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Thesis No	**108 : Draft a protocol for an observational study for anti-VEGF therapy in age related macular degeneration**

Observational Study Protocol

A prospective, observational, open-label, multicentre study to investigate the effectiveness of the routine treatment practice with ranibizumab in Greek patients with neovascular age-related macular degeneration.

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STATEMENT OF COMPLIANCE

This document is a clinical research protocol. The study will be conducted in compliance with the protocol and in accordance with the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP), with the ethical principles laid down in the Declaration of Helsinki and with the Greek National Organization of Medicines (EOF) regulations.

All personnel involved in the conduct of this study have completed human subjects protection training.

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List of Abbreviations

AE	Adverse Event
AMD	Age-related Macular Degeneration
APTC ATEs	Antiplatelet Trialists' Collaboration Arterial Thromboembolic Events
BCVA	Best Corrected Visual Acuity
CI	Confidence intervals
CMP	Clinical Monitoring Plan
CNV	Choroidal Neovascularization
CRF	Case Report Form
CRT	Central Retinal Thickness
CRO	Contract Research Organization
DBL	Database lock
EDC	Electronic Data Capture
EMA	European Medicines Agency
EOF	Greek National Organization of Medicines
EOS	End of Study
FDA	United States Food and Drug Administration
GCP	Good Clinical Practices
HRQoL	Health-Related Quality of Life
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IOP	Intraocular Pressure
IRB	Institutional Review Board
NEI-VFQ-25	National Eye Institute Visual Function Questionnaire-25
OCT	Optical Coherence Tomography
PRN	Pro re nata (as needed)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SPC	Summary of Product Characteristics
TE	Treat and extend
VA	Visual Acuity
VEGF A	Vascular Endothelial Growth Factor A

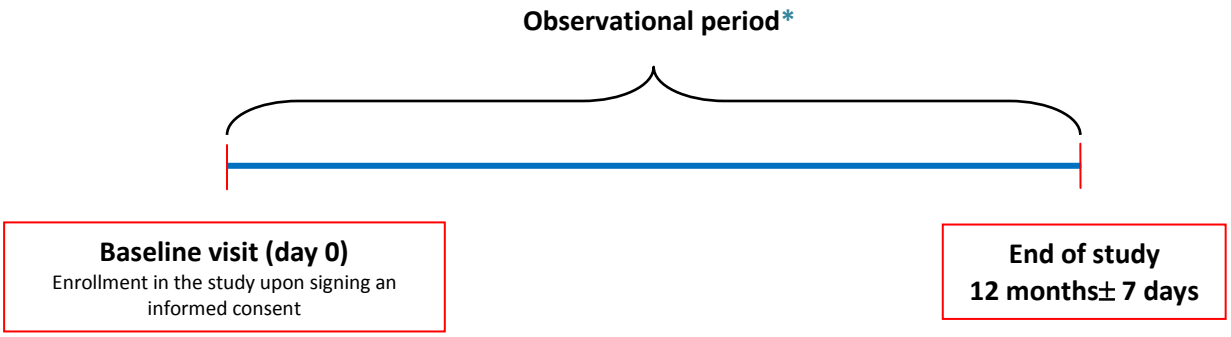
Protocol synopsis

Title:	An observational, prospective, cohort, multicenter, open label, single-arm 1-year study to investigate the effectiveness of the routine treatment practice of ranibizumab in Greek patients with neovascular age-related macular degeneration.
Background and rationale:	Ranibizumab- a recombinant, humanized, monoclonal antibody fragment that neutralizes all active forms of vascular endothelial growth factor A (VEGF-A) represents standard first line therapy for neovascular age-related macular degeneration (AMD). Although the efficacy and safety of ranibizumab have been studied and established in several phase III clinical trials, there is limited data in routine clinical conditions in Greece regarding the effectiveness, safety and treatment patterns in patients with neovascular AMD treated with intravitreal injections of ranibizumab. Therefore, a 12-month observational, prospective, cohort, multicenter, open label, single-arm study will be conducted in selected centers of Greece in order to collect data on the effectiveness and safety of ranibizumab and to describe treatment patterns in a study population of treatment naive patients with neovascular AMD treated with ranibizumab according to routine clinical practice in Greece.
Objectives:	**Primary Objective** • To evaluate the functional effectiveness of ranibizumab treatment in treatment naive patients with neovascular AMD in routine clinical practice in Greece as assessed by mean change in Visual Acuity (VA) from baseline to month 12 . **Secondary Objectives** • To evaluate the safety of ranibizumab as assessed by the type, frequency, and severity of ocular and non-ocular AEs over 12 months of treatment with intravitreal injections of ranibizumab for neovascular AMD in treatment naive patients in routine clinical practice in Greece. • To describe treatment patterns of ranibizumab administration for neovascular AMD in routine clinical practice in Greece. • To evaluate the anatomic effectiveness of ranibizumab treatment in treatment naive patients with neovascular AMD in routine clinical practice in Greece as observed in this 1-year study duration and assessed by mean change in Central Retinal Thickness (CRT) from baseline to month 12 .
Study design:	This study is an observational, prospective, cohort, multicenter, open label, single-arm 1-year study. The study will be conducted in ophthalmological clinics and practices throughout Greece. It is planned to collect valid documentations in 251 eligible patients with neovascular AMD. The decision upon treatment is made at the discretion of the attending physician, according to his/her medical practice.
Duration:	The overall planned duration of the study is anticipated to be 2 years. The enrollment period of subjects will continue until the required number of subjects will be enrolled or will last 12 months (whichever occurs earlier). Patients will be recruited from 10 Medical Retina Departments of outpatient Ophthalmology Clinics of public hospitals or private eye centers of Greece. Each enrolled patient will be followed for 12 months unless they discontinue or

	withdraw from the study. Every patient who successfully completes the study through Month 12 will be considered to have completed the study. A minimum of three visits per year is required in order to maintain a patient's participation in the study. The end of the study will be reached 12 months after last patient's first visit and enrollment or when study participation for all patients is completed. No extension study is been planned.
Population:	This study will include 251 consenting eligible patients who have been diagnosed with neovascular AMD, have not received any treatment for neovascular AMD and will be treated with ranibizumab as first treatment for exudative AMD.
Number of Sites:	Ten Medical Retina Departments of outpatient Ophthalmology Clinics of public hospitals or private eye centers of Greece.
Inclusion/ Exclusion criteria:	**Inclusion Criteria** Patients eligible for inclusion in this study must fulfill all of the following criteria: • Patients willing and able to provide written informed consent personally or by legal proxy. Written informed consent must be obtained before any study assessment is performed. • Patients willing to comply with all study procedures and to be available for the duration of the study. • Male or female ≥ 50 years old patients. • Patients with an eye diagnosed with neovascular AMD which has not received any treatment for neovascular AMD and is decided to initiate treatment with ranibizumab. • Patients where the decision of administration of ranibizumab for the treatment of neovascular AMD in accordance with the local summary of product characteristics (SPC) has been taken before the evaluation of the patient's eligibility for inclusion in the study. • One eye of each patient will be included in the study. If both eyes are eligible at Baseline visit the eye to be selected as the study eye is the one the investigator deems to be more appropriate for study treatment and the study, based on CNV lesion characteristics in addition to visual impairment. If the fellow eye requires treatment because of visual impairment due to neovascular AMD, it may also be treated at the discretion of the investigator. This eye, however, will not be included in the study. Any treatment administered in the fellow eye will be recorded in electronic CRF, but only the data for the study eye will be included in the analysis of outcomes of the study. **Exclusion Criteria** Patients fulfilling any of the following criteria are not eligible for inclusion in the study: • Simultaneous participation in a study that includes administration of any investigational drug or procedure with interventions outside of routine clinical practice. • Systemic treatment with any VEGF inhibitor within 6 months prior to study enrollment • Any prior therapy with any agent to treat neovascular AMD in the study eye. • Patients who have any contraindications to the administration of ranibizumab according to the product SPC. • Women of reproductive potential, defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during study period treatment (e.g. total abstinence, sterilization, barrier methods of contraception, hormonal contraception, intrauterine system). • Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test. • Men of reproductive potential unless using effective methods of contraception during study period (e.g. condoms).

Safety assessments:	All Serious Adverse Events (SAEs) reported to or noted by the physician from the time the patient signs the informed consent until 30 days after study discontinuation will be recorded in the SAE Case Report Form (CRF) and reported to the Sponsor within 24 hours of learning of its occurrence. All Adverse Events (AEs) will be captured on the AE CRF throughout the safety recording period (see Section 6 for details). AEs detected through ophthalmic examinations will be collected if available.
Clinical assessments and data collection:	Timing of actual patient visits is at the discretion of the treating physician. It is recommended all visits should be documented in the CRF within 1 month post visit. A minimum of three visits per year is required in order to maintain a patient's participation in the study. Data elements to be collected include demographics and medical history/ocular history (baseline only), relevant prior and concomitant medications, ophthalmologic examination, treatment information, VA, CRT as evaluated with Optical Coherence Tomography (OCT), SAEs/AEs. Physicians will be encouraged to follow-up with patients who have not been seen in the clinic for at least 3 months since the last visit to capture any AEs/SAEs that may have occurred since the previous visit, and again 3 months later if no subsequent visit occurs within the following 3 months.
Data analysis:	The primary effectiveness variable will be the mean change in VA with the 95% confidence interval (CI) from the baseline visit to month 12 in the study eye. Paired sample t test will be applied to compare the 12-month VA to Baseline VA. The secondary safety objective of this study will be assessed based on the number and proportion of patients experiencing any SAE/AE during the 12-month period which will be calculated and presented in frequency tables. Frequency tables will be presented for AEs by causality, seriousness, discontinuation of therapy, action taken and outcome. The treatment pattern variables will be evaluated using summary statistics; the number of ranibizumab injections administrated overall during treatment period, number of visits, reasons for re-treatment or not (i.e. VA deterioration, OCT abnormality etc), reasons for treatment termination and the average time interval (in weeks) between consecutive visits and injections will be summarized for the treated eye. Use of antimicrobial agents pre- or post-injection will be also evaluated. The mean change in CRT, as indicated by OCT measurements, from baseline to month 12, along with the 95% CI, will be calculated in order to evaluate the secondary effectiveness objective. Paired sample t test will be applied to compare the 12-month CRT to Baseline CRT.

Schematic diagram of study



*There will not be applied a strict, mandated visit schedule during the observational period.
The follow-up visits during observational period will take place at a frequency defined as per each investigator's discretion in line with their routine clinical practice.
At least another one visit between Day 0 and Month 12 is required in order to maintain a patient's participation in the study.

1 Introduction: Background information and scientific rationale

1.1 Background

Age-related macular degeneration (AMD) results in severe and irreversible loss of central vision and is the leading cause of legal blindness affecting 10-13% of adults over 65 years of age in the Western World (1-4). The disease has a profound effect on quality of life of affected individuals and represents a major socioeconomic challenge for societies due to the exponential increase in life expectancy and environmental risk factors.

The non-neovascular ("dry") form of AMD is the most common type and accounts for 85% of all AMD cases. This non-neovascular form is characterized by drusen and atrophic changes in the retinal pigment epithelium. The second and less common, neovascular ("wet") form of AMD is characterized by choroidal neovascularization (CNV). In CNV, the newly formed vessels have a tendency to leak blood and fluid, causing symptoms of scotoma and metamorphopsia. Furthermore, these new vessels are accompanied by proliferation of fibrous tissue leading to loss of photoreceptors within 3 to 24 months (5). The lesion can continue to grow, resulting in progressive, severe loss of central vision. Though the neovascular form of AMD is much less common, 80% to 90% of severe vision loss related to AMD is attributable to this form characterized by CNV (6, 7).

General ophthalmologic examination procedures, such as determination of best corrected visual acuity (BCVA), stereoscopic ophthalmoscopy and home monitoring between routine visits should be implemented in every patient diagnosed with dry AMD. Whenever neovascular AMD is suspected, advanced diagnostic measures such as fluorescein angiography and optical coherence tomography (OCT) must follow to confirm the diagnosis.

Although the pathogenesis of AMD is not fully understood, there is evidence from preclinical data that VEGF-A, a diffusible cytokine that promotes angiogenesis and vascular permeability, plays a key role in the pathophysiology of neovascular AMD and in the formation of CNV lesions (8).

The wide introduction of anti-VEGF therapy has led to an overwhelming improvement in the prognosis of patients affected by neovascular AMD, allowing recovery and maintenance of visual function in the vast majority of patients. Ranibizumab is a recombinant, humanized monoclonal antibody fragment that neutralizes all active forms of vascular endothelial growth factor A (VEGF-A). The binding of ranibizumab

to all isoforms of VEGF-A prevents the interaction of VEGF-A with its receptors (VEGFR1 and VEGFR2) on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation (9). Ranibizumab represents one of the standard first line therapies for neovascular AMD. Whereas previous treatments were effective in slowing the progression of disease and disability, ranibizumab has been shown not only to prevent vision loss but also to improve visual acuity (10-14).

The efficacy and safety of ranibizumab in the treatment of AMD have been established through several randomized, multicenter phase III clinical trials like ANCHOR (10, 15), MARINA (14, 16), PIER (17), SUSTAIN (18), CATT (19), HARBOR (20) and IVAN (21). These clinical trials proved that ranibizumab can efficiently block the pathophysiological process of AMD, restore retinal morphology and increase/maintain neurosensory function in most patients with neovascular AMD. They also demonstrated that intravitreal inhibition of VEGF-A with ranibizumab resulted in rapid improvement in visual acuity (VA) was superior to the respective control treatment and translated into improved visual function as measured with the National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25). Indicatively, MARINA study was a sham-controlled phase 3 trial where patients received monthly injections of 0.3 or 0.5 mg of ranibizumab or sham treatment continuously over 24 months (14, 16). At 12 months, 95% of ranibizumab-treated eyes compared with 62% of sham-treated eyes, lost <15 letters while VA improvement by >15 letters was found in 34% of eyes treated with ranibizumab at a dose of 0.5 mg (14). At 24 months, 90% of eyes in the 0.5 mg group continued to maintain stable vision without loss of >15 letters compared with 53% in the sham treated group. Furthermore, the two-year data of the MARINA study showed that 33% of eyes in the 0.5 mg dose group improved by >15 letters with 42% ending up with a VA of 20/40 or better (16).

Subsequent to the encouraging 1-year data from the ANCHOR and PIER trials and positive 2- year data from the MARINA trial, ranibizumab (Lucentis®), administered intravitreally at a dose of 0.5 mg (0.05 mL of 10 mg/mL ranibizumab solution), received approval for the treatment of all angiographic subtypes of CNV secondary to AMD from the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) in July 2006 and January 2007, respectively.

Fixed regimens were evaluated in the ANCHOR (10, 15), MARINA (14, 16) and PIER (17) studies in patients with classic or occult CNV. The pivotal MARINA and ANCHOR trials have shown that monthly intravitreal injections of ranibizumab not only maintain but also improve VA and function in patients with neovascular AMD (10, 14, 15). A randomized, double-masked, active control, multicenter phase III

trial EXCITE (22) demonstrated that participants who after three monthly, loading doses received quarterly injections of ranibizumab showed improvement of vision but not to the level achieved by the monthly regimen. The above trial found that intravitreal injections of ranibizumab given quarterly were clearly superior to treatment with sham (MARINA) or verteporfin photodynamic therapy (ANCHOR) in patients with CNV secondary to AMD but that treatment scheme was unable to deliver the same improvement in vision as the monthly treatment. Additionally, patients treated with ranibizumab reported large improvement in near vision, distance vision and vision-specific dependency as indicated by the NEI-VFQ-25 (23).

The topical route of administration of ranibizumab through intravitreal injection is considered to minimize potential systemic effects of anti-VEGF agents such as ranibizumab that are used to treat neovascular AMD. However, intraocular administered drugs could enter the systemic circulation, despite the presence of the blood-retinal barrier, which shields the retina from circulating blood. Several prospective, randomized clinical trials have demonstrated that intravitreal therapy with ranibizumab is generally well tolerated. However, within these trials, there is some circumstantial evidence that links systemic VEGF inhibition to systemic adverse events, particularly systemic thromboembolic events.

For instance, in the phase 3 ANCHOR and MARINA trials, intravitreal ranibizumab injections were associated with a low rate of ocular AEs, including endophthalmitis, uveitis, and transient increases in intraocular pressure (IOP). In these trials, a slightly increased but still low rate of systemic AEs, like Antiplatelet Trialists' Collaboration arterial thromboembolic events (APTC ATEs) (i.e. nonfatal myocardial infarction, stroke, and vascular death) and nonocular hemorrhages, were reported (14, 15).

Two-year data from the phase 3 MARINA trial showed that the rate of APTC ATEs was 4.6% in both ranibizumab dosage groups compared with 3.8% in the sham injection group, and serious nonocular hemorrhages were reported in 2.1% and 1.3% of patients in the 0.5-mg and 0.3-mg ranibizumab groups, respectively, compared with 0.8% of patients in the sham group (14). At 2-year follow-up of the phase 3 ANCHOR trial, APTC ATE rates were 5.0% of patients in the 0.5-mg group compared with 4.4% in the 0.3-mg group and 4.2% in the verteporfin photodynamic therapy group; serious nonocular hemorrhages were reported in 2.1% and 2.9% of patients in the 0.5-mg and 0.3-mg ranibizumab groups, respectively, compared with 0.7% of patients in the verteporfin group (15). The above mentioned data are indicative, and the systemic safety of ranibizumab is currently under investigation, with predominantly circumstantial evidence from clinical trials linking ranibizumab to systemic AEs.

1.2 Study Rationale

Real-life outcomes of ranibizumab treatment of neovascular AMD have consistently been found to be less favorable than clinical trial results (24-26). We have to distinguish between the *efficacy* and the *effectiveness* of an intervention. Efficacy trials are clinical trials that investigate whether an intervention produces the expected result under ideal circumstances. Effectiveness trials investigate whether a treatment results in the expected outcome under real, everyday routine clinical conditions and not under ideal circumstances (27).

Most of the times, the dosing regimens that are used in everyday clinical practice do not follow the treatment protocols of the above-mentioned multicenter clinical trials and differ among specialists, even in the same department.

Except from the strict, monthly administration of ranibizumab, many, more flexible regimens have been used among retina specialists. They all target to similar visual gain with fewer injections compared to monthly treatment. The official Summary of Product Characteristics (SPC) for ranibizumab in Europe recommends monthly intravitreal injections continued until maximum VA is achieved (9). One popular choice is treatment as-needed [Pro re nata (PRN)] where the first three consecutive monthly intravitreal injections are followed by a monthly monitoring with a VA and OCT-guided retreatment. More precisely, as retreatment criteria could be applied a 5-letter loss in VA and fluid as detected by OCT, a >100 μm increase in central retinal thickness (CRT), a new-onset classic CNV, a new macular haemorrhage or persistent macular fluid detected by OCT (28). Treat-and-extend (TE) is another flexible treatment strategy aiming to reduce retreatment rate and patient's overall visits. After three initial monthly ranibizumab TE scheme continues with monthly injections until no CNV activity is detected (i.e. subretinal or intraretinal fluid, loss of >5 letters in VA, or persistent/recurrent retinal haemorrhage). Thereafter, the interval to the next visit and injection is extended stepwise by 2 weeks to a maximum of 12 weeks. When any CNV activity is noted, this interval is shortened by 2 weeks (29).

A non-interventional study, conducted in Germany according to local guidelines, with 3 monthly intravitreal injections of 0.5 mg ranibizumab followed by a maintenance phase of 9 months with re-injections on PRN basis showed that treatment with intravitreal injections of ranibizumab led to a moderate improvement of functional, morphological outcomes in patients with neovascular AMD maintained by a small number of ranibizumab injections (4.3/year). However, these outcomes were

overall much less favorable than what was achieved in the pivotal ranibizumab studies with fixed monthly follow-ups and monthly intravitreal injections or injections as needed based on protocol's strict guidelines. In this study, treatment outcomes were likely to be affected by deficiencies in regular monitoring and by the insufficient implementation of optimal diagnostic and retreatment criteria. (30)

In Greece there is limited data of the effect and safety of individualized treatment of patients with neovascular AMD with ranibizumab in routine clinical practice. Consequently, this 1-year observational multicenter study attempts to collect data on the effectiveness and safety of individualized treatment with ranibizumab in everyday clinical practice in a representative sample of Greek patients with treatment naive neovascular AMD. The study also aims to evaluate treatment patterns of ranibizumab administration in these patients treated outside the highly standardized phase III clinical trials.

1.3 Potential risks and benefits

This study is designed as an observational study where enrolled patients are treated with ranibizumab according to the approved indication of neovascular AMD included in the local product SPC. Therefore, the benefit/risk associated with participation in this study is expected to be similar to the one described in the local product label.

2 Study Objectives

The main objectives of this observational cohort study are to evaluate effectiveness of ranibizumab and also to evaluate safety and to describe follow-up as well as treatment patterns in treatment naive patients with neovascular AMD in routine clinical practice in Greece for the total study population.

2.1 Primary Objective

- To evaluate the functional effectiveness of ranibizumab treatment in treatment naive patients with neovascular AMD in routine clinical practice in Greece as assessed by mean change in VA from baseline to month 12.

2.2 Secondary Objectives

- To evaluate the safety of ranibizumab as assessed by the type, frequency and severity of ocular and non-ocular AEs over 12 months of treatment with intravitreal injections of ranibizumab for the treatment of neovascular AMD in treatment naive patients in routine clinical practice in Greece.
- To describe treatment patterns of ranibizumab administration for neovascular AMD in routine clinical practice in Greece.
- To evaluate the anatomic effectiveness of ranibizumab treatment of neovascular in treatment naive patients with neovascular AMD in routine clinical practice in Greece as observed in this 1-year study duration and assessed by mean change in CRT from baseline to month 12.

2.3 Study outcome variables

2.3.1 Primary Outcome Variable

2.3.1.1 Effectiveness variable

The primary efficacy variable will be evaluated with a functional parameter which is the mean change in VA from baseline visit to month 12 of treatment for the treated eye. Visual acuity will be measured according to the method used by each participating physician in their routine practice. In order to integrate different VA assessment methods for analysis, VA assessments performed using Snellen fraction or decimal score will be converted into an approximate ETDRS letter score equivalent.

2.3.2 Secondary Outcome Variables

2.3.2.1 Safety variables

The safety objective of this study will be assessed based on the incidence, relationship and severity of ocular AE in the ranibizumab treated eye and of non-ocular AE during 12 months of follow-up.

2.3.2.2 Treatment pattern variables

The number of ranibizumab injections administrated overall during treatment period, the number of visits and the average time interval (in weeks) between consecutive visits and injections will be summarized for the treated eye.

The reasons for the decision to treat or not (i.e. VA deterioration, OCT abnormality etc) will also be summarized in each visit. Reasons for treatment termination will be also evaluated.

Any topical use of antimicrobial agents pre- or post-injection will be evaluated.

2.3.2.3 Effectiveness variables

Effectiveness of ranibizumab treatment will also be measured by mean change in CRT as an anatomic parameter. Mean change in CRT from baseline visit to month 12 of treatment for the treated eye will be evaluated as indicated by OCT measurements. For consistency and analyses' purposes, it is recommended that the same method of assessment and the same type of OCT machine be used throughout the study wherever possible.

3 Study Design

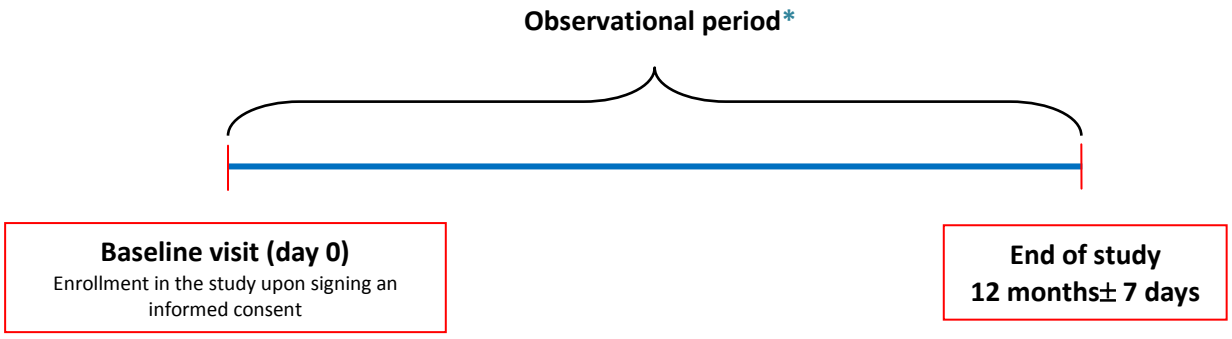
This study is designed as an observational, prospective cohort, multicenter, open label, single-arm 1-year study in treatment naive patients with neovascular AMD who initiate treatment with intravitreal injections of ranibizumab in Greece. The study will be conducted in 10 ophthalmological clinics and practices throughout Greece.

During the study period it is planned to collect valid documentations on treatment of neovascular AMD from a representative sample of approximately 251 patients who meet the specific for study eligibility criteria. All the collected data will be entered in electronic Case Report Forms (CRFs).

The study will be conducted by medical retina specialists who are employed in 10 Ophthalmology Departments of public hospitals or private eye centers of Greece. The patients will be enrolled in the study upon signing an informed consent. Eligibility criteria will be assessed, baseline characteristics information will be collected and entered in the electronic CRFs and patients will be treated according to local routine clinical practice. There will not be applied a strict, mandated visit schedule or a mandated treatment regimen in order to collect available data in a real life setting in patients with neovascular AMD who initiate treatment with intravitreal injections of ranibizumab. The follow-up visits will take place at a frequency defined as per investigator's discretion in line with the routine clinical practice of

participating investigators. It is advisable though that all visits during the study period to be documented in the CRF (Figure 1).

The overall planned duration of the study is anticipated to be 2 years. The enrollment period of subjects will continue until the required number of subjects will be enrolled or will last 12 months (whichever occurs earlier). Patients will be recruited from 10 Medical Retina Departments of outpatient Ophthalmology Clinics of public hospitals or private eye centers of Greece. Each enrolled patient will be followed for 12 months unless they discontinue or withdraw from the study. Every patient who successfully completes the study through Month 12 will be considered to have completed the study. A minimum of three visits per year is required in order to maintain a patient's participation in the study. The end of the study will be reached 12 months after last patient first visit and enrollment or when study participation for all patients is completed. No extension study is been planned.



*There will not be applied a strict, mandated visit schedule during the observational period.
The follow-up visits during observational period will take place at a frequency defined as per each investigator's discretion in line with their routine clinical practice.
At least another one visit between Day 0 and Month 12 is required in order to maintain a patient's participation in the study.

Figure 1: Schematic diagram of study design

This study is designed as a non-interventional study. Strict compliance with the following rules will ensure the non-interventional nature of this study:

- All the enrolled patients should be treated with ranibizumab for neovascular AMD according to the approved local SPC of ranibizumab.
- The decision of the investigator to administer ranibizumab to the patient with neovascular AMD will be taken based on current clinical practice and before evaluation of the patient's eligibility for inclusion in the study.
- Participating subjects will not undergo any other diagnostic, therapeutic or observational procedures than those usually applied by their ophthalmologist in line with routine clinical practice.

The study is mainly based on collection of primary data by the study investigators at a frequency determined by the individualized scheme of follow-up and treatment in routine clinical practice. Information on medical history and previous treatment of the disease will be collected from the patients' medical records. Prospective data will be obtained during the visits of the study through the interview with the patient, the routine ophthalmologic and imaging examinations and according to each investigator's clinical practice (See Section 5).

4 Population: study enrollment and withdrawal

The study population will consist of patients diagnosed with neovascular AMD at one eye and who have not received any prior treatment for the particular condition. The decision of the investigator to treat neovascular AMD with intravitreal injections of ranibizumab has to be taken before the evaluation of the patient's eligibility for inclusion in the study.

During the study period it is planned to collect valid documentations on treatment of neovascular AMD from a representative sample of approximately 251 patients who meet the specific for study eligibility criteria and are treated at outpatient level in 10 Ophthalmology Departments of public hospitals or private eye centers of Greece.

Eligible patients must fulfill **all** the inclusion criteria and **none** of the exclusion criteria.

4.1 Inclusion Criteria

Patients eligible for inclusion in this study must fulfill all of the following criteria:

- Patients willing and able to provide written informed consent personally or by legal proxy. Written informed consent must be obtained before any study assessment is performed.
- Patients willing to comply with all study procedures and to be available for the duration of the study.
- Male or female ≥ 50 years old patients.
- Patients with an eye diagnosed with neovascular AMD which has not received a ny treatment for neovascular AMD and is decided to initiate treatment with ranibizumab.
- Patients where the decision of administration of ranibizumab for the treatment of neovascular AMD in accordance with the local ranibizumab SPC has been taken before the evaluation of the patient's eligibility for inclusion in the study.
- One eye of each patient will be included in the study. If both eyes are eligible at Baseline visit the eye to be selected as the study eye is the one the investigator deems to be more appropriate for study treatment and the study, based on CNV lesion characteristics in addition to visual impairment. If the fellow requires treatment because of visual impairment due to neovascular AMD, it may also be treated at the discretion of the investigator. This eye, however, will not be included in the study. Any treatment administered in the fellow eye will be recorded in electronic CRF, but only the data for the study eye will be included in the analysis of outcomes of the study.

4.2 Exclusion Criteria

Patients fulfilling any of the following criteria are not eligible for inclusion in the study:

- Simultaneous participation in a study that includes administration of any investigational drug or procedure with interventions outside of routine clinical practice.
- Systemic treatment with any VEGF inhibitor within 6 months prior to study enrollment.
- Any prior therapy with any agent to treat neovascular AMD in the study eye.
- Patients who have any contraindications to the administration of ranibizumab according to the product SPC.
- Women of reproductive potential, defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during study period treatment (e.g. total abstinence, sterilization, barrier methods of contraception, hormonal contraception, intrauterine system).
- Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test.
- Men of reproductive potential unless using effective methods of contraception during study period (e.g. condoms).

4.3 Strategies for Recruitment and Retention

Each patient documented in the study will be selected only based on eligibility according to inclusion and exclusion criteria. No further selection should be applied. Patients will be recruited from outpatient ophthalmology clinics of 10 public hospitals or private eye centers of Greece. Data collection will occur as patients are enrolled into the study. Patients will be enrolled consecutively in order to avoid any selection bias and thus to increase the likelihood of representativeness.

No compensation will be provided for study participation.

4.4 Premature patient withdrawal from the study

Patients have the right to voluntarily withdraw from the study for any reason at any time. Patients may be considered withdrawn if they state an intention to withdraw, fail to perform at least three visits per year [including baseline and end of study (EOS) visits] or become lost to follow-up for any other reason.

Investigator has the right to terminate a subject's participation if he/she judges that this is for the best interest of the subject.

4.4.1 Reasons for Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request.

An investigator may terminate a study subject's participation in the study under the following circumstances:

- Withdrawal of study informed consent.
- If any medical condition, event or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Patient is not seen at the study center at least 3 times during the study period

4.4.2 Handling of Subject Withdrawals

If premature withdrawal occurs for any reason described above, the investigator must make every effort to determine the primary reason for a patient's premature withdrawal from the study and record this information on the Study Completion electronic CRF. However, patients are not obliged to justify their decision to withdraw.

For patients who are lost to follow-up (i.e. those patients whose status is unclear because they fail to appear for study visits without stating an intention to withdraw), the investigator should show "due diligence" by documenting in the source documents steps taken to contact the patient, e.g. dates of telephone calls, registered letters, etc.

Patient's withdrawal will not affect at any way their future medical care and treatment.

Patients who are prematurely withdrawn from the study will not be replaced by an equal number of newly enrolled patients.

4.5 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party. Should this be necessary, the patient should be seen as soon as possible and undergo all assessments for EOS as described by the study protocol. The investigator may be informed of additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the patient's interests. The principal investigator will be responsible to promptly inform institutional review boards (IRB) or independent ethics committees (IEC) of the early termination of the study and to provide the reason(s) for suspension or termination.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected significant or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable.
- Determination of futility.
- The study can be terminated at any time for any reason by the Sponsor.

5 Visit schedule, assessments and data collection

Table 5-1 lists all the data to be collected and entered in each patient's electronic CRF and indicates with an "X" the data when they are required to be collected (See also Figure 1).

There is no preset visit schedule, i.e. timing of actual patient visits is at the discretion of the treating physicians and sites will have the option of entering data from every patient visit during the study period. Physicians will be encouraged to follow-up with patients who have not been seen in the clinic for at least 3 months since the last visit to capture data as specified in Table 5-1.

If patients are not seen at least 3 times per year, they will be discontinued from the study.

All study visits should be documented in the electronic CRF. It is recommended that electronic CRFs are completed after every visit and at least within 1 month after the visit has occurred. However, whenever

any SAEs are detected, observed by or reported to the investigator at any time outside of a regularly scheduled visit, they have to be reported by the investigator according to Section 6.4.

Table 5-1 Recommended Data Collection Schedule

	Baseline	**Observation period**	**End of study**
Timing of data collection	**Day 0**	**At least one visit. Recommended at Every Visit.**	**12 months± 7 days**
Informed Consent	**X**		
Eligibility	**X**	**X**	
Demographics	**X**		
Non-ocular and ocular medical history/comorbidities	**X**		
Tobacco use history	**X**		
Prior and concomitant Systemic medications	**X**	**X**	**X**
Prior ocular treatments / Therapies	**X**	**X**	**X**
Visits performed between this and last documented visit that was not reported as part of the study		**X**	**X**
Ocular examination (e.g. Slit lamp examination, IOP)*	**X**	**X**	**X**
Visual acuity in ETDRS or Snellen (preferably BCVA)	**X**	**X**	**X**
Central retinal thickness	**X**	**X**	**X**
Ranibizumab treatment Given[#]	**X**	**X**	
Use of antimicrobials*	**X**	**X**	
SAEs[§]/AEs	**X**	**X**	**X**

* optional, according to the investigator's routine clinical practice

[#] treatment patterns according to each investigator's routine clinical practice

[§] whenever any SAEs are detected, observed by or reported to the investigator at any time outside of a regularly scheduled visit, have to be reported by the investigator according to Section 6.4.

5.1 Baseline Visit

5.1.1 Informed consent procedures

Eligible patients may only be included in the study after providing a written informed consent or, if incapable of doing so, after such consent has been provided by a legally acceptable representative of the patient. In cases where the patient's representative gives consent, the patient should be informed about the study to the extent possible given his/her understanding. If the patient is capable of doing so, he/she should indicate assent by personally signing and dating the written informed consent document or a separate assent form. Informed consent must be obtained before conducting any data collection (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the patient source documents.

5.1.1.1 Patient numbering

Each patient is identified by a unique patient identification code, which is only used for study purposes. This code is a number that results from the combination of his or her center number and patient number. Each study center will be identified with a two digit center number. The center number is assigned by the Contract Research Organization (CRO) to the study site.

Upon signing the informed consent form, the patient is assigned a patient number by the investigator. At each study site, the first patient is assigned patient number 1 and subsequent patients are assigned consecutive numbers (e.g., the second patient is assigned patient number 2, the third patient is assigned patient number 3 etc). Once assigned to a patient, a patient number will not be re-used.

For the duration of the study and afterwards, only the patient's investigator is able to identify the patient based on the patient identification code.

5.1.2 Verification of patient's eligibility

Determine patient's eligibility based on inclusion/exclusion criteria. See sections 4.1 and 4.2

5.1.2.1 Information to be collected on screening failures

Not applicable.

5.1.3 Patient demographics and other baseline characteristics

At baseline, patients' demographic variables and information about their medical and ocular history, co-morbidities and concomitant medication will be collected from the treating physician. The investigator collects data regarding patient's medical and ocular history from medical records if available, or else by interviewing the patient. More precisely, the following data elements will be collected at baseline:

- Date of visit (DD/MM/YYYY)
- Demographic data (e.g., date of birth, gender, race/ethnicity)
- Non-ocular medical history/comorbidities (based on historical data, current treatment, or clinical judgment), including
    - Cardiovascular/Cerebrovascular events, including
        - Myocardial infarction
        - Stroke
        - Thromboembolic event
    - Obesity
    - Family history of coronary artery disease
    - Hypercholesterolemia/hyperlipidemia
    - Hypertension
    - Diabetes
- Ocular Medical History
    - Date of diagnosis of neovascular AMD leading to inclusion in the study
    - Eye diagnosed with neovascular AMD
    - Type of CNV
    - Study eye selection in case of bilateral neovascular AMD
    - Any ocular surgeries and date of surgery
    - Any other eye diseases and conditions
- Smoking history

- Prior and concomitant systemic medications (all medications started prior to commencement of ranibizumab and in addition to ranibizumab respectively). Information on concomitant medication to be collected includes:
  - Name of drastic substance
  - Indication
  - Start date
  - Stop date or "continued"
  - Dosage
- Any prior or concomitant ocular treatments including
  - Indication
  - Eye(s) treated
  - Name of drastic substance
  - Start date
  - Stop date or "continued"
  - Dosage

All the relevant information has to be entered in the Demographics and Medical History/Comorbidities pages of the electronic CRFs. Drugs administered prior start of the study and other drugs continuing or started during the study period will be entered on the Prior and concomitant medications electronic CRF page.

5.1.4 Ocular examination

Ocular examination of the study eye including slit-lamp examination, fundoscopy and IOP measurement are considered optional and may according to the investigator's routine clinical practice. If available, the collected data will be entered in the Ocular Examination page of the electronic CRF.

5.1.5 Visual acuity

Effectiveness of ranibizumab treatment will be described by change in VA of the study eye from baseline. Effectiveness cannot be described if VA at baseline is missing. Therefore it is recommended to document VA in the Visual Acuity page of the electronic CRF at the Baseline visit.

Visual acuity (preferably BCVA) of the study eye will be measured according to the method used by the treating physician in the course of local routine care. Visual acuity will be captured in the electronic CRF, allowing use of ETDRS-like or Snellen charts. If a Snellen fraction or decimal score is entered, it will be converted into an approximate ETDRS equivalent letter score for the purpose of statistical analysis. Therefore, it is recommended that ETDRS-like sight charts are used if available.

For consistency and analyses, it is recommended that the same method of VA assessment be used throughout the study wherever possible. Progression or no progression of the disease activity will be evaluated at each visit from study entry.

5.1.6 Central retinal thickness as evaluated with OCT

In addition to VA, effectiveness of ranibizumab treatment will also be measured anatomically by change in CRT in the study eye as this is evaluated with OCT.

Effectiveness regarding this anatomical parameter cannot be evaluated if CRT value at baseline is missing. Therefore it is recommended to document it in the OCT page of the electronic CRF at the Baseline visit. Whenever CRT is measured with the OCT it is recommended to enter the data in the relevant page of each visit in the electronic CRF.

For consistency and analyses' purposes, it is recommended that the same method of assessment and the same type of OCT machine be used throughout the study wherever possible.

5.1.7 Treatment

This is an open-label, observational study to assess effectiveness of ranibizumab treatment of neovascular AMD in routine clinical practice. There is no therapy being prescribed or dispensed as part of this study. This observational study does not direct therapy nor recommends any treatment for neovascular AMD. All the patients enrolled will receive ranibizumab as treatment for neovascular AMD. The only study's recommendation for dose, frequency, or duration of treatment is that patients should be treated with ranibizumab according to the approved local ranibizumab SPC. The decision on the duration and frequency of treatment is solely at the discretion of the attending investigator.

In Greece Lucentis® is available as 10 mg/ml ranibizumab solution for injection in a single-use vial or as a 10 mg/ml solution for injection in a pre-filled syringe for single use. It is at the discretion of the investigator to chose or not the pre-filled syringe.

Information regarding ranibizumab administration during the study will be collected on the Drug Administration page of the electronic CRF. The volume injected and type of the drug formulation administered will also be recorded. Any topical administration of antimicrobial agents pre- or post-injection is optional, depending on the investigator's preference and will be reported.

5.1.8 Adverse events and serious adverse events

Safety evaluations will comprise of the monitoring and assessment of all systemic and ocular AE occurring during the study. Any relationship of an AE with the study drug will be assessed and indicated by the investigator.

These relevant data will be collected according to the schedule detailed in Table 5-1 and are required to be entered in the Adverse Event page of the electronic CRF. See section 6.

5.2 Observational period

There is no preset visit schedule during the observational period. The visit frequency should be dictated by the local standard of care as this is determined by each investigator individually at each site. At least one visit between Baseline and EOS visit is required to be performed and reported in order to maintain a patient's participation in the study. However, it is recommended to report the data from every visit that will take place during the observational period.

Typical information to be collected during a visit during observational period includes:

- Date of follow-up visit (DD/MM/YYYY)
- Confirmation of patient's eligibility based on inclusion/exclusion criteria.
- Concomitant medications (systemic and ocular)
- Any visits performed between this and last documented visit that was not reported as part of the study
    - Number of visits
    - Date of visit (precise or approximate)
    - Administration of treatment or not
- Ophthalmic examination
- Visual acuity

- Central retinal thickness as evaluated with OCT
- Administration of ranibizumab or not. The reason for administration or not of ranibizumab (i.e., VA, OCT findings, or a combination of the above) must be described on the electronic CRF. See section 5.1.7.
- Adverse events and serious adverse events. See section 6.

5.3 End of study visit

The EOS visit has to be performed at 12 months ± 7 days from baseline visit. Typical information to be collected at EOS visit includes:

- Date of follow-up visit (DD/MM/YYYY)
- Concomitant medications (systemic and ocular)
- Any visits performed between this and last documented visit that was not reported as part of the study
    - Number of visits
    - Date of visit (precise or approximate)
    - Administration of treatment or not
- Ophthalmic examination
- Visual acuity
- Central retinal thickness as evaluated with OCT
- Adverse events and serious adverse events. See section 6.

5.4 Withdrawal Visit

Not applicable.

5.5 Unscheduled Visit

Not applicable.

5.6 Other study procedures/evaluations

5.6.1 Laboratory Procedures/Evaluations

Not applicable.

5.6.2 Questionnaire Administration

Not applicable.

6 Safety monitoring and assessment

The safety recording period starts after providing informed consent from the patient.

6.1 Adverse event

An AE is the appearance of any sign(s), symptom(s), or medical condition(s) or the worsening of any pre-existing undesirable sign(s), symptom(s), or medical condition(s) occurring after signing the informed consent during the safety recording period even if the event is not considered to be related to ranibizumab.

The occurrence of AEs should be sought by non directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments provided by the patient. Clinically significant abnormal laboratory values or test results should be identified through a review of values outside of normal ranges or clinically notable ranges, significant changes from Baseline or the previous visit, or values which are considered to be non-typical in patient with underlying disease. Investigators have the responsibility for managing the safety of an individual patient and identifying clinically significant AEs.

Once an AE is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each routine visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to ranibizumab, the interventions required to treat it, and the outcome.

Physicians will be encouraged to follow-up with patients who have not been seen in the clinic for at least 3 months since the last visit to capture any AEs that may have occurred since the previous visit, and again 3 months later if no subsequent visit occurs within the following 3 months.

Information about common side effects and adverse reactions observed with ranibizumab can be found in the approved ranibizumab SPC. This information will be also included in the patient informed consent and should be discussed with the patient during the study as needed.

6.2 Reporting an adverse event

All AEs occurring during the safety recording period will be collected and -irrespective of any suspected causal association to ranibizumab administration- be recorded in the Adverse Event Report Form which is appended to the electronic CRF within 7 calendar days of awareness. Report of an AE should be accompanied by the following information:

- severity grade (mild, moderate, severe)
- location (non-ocular, left eye, right eye, both eyes)
- relationship to ranibizumab or to the procedure of the ocular injection (suspected or not suspected). Possibly related means that there is a reasonable possibility that the incident, experience, or outcome of the AE may have been caused by the ocular injection or ranibizumab.
- duration (start and end dates or if continuing at final examination)
- whether it constitutes an SAE
- action taken
    - no action taken (i.e., further observation only)
    - study drug dosage adjusted or temporarily interrupted
    - study drug permanently discontinued due to this AE
    - concomitant medication given
    - non-drug therapy given
    - patient hospitalized or patient's hospitalization prolonged.

6.3 Serious adverse event

As SAE defined an AE which:

- results in death
- is life-threatening (places the subject at immediate risk of death from the event as it occurred)

- results in persistent or significant disability or incapacity
- constitutes a congenital anomaly or birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting requirements; see Section 6.4.

6.4 Serious adverse event reporting

To ensure patient safety, **every SAE**, regardless of suspected causality, occurring after the patient has provided informed consent and until 30 days after the patient has stopped study participation (defined as time of last dose of ranibizumab taken during the study or last visit whichever is later) **must be reported to the Sponsor within 24 hours of learning of its occurrence**. Any SAEs experienced after this 30 day period should only be reported to the Sponsor if the investigator suspects a causal relationship to ranibizumab. Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form. The investigator must assess the relationship of any SAE to ranibizumab, complete the SAE Report Form in English and send by fax or e-mail the completed, signed form within 24 hours of learning of its occurrence to Sponsor. The original copy of the SAE Report Form and the fax confirmation sheet or email receipt confirmation must be kept with the case report form documentation at the study site. A SAE that is considered completely unrelated to a previously reported one should be reported separately as a new event.

The outcome of all reported SAEs (resolution, death etc.) will be followed up and documented. Recurrent episodes, complications or progression of the initial SAE must be reported as follow-up to the original episode, regardless of when the event occurs. Follow-up information is sent within 24 hours of the investigator receiving the follow-up information using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

If the SAE is unexpected, i.e. not previously documented in the local product SPC and is thought to be related to ranibizumab, the Sponsor may urgently require further information from the investigator for Health Authority reporting.

6.5 Pregnancies

A female patient must be instructed to stop taking ranibizumab if she becomes or intents to become pregnant during the study.

To ensure patient safety, all pregnancy cases in patients treated with ranibizumab must be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the Sponsor within 24 hours of learning of their occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal or newborn complications. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to ranibizumab of any pregnancy outcome.

Any SAE experienced during pregnancy must be reported on the Serious Adverse Event Report Form; see Sections 6.3 and 6.4.

7 Data review and database management

All activities described in this section will be performed by the CRO on behalf of the Sponsor. A CRO will be selected and assigned for the Electronic Data Capture (EDC) system development. The electronic CRFs will be part of the EDC system which allows documentation of all outcome variables and covariates by all participating sites in a standardized way.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by the CRO contractor.

7.1 Site monitoring

Clinical site monitoring is conducted to ensure that the rights of human subjects are protected, that the study is implemented in compliance with the protocol and in accordance with the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP), with the ethical principles laid down in the Declaration of Helsinki, with the Greek National Organization of Medicines (EOF) regulations and that the quality and integrity of study data and data collection methods are maintained. All visits according to CMP will take place at the study sites.

Before study initiation, at a site initiation visit, the CRO representative will review the protocol and CRFs with the investigators and their staff. Entrance of data to the electronic CRFs will be conducted by the treating investigator or by a designated person. During the study, the field monitor may visit the site according to the CMP, to evaluate study processes, check the completeness of patient records, the accuracy of entries on the electronic CRFs, the adherence to the protocol, to ICH guidelines for GCP, to the ethical principles laid down in the Declaration of Helsinki and to the EOF regulations. Documentation of SAEs and the progress of enrollment will be also evaluated. Key study personnel must be available to assist the field monitor during these visits.

The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, and the results of any other tests or assessments. All information on electronic CRFs must be traceable to these source documents in the patient's file. The investigator must also keep the original informed consent form signed by the patient (a signed copy is given to the patient). The investigator must give the monitor access to all relevant source documents to confirm their consistency with the electronic CRF entries. Checks of the consistency of the source data with the electronic CRFs are performed according to the study-specific CMP. No information in source documents about the identity of the patients will be disclosed.

Staff from the CRO contractor will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the CMP. Documentation of monitoring activities and findings will be provided to the Sponsor, the site study team and the study PIs. The Sponsor reserves the right to conduct independent audits as necessary. The observations and findings of an audit should be documented.

7.2 Data collection

Sites are required to enter study information in electronic CRFs. Designated investigator staff will enter the data required by the protocol into the electronic CRFs after appropriate training.

Automatic validation programs check for data discrepancies and, by generating appropriate error messages, allow the data to be confirmed or corrected before transfer of the data to the CRO. The Investigator must certify that the data entered into the electronic CRFs are complete and accurate.

7.3 Data management and quality control

The CRO working on behalf of the Sponsor will review the data entered into the electronic CRFs by investigational staff for completeness and accuracy and instruct the site personnel to make any required corrections or additions. Queries are sent to the investigational site using an electronic data query. Designated investigator site staff is required to respond to the query and confirm or correct the data.

7.4 Planned database locks

No interim analyses are planned in the study. Consequently, there will be no other database lock (DBL) than the final DBL.

8 Data Analysis

The statistical section below describes analyses as planned for the study. The statistical analyses will be performed by the designated CRO.

The study is not aimed to confirm or reject pre-defined hypotheses.

Continuous variables will be summarized with the use of descriptive statistical measures (mean value, standard deviation, median, minimum and maximum values) and categorical/distinct variables will be displayed as frequency tables (N, %).

If applicable, the analyses will be stratified by baseline factors. Baseline patient characteristics that may be used for subgroup analyses, providing that the number of patients per subgroup allow for meaningful inferences, include but are not limited to the following: age, smoking status, concomitant diseases, type of CNV etc.

The study eye will be defined as the eye for which treatment with ranibizumab is initiated and analyses will be done for the study eye only.

The analysis of adverse events will be based on observed data.

Complete analytical specifications will be fully detailed in the statistical analysis plan (SAP). The SAP will be finalized before study DBL and will specify any performed analyses that will be stratified for baseline factors.

A level of $p < 0.05$ will be considered to be statistically significant.

8.1 Analysis sets

The enrolled set will include all patients having signed the informed consent, and with at least a baseline assessment.

The Per Protocol Set will consist of all patients who complete the study without any major protocol deviations.

The study eye set will include all study eyes treated with ranibizumab.

In accordance to the non-interventional design of the study, all statistical analyses with the exception of the primary objective will be performed in all eligible subjects with available data who have been enrolled into the study, regardless of whether or not they have finally completed their projected participation in the study.

8.2 Patient demographics and other baseline characteristics

Descriptive statistics will be provided for patient demographics and all baseline characteristics (including the baseline values of the primary and secondary efficacy variables). Relevant medical history (ocular and non-ocular), current medical conditions will be tabulated by site (non-ocular, ocular (study/fellow eye). Other relevant baseline information like tobacco use will be listed and summarized as appropriate with descriptive statistics.

Statistical analyses will be based on the enrolled set.

8.3 Primary Effectiveness Outcome Analyses Variables

The analysis of the primary objective of the study will be performed in the per protocol set with available data pertaining to the study primary endpoint, i.e. baseline and 12-month VA data.

The mean change in VA from baseline to month 12 along with the 95% confidence interval (CI) will be calculated for subjects completing 12 months of follow-up. Paired sample t test will be applied to compare the 12-month VA to Baseline VA.

8.4 Analysis of secondary variables

8.4.1 Safety variables

Non-ocular adverse events by patient and ocular adverse events by study eye will be evaluated. The number and proportion of patients experiencing any serious AE and the number and proportion of patients experiencing any non-serious AE will be calculated and presented in frequency tables.

Frequency tables will be presented for AEs by causality, seriousness, discontinuation of therapy, action taken and outcome.

The safety objective will be interpreted with respect to the related number of treatments.

8.4.2 Treatment pattern variables

The treatment pattern variables will be evaluated using summary statistics applied on the enrolled set.

The number of ranibizumab injections administrated overall during treatment period, the number of visits and the average time interval (in weeks) between consecutive visits and injections will be summarized for the treated eye.

The reasons for the decision to treat or not (i.e. VA deterioration, OCT abnormality etc) will also be summarized in each visit. Reasons for treatment termination will be also evaluated.

Any topical use of antimicrobial agents pre- or post-injection will be evaluated.

8.4.3 Effectiveness variables

Effectiveness of ranibizumab treatment will also be measured by mean change in CRT as an anatomic parameter. Statistical analyses will be performed in the enrolled set and will be based on observed data and, in case of missing data, Last Observation Carried Forward method.

The mean change in CRT, as indicated by OCT measurements, from baseline to month 12 along with the 95% CI will be calculated. Paired sample t test will be used to compare the 12-month CRT to Baseline CRT.

8.5 Sample size calculation

The sample size has been estimated based on the primary outcome of the study, i.e., the mean change of VA from baseline to 12 months after the initiation of treatment with ranibizumab.

The sample size calculation was conducted with the precision-based sample size calculation.

The 95% CI of the mean change in VA from Baseline to Month 12 was estimated with a precision of about ±2 letters (i.e. with a margin of error of 2 letters), that means that we required the 95% CI to be no wider than 4 letters. A search in published data indicated that, based on the results from the phase IV TWIN Study, the estimated standard deviation for the change in VA noted at 12 months of treatment with ranibizumab 0.5 mg in daily clinical practice was 15.4 letters (31).Thus we would have sufficient precision if we enrolled at least 228 patients.

In order to compensate for a potential dropout rate of 10%, a total number of 251 patients should be enrolled in the study.

8.6 Power for analysis of key secondary variables

Not applicable.

8.7 Interim analyses

No interim analyses are planned for the study.

9 Ethical and regulatory considerations

9.1 Regulatory and ethical compliance

This study is an observational study where the treatment decision falls within current practice and the prescription of the medicines is clearly separated from the decision to include the patient in the study. No additional diagnostic or monitoring process is required for participation or during the study.

The study was designed and will be conducted in compliance with the protocol and in accordance with the ICH guidelines for GCP, with the ethical principles laid down in the Declaration of Helsinki and with the EOF regulations.

Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing patient data.

9.2 Responsibilities of the investigators and IRB/IEC

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted IRB or IEC before study start. A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC must be given to the Sponsor before study initiation where applicable. Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to the CRO's monitors, auditors, designated agents of the Sponsor, IRBs or IECs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform the Sponsor and/or CRO immediately that this request has been made.

Any amendment to the protocol will require review and approval by the IRB/IEC before the changes are implemented in the study.

9.3 Informed consent procedures

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to participants and their families, if applicable. A consent form written in Greek language, must comply with the ICH GCP guideline and regulatory requirements and describe in detail the study procedures and risks. The consent form will be provided by the CRO to the study sites after IRB- or IEC-approved and be given to the participant. The participant is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The process of obtaining informed consent should be documented in the patient source documents.

9.4 Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff and the Sponsor and its agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

The CRO's monitors, auditors, designated agents of the Sponsor, IRBs or IECs, and regulatory authorities may inspect all study documents and patient records and source documents required to be maintained by the investigator. The clinical study site will permit access to such records.

10 Data handling and record keeping

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study participants and source documentation.

10.1 Storage of source documents and archiving

Study staff will maintain appropriate medical and research records for this study, in compliance with ICH GCP guidelines and with topical regulatory and institutional requirements for the protection of confidentiality of subjects. Study staff will permit authorized representatives of CRO and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

After DBL, the investigator will receive a CD-ROM or paper copies of the patient data entered in the electronic CRFs for archiving at the study site.

The PI will make sure that all relevant documents and source documents of this observational study will be stored at a secure and locked place in the site after end or discontinuation of the study according to the local regulation.

11 Protocol adherence

This observational study protocol does not direct therapy or dictate any treatment other than that patients be treated in accordance with the ranibizumab approved local SPC.

11.1 Protocol Amendments

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the Sponsor, Health Authorities and the IRB or IEC, where required. Only amendments that are required for patient safety may be implemented prior to IRB or IEC approval. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any patient included in this study, even if this action represents a

deviation from the protocol. In such cases, the Sponsor and the CRO should be notified of this action and the IRB or IEC at the study site should be informed within 10 working days.

11.2 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol. The noncompliance may be on the part of the subject, the investigator or study staff. Investigators ascertain they will apply due diligence to avoid protocol deviations. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly. All significant protocol deviations will be recorded and reported in the clinical study report.

12 Publication of results

Sponsor assures that the key design elements of this protocol will be posted in a publicly accessible database. In addition, upon study completion and finalization of the study report the results of this trial will be either submitted for publication and/or posted in a publicly accessible database of clinical trial results. No individual investigator may publish on the results of this study without prior approval from the sponsor.

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