Moorfields Eye Hospital
NHS Foundation Trust

Moorfields Eye Hospital
NHS Foundation Trust
162 City Road, London EC1V 2PD
Phone: 020 7253 3411

**Title Page**

| | |
|---|---|
| **Full title of study** | Can deep phenotyping using retinal images predict response to intravitreal aflibercept therapy in patients with neovascular age-related macular degeneration? |
| **Short title** | **PRECISE Study** |
| **Version and date of protocol** | Protocol Version 2.1 dated 24-02-2021 |
| **Sponsor** | Moorfields Eye Hospital NHS Foundation Trust |
| **EudraCT no** | None |
| **Intervention** | None |
| **Placebo** | N/A |
| **Study sites(s)** | 12 NHS sites in England and Ireland |
| **Chief investigator** | Prof Sobha Sivaprasad<br>Moorfields Eye Hospital<br>162, City Road,<br>London EC1V 2PD<br>Sobha.sivaprasad@nhs.net |
| **Trial Statistician and Co-Lead** | Professor A Toby Prevost<br>Kings College London<br>Email: toby.prevost@kcl.ac.uk |
| **Funder** | Boehringer Ingelheim<br>Binger Str. 173, 55216<br>Ingelheim am<br>Rhein, Germany |
| **Sponsor Representative** | Lauren Leitch-Devlin, Moorfields Eye Hospital<br>162, City Road, London<br>EC1V 2PD |

## Signatures

The Principal Investigator and the RMC have discussed this protocol. The investigators agree to perform the investigations and to abide by this protocol except in case of medical emergency (see MEH SOP for the recording and reporting of deviations, violations, potential serious breaches, serious breaches and urgent safety measures) or where departures from it are mutually agreed in writing.

The investigator agrees to conduct the study in compliance with the protocol, GCP, the Data Protection Legislation, the Trust Information Governance Policy, the Research Governance Framework (2005), the Sponsor's SOPs, and other regulatory requirements as appropriate.

**Chief investigator**

Prof Sobha Sivaprasad

Moorfields Eye Hospital

24/02/2021

Signature Date

**Sponsor Representative**

Miss Lauren Leitch-Devlin

Moorfields Eye Hospital

24/02/2021

Signature Date

Contents

**List of Abbreviations:**

| Abbreviation | Description |
|---|---|
| **AI** | Artificial Intelligence |
| **AMD** | Age-related Macular Degeneration |
| **anti-VEGF** | anti-Vascular Endothelial Growth Factor |
| **APR** | Annual Progress Report |
| **AUC** | Area Under the Curve |
| **CI** | Confidence Interval |
| **CNV** | Choroidal Neo-Vascularisation |
| **EDC** | Electronic Data Capture |
| **GCP** | Good Clinical Practice |
| **HRA** | Health Research Authority |
| **MEH** | Moorfields Eye Hospital |
| **NHS** | National Health Service |
| **NICE** | National Institute for health and Care Excellence |
| **OCT** | Optical Coherence Tomography |
| **OCTA** | Optical Coherence Tomography Angiography |
| **PI** | Principal Investigator |
| **PIN** | Patient Identification Number |
| **PIS** | Participant Information Sheet |
| **REC** | Research Ethics Committee |
| **RMC** | Research Management Committee |
| **ROC** | Receiver Operating Characteristic |
| **SHRM** | Subretinal Hyper-Reflective Material |
| **SmPC** | Summary of Product Characteristics |
| **SOP** | Standard Operating Procedure |
| **UK** | United Kingdom |

**List of 12 sites:**

**Moorfields Eye Hospital NHS Foundation Trust**
162, City Road
London, EC1V 2PD

**James Paget University Hospital NHS Trust**
Lowestoft Road
Gorleston on Sea
Great Yarmouth, Norfolk, NR31 6LA

**Frimley Health NHS Foundation Trust**
Frimley Park Hospital
Portsmouth Road
Frimley, GU16 7UJ

**Royal Liverpool and Broadgreen University Hospitals NHS Trust**
Royal Liverpool University Hospital
Prescot Street
Liverpool, L7 8XP

**King's College Hospital NHS Foundation Trust**
Denmark Hill
London, SE5 9RS

**Brighton and Sussex University Hospitals NHS Trust**
Sussex Eye Hospital
Eastern Road
Brighton, BN2 5BF

**Bradford University Hospitals NHS Foundation Trust**
Duckworth Lane
Bradford, BD9 6RJ

**Royal Victoria Infirmary**
Queen Victoria Road
Newcastle upon Tyne, NE1 4LP

**York Teaching Hospital NHS Foundation Trust**
The York Hospital, Wigginton Road,
York, YO31 8HE

**The Leeds Teaching Hospitals NHS Trust**
St James's University Hospital
Beckett Street, Leeds, LS9 7TF

**South Tyneside and Sunderland NHS Foundation Trust**
Sunderland Eye Infirmary
Queen Alexandra Road
Sunderland, SR2 9HP

**Belfast Health and Social Care Trust**
Royal Victoria Hospital
Grosvenor Road
Belfast, BT12 6BA

**Summary**

| | |
|---|---|
| **Title** | Can deep phenotyping using retinal images predict response to intravitreal aflibercept therapy in Patients with Neovascular Age-Related Macular Degeneration (AMD)? |
| **Short title** | PRECISE Study |
| **Study intervention** | None |
| **Objectives** | **Primary objective**: To analyse OCT and OCTA retinal image scans using artificial intelligence to identify good, partial and poor responders to intravitreal aflibercept therapy for wet age related macular degeneration and compare with human grading outcome.<br>**Secondary objective**:<br>To identify the imaging markers in order of accuracy to predict response to intravitreal aflibercept therapy treatment in neovascular AMD.<br>To develop an algorithm of imaging markers that will stratify patients into good, partial and poor responders.<br>To create a statistical model with the markers identified by human and artificial intelligence that can be used in routine clinical practice to accurately predict response to treatment |
| **Type of study** | Multi-centre study |
| **Study design and methods** | This is a multi-centre study that will look at imaging markers to predict treatment response to intravitreal aflibercept therapy in at least 2000 patients. Informed consent will be obtained from all patients who had (retrospective part) or are undergoing (prospective part) Heidelberg OCT with or without OCTA to export and use their anonymised imaging data for this study. The dates of 3 intravitreal aflibercept loading injections, visual acuity at these visits and next visit up to 10 weeks after the loading dose; and routine demographic data of age, gender and smoking data (if available) will be collected. |
| **Study duration per participant** | One-off consent to use their anonymised retinal imaging scans from each eye until 10 weeks after the 3 monthly loading injections |

| | |
|---|---|
| **Estimated total study duration** | 28 months |
| **Planned study sites** | 12 NHS sites |
| **Total number of participants planned** | 2000 subjects |
| **Main inclusion criteria** | Patients with newly-diagnosed neovascular AMD who are being treated with aflibercept therapy during the monthly loading phase (3 injections) and reviewed up to 10 weeks after the loading phase. |
| **Statistical methodology and analysis** | The primary outcome is to evaluate the AUC, sensitivity, and specificity of the Artificial Intelligence (AI) versus the human graders in detecting presence of a dry macula at the final visit. Accepted markers from OCT and blood flow-based OCTA markers will be then assessed statistically together with sex and age and other routinely available baseline data as potential predictors.<br>Half of the data will be used to develop an assessment of the biomarkers and their combinations. From a logistic regression framework, an organised sequence of statistical models will provide parameter estimates within each model from which will be derived the predicted patient probabilities predicting the primary outcome based on the other half of the data from patients in an independent validation. The performance of the predictive ability of each model will be measured by using the model's predicted probabilities within the area under the curve (AUC) of the receiver operating characteristic (ROC) curve.<br>All hypotheses tested will be 2-sided, and a *P* value of less than 0.05 will be considered statistically significant. No adjustment for multiple comparisons will be made because the study is restricted to a small number of planned comparisons. |

# 1. Introduction

## 1.1 Background

Regular intravitreal aflibercept therapy injections into the eye are the most common treatment for neovascular (wet) AMD in the UK, but at enormous cost to the National Health Service. It is a potent anti-vascular endothelial growth factor, but it is not universally successful. The 2013 UK Royal College of Ophthalmologists guidelines state that licensed anti-VEGF treatment will only improve vision in a third of patients; the majority will maintain vision, and some 10% will not respond to therapy. [1] Aflibercept therapy typically begins with 3 injections given at monthly intervals (loading dose), followed by regular review for up to and often beyond two years. It is clear that some patients respond to anti-VEGF very well after the first 3 injections, with retina being completely “dry”, that is the absence of any retinal fluid in the subfoveal area. Newer therapies need not be tested in this group of good responders. Furthermore, the visual outcome does not change much after the first 3 months, providing the patients are treated regularly as in the SmPC. Moreover, if the retina is completely dry, it is unlikely additional therapy can improve the visual outcome. In previous studies, it was suggested that these good responders account for about 35-40% of all patients in clinical trials. Recent data suggests that it might be as high as 50% as patients are now presenting earlier to retinal clinics. Hence, this group of excellent responders do not need the development of new therapies, and the new molecules should be targeting the groups of patients with the highest unmet needs.

However, at present, it is impossible to predict the good responders to aflibercept therapy in order to provide patients with the correct drug choice at baseline.

Currently, there are several widely accepted biomarkers on OCT that signify disease activity. [2] These signs prompt the ophthalmologist to commence or resume treatment with intravitreal aflibercept therapy. However, there is a paucity of biomarkers that predict the response to treatment from baseline. [3,4] Recently subretinal fluid has been shown to be a predictor of good outcome in one study. [5]These biomarkers are mostly based on small-scale studies involving a relatively small number of patients and require further validation.

OCTA is a novel and non-invasive technique for demonstrating the microvascular blood flow within the macula. It produces depth-resolved evaluation of the reflectance data from retinal tissue, providing a three-dimensional volume of information. [6] Several studies have identified different patterns of CNV in AMD patients, and various descriptors have been applied to the different morphologies of the CNV lesion. [7] Nevertheless, despite these novel findings, none of them have been studied in the prediction of response to intravitreal aflibercept therapy treatment.

Using the combination of OCT and OCTA images from medical retinal clinics in the UK, this study will aim to characterise imaging biomarkers using artificial intelligence and otherwise, and study the predictive value of these markers at baseline to identify good responders to intravitreal aflibercept treatment. The rest of the patients can be identified as partial and poor responders and we will be able to define this group better for eligibility criteria for new drugs for this indication.

### 1.2 Rationale and risks/benefits

The rationale of this study is to be able to precisely identify and predict people who will be good responders after 3 loading injections of intravitreal aflibercept from the partial and poor responders so that we can define a patient cohort of partial and poor responders who will benefit from alternate therapies that are being evaluated for neovascular AMD.

Several biomarkers have been shown to be predictors of poor response to intravitreal aflibercept therapy in AMD patients, in a few small-scale studies. These biomarkers include subretinal hyperreflective material (SHRM) and the type 2 subtype of CNV. In the Comparison of Age-related Macular Degeneration Treatments Trial (CATT) study, SHRM existed in 77% of eyes with neovascular AMD before intravitreal aflibercept therapy treatment. [4] After 2 years of treatment, eyes with a macular scar were more likely to have SHRM than other eyes, and eyes with greater SHRM dimensions were associated with worse visual acuity. An observational study by Chae et al. involving 154 eyes, showed that type 1 vs type 2 lesions had better vision after 24, 36, and 48 months of treatment. [3] Recently, a study using machine learning suggested that subretinal fluid on OCT may have a protective effect, whereas intraretinal fluid may predict poor response to intravitreal aflibercept therapy treatment. [5]

Studies on OCTA have identified different morphological patterns of CNV, including terms like “sea fan”, “medusa”, “tangle”, and “dead tree”. [7] Al-Sheikh et al. showed the correlation between some of these morphologies and the level of activity of the CNV. [8] Only one study showed use of this modality for the prediction of response to treatment. Using OCT-A, Kwashima et al. were able to categorise SHRM into two types: vascular and avascular, according to the vascularity seen on OCTA. They have shown that vascular SHRM is predictive of lower response to intravitreal aflibercept therapy. [9]

Our study will use a relatively large cohort of patients and will analyse multiple characteristics of each imaging modality in order to come up with more biomarkers for the predictability of treatment response in AMD. We expect the large sample size to assist in the validity of the findings. We also aim to analyse the combined use of these modalities to develop an algorithm to predict response.

## 1.3 Objectives

Primary objective:

To analyse OCT and OCTA retinal image scans using artificial intelligence to identify good, partial and poor responders to intravitreal aflibercept therapy for wet age related macular degeneration and compare with human grading outcome.

Secondary objective:

To identify the imaging markers in order of accuracy to predict response to intravitreal aflibercept therapy treatment in neovascular AMD.

To develop an algorithm of imaging markers that will stratify patients into good, partial and poor responders.

To create a statistical model with the markers identified by human and artificial intelligence that can be used in routine clinical practice to accurately predict response to treatment.

## 2. Overall study design

This is a multicentre study that will evaluate imaging biomarkers (OCT and OCTA) as predictors for response to intravitreal aflibercept therapy in treatment naïve neovascular AMD in at least 2000 patients across 12 NHS sites. This study involves both retrospective and prospective data collection in 4 visits. (Please see schedule of assessments). All consenting patients who have had first 3 monthly loading injections and meet the inclusion and exclusion criteria will be included in the study for retrospective data collection. These can be any patient since the NICE approval of aflibercept for AMD in UK.

Those with a new diagnosis of neovascular AMD and meeting the criteria will consent for the prospective part of the study. This group of patients will have 3 loading injections of intravitreal aflibercept at monthly intervals as per standard NHS care. They undergo mandated Heidelberg OCT at baseline and 4th visit (8-10 weeks after the last loading injections), the OCT-A is mandatory at the baseline visit only. They will have Heidelberg OCT at the 2nd and 3rd visits with OCTA being optional at these visits and visit 4. Patients who are in the midst of receiving the loading injections will need to wait for the completion of the loading injections and then will be enrolled as being in the retrospective part of the study at the next appointment. If the patient is not compliant for obtaining good quality OCT and OCTA images, they will not be enrolled into the study. If new patients are identified, they will consent before their first treatment for their data and OCT and OCTA (optional) images be obtained for this study until 8-10 weeks after the loading injections.

For both parts of the study, in addition to the images; dates of 3 loading intravitreal injections, visual acuity at all 4 visits and routine demographic

data like age, gender and smoking history (if available) will be collected. For the purpose of this study only the images and vision of the study eye will be collected. All anonymised images will be sent to Moorfields Eye Hospital and then to University College London and IBM, Australia and Europe to be graded by imaging experts and for machine learning respectively. The grading will be classified as good responder (macula is dry), partial responder (macula is not completely dry, but better than baseline) and poor responder (macula is not dry and is the same or worse than baseline). More detailed phenotyping may be done to evaluate prognosis.

***Selection of study eye:***

For the retrospective part, if both eyes meet the eligibility criteria both eyes can be enrolled into the study but a new patient identification number (PIN) must be generated for each eye. For the prospective part, only one eye will be designated as the study eye as per investigator's discretion. If the fellow eye converts during the study or after the study, this eye can be included in the study with a new PIN. In either retrospective or prospective part, a new consent form has to be signed by the patient for inclusion of the fellow eye.

## 3. Study population:

### 3.1 Inclusion criteria

Inclusion criteria for both retrospective and prospective parts:

1. Adults who are ≥ 50 years and ≤ 100 years
2. Treatment naïve neovascular AMD at baseline
3. Media clarity, pupillary dilation and patient cooperation for adequate imaging.
4. Ability to give informed consent

Inclusion criteria for retrospective part only in addition to above:

1. Have received 3 loading injections of intravitreal aflibercept therapy at monthly intervals as per standard care
2. Review up to 10 weeks after the 3rd loading dose with or without injection at this visit.
3. Had Heidelberg OCT at least at baseline and after the loading phase but ideally 4 Heidelberg OCTs for the 4 visits.
4. Heidelberg OCTA images if available for baseline and any visit thereafter (2nd, 3rd or 4th visit) provided there is a baseline OCTA (*optional criteria*)

### 3.2 Exclusion Criteria

1. Co-existent ocular disease: Any other ocular condition that, in the opinion of the investigator, might affect or alter visual acuity during the course of the study.

2. Any patient who has opted out of their information being used for research nationally or locally at any site.

### 3.3 Recruitment:

Newly or previously diagnosed neovascular AMD who have not already opted out of participating in research either directly at any site or through the National Data Opt-Out will be identified from clinics directly or from databases in medical retina clinics. An invitation letter and patient information sheet will be sent to the eligible patient by post or they will be approached in clinic at their routine appointment**.**

We plan to recruit at least 2000 patients with an additional 1000 with OCTA scans if possible. Recruitment will be completed within 18 months. This is based on our previous recruitment rates for studies for the same indication.

### 3.4 Withdrawals:

Withdrawals are not applicable for the retrospective part of the study. For the prospective part, the patient can be withdrawn by the investigator if:

1. The patient has missed visits / lost to follow up
2. Any ocular or systemic disease that according to the investigator is a contraindication for aflibercept injection or hinders imaging

Screen failures are not applicable for both parts of the study, as the patients are only enrolled after establishing eligibility.

### 3.5 Patient identification number (PIN):

A unique PIN will be generated by registering the patient on the electronic database after consent has been signed. Once allocated, the PIN number will identify the subject throughout the study including the anonymised transfer of images.

## 4. Study procedures and schedule of assessments

### 4.1 Informed consent procedure

The investigator will provide the patient information sheet (PIS) and consent form by post or in person after checking whether the patients have opted out through the national data opt-out or locally before informed consent is obtained.

Patients will be given sufficient time as they may require after receiving the PIS to consider taking part in the study. They will also be informed that they are under no obligation to participate. The Investigator or designee will also answer all the queries in person or by telephone.

A copy of the original signed Informed Consent will be given or sent to the participant. A copy of the original signed Informed Consent will be placed in the patients notes. The original signed form will be retained at the study site in the project site file.

Investigators will be able to obtain consent by telephoning the patient (using the specific telephone informed consent form) and taking consent over the phone for the retrospective part of the study. Investigators or delegated study team members will also need to document the consent process in the patients' health records.

In the event that a participant loses capacity to consent while being treated in the study, he/she would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to you. This does not apply if you had provided a one off consent for use of previous data collected.

### 4.2 Randomisation procedures

None

### 4.3 Emergency unblinding

Not applicable

### 4.4 Schedule of Assessments

All potential participants will be consented before we collect the anonymised images. Data will be collected from each patient as shown in the table. The images and any other data will be identified by a study PIN number only. The three loading injections should be a month apart (baseline, 2nd and 3rd visit) and the 4th review visit should be 8-10 weeks after the 3rd loading dose

| | **Baseline** | **2nd visit** | **3rd visit** | **4th visit** |
|---|---|---|---|---|
| **Demographics (age, and gender)** | X | | | |
| **Smoking history (optional)** | X | | | |
| **Visual acuity of study eye** | X | X | X | X |
| **Date of Aflibercept injection** | X | X | X | |
| **Heidelberg OCT** | X | (X) | (X) | X |
| **Heidelberg OCTA (retrospective part)** | (X) | (X) | (X) | (X) |
| **Heidelberg OCTA (prospective part)** | X | (X) | (X) | (X) |

X – mandated (X) - optional

### 4.5 Definition of end of study

The last patient arriving for a fourth visit will be defined as the date of end of the study.

## 5. Name and description of the intervention used in the study

Collection and transfer of anonymised Heidelberg OCT and OCTA images from neovascular AMD patients who were treated with 3 loading aflibercept intravitreal injections will be done using the below specifications

### 5.1: Instrument and Software requirements

Software:

HRA2 systems require software version 5.6 or higher.
Spectralis systems require software version 5.4 or higher.
Heyex 6.7a OCTA or higher
HEYEX 2 server installed in Heidelberg Engineering device

Hardware:

Heidelberg™ Spectralis with OCT2 Module installation

### 5.2: Study visit masking:

All identifiable patient information from the images must be removed; such as the names of the patient and any other information that can link to identify the patient or investigator site.

NB: Study site should keep a log of the patients linked to the anonymised patient identification number.

In the Spectralis machine, click on the ‘New patient’ icon and complete the details as follows

a. Last Name: Enter study name “Precise”
b. First Name: Enter centre number and patient study number – (e.g. 01-001)
c. Date of Birth: Enter 01/01/1900
d. Gender: must be entered.
e. Patient ID: Enter Patient study number (e.g. 001)
f. Disregard ‘More data’ and ‘Memo’
g. On the “Examination Data” box - Select Device Type: Spectralis OCT
h. Operator: Select from Drop down list
i. Study: Select from drop down list or add new study by clicking OK and enter PRECISE Study

### 5.3: SD-OCT IR Dense Volume scan setting

Acquire the images using the following settings for study eye

- Centre on the fovea
- Field of view: Lens 30°
- Resolution: High Speed (HS)
- ART mean: At least 15 frames – Do not exceed 25 frames for patient comfort
- Normalized setting: activated/on
- IR mode

| **Final scan settings** | |
|---|---|
| 20°x20°, HS | 512 A scans |
| 49 sections , 120microns | ART 15 minimum |

### 5.4: OCTA Scan settings

Mandated scans will be obtained at baseline and visit 4 in the prospective part and optional scans at visit 2 and 3. The following 2 scans are obtained for the study eye only with the following settings. If the patient is not compliant with both scans at baseline, only scan 1 will be performed at subsequent visits.

Scan 1

OCTA setting: 512 A scans - 20°x 20° HS, ART 5 , 512 sections – 11 microns (30-40 seconds time to perform approximately)

Scan 2

OCTA setting: 512 A scans 10° x 10° HR, ART 5, 512 sections - 6 microns (50-60 seconds time to perform approximately)

### 5.5: Exporting of images

The final step of exporting images will be facilitated by HEYEX 2 or NHS complaint encrypted device. HEYEX 2 is a complete ophthalmic image management solution designed to streamline work flow and enhance data security. All anonymised images are transferred to Moorfields Eye Hospital and then to University College London and IBM, Australia and Europe.

The OCT and OCTA scans will be manually inspected for treatment response at Moorfields Eye Hospital and appropriately graded using detailed grading format for each imaging biomarker.

The OCT and OCTA images will be graded wet or dry and sent for machine learning process to IBM Australia and Europe where the images may require to be transferred between IBM Australia and IBM Europe depending on the expertise required.

Roles of collaborating sites:

1. 12 NHS sites will consent patients for use of their data for this study. The data including retinal images will be exported to Moorfields Eye Hospital through the HEYEX system or encrypted NHS compliant encrypted device.
2. The retinal images will then be transferred to UCL data scientists for data checks and eligibility of quality of images for artificial intelligence using analytical tools and inspection. The images will be broadly classified as those that did and did not pass the quality checks for reporting purposes. They will also divide the dataset into test set and validation set.
3. After quality check, the retinal images and linked data will be sent back to Moorfields to be sent to IBM Australia and Europe to develop and validate the AI of the treatment response. This will form the AI test and validation set. The retinal images will also be graded by trained retinal fellows in Moorfields using a detailed grading format that will be developed with the help of the data scientists in UCL and the medical statistics team in Imperial. This set is the human graders test and validation set.
4. On receiving the data from human graders and IBM, the data scientists in UCL with experience in big data, machine learning and analytical tools will work with the Medical Statistics team to incorporate the data and grading obtained from retinal images graded by human graders to develop an algorithm on treatment response. This algorithm will be tested for accuracy in different scenarios, clear media, age and gender stratified to contribute to the secondary outcomes of the study.
5. The Medical statistics team will work with the grading outcomes received from the data scientists, clinicians and IBM to develop the diagnostic accuracy models in whole population and in stratified populations comparing AI with human graders.

## 6. Recording and reporting of adverse events and reactions

All adverse events and serious adverse events related to this project in terms of image capture will be collected and reported. OCT and OCTA are non-invasive diagnostic techniques that utilise reflected light waves. There are no known side effects or complications related to this technique.

## 7. Data management

### 7.1 Confidentiality

All data will be handled in accordance with the Data Protection legislation. The database will not bear the subject's name or other personal identifiable data.

The patients are selected from routine care records, and the Study site coordinator will record each patient as a PIN number generated by the study database for each patient. The PIN Number of each patient identified by hospital number will be kept in

the site file and kept in locked research office rooms. The images will be identified only by the study PIN number without any metadata or links to the original machine for transfer and analysis at Moorfields Eye Hospital and then to University College London and IBM, Australia and Europe.

The study consent will explicitly mention that the fully anonymised OCT and OCTA images will be exported to outside Europe and therefore be subject to different data protection laws. The images will also be shared with commercial entities who have the scientific expertise to deal with predictive modelling and deep phenotyping of retinal images using artificial intelligence and other computational tools. The images are fully anonymised by the Heidelberg system and so no metadata or identifiers will be possible.

### 7.2 Data collection:

The data collection tool for this study will be through an electronic data capture (EDC) system. Data required according to this protocol will be recorded by site personnel at their respective sites. All access to the EDC system is through a password-protected security system. The generation of PIN also happens through this system. The EDC contains an audit trail that captures any changes made to the data field, including who made the change, the reason why the change was made and the date and time it was made. This information will be available for both the personnel at the investigator's sites and the sponsor designees.

### 7.3 Record keeping and archiving

All paper-based site files containing patient identifiable data will be retained for 5 years to comply with the NHS Records Management Code of Practice 2016.

Original digital documents will be kept within the NHS environments at the respective data collection sites. Copies of fully anonymised digital documents and raw image files will be shared with Moorfields Eye Hospital and University College London and external collaborative partners (IBM) via encrypted connection via HEYEX2 or secure NHS compliant encrypted devices. Documents will be stored and shared via hardware encrypted devices compliant with digital NHS.

Principal Investigators are responsible for the secure archiving of study documents and the study data. The study data needs to be archived for 5 years. Investigators should provide archiving details to the Chief Investigator/delegate and will be instructed that authorisation from the Chief Investigator should be obtained before study data or study documentation is destroyed.

## 8. Statistical Considerations

### 8.1 Sample size calculation

With a total of 2000 patients, then after allowing for 10% missing data in variables in the modelling, 900 will be involved in the development phase and 900 in the

validation phase of the modelling. Data from the Royal College of Ophthalmologist suggests that up to 10% of patients do not respond to treatment, and another 30% may improve vision after treatment, and the rest may maintain the vision. Therefore, the number of patients with events of improved vision is expected to be 270 in each phase. The rule of thumb of ten events per variable in a logistic regression model is commonly applied, although a higher quality criterion of 20 can be appropriate to apply[10]. This allows room for up to 13 predictors to be assessed in modelling improvement in vision and a smaller model of up to 9 predictors of no response to treatment.. This would then allow the recommended backwards elimination approach to model selection to be considered. For the endpoint of dry macula at the end of follow-up. 270 patients with the event and 630 without the event, provide precise estimates of sensitivity (95% confidence interval width of 10.3%) and specificity (95% CI width 6.8%), calculated where these are each observed to be 75%. There is also adequately precise estimation of the area under the ROC curve (95% CI width <0.078 for AUCROC>0.7)[11]. An anticipated reduction in the sample size from 2000 to between 200 and 400 with OCTA based markers would mean that these markers can each be assessed in a single phase analysis adjusting for a limited number of other predictors identified in the main analysis, and which will inform the relative value of OCTA markers for taking forward in future validation studies..

On the other hand, the deep knowledge techniques required of a minimum of 500 patients eye information on each group for the machine to learn and determine the potential difference in the two groups of interest (responders and non responders). Additionally it is needed a minimum of other 500 patients eye information to validate the results. Since one of the objectives is to quantify features available by OCT, and there are two different imaging scans currently available OCT and OCTA where OCTA may provide more detailed information, it will be needed to consider separately these two groups of patients in the sample size calculations. Based on this considerations, the number of patients needed is 2000 patients.

### 8.2 Statistical analysis plan

The hypothesis determined is that the artificial intelligence system (AI) will be at least comparable to the human graders' performance in detecting dry macula.

The primary outcome is the presence of a dry macula at the final visit. Accepted markers from OCT and blood flow-based OCTA markers will be then assessed statistically together with sex and age and other routinely available baseline data as potential predictors.

Primary outcome:

- To evaluate the AUC, sensitivity, and specificity of the AI vs the human graders in detecting dry macula

Secondary outcomes:

- The analyses will be repeated excluding patients who appeared in training set

and the primary validation set
- Performance of the AI will be evaluated using higher-quality images with no media opacity (eg, cataracts) as noted by professional graders
- AUC subgroups will be computed stratified by age and sex.
- The analysis will be repeated by calculating the AUC, sensitivity, and specificity of the AI and the proportion of concordant and discordant eyes on the external validation datasets, compared with the reference standards

Half of the data on patients within the database will be used to develop an assessment of the biomarkers and their combinations. From a logistic regression framework, an organised sequence of statistical models will provide parameter estimates within each model from which will be derived the predicted patient probabilities predicting the primary outcome based on the other half of the patients in an independent validation. The performance of the predictive ability of each model will be measured by using the model's predicted probabilities within the area under the curve (AUC) of the receiver operating characteristic (ROC) curve.[11]

The performance will be further demonstrated in terms of sensitivity and specificity at thresholds where these are equal, and where the sensitivity and specificity exceed each other. Positive and negative predictive values will be presented and then used in combination with the prior probability (prevalence) of dry macula at final visit to provide updated posterior probabilities.

The modelling process will initially exclude OCTA based biomarkers. These will be brought in on the reduced dataset of patients with this measured. Further models will utilise OCT at the 2$^{nd}$ as well as the baseline visit to explore whether changes in biomarkers during the first month may improve the prediction over and above baseline.

The rates of false positives (predicting success when macula is not dry) and false negatives (predicting failure when macula is dry) from the models will be assessed by sex, age and baseline visual acuity. 95% confidence intervals will be presented for rates. P-values will be presented for predictors of the primary outcome, and for testing the paired difference between the AUC of ROC curves. [12] A detailed statistical analysis plan will be developed using the principles of developing and validating prognostic models [12,13] and methods for accounting for the distribution of biomarkers and dealing with any detection limits and missing data.

All hypotheses tested will be 2-sided, and a P value of less than 0.05 will be considered statistically significant. No adjustment for multiple comparisons will be made because the study is restricted to a small number of planned comparisons.

### 8.3 Randomisation

Not applicable

### 8.4 Interim analysis:

None

## 9. Direct Access to Source Data/Documents

The investigator(s)/ institution(s) will permit study-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data/documents. Study participants are informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes. Only the patients' routine clinical care team will have access to the patient records and the research co-ordinator at each site will have the link of the study numbers to the patient records which will be kept in locked rooms at each research site.

## 10. Ethics and regulatory requirements

The sponsor will ensure that the study protocol, patient information sheet, consent form, and submitted supporting documents have been approved by the main research ethics committee/HRA, prior to any patient recruitment. The protocol and all agreed substantial protocol amendments will be documented and submitted for ethical and regulatory approval prior to implementation.

Before the sites can enrol patients into the study, the Principal Investigator or designee must be granted permission from each host organisation prior to the start of the study at the site concerned. Each NHS organisation involved in the study must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed following an assessment of capability/capacity. It is the responsibility of the Principal Investigator or designee at each site to ensure that all subsequent amendments gain the necessary approval. This does not affect the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual patients. Within 90 days after the end of the study, the PI and sponsor will ensure that the main REC are notified that the study has finished. If the study is terminated prematurely, those reports will be made within 15 days after the end of the study.

The CI will supply a summary report of the clinical study to the main REC within 1 year after the end of the study.

### Annual progress reports

An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended. The chief investigator will prepare the APR.

## 11. Monitoring plan for the study

The study will be monitored according to the monitoring plan agreed by the Sponsor. Authorized representatives of the Sponsor or regulatory authority

representatives may conduct on-site visits to review, audit and copy study-related documents. These representatives will meet with the investigator(s) and appropriate staff at mutually convenient times to discuss study-related data and questions.

## 12. Finance

This Investigator Initiated Grant is funded by Boehringer Ingelheim.

## 13. Insurance

NHS Indemnity covers the Sponsor's liability for the design, management and conduct of this study.

## 14. Publication policy

Authorship and manuscript composition will reflect joint cooperation between multiple investigators. Authorship will be established prior to the writing of the manuscript.

## Statement of compliance

The study will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement(s).

## 15. References:

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9. Kawashima Y, Hata M, Oishi A, et al. Association of Vascular Versus Avascular Subretinal Hyperreflective Material With Aflibercept Response in Age-related Macular Degeneration. *Am J Ophthalmol*. 2017;181:61-70. doi:10.1016/j.ajo.2017.06.015

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13. Altman DG, Vergouwe Y, Royston P, Moons KGM. Prognosis and prognostic research: validating a prognostic model. *BMJ*. 2009;338. doi:10.1136/bmj.b605.

## ***Appendix 1 - Anonymisation/Pseudonymisation Process***

1. The data to be exported to IBM Watson and UCL will include Patient Study Number, Age, Study Eye, Smoker/ Non-smoker, Ethnic group, medical history, if FFA done at baseline the lesion subtype, Visual acuity, OCT and OCTA qualitative and quantitative parameters at baseline and week 16 in study eye and non-study eye. OCT parameters at week 4, 8 and 12 are optional.

2. Table of data below shows the data before, during and after anonymisation: with columns listing all fields in the dataset, with one column showing original

data, a column for the procedure and a final column with the data as it will be sent.

| Original Column | | Procedure | Final Column | |
|---|---|---|---|---|
| PatKey | Hospital Number | Anonymisation through has function (MD5) | Study ID | Number, Integer |
| Eye | Number, Categorical 0=right, 1= left | | eye | Number, categorical 0=right, 1=left |
| Appointment date | Date Xx/xx/xxx | Anonymisation by replacement of days relative to first date | Follow-up weeks | Baseline, week 4, 8, 12 and 16 |
| Date of Birth | Date Xx/xx/xxx | Anonymisation be replacing date of birth with age at baseline | Baseline age | Number, integer |
| OCT and OCTA and FFA Heidelberg Engineering | Hospital Number, name and date of birth | Anonymisation using Heidelberg Batch Anonymisation tool | Study ID | Number, Integer |

4. The data will be anonymised before entry into the study database after research receives it. The anonymised retinal images (OCT and OCTA images) will be linked to the study database only by studyID number. The data will be fully anonymised before it leaves the Trust so the data cannot be reconstructed.

5. The data exports to IBM Australia and Europe will be sent by encrypted batch(es).

6. The data manager will check each batch of data manually to ensure that the data has been fully anonymised and the checks will be documented in the monitoring report.