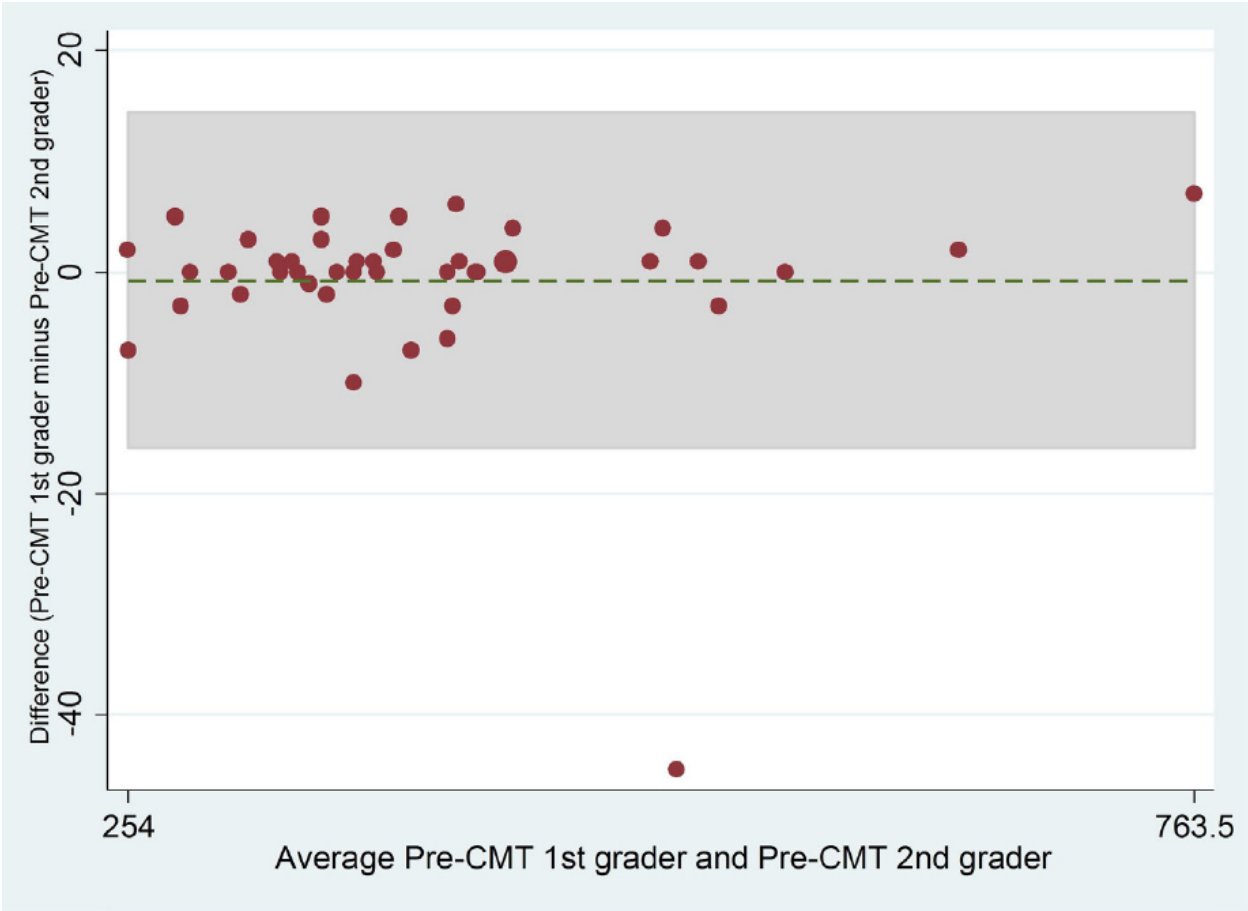
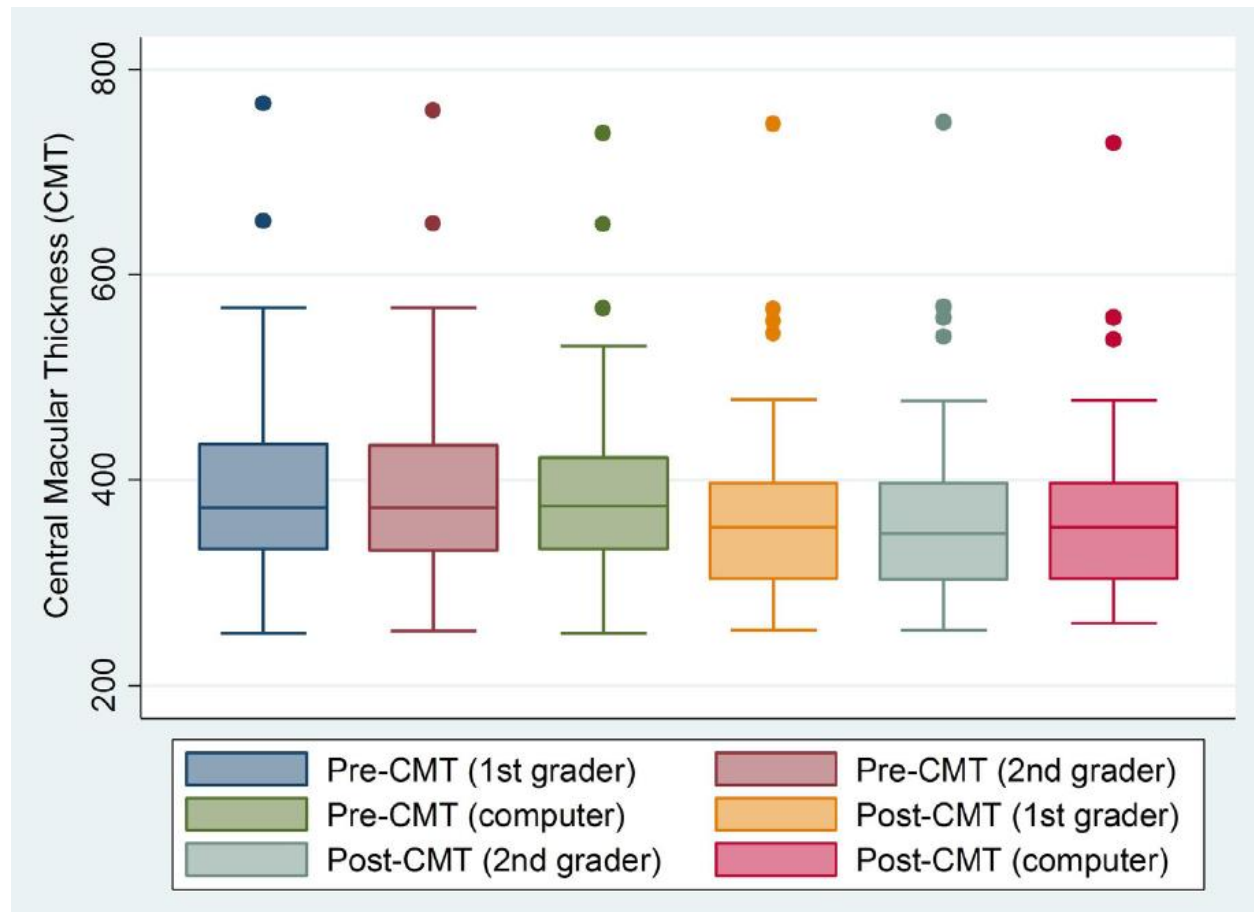


Figure 2 Box plot illustrative of pre-CMT and post-CMT measurements made by the two graders. CMT, central macular thickness.



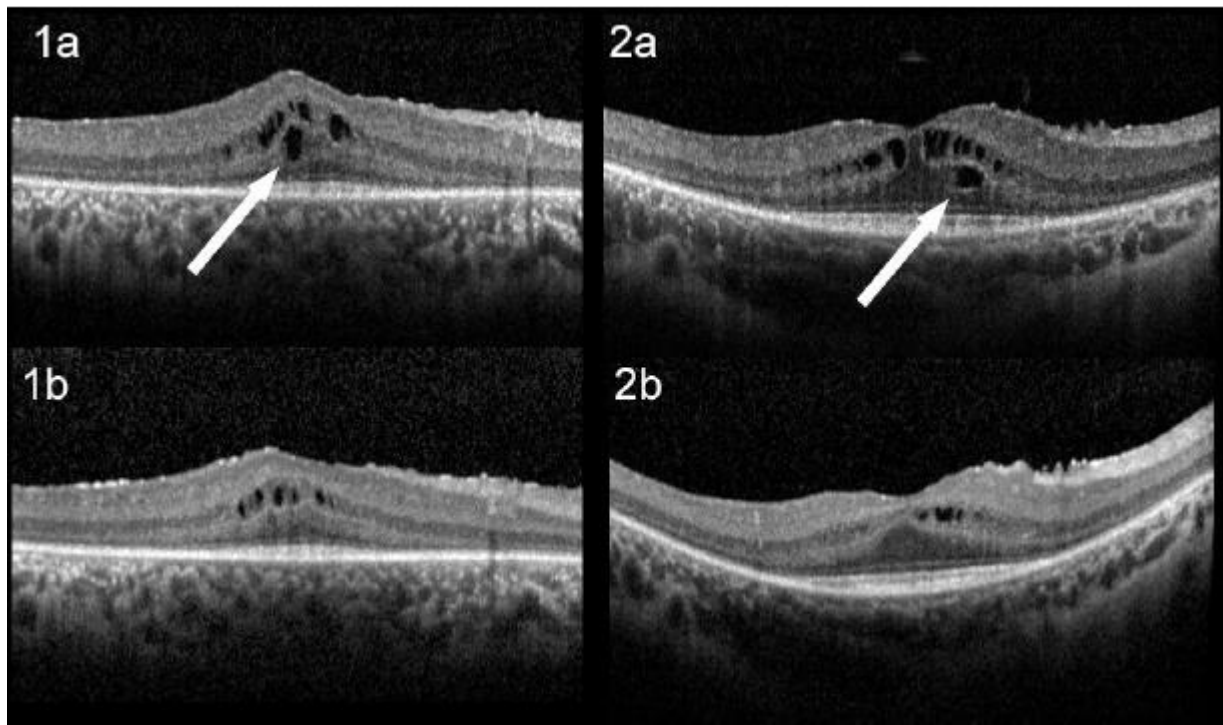
Out of 43 eyes that were graded, 13 (30.2%) were classed as ‘responders’ having achieved a CMT reduction following treatment of at least 11%. All 13 responders demonstrated ONL fluid on their pre-treatment OCT and the presence of fluid (in any layer) directly under the fovea. ERM was ‘definitely present’ in 8 out of 13 (61.5%) responders and ‘questionably present’ in 2 out of 13 (15.4%) responders. No responder demonstrated VMA, VMT or a FTMH on their pre-treatment OCT scan. ELM was considered to be present (intact or disrupted) within 1200 microns of the fovea and present directly under the fovea in all but 1 responder (92.3%). Best corrected visual acuity (BCVA) improved by at least 10 ETDRS letters in 2 out of 13 (15.4%) responders. No responders demonstrated a loss of 10 or more

ETDRS letters. The remaining 11 out of 13 (84.6%) responders demonstrated no change in their BCVA following treatment. Interestingly, 4 out of 13 (30.8%) responders demonstrated total clearance of ONL fluid on their post-treatment OCT scan whilst INL cysts remained

Out of 30 non-responders, 20 (66.7%) eyes had ONL fluid on their pre-treatment OCT compared to 10 (33.3%) eyes without ONL fluid on their pre-treatment OCT. Sixteen out of 30 (53.3%) non-responder eyes had fluid (in any layer) directly under the fovea. Interestingly, there were three non-responders that demonstrated total clearance of ONL fluid on their post-treatment OCT scan whilst INL cysts remained (see Figure 3). VMA was definitely present in 7 out of 30 (23.3%) non-responders and questionably present in 2 out of 30 (6.7%) non-responders. ELM was considered to be present (intact or disrupted) within 1200 microns of the fovea in 26 out of 30 (86.7%) non-responder eyes, however, was only present directly under the fovea in 20 out of 30 (67.0%) eyes. BCVA improved by at least 10 ETDRS letters in 2 out of 30 (6.7%) non-responders. No non-responders demonstrated a loss of 10 or more ETDRS letters. The remaining 28 out of 30 (93.3%) non-responders demonstrated no change in their BCVA following treatment.

Of note, all 4 patients (2 responders and 2 non-responders) who gained at least 10 ETDRS letters of BCVA, demonstrated ONL fluid on their pre-treatment OCT scans.

Figure 3 Two examples where ONL cysts have disappeared following treatment with CAIs, yet INL cysts remain. 1a and 2a: pretreatment SD-OCT scans of two separate patients who were classed as a ‘non-responder’ (the white arrows demonstrate the presence of ONL fluid cysts). 1b and 2b: post-treatment SD-OCT scans with absence of ONL fluid cysts, yet INL cysts remain. CAIs, carbonic anhydrase inhibitors; INL, inner nuclear layer; ONL, outer nuclear layer; SD-OCT, spectral domain optical coherence tomography.



Discussion

In keeping with Makiyama et al ⁹, this study observed an overall higher frequency of INL compared to ONL fluid in patients with RP-CMO ⁹. It was interesting to note that 100% of responders demonstrated ONL fluid on their pre-treatment OCT; however, not every patient with pre-treatment ONL fluid responded. The presence of ERM graded as either ‘questionably present’ or ‘definitely present’ was similar in both groups (10 out of 13 (77.0%) responder eyes versus 24 out of 30 (80.0%) non-responder

eyes) and therefore does not appear to significantly affect response to treatment in our cohort. In contrast, the presence of VMA graded as either 'questionably present' or 'definitely present' was greater in the non-responder group (0 out of 13 (0.0%) responder eyes versus 9 out of 30 (30.0%) non-responder eyes) and may therefore play a role in limiting response to treatment as being indicative of a tractional component that would not be expected to respond to CAIs.

Overall, there were seven patients (including 'responders' and 'non-responders') that demonstrated total clearance of ONL fluid on their post-treatment OCT scan despite persistence of INL cysts. Our working hypothesis to explain this response includes the closer anatomical proximity of ONL fluid to the RPE where the action of CAI's take place. A greater percentage of eyes in the responder group demonstrated at least 10 ETDRS letter improvement of BCVA compared to the non-responder group (15.4% of responders versus 6.7% of non-responders). It is not uncommon for anatomical improvement to occur without significant functional improvement¹⁸. Factors such as underlying photoreceptor loss and/or chronicity of RP-CMO may have an effect on visual outcome^{8,18}. Whilst it was noted that patients with visual gain demonstrated ONL fluid on their pre-treatment OCT scans, a larger study with appropriate statistical analysis is required to be able to qualify whether this observation is significant. In 2015, Liew et al¹⁰ conducted the largest retrospective study to date involving 81 patients (157 eyes) with RP-CMO. A positive response to treatment was only observed in 53% of patients (40% of eyes) using topical dorzolamide and 41% of patients (28% of eyes) using oral acetazolamide¹⁰. CAIs are also associated with side-effects such as: tingling/numbness of the limbs, fatigue, renal stones, aplastic anaemia, hypokalaemia and cardiac arrhythmia¹¹. To this end, it would be valuable to identify factors that might help predict response of RP-CMO to CAI treatment in order to tailor patient care appropriately. Whilst this study might suggest the presence of ONL fluid on pre-treatment OCT scan to

be a positive prognostic factor in the treatment of RP-CMO, we are limited by design and numbers to be able to provide accurate statistical analysis and it must therefore be interpreted as observation only

There is currently no level 1 evidence for the treatment of RP-CMO. The following recommendations are therefore based on expert opinion:

- 3) Initial treatment using a topical CAI and review after 4-6 months.
- 4) a) After 4-6 months, if there has been minimal (but not significant) reduction of CMT or improvement of BCVA, options may include switching to a different topical CAI agent or oral CAI.
b) After 4-6 months, if there has been no reduction of CMT or improvement of BCVA or if the spatial distribution of cysts are located only in the INL, consideration should be given to trialling alternative therapeutic options, such as: topical non-steroidal anti-inflammatory agents or considering intravitreal anti-VEGF (although these latter agents are currently unlicensed for RP-CMO).

Although there was only a limited vision-improving effect observed in this study, we still believe in treating RP-CMO to achieve anatomical improvement to prevent irreversible structural damage as well as to potentially decelerate underlying photoreceptor loss⁸.

Studies currently being undertaken for the treatment of RP-CMO include: The AMOUR study to assess the safety and efficacy of aflibercept for RP-CMO (clinicaltrials.gov identifier NCT02661711).

Our study has several limitations including the small cohort size, with further studies with larger patient numbers required in order to support our findings of OCT phenotypes that may help predict treatment

response in patients with RP and thereby better inform patient counselling and management in this highly genetically heterogenous retinal dystrophy.

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Citation:

Strong S, Liew G, Michaelides M. Retinitis pigmentosa-associated cystoid macular oedema: pathogenesis and avenues of intervention. Br J Ophthalmol. 2017;101(1):31-7.

Full title:

Retinitis Pigmentosa-associated Cystoid Macular Oedema: Pathogenesis and Avenues of Intervention

Abstract

Hereditary retinal diseases are now the leading cause of blindness certification in the working age population (age 16-64 years) in England and Wales, of which Retinitis Pigmentosa (RP) is the most common disorder.

RP may be complicated by cystoid macular oedema (CMO), causing a reduction of central vision. The underlying pathogenesis of RP-associated CMO (RP-CMO) remains uncertain, however, several mechanisms have been proposed, including: 1) Breakdown of the blood-retinal barrier, 2) Muller cell oedema and dysfunction, 3) Anti-retinal antibodies, and 4) Vitreous traction. There is limited data on efficacy of treatments for RP-CMO. Treatments attempted to date include, oral and topical carbonic anhydrase inhibitors, oral, topical, intravitreal and periocular steroids, topical non-steroidal anti-inflammatory medications, photocoagulation, vitrectomy with internal limiting membrane peel, oral lutein, and intravitreal anti-vascular endothelial growth factor injections. This review summarises the evidence supporting these treatment modalities.

Successful management of RP-CMO should aim to improve both quality and quantity of vision in the short term and may also slow central vision loss over time.

Introduction

Cystoid macular oedema (CMO) may complicate Retinitis Pigmentosa (RP) and has been reported to occur in 10 - 50% of patients (1-4).

Hereditary retinal diseases are now the leading cause of blindness certification in the working age population (16-64 years) in England and Wales, of which RP is the most common disorder (5). RP causes nyctalopia and progressive peripheral visual field loss, with particular disability experienced when disease progression results in central visual compromise. One important treatable cause of central vision loss is RP-associated CMO (RP-CMO) (6). A better understanding of the underlying mechanisms and response to treatments of RP-CMO is required to facilitate better targeted and more efficacious therapies. In this review we will discuss the pathogenesis of RP-CMO and the multiple avenues of intervention that have been investigated or being considered.

Pathogenesis

No single aetiology has been attributed to the overall cause of RP-CMO. Whilst we describe several individually proposed mechanisms, it is plausible that RP-CMO may result from a combination of these.

- Breakdown of the blood-retinal barrier

The blood-retinal barrier (BRB) exists to maintain homeostasis via the highly selective diffusion and active transport of molecules into and out of the retina thus preventing extravascular accumulation of fluid within the retina (7). This is achieved in two ways: (i) an outer barrier of apical tight junctions between retinal pigment epithelial (RPE) cells (8, 9), and (ii) an inner barrier of tight junctions between vascular endothelial cells (10). CMO can occur from BRB breakdown secondary to RPE and/or endothelial damage/dysfunction.

Studies have investigated whether one barrier is more affected than the other in order to better focus potential therapies. Viores et al used immunolocalisation of endogenous albumin to highlight areas of extravasation in normal eyes compared to those with RP (11). In eyes with RP alone, albumin leakage was greatest from the inner barrier (11). In RP-associated with other ocular complications (e.g. aphakia, glaucoma), leakage varied between the inner and outer barriers. No correlation was found between severity of photoreceptor degeneration and albumin leakage (11) suggesting that therapies for RP-CMO could be used regardless of underlying disease status.

The release of 'toxic products' from degenerating retina/RPE may cause RP-CMO by disrupting the BRB (12). In keeping with CMO observed in other disorders, RP-CMO has been associated with release of vascular endothelial growth factor (VEGF), adenosine (7), prostaglandins (13), histamine (14), Insulin-like growth factor 1 (15), tumour necrosis factor alpha and interleukin 1 alpha and beta (16).

- Failure (or dysfunction) of the pumping mechanism in the RPE

An important function of the RPE is to pump fluid out from the sub-retinal space in order to maintain the negative hydrostatic pressure required for adhesion between RPE and photoreceptors (17). Under

normal conditions, Cl⁻ enters the RPE cell via Na⁺/K⁺ ATP-ase located on the apical membrane and exits via Cl⁻ channels on the basolateral membrane. It is this active transport that drives water through aquaporin channels from the sub-retinal space into the choriocapillaris. It may be that failure (or dysfunction) of this pumping mechanism occurs in RP, thus resulting in RP-CMO. The presence of CMO has been suggested to result in loss of polarised distribution of membrane-bound carbonic anhydrase (CA) IV in the RPE, which further contributes to RP-CMO (10).

- Muller cell oedema and dysfunction

The Muller cell is essential for visual transduction and retinal homeostasis, including fluid dynamics. Water enters the retina by two routes: directly from the blood, coupled to glucose up-take and/or as a by-product of aerobic metabolism; with the bidirectional movement of water osmotically coupled to the transport of osmolytes such as potassium (18).

Potassium ions are released by activated retinal neurons. To prevent a build-up and potential excitotoxicity, potassium is passively taken up into Muller cells via inwardly-rectifying channels (Kir2.1) with release of potassium occurring via Kir4.1 channels (18). Kir channels consist of two transmembrane regions with cytosolic NH₂ and COOH termini connected by a pore-forming loop. Molecules such as Mg²⁺ and polyamines are able to physically block this channel pore from allowing outward movement of K⁺, while still able to accept inwards movement of K⁺ (19). Under pathological conditions such as inflammation and oxidative stress, Kir4.1 channels redistribute, becoming more evenly spread throughout the Muller cell. Kir2.1 channels do not redistribute, however, resulting in intracellular potassium overload, increased osmotic pressure within the Muller cell, reduction in water efflux and ultimate Muller cell swelling (18).

Makiyama et al used optical coherence tomography (OCT) to investigate the prevalence and spatial distribution of cystoid spaces (CS) in patients with RP. Seventy-four of 275 patients (27%) demonstrated RP-CMO in at least one eye. Inner nuclear layer (INL) CS were observed in 99% of eyes with CMO. The outer nuclear layer (ONL)/outer plexiform layer was involved in 28% and ganglion cell layer involved in 7% (20). Muller cell bodies reside in the INL, which supports the hypothesis of Muller cell swelling and dysfunction. Interestingly, 79% of CS were located in areas of relatively well-preserved outer retina (20); in keeping with the observation that CMO is seen more commonly in less advanced RP compared to late stage RP.

- Anti-retinal antibodies

Serum levels of immunoglobulins G, A and M have been investigated in 52 patients with RP compared to 40 controls. Higher levels of IgM were found in patients with RP compared to controls (21). Spiro et al, however, found no difference in IgM levels between 75 patients with RP and 51 controls (22). Spalton et al performed immunological studies on 17 RP patients with central and/or peripheral vascular leakage observed on fluorescein angiogram (FA). Five out of 17 patients had raised IgM unrelated to degree of vascular leakage. All patients demonstrated positive immunofluorescence to rat photoreceptors at 1:5 dilution of serum, however, this could be attributed to cross reactivity of smooth muscle antibodies with photoreceptor contractile organelles (12).

Anti-retinal antibodies have been prospectively studied in 30 RP patients with CMO compared to 30 RP patients without. Anti-retinal antibodies were found in 27 of 30 RP-CMO patients compared to 4 of 30 RP patients without CMO (23). Nevertheless, the role of anti-retinal antibodies in RP progression or RP-CMO remains unclear, with many unanswered questions including whether they are a secondary

consequence of the degenerative process, the wide range of auto-antibodies identified, and the high prevalence in normal controls (23, 24).

- Vitreous traction

It has been suggested that vitreous traction and epiretinal membrane contributes to RP-CMO by causing mechanical damage to Muller cells, an inflammatory reaction with subsequent capillary dilatation and leakage (25, 26). Schepens et al and Takezawa et al have reported cases of RP-CMO in the presence of vitreous traction (26, 27).

Diagnosis and monitoring of RP-CMO

Prior to the advent of OCT, monitoring RP-CMO included slit-lamp biomicroscopy together with FA. OCT has since been shown to be more sensitive in detecting macular oedema compared to biomicroscopy in patients with diabetic retinopathy and RP-CMO (28, 29). OCT can detect CS in RP-CMO even when little, or no leakage is demonstrated on FA (29, 30) and being non-invasive is ideal for monitoring RP-CMO. No studies have been performed using OCT-A to investigate RP-CMO.

RP-CMO is not always associated with a reduction in visual acuity (VA) (31). Oishi et al found no correlation between total macular thickness and VA (32). The integrity of the inner segment ellipsoid layer, however, has been shown to correlate well with VA and inform the likely prognosis (31-33). Central vision loss in RP-CMO may be due to retinal thinning (from atrophy), thickening (from CMO), or a combination of both (34). Anatomical but not functional improvement following treatment could be due to underlying retinal dysfunction/loss.

Automated static perimetry may also be useful for monitoring RP-CMO given the documented correlation between changes in retinal thickness due to CMO and retinal sensitivity (35-37).

Inheritance patterns and specific associations

Whilst pedigree structures may be informative, definitive conclusions on mode of inheritance can only be made with a molecular diagnosis.

In a retrospective cohort study, CMO was present in 133/581 (23%) Italian patients with RP. This appeared to be significantly associated with autosomal dominant (AD) inheritance (2). In contrast, Liew et al constructed pedigrees for RP-CMO patients in a retrospective cohort and found 55/81 (68%) patients with autosomal recessive (AR) inheritance (4 of whom were molecularly proven) (38).

A family with AD-RP associated with the p.P347L variant in rhodopsin (*RHO*) has been reported, where all four children had bilateral CMO, suggesting this *RHO* mutation may be associated with early-onset CMO (39).

RP-CMO has been associated with female gender (2) and does not appear to be age-related (1).

Avenues of Intervention

Despite RP being the most common inherited retinal degeneration in the working age population, it remains a rare condition with only a portion of these patients developing CMO (5). It can therefore be a

challenge to set up clinical trials targeting RP-CMO and most evidence to date therefore comprises of case reports/series and small prospective/retrospective studies.

We conducted a Pubmed search to include all reports/studies reviewing interventional treatment for RP-CMO published between 1975 and 2016. The search strategy involved the terms 'retinitis pigmentosa', 'rod cone dystrophy', 'retinal dystrophy', 'inherited retinal dystrophy' and 'macular oedema'. We identified 203 publications the abstracts of which were retrieved and reviewed. Inclusion criteria included prospective and retrospective reports/studies using a drug and/or procedure to treat RP-CMO. We also included patients with syndromic RP such as Usher syndrome and those with Coats-like exudation. We excluded patients with MFRP (membrane-type frizzled-related protein)-related nanophthalmos-retinitis pigmentosa-foveoschisis-optic disc drusen syndrome due to its complexity. We identified 45 reports/studies that met our inclusion criteria and retrieved these articles. Whilst preparing this manuscript, a case report published by the authors' became available on-line, which was also included in the studies reviewed, bringing the total up to 46.

Most of the evidence published is in the form of small cohort and case control studies. These may be influenced by publication bias as negative reports are unlikely to be published. There are no RCTs to date which may help alleviate this concern. We did not construct funnel plots or perform other statistical tests for publication bias due to the small number of studies and the highly heterogeneous populations and study designs.

Significant studies that describe interventions in detail or highlight important / interesting points are mentioned in the text. Supplementary table 1 provides an overview of all studies that met our inclusion criteria.

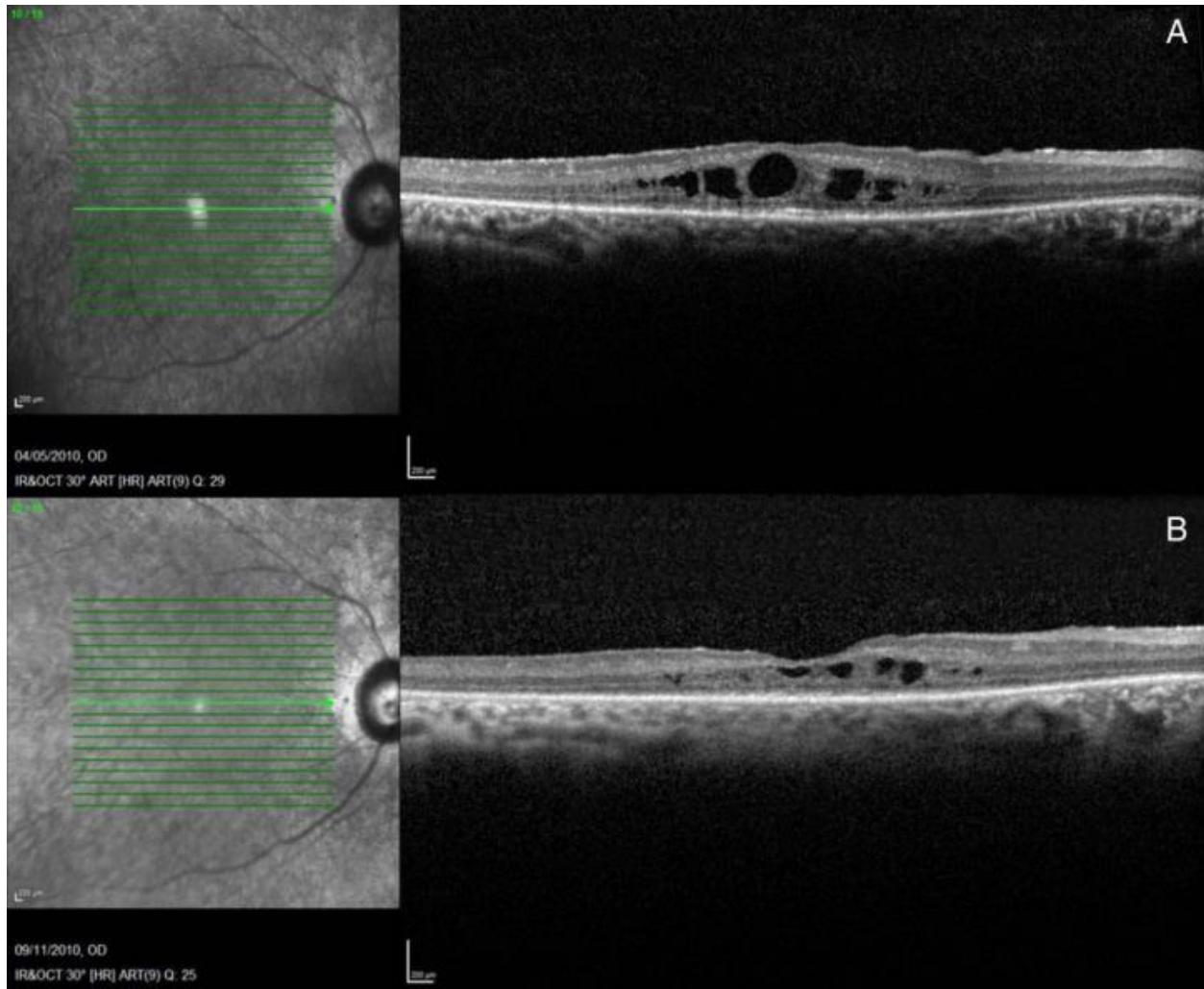
Pharmacological

- Carbonic Anhydrase Inhibitors: Oral and Topical

. Inhibition of CA IV results in acidification of the sub-retinal space, increased chloride ion transport, with subsequent movement of water across the RPE into the choroid (10).

Several studies have shown RP-CMO improvement following treatment with CA inhibitors (CAIs) (38, 40-53). In the largest retrospective study to date involving 81 patients (157 eyes) with RP-CMO, objective improvement on OCT was observed in 53% of patients (40% of eyes) using topical dorzolamide and in 41% of patients (28% of eyes) using oral acetazolamide (see figure 1) (38). VA improved from 6/15 to 6/12 in most patients. AR-RP and greater initial central macular thickness (CMT) predicted better response to treatment.

Figure 1 Spectral domain optical coherence tomography of a man aged 30 years with autosomal recessive retinitis pigmentosa and cystoid macular oedema. (A) Before treatment was initiated (visual acuity (VA) 6/18). (B) Following 6 months usage of topical carbonic anhydrase inhibitors (VA 6/12).



In another retrospective study, 33 eyes (51%) of 20 patients with RP-CMO using topical dorzolamide demonstrated CMT reduction of at least 11% (46). While there are other reports in keeping with these findings (42), some studies have documented improvement of anatomy or leakage on FA only, with little or no corresponding improvement in VA (47-49).

In a prospective, crossover study, 9 out of 17 patients using oral methazolamide demonstrated angiographic improvement of CMO, however, VA improved in at least one eye, by at least 2 lines in only 3 patients (43). Another prospective, masked, crossover study using oral acetazolamide versus placebo

observed VA improvement of at least one line, in at least one eye in 10 out of 12 patients. Three of these patients initiated on placebo only demonstrated improvement once switched to acetazolamide (44).

Location of CS in RP-CMO may influence response to treatment with CAIs. Acetazolamide cannot readily enter the neurosensory retina making it potentially less effective at reducing INL CS (54). However, with good access to the RPE basolateral membrane (41), acetazolamide may be more effective at reducing ONL CS (55).

Rebound CMO has been observed after stopping CAIs (42, 47). Importantly, a restored response has been demonstrated after the re-introduction of CAI treatment following a period of discontinued usage (between 1-6 months) in 3 patients with rebound RP-CMO (53).

Oral CAI's have more side-effects than topical CAIs ranging from fatigue, loss of appetite and limb paraesthesia, to the development of kidney stones, aplastic anaemia and electrolyte disturbance including hypokalaemia with potential associated cardiac arrhythmia (56).

- Steroids: Oral, Periocular and Intravitreal

Steroids reduce the synthesis and release of pro-inflammatory cytokines, including: prostaglandins, leukotrienes, VEGF and intercellular adhesion molecule 1 (57-60). This, together with suppression of inflammatory cell proliferation and migration contributes to improved BRB function and reduction of CMO. Indeed, steroids have been trialled and observed to improve VA and / or CMT in RP-CMO (4, 6, 61-75)..

A one year pilot study using oral deflazacort in 10 patients with RP-CMO reported significant improvements in near VA, retinal sensitivity, and angiographic findings (65). No ocular or systemic side-effects were recorded.

Five patients with RP-CMO underwent unilateral intravitreal injection of 4mg (0.1ml) of triamcinolone acetonide (IVTA). CMT improved from 418 μ m (range 376–626 μ m) at baseline, to 224 μ m (range 214–326 μ m) at 1-month, 275 μ m (range 215–584 μ m) at 3-months and 312 μ m (range 239–521 μ m) at 6-months. VA improved in 2 patients by 1-month but was not maintained. CMO recurrence occurred in 2 patients between 3-6 months (4).

In comparison, a prospective, non-randomised trial compared 20 eyes of 20 RP-CMO patients treated with IVTA with 20 eyes of 20 RP-CMO patients who declined treatment. All treated patients showed anatomical improvement, greatest at 3-months post-injection. No statistical improvement in VA was observed. At day-1 post-IVTA, 10 eyes (50%) had a raised IOP (>21mmHg) including 2 patients (10%) measuring between 30-35mmHg. All IOPs returned to baseline within 6 months (6).

A case report of bilateral RP-CMO refractory to IVTA, reported CMO reduction and VA improvement following bilateral sub-tenon injections of triamcinolone (63). CMO recurred in one eye at 3-months. Intravitreal dexamethasone (DEX implant; Ozurdex, Allergan, Inc., Irvine, CA) has also been used in 3 patients with RP-CMO refractory to oral CAI's and/or sub-tenon triamcinolone and/or topical non-steroidal anti-inflammatory. Two patients required re-treatment at 3-months due to recurrence (73). Another case report demonstrated similar outcomes with initial improvement of CMO and VA, but recurrence at 2-3 months (69).

The use of regular steroids in RP-CMO is significantly limited by their side-effect profile. Ozurdex appears to have a lower incidence of cataract and raised intra-ocular pressure (IOP) compared to IVTA in treatment of retinal vein occlusion-CMO (76, 77), however, the incidence in RP-CMO is unknown.

- Combination of topical Non-Steroidal Anti-Inflammatory (NSAID) together with topical Steroid or topical CAI

A case report using topical steroid (prednisolone acetate 1%) together with topical NSAID (ketorolac trometamol 0.5%) in an 85-year-old lady with unilateral RP-CMO demonstrated CMO resolution and VA improvement at 3-months. CMO recurrence on cessation required re-treatment to good effect (78).

A recent prospective study of 18 patients, randomised 15 eyes to topical ketorolac and 13 eyes to topical dorzolamide for 12-months. No significant change in CMT was observed in either group. VA improved in both groups at 6-months, however, this improvement was lost in the dorzolamide group at 12-months (79).

- Intravitreal Anti-Vascular Endothelial Growth Factor (anti-VEGF)

The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (80). In addition to promoting angiogenesis, VEGF-A reduces endothelial barrier function and increases permeability of choroidal vessels, both of which cause CMO (80).

Salom et al (2008) identified markedly lower levels of VEGF-A in the aqueous humour of 16 eyes of 16 patients with RP (94.9 +/- 99.8 (mean +/- SD) pg/mL) compared to the same number of controls (336.5 +/- 116.8 pg/mL). Relative hyperoxia of the inner retina due to photoreceptor cell death may reduce

VEGF production from retinal cells such as pericytes, endothelial cells, glial cells, Muller cells, and ganglion cells (81).

While no studies have assessed vitreous levels of VEGF in patients with RP or RP-CMO, anatomical and/or functional improvement of RP-CMO has been observed following intravitreal anti-VEGF medication (82-87). Anti-VEGF agents are routinely used to treat CMO and neovascularisation (NV) in multiple retinal diseases. NV is rare in RP; Triolo et al reported choroidal NV in 3 of 176 eyes (2%) with RP (88). Indeed, intravitreal bevacizumab (Avastin, **Genentech**/Roche, South San Francisco, California) has been observed to improve RP-choroidal NV (89, 90). While VEGF may contribute to RP-CMO formation, anti-VEGF medication remains under review as a treatment modality.

- Pegaptanib

A case report using a single intravitreal injection of 0.3mg pegaptanib (**Macugen, Eyetech Pharmaceuticals**, Inc. and **Pfizer** Inc, New York, NY) together with oral acetazolamide in a 33-year-old male with RP-CMO demonstrated improvement of CMT and VA, which was maintained at 4-months (82).

- Bevacizumab

Bevacizumab has been used off-label to treat RP-CMO with varying results. Melo et al observed neither anatomical nor functional improvement in 2 eyes of 2 patients following treatment with a single injection of intravitreal 1.25mg bevacizumab: Case 1 maintained VA of 20/200 both pre and post-bevacizumab injection with no further improvement following IVTA. Case 2 had a baseline VA of 20/100, which worsened at 1-month post-bevacizumab to 20/200. Due to worsening cataract, the patient subsequently underwent phacoemulsification plus IVTA and VA at 3 months post-op improved to 20/25

(91). In contrast, Yuzbasioglu et al performed an average of 3.3 (range, 1-8) injections of 1.25mg bevacizumab over 10.3 (range, 6-14) months in 13 eyes of 7 patients and observed a reduction of CMT from 370.15 μ m (range, 245-603 μ m) at baseline to 142.53 μ m (range, 124-168 μ m). Pre- and post-treatment visual acuity ranges were 5/400 - 20/100 and 20/200 - 20/63, respectively (83).

- Ranibizumab

Artunay et al enrolled 30 eyes of 30 patients with RP-CMO into a prospective, controlled interventional study of 0.5mg intravitreal ranibizumab (LUCENTIS; Genetech, South San Francisco, California, USA). Thirteen eyes (87%) in the treatment group demonstrated a significant reduction of CMO at 6-months. No statistically significant difference in VA was seen between the groups (84).

- Aflibercept

Improvement of CMT and VA following a single unilateral intravitreal injection of aflibercept (EYLEA; Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA and Bayer Healthcare Pharmaceuticals, Berlin, Germany) in a 52-year-old patient with RP-CMO was observed and maintained at 6-months (85). We have observed similar responses (86) and have commenced a prospective study to determine safety and efficacy of intravitreal aflibercept in RP-CMO (ClinicalTrials.gov identifier NCT02661711). Aflibercept may be superior to other anti-VEGF medications due to its intermediate size (115 kDa) and higher binding affinity.

Nutritional

- Oral Lutein

Lutein and zeaxanthin are carotenoid pigments that contribute to the formation of macular pigment which is thought to be protective against oxidative damage (92). A 48-week study tested the effect of oral lutein on CMT in 39 patients with RP. Lutein was found to have no statistically significant effect on CMT in RP patients with, or without CMO (3).

- Oral omega-3 fatty acid

Oral omega-3 has been used in RP to investigate whether loss of cone or rod ERG function can be slowed. A systematic review carried out by Hodge et al (2006) summarised trends in improvement have been seen in some retinitis pigmentosa outcomes (93). A placebo controlled RCT found no effect of omega 3 fatty acid supplementation on the primary endpoint of ERG changes in RP. However, beneficial changes in secondary endpoints were observed, namely slowed progression of visual field loss and dark adapted thresholds (94, 95).

- Oral intake of iodine

While oral iodine has not been trialled specifically for RP-CMO, higher urinary iodine concentration has been observed to be significantly associated with reduced macular swelling in non-smoking adults with RP-CMO, suggesting a potential role for limiting iodine intake (96).

Surgical

- Laser

In 1987, grid laser photocoagulation was undertaken in one eye of 16 patients with bilateral RP-CMO. Six treated eyes gained one or more lines of vision, while none of the untreated eyes did. Seven

untreated eyes lost one or more lines of vision, while none of the treated eyes did. Thirteen of 16 eyes showed decreased fluorescein leakage after treatment (97). Laser may remove hypoxic degenerating retina thus reducing VEGF production (98).

- Vitrectomy

In 2003, vitrectomy combined with inner limiting membrane peel was carried out on 12 eyes of 8 patients with RP-CMO refractory to treatment with acetazolamide. The presence or absence of pre-operative vitreo-macular traction was not confirmed. Mean CMT improved from 478 μ m (range, 380 - 570 μ m) pre-operatively to 260 μ m (range, 177 - 424 μ m) at 6-months. Mean VA improved from 20/115 (range, 20/60 – 20/400) pre-operatively to 20/45 (range, 20/30 – 20/100) at 6-months (25).

Conclusions and Future Directions

RP-CMO commonly complicates RP, however, its exact underlying pathogenesis remains uncertain. Proposed mechanisms which are most likely to be involved include breakdown of the BRB and/or RPE pump mechanism failure and /or Muller cell oedema and dysfunction. When CS are present they are most commonly located in the INL, suggesting that inner BRB dysfunction may have a greater role than the outer BRB, in development of RP-CMO. A better understanding of these mechanisms will facilitate better targeted and likely more efficacious and durable therapies.

Setting up clinical trials for RP-CMO, however, remains a challenge due to its low prevalence, the highly variable course of disease progression, lack of clearly accepted endpoints, multiple underlying

mutations, and very slow progression to visual loss. With the majority of studies producing levels of evidence between 3 and 4 and no large RCTs, we remain in a position where there are still no studies providing high level evidence for treatments for RP-CMO. In the absence of RCTs, the effect of known and unknown confounders affecting the findings cannot be excluded. The majority of studies did not have a control group (level 4 evidence) which limits the validity of findings as these may be a result of natural history rather than the intervention. Many studies were retrospective which may be affected by recall bias. This review has highlighted the lack of high quality evidence for treatments of RP-CMO. Whilst concrete conclusions cannot be drawn, the evidence available to us suggests that topical CAIs may be used as first-line approach in the treatment of RP-CMO. Consideration should be given to the possibility of side-effects and potential for rebound CMO. Oral CAIs may be a second line agent but there is the risk of more side effects.

As there are currently no treatments for the underlying retinal degeneration in RP and given the potentially reversible nature of RP-CMO, there is a real need to better understand disease mechanisms and undertake prospective clinical trials of therapeutic agents to provide the evidence base to improve treatment of RP-CMO. Successful management of CMO should aim to both improve quality and quantity of vision in the short term and slow the rate of vision loss over time.

Acknowledgments

This work was supported by grants from the National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital National Health Service Foundation Trust and UCL Institute of Ophthalmology, Fight For Sight (UK), The Macular Society (UK), Moorfields Eye Hospital Special Trustees, Moorfields Eye Charity, by a multiuser equipment grant from The Wellcome Trust [099173/Z/12/Z], the Foundation Fighting Blindness (FFB; USA), Bayer UK, and Retinitis Pigmentosa Fighting Blindness. Prof. Michaelides is a recipient of an FFB Career Development Award.

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Supplementary table 1: A summary to date of the pharmacological, nutritional and surgical interventional studies for RP-CMO

Authors	Year published	Study design	N (pts)	Intervention	Results	Comments	Level of evidence*
Carbonic Anhydrase Inhibitors							
Liew <i>et al</i>	2015	Retrospective cohort	81	125 eyes of 64 patients received topical dorzolamide, 32 eyes of 17 patients received oral acetazolamide 250mg BD or 500mg OD	CMT reduction greater in dorzolamide group than acetazolamide group VA improvement both groups		3B

Ginead <i>et al</i>	2010	Retrospec tive case series	32	Topical dorzolamide 2% TDS or BD for 6 - 58 months	CMT improvement more than VA improvement		4
Fishman <i>et al</i>	1994	Prospecti ve, placebo- controlled , double- masked, crossover design	17	Methazolamide or placebo taken for 3 weeks Sub-group received additional 3/12 methazolamide treatment	Angiographic improvement more than VA improvement No change in VA with extended methazolamide treatment		3B
Grover <i>et al</i>	2006	Prospecti ve, non- randomis ed	15	Topical dorzolamide TDS for at least 4 weeks BE	CMT improvement more than VA improvement	Recurrence in 4 patients	4
Fishman <i>et al</i>	1989	Prospecti ve, masked, cross- over	12	Oral acetazolamide or placebo for 2 week periods	BCVA improved, CMT reduced. Improvement angiographically in almost 50%	500 mg/day acetazolamide more effective than 250 mg/day	3B
Ikeda <i>et al</i>	2013	Prospecti ve	10	Topical dorzolamide 1%	CMT reduction with majority effect lasting 18	Recurrence in 5 eyes	4

				TDS BE for 18 months	months. Macular sensitivity improvement No significant change in BCVA	between 12-18 months	
Ikeda <i>et al</i>	2012	Prospective	9	Topical dorzolamide 1% TDS BE for 6 months	CMT reduction greater than macular sensitivity improvement No difference in BCVA		4
Fishman <i>et al</i>	2007	Prospective cohort	8	Topical dorzolamide 2% TDS BE for 7 - 15 months	CMT improvement more than VA improvement	Patients previously included in study by Grover <i>et al</i> 2006 Two patients experienced rebound CMO	4
Orzalesi <i>et al</i>	1993	Prospective pilot	7	Oral acetazolamide for	VA improvement	Effect independent	4

				2 weeks then reduced / maintained / increased according to response Follow-up between 3 weeks – 16 months		of reduction of CMO on FFA	
Apushkin <i>et al</i>	2007	Prospective cohort	6	500 mg oral acetazolamide	CMO improved at 3-5 weeks	Recurrence at 8-12 weeks	4
Grover <i>et al</i>	1997	Prospective, double-masked, crossover	5	Topical dorzolamide or placebo given for 4 weeks followed by crossover treatment for 4 weeks Oral acetazolamide then given for 2 weeks	VA no change using dorzolamide VA improvement using acetazolamide CMO and FFA improvement more marked using acetazolamide	4 week flush-out period in between each phase	3B

					than dorzolamide		
Pacella <i>et al</i>	2014	Case reports	3	Topical dorzolamide BE	CMT improvement within 7 - 20 days No significant change in VA observed		4
Thobani & Fishman	2011	Retrospec tive case series	3	Only 2 patients with RP and CMO. Recurrence whilst taking 500mg oral acetazolamide. Re-introduced to treatment after period of discontinuation	Improvement of macular oedema. VA not mentioned in RP patients.		4
Fishman <i>et al</i>	1993	Prospecti ve	3	Oral methazolamide 50mg BD	Slight improvement BCVA	Recurrence at 6-12 weeks	4

Chen <i>et al</i>	1990	Case study	1	Oral acetazolamide	BCVA improvement RE only Improvement of CMO BE		4
Steroids							
Wang <i>et al</i>	2003	Retrospective chart review	50	Only 2 of these patients were diagnosed with RP-CMO. Single bilateral intravitreal injection of triamcinolone given.	Improvement in CMT and VA.		4
Scorolli <i>et al</i>	2007	Prospective, nonrandomized, comparative trial	40	20 eyes received a unilateral single intravitreal injection of triamcinolone acetate, 20 eyes who declined were used as controls	No significant change in BCVA CMT reduction	IOP increased after the first day, at 1 month, and at 3 months in both groups but no significant	3B

				1 year follow-up		increase afterwards	
Giusti <i>et al</i>	2002	Pilot	10	Treated for 1 year with oral deflazacort	Near VA, FFA and perimetry improved significantly Distance VA varied only slightly		4
Ozdemir <i>et al</i>	2005	Prospective small series	5	IVTA given Follow up 6-8 months	CMT improvement No improvement VA seen	Refractory to oral acetazolamide Recurrence in 3 patients between 3 – 6 months	4
Srouf <i>et al</i>	2013	Prospective	3	Intravitreal dexamethasone implant (Ozurdex) Follow-up for 6 months	CMT and VA improvement	Refractory to oral acetazolamide, sub-tenon triamcinolone, topical NSAID Recurrence at 3 months in 2	4

						patients requiring re-treatment	
Schaal <i>et al</i>	2016	Case study	1	Right eye received a sub-tenon injection of triamcinolone. A 2 nd sub-tenon injection was performed in the right eye 8 months following the 1 st injection.	VA and CMT improvement 2-weeks post 1 st injection. Mild rebound CMO at 8 months following 1 st injection.	Refractory to oral acetazolamide 500mg OD for 2 years and topical 2% dorzolamide TDS together with topical 0.09% bromofenac OD for 1 year. IOP increase at 4 months controlled with 0.5% timolol BD.	4
Ahn <i>et al</i>	2014	Case study	1	0.7mg intravitreal Ozurdex	BCVA improved. OCT central thickness improved.	Refractory to oral acetazolamide and	4

						intravitreal bevacizumab	
Patil L & Lotery A.J	2014	Case study	1	Treated with 0.7mg of intravitreal injection of dexamethasone implant (Ozurdex)	VA and CMT improvement, maintained after 10 months Exudation at the disc and the inferior retina resolved.	Patient from De Salvo 2011 study. RE RP- CMO and Coats'-like exudative RD. Refractory to oral acetazolamide , topical dorzolamide, orbital floor injection of depo- medrone and CMO recurrence following initial success with cryotherapy.	4

Alhassan, M & Quintyn, J.C	2013	Case study	1	Single unilateral intravitreal dexamethasone implant (Ozurdex)	Bilateral BCVA improvement and CMT reduction at 1 month	Refractory to oral acetazolamide 500mg OD and topical brinzolamide BD	4
Barge <i>et al</i>	2013	Case study	1	Bilateral intravitreal injections of triamcinolone (IVTA) before sub-tenon depot of triamcinolone	BCVA improved CMT reduced IOP raised BE	Refractory to oral acetazolamide and topical ketoralac Recurrence at 2-5 months post-IVTA	4
Buchaim <i>et al</i>	2013	Case study	1	Bilateral intravitreal dexamethasone implant (Ozurdex)	BCVA improved No CMO at 4 months	Previously received 19 IVTAs RE and 13 IVTAs LE but with decreasing therapeutic effect	4

Saati <i>et al</i>	2013	Case study	1	Bilateral intravitreal dexamethasone implant (Ozurdex)	CMO resolution at 1/52 VA improvement	Refractory to topical dorzolamide Recurrence BE between 2-3 months	4
Urban <i>et al</i>	2009	Case study	1	4 x unilateral intravitreal triamcinolone repeated every 4 months	FT reduction within 3 months BCVA improvement Endophthalmitis following 4th IVT diagnosed day of RTA Traumatic inferior RD, migration of triamcinolone into sub-retinal space. Scleral buckling and vitrectomy performed	Intolerant to oral acetazolamide Previous autologous plasmin enzyme–assisted vitrectomy without ILM peel	4

					Resolution of CMO observed, however, BCVA remained at 20/100.		
Kim <i>et al</i>	2006	Case study	1	2 x Intravitreal triamcinolone (4mg/0.1ml) RE cataract surgery at 3 months post-1 st IVTA	1 st IVTAs resolution of CMO by 1/12. No significant change in VA 2nd IVTAs resolution of CMO by 2 weeks	Refractory to oral acetazolamide 500mg for 1 year and sub-tenon triamcinolone 40mg Recurrence in BE at 11 months post 1 st IVTA.	4
Minella <i>et al</i>	2006	Case study	1	IVTA Follow-up for 6 months	CMT reduced No significant change FERG amplitude/phase VA showed a significant		4

					tendency to improve		
Sallum <i>et al</i>	2003	Case study	1	Bilateral IVTAs	CMO resolution between 30 - 40 days VA improvement LE only	Refractory to oral acetazolamide Recurrence at 6 months	4
Saraiva <i>et al</i>	2003	Case study	1	Bilateral IVTAs	CMO reduction VA improvement LE only	Refractory to oral acetazolamide Recurrence at 6 months	4
Non-steroidal anti-inflammatory together with steroid or CAI							
Lemos-Reis <i>et al</i>	2015	Prospective, randomized and interventional	18	15 eyes received topical ketorolac and 13 eyes received topical dorzolamide for 12 months	No significant change in CMT in either group Improvement BCVA both groups at 6/12 but reduced in dorzolamide group at 1 year		3B

Park <i>et al</i>	2013	Case study	1	Topical prednisolone and ketorolac 3 months	VA improvement and resolution of CMO at 3 months	Treatment re-introduced at 6 months due to recurrence of CMO	4
Intravitreal anti-vascular endothelial growth factor							
Artunay <i>et al</i>	2009	Prospective cohort	30	15 eyes received intravitreal ranibizumab, 15 eyes remained off treatment	CMT improved but no change in BCVA at 6 months	Refractory to oral acetazolamide	3B
Yuzbasioglu <i>et al</i>	2009	Prospective	7	Intravitreal bevacizumab	CMT and VA improvement		4
Melo <i>et al</i>	2007	Case reports	2	Single unilateral intravitreal bevacizumab 1st patient received IVTA at 3/12 post IVT 2nd patient underwent cataract	Unchanged CMT, VA same or worse following IVT 1st patient CMT improved following IVTA 2nd patient: VA reduced due to	Previously received 2 or 3 IVTAs with transient VA improvement	4

				surgery/IVTA at 1 month post IVT	lens opacity. VA and CMT improvement documented at 3 months post-cataract surgery		
Strong <i>et al</i>	2016	Case report	1	Bilateral intravitreal injections of Eylea	CMT improvement	Refractory to topical dorzolamide. Minimal response previously to intravitreal ranibizumab.	4
Moustafa <i>et al</i>	2015	Case study	1	Single unilateral intravitreal injection of aflibercept	VA and CMT improvement No significant multifocal ERG changes	Improvements maintained at 6 months	4
Shah <i>et al</i>	2010	Case study	1	3 x Intravitreal unilateral injections of ranibizumab	CMT reduction BCVA improvement after 3 rd IVT	Unable to tolerate PO acetazolamide	4

						Refractory to topical dorzolamide Recurrence at 3 months after 3 rd IVT	
Querques <i>et al</i>	2009	Case study	1	Single unilateral intravitreal injection of pegaptanib sodium 0.3mg given whilst PO acetazolamide continued	VA and CMT improvement No recurrence of CMO seen at 4 months post-IVT	Refractory to oral acetazolamide	4
Oral lutein							
Adackapara <i>et al</i>	2008	Prospective	39	Patients already enrolled in phase I/II clinical trial with double-masked, placebo-lutein, crossover design	No significant effect on CMT in patients with, or without CMO	19 / 39 patients had RP-CMO, 20 /39 patients had RP without CMO	3B
Laser							

Newson <i>et al</i>	1987	Prospective pilot	16	Unilateral grid photocoagulation performed Follow-up between 4 - 21 months	Decreased dye accumulation on FFA VA better in treated compared to untreated eyes		4
Vitrectomy							
Garcia-Arumi <i>et al</i>	2003	Prospective non-comparative case series	8	Pars plana vitrectomy with ILM peel	VA and CMT improvement	Refractory to oral acetazolamide 250mg BD for 1 month	4
Other							
Siqueira <i>et al</i>	2013	Case report within phase 2 study	1	Unilateral intravitreal autologous BM-derived hematopoietic stem cell transplantation	CMO resolution and VA/macular sensitivity improvement	Refractory to oral acetazolamide and topical dorzolamide	4
De Salvo <i>et al</i>	2011	Case study	1	Cryotherapy applied to infero-	Slight increase BCVA with mild	Coats'-like exudative RD.	4

				temporal quadrant of RE retina	residual CMO at 6 months	Refractory to oral acetazolamide , topical dorzolamide and orbital floor injection depomedrone	
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*Oxford Centre for evidence-based medicine – Levels of evidence (March 2009)

Citation:

Strong SA, Gurbaxani A, Michaelides M. Treatment of Retinitis Pigmentosa-Associated Cystoid Macular Oedema Using Intravitreal Aflibercept (Eylea) despite Minimal Response to Ranibizumab (Lucentis): A Case Report. Case Rep Ophthalmol 2016;7:389-97.

Full title:

Treatment of Retinitis Pigmentosa-associated cystoid macular oedema using intravitreal aflibercept (Eylea) despite minimal response to ranibizumab (Lucentis) : A case report

Abstract:

Background: We present an interesting case of bilateral Retinitis Pigmentosa (RP)-associated cystoid macular oedema that responded on two separate occasions to intravitreal injections of aflibercept, despite previously demonstrating only minimal response to intravitreal ranibizumab. This unique case would support a trial of intravitreal aflibercept for the treatment of RP-associated cystoid macular oedema.

Case presentation: A 38-year-old gentleman from Dubai presented to the UK with a 3-year history of bilateral RP-associated cystoid macular oedema. Previous treatment with topical dorzolamide, oral acetazolamide and intravitreal ranibizumab had demonstrated only minimal reduction of cystoid macular oedema. Following re-confirmation of the diagnosis by clinical examination and optical coherence tomography imaging, bilateral loading doses of intravitreal aflibercept were given. Central macular thickness reduced and the patient returned to Dubai. After 6-months, the patient was treated

with intravitreal ranibizumab due to re-accumulation of fluid and the unavailability of aflibercept in Dubai. Only minimal reduction of central macular thickness was observed. Once available in Dubai, intravitreal aflibercept was administered bilaterally with further reduction of central macular thickness observed. Visual acuity remained stable throughout.

Conclusions: This is the first case report to demonstrate a reduction of RP-associated CMO following intravitreal aflibercept despite inadequate response to ranibizumab on two separate occasions. Aflibercept may provide superior action to other anti-VEGF medications due to its intermediate size (115 kDa) and higher binding affinity. This is worthy of further investigation in a large prospective cohort over an extended time to determine the safety and efficacy of intravitreal aflibercept for use in this condition.

Keywords:

Aflibercept, Cystoid Macular Oedema, Eylea, Retinitis Pigmentosa, Case report

Background:

Retinitis Pigmentosa (RP) is the most prevalent inherited retinal disease (IRD), with IRD now representing the commonest cause of visual impairment registration in the working age population and the second commonest in childhood in the UK [1]. Typical symptoms of RP include nyctalopia, photopsia and progressive visual field loss, however, vision can also be affected by cataracts and/or cystoid macular oedema (CMO).

Around 20% of RP patients develop CMO, the pathogenesis of which is not clearly understood.

Suggested mechanisms include: anti-retinal antibodies [2], retinal pigment epithelium (RPE) dysfunction [3], Muller cell oedema [4] and vitreous traction [5].

Many treatments have been attempted for RP-associated CMO, including: grid laser, vitrectomy, oral lutein, intravitreal dexamethasone, intravitreal triamcinolone, topical carbonic anhydrase inhibitors, oral carbonic anhydrase inhibitors, oral corticosteroids, topical non-steroidal anti-inflammatory medication and topical steroid [6-10]. However, all of the aforementioned treatments have limited and highly variable efficacy. This, together with several side-effects of these medications that markedly restrict their use, has led to the search to find alternative therapies that are both well tolerated and are more consistently effective.

Intravitreal anti-vascular endothelial growth factor (anti-VEGF) medication is now licenced for use within the UK for CMO secondary to macular degeneration, diabetic retinopathy and retinal vein occlusion.

While the pathogenesis of RP-associated CMO is still unclear, it is believed that VEGF may play a role in the development of CMO. It has therefore been suggested as an alternative treatment for RP-associated CMO.

Limited data has been published regarding the use of intravitreal anti-VEGF medication for RP-associated CMO. Querques et al (2009) observed improvement of visual acuity and macular thickness in a patient with refractory RP-associated CMO taking oral acetazolamide, one month following a single injection of intravitreal pegaptanib (MACUGEN; EyeTech Pharmaceutical, Inc., New York, USA). No recurrence of CMO was seen at 4 months post-injection [11]. Melo et al (2007) observed no improvement in 2 patients with RP-associated CMO treated with a single injection of intravitreal

bevacizumab (AVASTIN; Genentech, South San Francisco, California, USA) [12], however, Yuzbasioglu et al (2009) documented improvement of macular thickness and visual acuity in all 13 eyes of 7 patients [13].

Artunay et al (2009) treated 15 eyes with RP associated CMO with intravitreal ranibizumab and compared them with 15 eyes of similar patients who refused treatment. A significant improvement in macular thickness was observed in those patients treated with intravitreal ranibizumab [14].

In a recent case report, a single unilateral intravitreal injection of aflibercept was given to a patient with RP associated CMO. Improvement in both visual acuity and macular thickness was seen at one month post-injection as well as maintenance of this improvement documented at 6 months [15]. Aflibercept is a recombinant fusion protein consisting of portions of the extracellular domains of human VEGF receptors 1 and 2 fused to the Fc portion of human IgG1. This unique design is what sets aflibercept apart from other intravitreal anti-VEGF medications by enabling its action as a decoy receptor.

Case presentation:

In August 2013, a 38-year-old gentleman from Dubai, United Arab Emirates was seen by a medical retina specialist in the UK. He was previously diagnosed with Autosomal Recessive Retinitis Pigmentosa (RP) in Dubai and bilateral CMO had been documented over the last 3 years. The patient had undergone uncomplicated bilateral cataract surgery with the insertion of posterior chamber intraocular lenses in 2004. There was no other relevant past medical history. Family history revealed parental consanguinity, with his parents being first cousins.

He had previously received topical dorzolamide and a 3 week trial of oral acetazolamide (Diamox) 250mg three times per day, with no significant improvement in the degree or extent of CMO. Bilateral injections of ranibizumab (LUCENTIS; Genentech, South San Francisco, California, USA) had been performed once a month for three months in Dubai in 2013 with only minimal response observed. At the time of the consultation in the UK, the patient was no longer receiving topical or oral treatment for CMO.

On examination, BCVA was 6/18 in the right eye and 6/36 in the left eye. Visual field testing to confrontation revealed constricted fields of 10 to 20 degrees in both eyes. Fundoscopy revealed bilateral dense bone-spicules, bilateral CMO, attenuated retinal vessels and pale optic discs. Spectral domain optical coherence tomography (SDOCT) revealed marked bilateral CMO with central macular thickness (CMT) of 394 and 414 microns in the right and left eye respectively (Figure 1, A and E).

The anti-VEGF medication selected for use in this gentleman was aflibercept (EYLEA; Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA and Bayer Healthcare Pharmaceuticals, Berlin, Germany). This was due to there being only a minimal response observed following treatment with ranibizumab as well as consideration that its effects may be longer lasting than other anti-VEGF medication. The risks and benefits of treatment with aflibercept together with its off-label use were discussed with the patient. It was also highlighted that there was a limited evidence base for its usage in RP-associated CMO.

Informed consent was taken and bilateral intravitreal injections of 0.05ml aflibercept (40mg/ml) given via standard aseptic technique. There were no peri-operative complications. Post-operative chloramphenicol drops were prescribed.

One month after treatment, BCVA improved to 6/12 in the right eye but remained 6/36 in the left eye. The patient did not notice any subjective improvement. SDOCT revealed markedly less CMO in both eyes, with CMT of 263 and 243 microns in the right and left eye respectively (Figure 1, B and F). A second uncomplicated intravitreal injection of aflibercept was undertaken bilaterally.

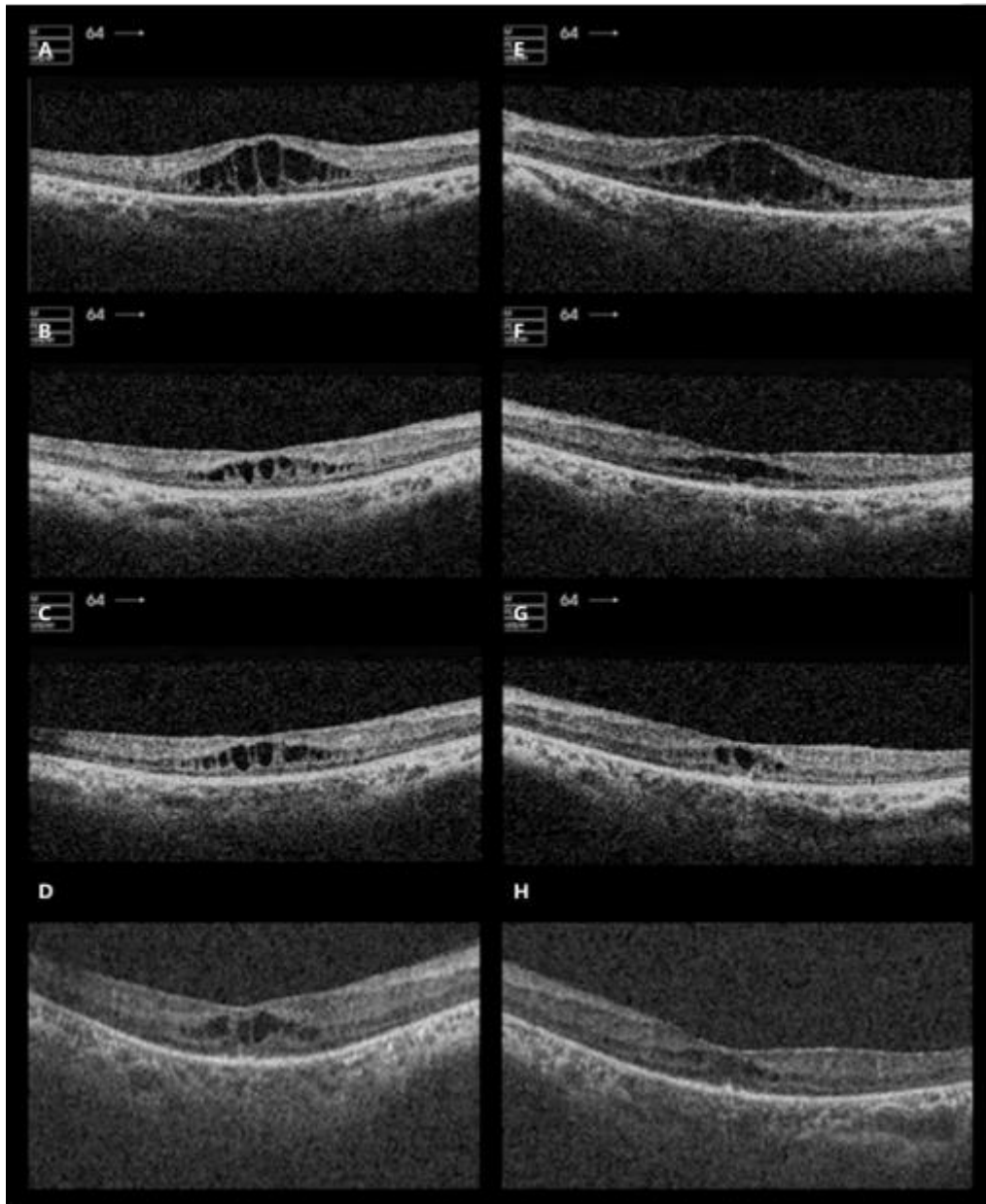
One month after his 2nd injection with aflibercept, BCVA remained at 6/12 in the right eye and 6/36 in the left eye. At this visit the patient reported a subjective improvement of vision. SDOCT revealed a similar level of CMO bilaterally compared to the previous visit. CMT was 268 and 239 microns in the right and left eye respectively (Figure 1, C and G).

The patient then returned to the United Arab Emirates where he was seen by the medical retina team at Moorfields Eye Hospital, Dubai in December 2013. BCVA was 6/18 in the right eye and 6/24 in the left eye. SDOCT revealed a similar level of CMO bilaterally (Figure 1, D and H) despite his last aflibercept injection being 8 weeks prior. CMT was recorded as 253 and 224 microns in the right and left eye respectively. As the patient was stable, the decision was taken not to treat with an alternative anti-VEGF since aflibercept was due to be made available for use in Dubai from January 2014.

Fig. 1. Optical coherence tomography of both eyes before and after intravitreal injections of aflibercept given in the UK.

Optical coherence tomography in the right eye before injection with aflibercept (Figure 1A), one month after 1st injection of aflibercept (Figure 1B), one month after 2nd injection of aflibercept (Figure 1C), 8 weeks after 3rd injection with aflibercept (Figure 1D). Optical coherence tomography in the left eye before injection with aflibercept (figure 1E), one month after 1st injection of aflibercept (Figure 1F), one

month after 2nd injection of aflibercept (figure 1G), 8 weeks after 3rd injection with aflibercept (Figure 1H).

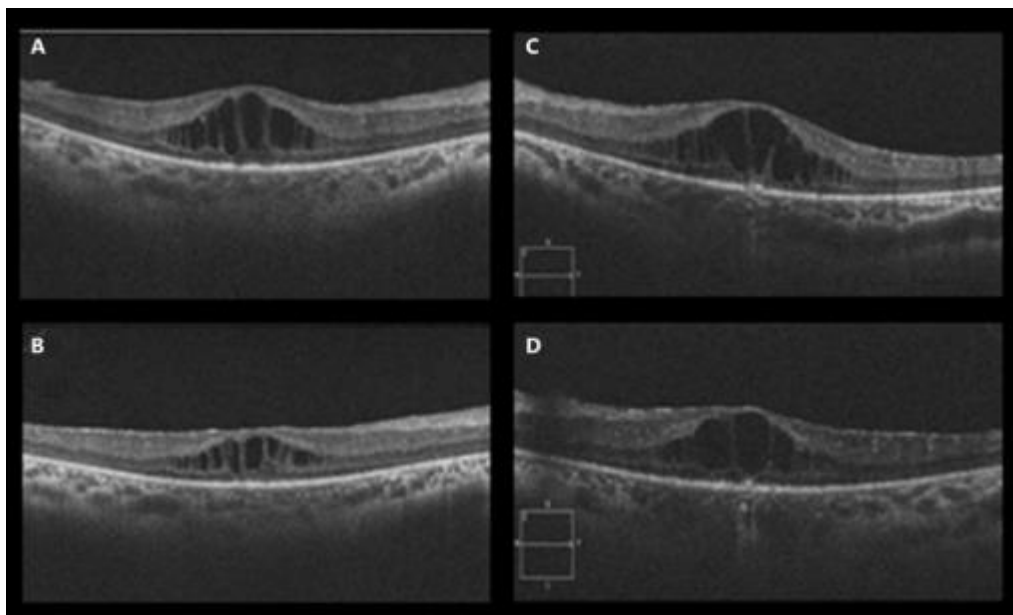


Unfortunately there was an unexpected delay in aflibercept being made available for use in Dubai. In March 2014 (5 months after the patient's last injection with aflibercept) BCVA was 6/18 right eye and

6/36 in the left eye. SDOCT revealed a significant increase of CMO bilaterally, with CMT of 385 and 434 microns in the right and left eye respectively (Figure 2, A and C). In order not to delay treatment any further, the decision was taken to perform 3 monthly loading doses of ranibizumab bilaterally.

The response to ranibizumab was markedly less pronounced compared to aflibercept. In May 2014, BCVA remained at 6/18 in the right eye and had decreased to 6/48 in the left eye. SDOCT revealed bilateral CMO, with CMT of 304 and 342 microns in the right and left eye respectively (Figure 2, B and D). The decision was taken not to undertake further injections with ranibizumab.

Fig. 2. Optical coherence tomography of both eyes before and after intravitreal injections of ranibizumab given in Dubai. Optical coherence tomography in the right eye immediately before injection with ranibizumab (Figure 3A) and on the day he received his 3rd injection with ranibizumab (Figure 3C). Optical coherence tomography in the left eye immediately before injection with ranibizumab (Figure 3B) and on the day he received his 3rd injection with ranibizumab (Figure 3D).



In August 2014, the patient had an increased amount of CMO. SDOCT measured 452 and 513 microns in the right and left eye respectively (Figure 3, A and E). Fortunately, aflibercept became available for use in Dubai and the patient received treatment with 3 monthly loading doses of aflibercept bilaterally.

A good response was noted once again. In October 2014, BCVA had improved to 6/18 in the right eye and 6/36 in the left eye. SDOCT showed a marked reduction in CMO (Figure 3, B and F) measuring 248 and 226 microns in the right and left eye respectively.

The patient continued to receive aflibercept injections in January 2015, March 2015, June 2015 and September 2015 and CMT remained stable (Figure 3, C and G). In September 2015, BCVA was 6/15 in the right eye, 6/36 in the left eye, with stable CMT of 250 and 194 microns in the right and left eye respectively (Figure 3, D and H). Figure 4 summarises the effect of anti-VEGF medications on CMT over time.

Fig. 3. Optical coherence tomography of both eyes before and after intravitreal injections of aflibercept given in Dubai.

Optical coherence tomography in the right eye immediately before injection with aflibercept (Figure 4A), immediately before the 3rd injection with aflibercept (Figure 4B), two months following the 4th injection with aflibercept (Figure 4C) and 3 months following the 6th injection with aflibercept (Figure 4D). Optical coherence tomography in the left eye immediately before injection with aflibercept (Figure 4E), immediately before the 3rd injection with aflibercept (Figure 4F), two months following the 4th injection with aflibercept (Figure 4G) and 3 months following the 6th injection with aflibercept (Figure 4H).

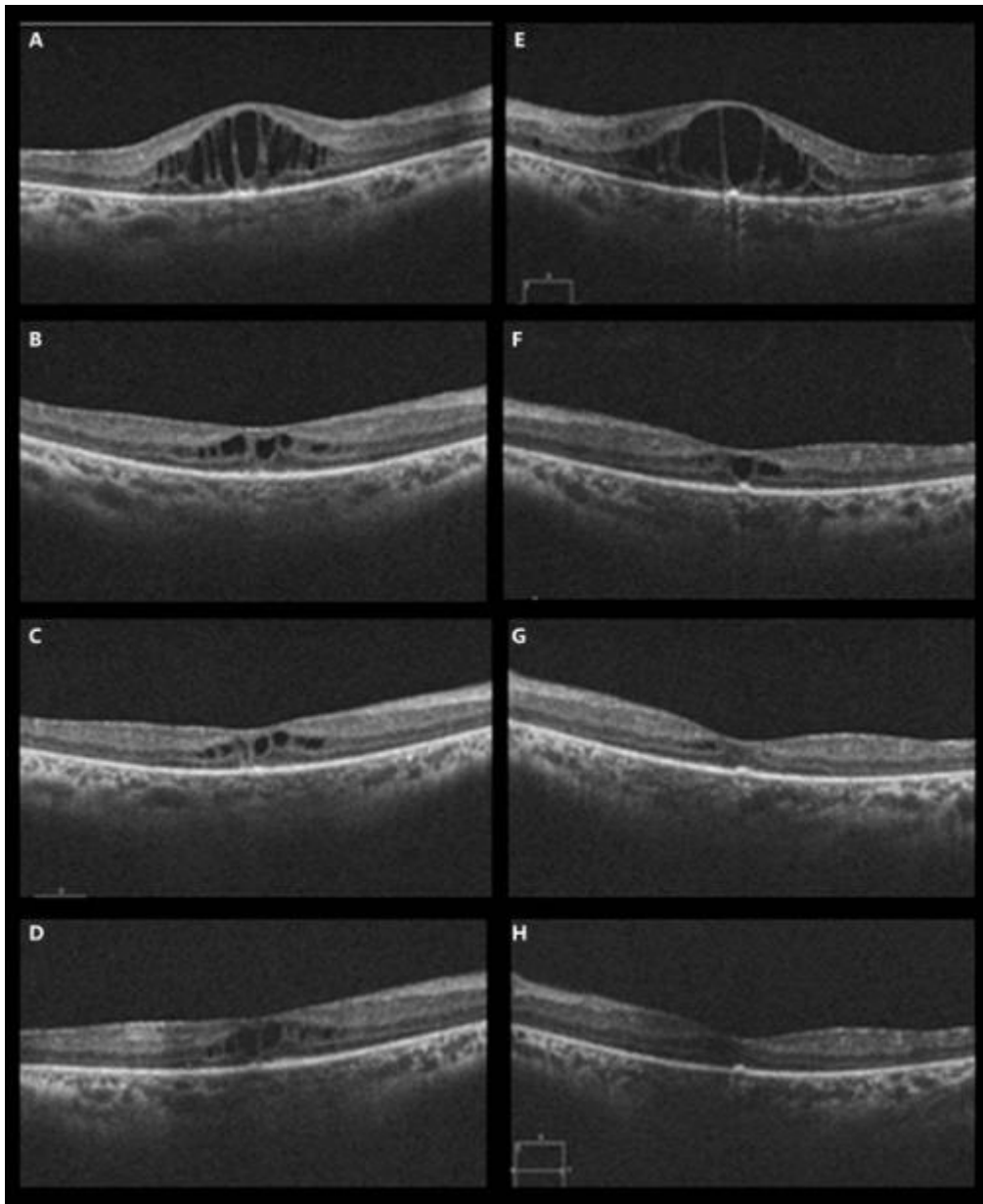
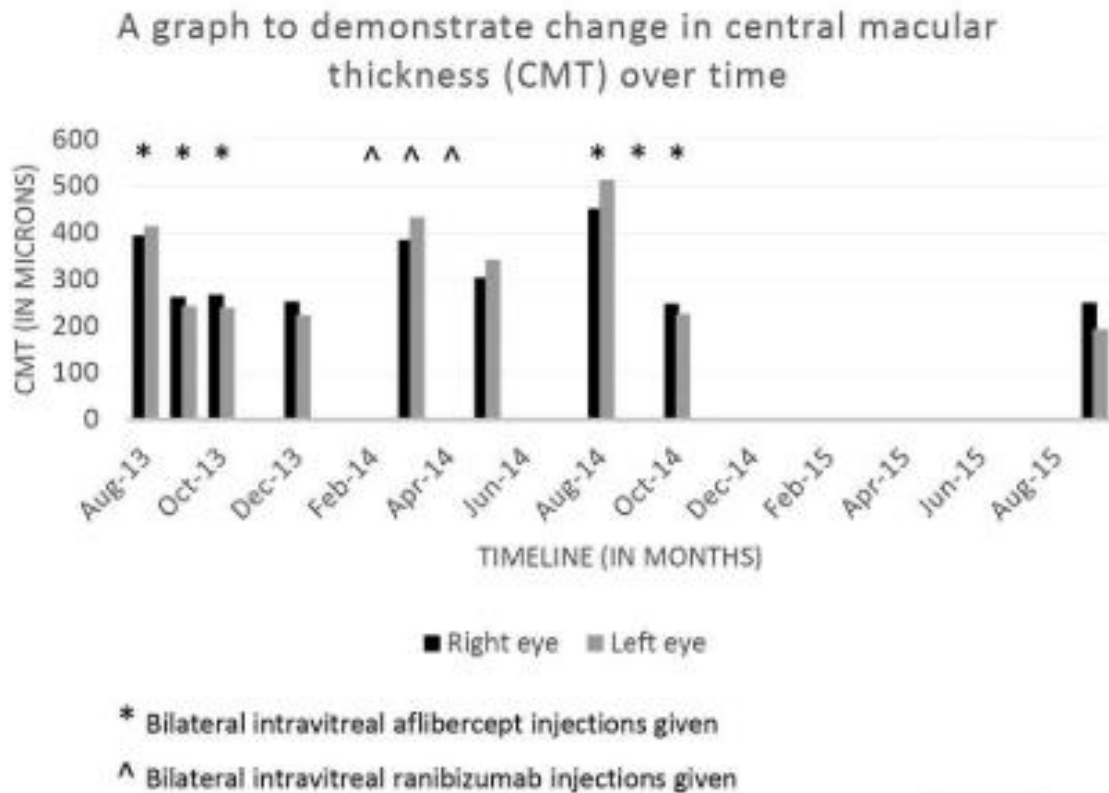


Fig. 4. The effect of anti-VEGF medications on CMT over time is presented.



Conclusions

There are currently no proven treatments for RP-associated CMO. The use of medication such as carbonic anhydrase inhibitors has demonstrated inconsistent efficacy and unwanted side effects. FDA approval of anti-VEGF medication has now extended to include CMO secondary to age-related macular degeneration, diabetic retinopathy and retinal vein occlusion. While the pathogenesis of RP-associated CMO is not entirely understood, VEGF may play a role in the formation of RP-associated CMO thereby representing a potential target for treatment. RP is not an ischaemic condition and if anything, the natural bone spicule formation secondary to photoreceptor cell death results in an overall reduction of oxygen consumption by the retina. We hypothesise that a localised source of VEGF produced, for

example, by Muller cells under pathological conditions contributes to CMO formation whilst also explaining why it is rare to find reports of peripheral neovascularisation in RP.

This is the first case report to demonstrate a reduction of RP-associated CMO following intravitreal aflibercept despite inadequate response to ranibizumab. Aflibercept may provide superior action to other anti-VEGF medications due to its intermediate size (115 kDa) and higher binding affinity. This is worthy of further investigation in a large prospective cohort over an extended time to determine the safety and efficacy of aflibercept for use in this condition.

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- Strong, SA and Michaelides, M. An update on inherited retinal disorders (part 2): Approaches to therapy for IRDs. *Eye News.* 2017;23(4):22-6.
- Strong, SA and Michaelides, M. An update on inherited retinal disorders (part 1) – overview and assessment of inherited retinal disease. *EyeNews* 2016;23(3).